European Commission

Combined Draft Renewal Assessment Report prepared according to Regulation (EC) N° 1107/2009
and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

LENACIL

Volume 1

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.

Table of contents

Level 1

LENACIL

1 STATEMENT OF SUBJECT MATTER AND PURPOSE FOR WHICH THIS REPORT HAS BEEN PREPARED AND BACKGROUND INFORMATION ON THE APPLICATION

1.1 CONTEXT IN WHICH THIS DRAFT ASSESSMENT REPORT WAS PREPARED

1.1.1 Purpose for which the draft renewal assessment report was prepared

The Draft Renewal Assessment Report (DRAR) has been prepared in the framework of the evaluation of the application for **renewal of the approval** of lenacil according to Reg. (EU) No 844/2012. Lenacil is part of the AIR-3 programme.

A proposal for **MRL setting** is not included.

A fully revised proposal for **Classification & Labelling** is not included, because relatively recently, a CLH report (dated October 2012) had already been submitted by Belgium to the Committee for Risk Assessment (RAC). The CLH report was made publicly available on 15 May 2013 and the RAC opinion on the proposed harmonized classification and labelling was adopted on 5 December 2013 (CLH-O-0000002461-82-02/F). The **classification** for lenacil was confirmed by the ECHA and adopted with Commission Reg. (EU) No 2015/1221 of 24 July 2015, amending Reg. (EC) No 1272/2008.
A change of classification and labelling is not deemed necessary by the RMS for most of the parameters. Only for the aquatic environment, acute, a slight change was identified: lenacil remains classified as aquatic acute category 1, but the M-factor should be changed from 10 to 100. This is only a minor change, which is not considered by the RMS to necessitate the initiation of a procedure to update the Harmonised Classification

1.1.2 Arrangements between rapporteur Member State and co-rapporteur Member State

The RMS is Belgium (BE), the co-RMS is Austria (AT). Before submission of the DRAR to the EFSA, draft versions have been provided to AT for commenting. Comments have been taken into account and agreements or disagreements have been indicated in the DRAR, where relevant.

1.1.3 EU Regulatory history for use in Plant Protection Products

- Lenacil was first reviewed in the EU as part of the third stage (part B) of the work programme for review of existing active substances as provided for in art. 8(2) of **Council Directive 91/414/EEC** (and amending regulations Reg. (EC) No 451/2000 and Reg. (EC) No 1490/2002). In that context, Schirm GmbH (transferred from Du Pont de Nemours S.A.S.) was the main data submitter and the designated Rapporteur Member State (RMS) was Belgium (BE, 2007).

  Lenacil was first approved in the EU by means of **inclusion in Annex I** of Council Directive 91/414/EEC on 1 January 2009 (by Commission Directive 2008/69/EC), without having undergone a peer review organised by EFSA. This decision was pursuant to Article 11b of Reg. (EC) No 1490/2002, as last amended by Reg. (EC) No 1095/2007. It was based on an examination by the European Commission of the Draft Assessment Report and the recommendations of the RMS and taking into account comments from other Member States. On the basis of this examination, it was concluded that there are clear indications that it may be expected that the active substance lenacil does not have any harmful effects on human or animal health or on groundwater or any unacceptable influence on the environment, as defined by the criteria set out in Annex VI of Reg. (EC) No 1095/2007. For further details, reference is made to the EU Review Report for the active substance lenacil (SANCO/833/08 – rev.2, 7 March 2008).

- Following the inclusion in Annex I of 91/414/EEC, a full **scientific EU peer review** was organized by EFSA, resulting in an EFSA conclusion on the peer review of the active substance lenacil (**EFSA, 2009**).

  Taking into account the EFSA conclusions, the inclusion of lenacil in Annex I of 91/414/EEC was confirmed by Commission Directive 2010/39/EU, though with **amendment of the specific provisions and conditions**, in particular a **request to submit further confirmatory information**. For further details, reference is made to the revised EU Review Report for the active substance lenacil (SANCO/833/08 – rev. 3, 11 May 2010).

- Lenacil has been deemed to be approved under **Reg. (EC) No 1107/2009**, in accordance with Commission Implementing Reg. (EU) No 540/2011, as amended by Commission Implementing Reg. (EU) No 541/2011.

- The requested **confirmatory information** (see above), which concerned Environmental Fate and Behaviour (E-Fate) and Residues, was submitted by Schirm GmbH, assessed by RMS Belgium (BE, 2012; BE, 2013) and subjected to an EU peer review. EFSA delivered a conclusion on the peer review of the confirmatory information related to E-Fate (**EFSA, 2013**), while for residues, no specific EFSA conclusion is available (only a reporting table collating all comments and RMS's responses). Eventually, it was agreed by the European Commission and Member States that the request for confirmatory information had been satisfactorily addressed: see revised EU Review Report for the active substance lenacil (SANCO/833/08 – rev. 4, 16 May 2014).

- During the EU review process, lenacil was proposed for classification as Carcinogen Category 2. Thus, also the following specific provisions were included in Regulation (EU) No 540/2011: *"If a decision on the classification of lenacil under Regulation (EC) No 1272/2008 of the European Parliament and of the Council identifies the need for further information on the relevance of the metabolites IN-KE 121, IN-KF 313, M1; M2, M3, Polar B and Polars, the Member States concerned shall request the submission of such information. They shall ensure that the notifier provides that information to the Commission within six months from the notification of such a classification decision."*

  The **classification** proposal (Carc. 2 – H351) for lenacil was confirmed by the ECHA and adopted with Commission Reg. (EU) No 2015/1221 of 24 July 2015, amending **Reg. (EC) No 1272/2008**. Further **confirmatory information on the toxicological relevance of groundwater metabolites** was submitted by the applicant DuPont and assessed by RMS Belgium (BE, 2016). The RMS's assessment was subjected to a consultation process (commenting by MSs, the applicant and EFSA), which was summarized in the EFSA Technical Report (**EFSA, 2016**).
  At the moment of finalizing the first version of the DRAR, a decision related to this additional assessment had not yet been taken at SCPAFF level.

- Existing **EU MRLs** for lenacil have not yet been reviewed by EFSA in the framework of art.12 of **Regulation (EC) No 396/2005**. The MRL review is foreseen to be (re)launched after renewal of a.s. approval of lenacil.

- Note with regard to **applicant** and **data ownership**: DuPont de Nemours (Deutschland) GmbH applied in due time (21-12-2015) for the renewal of approval of lenacil (cf. SANCO/10148/2014). On November 1, 2017, a significant portion of DuPont's Crop Protection business was acquired by FMC Corporation, including the assets associated with the active substance lenacil. By letters dated 22 February 2018 and 5 December 2018, FMC notified to the European Commission (and MSs) that the responsibilities of EU approval activities for *a.o.* the active substance lenacil, as well as all other associated assets (incl. including registration, trademarks, patents, data and know-how), have been transferred to FMC. Therefore, the applicant has changed to FMC Agricultural Solutions A/S (which is a secondary name to Cheminova A/S), Thyborønvej 78, 76373 Harboøre, Denmark. Data owner for all data submitted on lenacil is FMC International Switzerland Sàrl (FISSarl), Quai de l'Ile 13, CH-1204 Geneva, Switzerland. Thus, FMC is to be regarded as the main data submitter.

- The initial expiry date of approval for lenacil (31/12/2018) was extended with 1 year (to 31/12/2019) by Commission Implementing Regulation (EU) No 2018/1796, to allow for sufficient time to finalise the EU peer review and decision making on a possible renewal of the approval.

1.1.4 Evaluations carried out under other regulatory contexts

Lenacil has not yet been evaluated by the **JMPR** (Joint FAO/WHO Meeting on Pesticide Residues) or **JMPS** (Joint Meeting on Pesticide Specifications).

1.2 APPLICANT INFORMATION

1.2.1 Name and address of applicant(s) for approval of the active substance

FMC Agricultural Solutions A/S
Thyborønvej 78,
7673 Harboøre,
Denmark

Previously:
DuPont de Nemours (Deutschland) GmbH

Bereich Pflanzenschutz
Hugenottenallee 175
63263 Neu-Isenburg
Germany

Note: Lenacil from DuPont was totally acquired by FMC, who is thus to be regarded as the main data submitter for the renewal of the active substance.
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).

1.2.2 Producer or producers of the active substance

Lenacil is manufactured by the registered source (see Vol. 4) for :

FMC International Switzerland Sárl
Quai de I´lle 13
CH-1204 Geneva
Switzerland

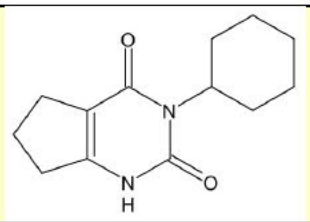
Previously:
DuPont International Operations Sàrl
2, chemin du Pavillon
P.O. Box. 50
CH-1218 Le Grand-Saconnex
Geneva, Switzerland

1.2.3 Information relating to the collective provision of dossiers

Not relevant.

1.3 IDENTITY OF THE ACTIVE SUBSTANCE

1.3.1 Common name proposed or ISO-accepted and synonyms	Lenacil
1.3.2 Chemical name (IUPAC and CA nomenclature)	
IUPAC	3-cyclohexyl-1,5,6,7-tetrahydrocyclopentapyrimidine-2,4(*3H*)-dione
CA	3-cyclohexyl-6,7-dihydro-1H-cyclopentapyrimidine-2,4(*3H,5H*)-dione
1.3.3 Producer's development code number	DPX-B0634 (synonym : B10048563)
1.3.4 CAS, EEC and CIPAC numbers	
CAS	2164-08-1
EEC	218-499-0 (EINECS)
CIPAC	163
1.3.5 Molecular and structural formula, molecular mass	
Molecular formula	$C_{13}H_{18}N_2O_2$

Structural formula	
Molecular mass	234.3 g/mol
1.3.6 Method of manufacture (synthesis pathway) of the active substance	Confidential information, for details please refer to the confidential Vol.4.
1.3.7 Specification of purity of the active substance in g/kg	The minimum purity is 975 g/kg. For details please refer to the confidential Vol.4.
1.3.8 Identity and content of additives (such as stabilisers) and impurities	
1.3.8.1 Additives	Confidential information, for details please refer to the confidential Vol.4.
1.3.8.2 Significant impurities	Confidential information, for details please refer to the confidential Vol.4.
1.3.8.3 Relevant impurities	Impurities (potentially) relevant have been identified. For details please refer to the confidential Vol.4.
1.3.9 Analytical profile of batches	Confidential information, for details please refer to the confidential Vol.4.

1.4 INFORMATION ON THE PLANT PROTECTION PRODUCT

1.4.1 Applicant	**FMC Agricultural Solutions A/S** Thyborønvej 78, 7673 Harboøre, Denmark **Previously:** **DuPont de Nemours (Deutschland) GmbH** Bereich Pflanzenschutz Hugenottenallee 175 63263 Neu-Isenburg Germany (a) Contact: [redacted] Telephone No: [redacted] E-mail: [redacted] (b) Alternative: [redacted] Telephone No.: [redacted] E-Mail: [redacted]

1.4.2	**Producer of the plant protection product**	Manufacturer of the Plant Protection Product (legal entity) **FMC International Switzerland Sárl** Quai de I´lle 13 CH-1204 Geneva Switzerland Affiliates or representatives: **FMC Agricultural Solutions A/S** Thyborønvej 78, 7673 Harboøre, Denmark Contact person: [redacted] Telephone No: [redacted] E-mail: [redacted] Location of the manufacturing site of the Plant Protection Product CONFIDENTIAL information – please refer to Vol. 4. Manufacturer of the lenacil (legal entity) **FMC International Switzerland Sárl** Quai de I´lle 13 CH-1204 Geneva Switzerland Affiliates or representatives: FMC Agricultural Solutions A/S Thyborønvej 78, 7673 Harboøre, Denmark Contact person: [redacted] Telephone No: [redacted] E-mail: [redacted] Location of the manufacturing plant: CONFIDENTIAL information – please refer to Vol. 4.
1.4.3	**Trade name or proposed trade name and producer's development code number of the plant protection product**	Code number: DPX-B0634 500 SC, LENACIL 500 G/L SC Trade names: VENZAR® 500SC VENAR® SC
1.4.4	**Detailed quantitative and qualitative information on the composition of the plant protection product**	

1.4.4.1 Composition of the plant protection product

Pure active substance

Content of pure a.s. lenacil:	500 g/L	43.86 % w/w *
Limits (±5%):	475 – 525 g/L	41.67 – 46.05 % w/w *

Technical active substance

Content of technical a.s. lenacil:	512.82 g/L	44.98 % w/w *
Limits (±5%):	487.2 – 538.5 g/L	42.74 – 47.24 % w/w *

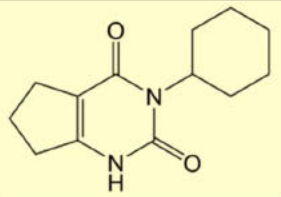
* contents are calculate with a product density of 1.140g/cm³

At a minimum of the technical active substance of 97.5% w/w.

Safeners, synergists and co-formulants

CONFIDENTIAL information – please refer to Vol. 4.

1.4.4.2 Information on the active substances

Type	**Name/Code Number**
ISO common name	LENACIL
IUPAC name	3-cyclohexyl-1,5,6,7-tetrahydrocyclopentapyrimidine-2,4(3H)-dione
CA name	3-cyclohexyl-6,7-dihydro-1Hcyclopentapyrimidine-2,4(3H,5H)-dione
CAS No	2164-08-1
EC No	218-499-0 (EINICS)
CIPAC No	163
Molecular formula	$C_{13}H_{18}N_2O_2$
Structural formula	
Molecular mass	234.29 g/mol
Salt, ester anion or cation present	The active substance is not present in the formulation in the form of a salt, ester, anion or cation.

1.4.4.3 Information on safeners, synergists and co-formulants

CONFIDENTIAL information – please refer to Vol. 4.

1.4.5 Type and code of the plant protection product

Type: Suspension concentrate [Code: SC].

1.4.6 Function

Herbicide

1.4.7 Field of use envisaged

Agriculture (field conditions only)

1.4.8 Effects on harmful organisms

Potent direct inhibitor of photosynthesis activity at chloroplast level.

1.5 DETAILED USES OF THE PLANT PROTECTION PRODUCT

1.5.1 Details of representative uses (lenacil*)

Crop and/or situation (a)	Member State	Product Name	F G I (b)	Pests or group of pests controlled (c)	Formulation		Application				Application rate per treatment			PHI (days) (l)	Remarks (m)
					Type (d-f)	Conc of a.i. (i)	Method kind (f-h)	Growth stage and season (j)	Number min max (k)	Interval between applications (min)	g a.i./hl min max (g/hL)	Water L/ha min-max	g a.i./ha min max (*) (g/ha)		
Sugar and fodder beet (BEAVA, BEAVC)	EU Central Zone	Lenacil 500 g/L SC	F	Annual dicotyledonous weeds (3ANDIT)	SC	500 g/L	Medium-low volume spraying, broadcast or band application	BBCH 10-31	1-4	7	31.25 – 500	100-400	125-500	None**	Maximum of 0.5 kg a.s./ha per season
Sugar and fodder beet (BEAVA, BEAVC)	EU South Zone	Lenacil 500 g/L SC	F	Annual dicotyledonous weeds (3ANDIT)	SC	500 g/L	Medium-low volume spraying, broadcast or band application	BBCH 10-31	1-4	7	31.25 – 500	100-400	125-500	None**	Maximum of 0.5 kg a.s./ha per season

** PHI is covered by conditions of use and/or growing period between application and harvest.

(a) For crops, the EU and Codex classifications (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure)
(b) Outdoor or field use (F), greenhouse application (G) or indoor application (I)
(c) *e.g.* biting and sucking insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) CropLife International Technical Monograph no 2, 6th Edition. Revised May 2008. Catalogue of pesticide
(f) All abbreviations used must be explained
(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant- type of equipment used must be indicated
(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants. In certain cases, where only one variant is synthesised, it is more appropriate to give the rate for the variant.
(j) Growth stage range from first to last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of applications possible under practical conditions of use
(l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha
(m) PHI - minimum pre-harvest interval

1.5.2 Further information on representative uses

Please refer to GAP table under section 1.5.1.

1.5.3 Details of other uses applied for to support the setting of MRLs for uses beyond the representative uses

Not applicable.

1.5.4 Overview on authorisations in EU Member States

The currently authorized uses of lenacil formulations were provided within the document D2 of the renewal dossier and are as follows:

Austria	Crops: Sugar and fodder beet Pests controlled: Broad leaved weeds (BLW) Application: max 500 g a.s./ha, 1-3 applications, BBCH10-31
	Crops: Sugar beet Pests controlled: Broad leaved weeds (BLW) Application: 1 × max 2000 g a.s./ha, post-emergence
	Crops: Spinach Pests controlled: Broad leaved weeds (BLW) Application: 1 × max 2000 g a.s./ha, pre-emergence
Belgium	Crops: Sugar and fodder beet Pests controlled: BLW Application: max 500 g a.s./ha, 1-4 applications, from BBCH10-31
	Crops: Winter spinach (minor uses) Pest controlled: annual BLW Application: max 400 g a.s./ha, 1 application, from BBCH00-09
	Crops: potted ornamental trees and shrubs Pest controlled: weeds & mosses Application: max 500 g a.s./ha, 1 application
Czech Republic	Crops: Sugar and fodder beet Pests controlled: BLW Application: 3 x 160 g a.s./ha, BBCH12-31
Cyprus	Crops: Sugar beet, Pests controlled: BLW Application: max 800 g a.s./ha, 1 application, pre-emergence
	Crops: Spinach, fodder beets Pests controlled: BLW Application: max 1600g a.s./ha, 1 application, pre-emergence
	Crops: red beet Pests controlled: BLW Application: max 1400 g a.s./ha, 1 application, pre-emergence
France	Crops: Sugar and fodder beet Pests controlled: BLW Application: max 800 g a.s./ha, 1-4 applications, post-emergence
	Crops: Spinach Pests controlled: BLW Application: max 800 g a.s./ha, 1 application, pre-emergence
	Crops : Perfum, medicinal, food and aromatic plants, herbs Pests controlled : BLW Application : max 500 g/ha, 1 application, PHI 30 days
	Crops : Seeds production (fodder beet, lamb's lettuce) Pests controlled : BLW Application : max 500 g/ha, 1 application
Greece	Crops: Sugar beet, Pests controlled: BLW Application: max 800 g a.s./ha, 1 application, pre-emergence
	Crops: Spinach, fodder beets Pests controlled: BLW Application: max 1600g a.s./ha, 1 application, pre-emergence

	Crops: red beet Pests controlled: BLW Application: max 1400 g a.s./ha, 1 application, pre-emergence
Ireland	Crops: Sugar and fodder beet Pests controlled: BLW Application: Max. 440 g a.s./ha, post-emergence max BBCH39
Italy	Crops: Sugar and fodder beet Pests controlled: BLW Application: post-emergence from BBCH 10 to 39: • in one shot application: 1 × 400-480 g a.s./ha, • or in split applications with 7-14 dd interval: 4 x 120 g a.s./ha or 3 x 160 g a.s./ha or 2 x 240 g a.s./ha. * first Venzar re-authorization under 91/414 has not yet concluded in Italy and it will take another 8-10 months. We based the file correction on the label proposed for re-authorization under 91/414, label that is pending since 2013. The current Italian label is completely obsolete.
Poland	Crops: Sugar beet Pests controlled: BLW Application: 2 × 250 g a.s./ha, First application post-emergence and second after 7-10 days.
Portugal	Crops: Sugar beet Pests controlled: BLW Application: 1 x max 640 a.s./ha, pre-emergence or 1 × max 400 g a.s./ha, post-emergence
Romania	Sugar and fodder beet Pests controlled: BLW Rates of applications: max 500 gai/ha/year, max 250 g/ha/aplicare; Number of applications: 1-4 applications; Timings of applications: post-emergent, BBCH10-31; Application interval: minim 7 days
Slovakia	Crops: Sugar and fodder beet Pests controlled: BLW Application: 1 x max 500 gai/ha or split 1-4 applications, BBCH12-31
Spain	Crops: Sugar and fodder beet, garden beet Pests controlled: BLW Application: 1 × max 640 g a.s./ha, BBCH14-39
	Crops: Spinach and chard Pests controlled: BLW Application: 1 × max 640 g a.s./ha, pre-emergence
United Kingdom	Crops: Sugar and fodder beet, redbeet Pests controlled: BLW Application: 1 × max 2200 g a.s./ha, pre-emergence or 3 × max 176 g a.s./ha, max BBCH10-39

Level 2

LENACIL

2 SUMMARY OF ACTIVE SUBSTANCE HAZARD AND OF PRODUCT RISK ASSESSMENT

Note RMS (December 2018): As regards

- The physical chemistry
- The mammalian toxicology and metabolism
- The environmental fate and behaviour,

no new data, relevant for a revisal of the recently (2013) harmonised classification and labelling, indicate a need for a revisal by ECHA

Only for ecotoxicology, the hazard assessment of aquatic organisms indicate the need to revise the M-factor, and further consideration at ECHA to adapt the harmonised C&L may be appropriate. Therefore, the CLH as inserted into the current Volume 1, for the three first sections, is given for information, and only the ecotoxicological assessment is relevant for any modification of the CLH.

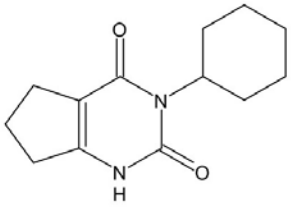
Summary of methodology proposed by the applicant for literature review and for all sections

No peer reviewed open literature data were submitted for the physical and chemical properties.

2.1 IDENTITY

2.1.1 Summary or identity

Lenacil, chemical name (IUPAC) 3-cyclohexyl-1,5,6,7-tetrahydrocyclopentapyrimidine-2,4(*3H*)-dione is a post-emergence herbicide (part of the uracil class of herbicides). Its chemical structure is presented here below:



New 5 batch analyses based on large scale production have been submitted for the purpose of renewal (please refer to the Vol. 4). The minimum purity proposed by the notifier is 975 g/kg. That min. purity and the proposed specification is the same as for first approval of lenacil. For details on the proposed specification of the current production (notifier/RMS) and the proposal regarding the reference specification, please refer to Vol. 4.

2.2 PHYSICAL AND CHEMICAL PROPERTIES [EQUIVALENT TO SECTION 7 OF THE CLH REPORT TEMPLATE]

The pure active substance lenacil is a light beige solid with a characteristic odour, which starts to decompose at ~ >270°C. It is very slightly volatile (vapour pressure = 1.7×10^{-9} Pa and Henry's law constant = 1.3×10^{-7} Pa m^3 mol^{-1}). Lenacil is not surface active. Lenacil is a weak acid with a pK_a of 10.7, and has a low water solubility, which does not vary considerably in the pH range 5 to 9 (3 to 4 mg/L at 20°C). The partition coefficient Log P_{ow} (1.7 at pH 4 and 1.69 at pH 7; 1.25 at pH 10; at 25°C) does not indicate any risk for bioaccumulation in the environment. Lenacil (as manufactured) is not self-igniting, not highly flammable, not explosive and does not exhibit oxidising properties and hence, it does not need to be classified for physical and chemical hazards.

2.2.1 Summary of physical and chemical properties of the active substance

Table 2.2.1: Summary of physicochemical properties of the active substance

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Light beige solid (99% purity) Fine powder, light beige solid (98.6% purity)	ACD 025/014039 Comb, A.L. 2002a Hamroll, K. 2003	Visual assessment
Melting/freezing point	Melting point not determinable; decomposes >270°C (99% purity)	ACD 025/014039 Comb, A.L. 2002a	Measurement according to EEC-Method A1 but decomposition starts at 270°C
Boiling point	Boiling point not determinable; decomposes >270°C (99% purity)	ACD 025/014039 Comb, A.L. 2002a	Test not performed since the test substance was observed to decompose prior to melting
Relative density	1.31kg/L	ACD 025/014039 Comb, A.L. 2002a	Measured – EEC-Method A3 (Pycnometer solvent displacement)
Vapour pressure	1.7×10^{-9} Pa at 25 °C (99% purity)	ACD 025/014039 Comb, A.L. 2002a	Measured - EEC-method A4 (vapour pressure balance method)
Surface tension	62.5 mN/m (90% saturated solution, 24°C, purity 99%) Lenacil is not surface active	ACD 025/014039 Comb, A.L. 2002a	Measured - EEC-method A5
Water solubility	At 20°C (99% purity) pH 5: 2.9 mg/L pH 7: 2.9 mg/L pH 9: 3.6 mg/L	CEMS-2787 Bell, A. (2005)	Measured - EEC-method A6 (column elution)
Partition coefficient n-octanol/water	At 25°C (99% purity) pH 4 : Log Pow = 1.70 pH 7 : Log Pow = 1.69 pH 9 : Log Pow = 1.25	ACD 025/014039 Comb, A.L. 2002a	Measured - EEC-method A8 (HPLC method)
Henry's law constant	1.3×10^{-7} Pa m^3 mol^{-1} at 25°C	ACD 025/014039 Comb, A.L. 2002a	Estimated (calculation)
Flash point	Not applicable	Not applicable	Not applicable Decomposition starts at 270°C (purity 99%).

Property	Value	Reference	Comment (e.g. measured or estimated)
Flammability	(98.6% purity) Lenacil technical ignited and propagated a flame over 200 mm in 8 minutes and 26 seconds. Lenacil is not highly flammable.	ACD 024/013898 Comb, A.L. 2002b	Measured-EEC-method A10 (burning rate test)
Explosive properties	(98.6% purity) Hoenen test: yellow flame, tubes recovered intact, no explosion. Fall Hammer test: no evidence of explosion or decomposition. Friction test: no sign of ignition or explosion but slight decomposition indicated by dark mark on porcelain plate. Lenacil is not explosive	ACD 024/013898 Comb, A.L. 2002b	Measured - EEC-method A14 (thermal, shock and friction)
Self-ignition temperature	(98.6% purity) Lenacil is not self-igniting below 400°C.	ACD 024/013898 Comb, A.L. 2002b	Measured - EEC-method A16 (relative self ignition)
Oxidising properties	- Reference mixture (Barium nitrate/cellulose 3:2) burned vigorously to completion in 56 seconds; - Neither mixture of 2:1, 1:1 or 1:2 test substance/cellulose burned to completion. Lenacil is not oxidizing Additional statement of notifier: *"[…] an examination of the structural formula in accordance with 1.1 of method A17 also establishes that lenacil is not likely to react exothermically with a combustible material. […]"*	ACD 024/013898 Comb, A.L. 2002b	Measured - EEC-method A17 + statement
Granulometry	No data		
Solubility in organic solvents and identity of relevant degradation products	At 20°C (98.9% purity) Hexane: 1.3 mg/L Toluene: 80 mg/L Acetonitrile: 230 mg/L Ethylacetate: 500 mg/L Acetone: 690 mg/L Methanol: 1500 mg/L Dichloromethane: 2000 mg/L	AMR 2377-92 McOuage J. D. 1992	Measured - The method is based on the method EEC A6 (flask method)
Dissociation constant	pKa = 10.7 at 25°C (99% purity)	ACD 025/014039 Comb, A.L. 2002a	Measured - OECD 112
Viscosity	Not applicable (lenacil is solid)		

<table>
<tr><th>Property</th><th>Value</th><th>Reference</th><th>Comment (e.g. measured or estimated)</th></tr>
<tr><td>Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity</td><td>(99% purity)

UV/VIS absorbance characteristics :
<table><tr><th></th><th>λ_{max} (nm)</th><th>ε (L.mol^{-1}.cm^{-1})</th></tr><tr><td>Neutral water/acetonitrile 3 : 1 v/v</td><td>271
at λ = 290 nm</td><td>7880
1760*</td></tr><tr><td>Acidic 0.133M HCl / acetonitrile 3 : 1 v/v</td><td>271
at λ = 290 nm</td><td>7990
1760*</td></tr><tr><td>Alkaline 0.133M NaOH / acetonitrile 3 : 1 v/v</td><td>227
291</td><td>7220
10100</td></tr></table>*calculated estimation by RMS on the basis of the provided UV absorption spectra

For details on IR, NMR and MS spectra, please refer to Vol.3 CA-B.2.4</td><td>ACD 025/014039
Comb, A.L. 2002a</td><td>Measured (OECD 101 for UV/VIS)</td></tr>
</table>

2.2.1.1 Evaluation of physical hazards [equivalent to section 8 of the CLH report template]

2.2.1.1.1 Explosives *[equivalent to section 8.1 of the CLH report template]*

Table 2.2.1.1: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EEC-method A14 (thermal, shock and friction)	(98.6% purity) Koenen test: yellow flame, tubes recovered intact, no explosion. Fall Hammer test: no evidence of explosion or decomposition. Friction test: no sign of ignition or explosion but slight decomposition indicated by dark mark on porcelain plate. Lenacil is not explosive	/	ACD 024/013898 Comb, A.L. 2002b

2.2.1.1.1.1 Short summary and overall relevance of the provided information on explosive properties

Lenacil was submitted to the effect of a flame (thermal sensitivity), to shock and friction according to EEC-Method A14. The test was performed under GLP. No explosion occurred at the conditions of the thermal (Koenen test apparatus), shock (fall hammer test apparatus) and friction test, respectively.

2.2.1.1.1.2 Comparison with the CLP criteria

The test required for CLP classification regarding explosive properties is the United Nations Recommendations on the Transport of Dangerous Goods (UN RTDG) Manual of Tests and Criteria ST/SG/AC.10/11/ Rev. 5 – Part I (Test series), section 11. However, since no sign of explosion were observed during the test according to EEC-Method A14, and taking into account that:

- the Lenacil's oxygen balance is <-200 (calculation),
- the thermal stability was tested via DSC (KCA 2.1 melting point) showing only endothermic effects in traces at approx. 310 °C.

there is sufficient information to reasonably assume that Lenacil does not exhibit explosive properties.

2.2.1.1.1.3 Conclusion on classification and labelling for explosive properties

Lenacil does not exhibit explosive properties.

2.2.1.1.2 Flammable gases (including chemically unstable gases) ***[equivalent to section 8.2 of the CLH report template]***

Table 2.2.1.1.2: Summary table of studies on flammable gases (including chemically unstable gases)

Method	Results	Remarks	Reference

Not relevant.

2.2.1.1.2.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)

.

Not relevant.

2.2.1.1.2.2 Comparison with the CLP criteria

Not relevant.

2.2.1.1.2.3 Conclusion on classification and labelling for flammable gases

Not relevant.

2.2.1.1.3 Oxidising gases ***[equivalent to section 8.3 of the CLH report template]***

Table 2.2.1.1.3: Summary table of studies on oxidising gases

Method	Results	Remarks	Reference

Not relevant.

2.2.1.1.3.1 Short summary and overall relevance of the provided information on oxidising gases

Not relevant.

2.2.1.1.3.2 Comparison with the CLP criteria

Not relevant.

2.2.1.1.3.3 Conclusion on classification and labelling for oxidising gases

Not relevant.

2.2.1.1.4 Gases under pressure ***[equivalent to section 8.4 of the CLH report template]***

Table 2.2.1.1.4: Summary table of studies on gases under pressure

Method	Results	Remarks	Reference

2.2.1.1.4.1 Short summary and overall relevance of the provided information on gases under pressure

Not relevant.

2.2.1.1.4.2 Comparison with the CLP criteria

Not relevant.

2.2.1.1.4.3 Conclusion on classification and labelling for gases under pressure

Not relevant.

2.2.1.1.5 Flammable liquids *[equivalent to section 8.5 of the CLH report template]*

Table 2.2.1.1.5: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference

Not relevant.

2.2.1.1.5.1 Short summary and overall relevance of the provided information on flammable liquids

Not relevant.

2.2.1.1.5.2 Comparison with the CLP criteria

Not relevant.

2.2.1.1.5.3 Conclusion on classification and labelling for flammable liquids

Not relevant.

2.2.1.1.6 Flammable solids *[equivalent to section 8.6 of the CLH report template]*

Table 2.2.1.1.6: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
Measured- EEC-method A10 (burning rate test)	(98.6% purity) Lenacil technical ignited and propagated a flame over 200 mm in 8 minutes and 26 seconds. Lenacil is not highly flammable.	/	ACD 024/013898 Comb, A.L. 2002b

2.2.1.1.6.1 Short summary and overall relevance of the provided information on flammable solids

Lenacil was submitted to the burning rate test according to EEC-Method A10. The test was performed under GLP. The lenacil ignited and propagated a flame over 200 mm in 8 minutes and 26 seconds. This is more than the 4 min. as reported in EEC-Method A10 and therefore more than the 2 min. as recorded in Test N.1.

2.2.1.1.6.2 Comparison with the CLP criteria

Screening tests of EC method A.10 and the corresponding UN test method N.1 are comparable. EEC-Method A.10 requires a burning rate > 4min (as criterion to omit the full test) and thus covers the 2 min burning rate required in the UN method.

2.2.1.1.6.3 Conclusion on classification and labelling for flammable solids

Lenacil is not expected to be highly flammable.

2.2.1.1.7 Self-reactive substances *[equivalent to section 8.7 of the CLH report template]*

Table 2.2.1.1.7: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference

Data lacking.

2.2.1.1.7.1 Short summary and overall relevance of the provided information on self-reactive substances

Data lacking.

2.2.1.1.7.2 Comparison with the CLP criteria

Data lacking.

2.2.1.1.7.3 Conclusion on classification and labelling for self-reactive substances

Data lacking.

2.2.1.1.8 Pyrophoric liquids *[equivalent to section 8.8 of the CLH report template]*

Table 2.2.1.1.8: Summary table of studies on pyrophoric liquids

Method	Results	Remarks	Reference

Not relevant

2.2.1.1.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

Not relevant

2.2.1.1.8.2 Comparison with the CLP criteria

Not relevant

2.2.1.1.8.3 Conclusion on classification and labelling for pyrophoric liquids

Not relevant

2.2.1.1.9 Pyrophoric solids *[equivalent to section 8.9 of the CLH report template]*

Table 2.2.1.1.9: Summary table of studies on pyrophoric solids

Method	Results	Remarks	Reference

Data lacking.

2.2.1.1.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

Data lacking.

2.2.1.1.9.2 Comparison with the CLP criteria

Data lacking.

2.2.1.1.9.3 Conclusion on classification and labelling for pyrophoric solids

Data lacking.

2.2.1.1.10 Self-heating substances *[equivalent to section 8.10 of the CLH report template]*

Table 2.2.1.1.10: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EEC-method A16 (relative self ignition)	(98.6% purity) Lenacil is not self-igniting below 400°C	/	ACD 024/013898 Comb, A.L. 2002b

2.2.1.1.10.1 Short summary and overall relevance of the provided information on self-heating substances

Lenacil was submitted to the self-ignition test according to EEC-Method A16. The test was performed under GLP. Lenacil is not self-igniting below 400°C. A slight sample temperature fluctuation was observed around 300°C, which is consistent with the decomposition of the test substance.

2.2.1.1.10.2 Comparison with the CLP criteria

Test N.4: test method for self-heating substances (UN RTDG Manual of Tests and Criteria ST/SG/AC.10/ 11/Rev. 5 – Part III, section 33.3.1.6) is recommended to determine the self-heating classification under the CLP. The test performed according to EEC-Method A16 can only be seen as a preliminary and indicative test. Since Lenacil was found not self-igniting below 400°C, it is expected that it will not exhibit self-heating properties.

2.2.1.1.10.3 Conclusion on classification and labelling for self-heating substances

Lenacil is not expected to exibit self-heating properties.

2.2.1.1.11 Substances which in contact with water emit flammable gases *[equivalent to section 8.11 of the CLH report template]*

Table 2.2.1.1.11: Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference

Data lacking.

2.2.1.1.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

Data lacking.

2.2.1.1.11.2 Comparison with the CLP criteria

Data lacking.

2.2.1.1.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

3

Data lacking.

2.2.1.1.12 Oxidising liquids *[equivalent to section 8.12 of the CLH report template]*

Table 2.2.1.1.12: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference

Not relevant

2.2.1.1.12.1 Short summary and overall relevance of the provided information on oxidising liquids

Not relevant

2.2.1.1.12.2 Comparison with the CLP criteria

2.2.1.1.12.3 Conclusion on classification and labelling for oxidising liquids

Not relevant

2.2.1.1.13 Oxidising solids *[equivalent to section 8.13 of the CLH report template]*

Table 2.2.1.1.13: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
Measured - EEC-method A17 + statement	Lenacil is not oxidizing	/	ACD 024/013898 Comb, A.L. 2002b

2.2.1.1.13.1 Short summary and overall relevance of the provided information on oxidising solids

The oxidising properties of Lenacil have been investigated according to the EEC-method A17 (preliminary test) and the study was GLP. The burning rates of the reference mixture and the test item mixture have been compared and the following results were obtained:

- Reference mixture (Barium nitrate/cellulose 3:2) burned vigorously to completion in 56 seconds;
- Neither mixture of 2:1, 1:1 or 1:2 test substance/cellulose burned to completion.

Additionally, the notifier provided a statement : *"[...] an examination of the structural formula in accordance with 1.1 of method A17 also establishes that lenacil is not likely to react exothermically with a combustible material. [...]"*

2.2.1.1.13.2 Comparison with the CLP criteria

The test according to EEC-method A17 is not fully equivalent to the test required for CLP classification (Test O.1: Test for oxidizing solids (UN RTDG Manual of Tests and Criteria ST/SG/AC.10/11/Rev. 5 – Part III, section 34.4.1; i.e. the reference mixture recommended is different) but based on the obtained results and considering the structure of Lenacil, it can be reasonably assumed that Lenacil does not exhibit oxidizing properties.

2.2.1.1.13.3 Conclusion on classification and labelling for oxidising solids

Lenacil does not exhibit oxidizing properties.

2.2.1.1.14 Organic peroxides *[equivalent to section 8.14 of the CLH report template]*

Table 2.2.1.1.14: Summary table of studies on organic peroxides

Method	Results	Remarks	Reference

Data lacking. Not relevant.

2.2.1.1.14.1 Short summary and overall relevance of the provided information on organic peroxides

Data lacking. Not relevant.

2.2.1.1.14.2 Comparison with the CLP criteria

Data lacking. Not relevant.

2.2.1.1.14.3 Conclusion on classification and labelling for organic peroxides

Data lacking. Not relevant.

2.2.1.1.15 Corrosive to metals *[equivalent to section 8.15 of the CLH report template]*

Table 2.2.1.1.15: Summary table of studies on the hazard class corrosive to metals

Method	Results	Remarks	Reference

Data lacking.

2.2.1.1.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

Data lacking.

2.2.1.1.15.2 Comparison with the CLP criteria

Data lacking.

2.2.1.1.15.3 Conclusion on classification and labelling for corrosive to metals

Data lacking.

2.2.2 Summary of physical and chemical properties of the plant protection product

All studies have been performed in accordance with the current requirements and the results are deemed to be acceptable. The appearance of the product is that of an opaque white, homogenous liquid; free from visible suspended matter and sediment. It is not explosive, has no oxidising properties. It is not flammable and has an auto-ignition temperature of 530°C. It has a pH value of 7.58 at 20°C. There is no effect of low and high temperature on the stability of the formulations, since storage after 7 days at 0°C and 2 weeks at 54°C (in the commercial packaging) neither the active ingredient content nor the technical properties (at 0.062% v/v and 1% v/v) were changed. The stability data of 1 year and 2 years at ambient temperature (at 0.062% v/v and 1% v/v, in commercial packaging material) indicate a shelf life of at least 2 years at ambient temperature. A 3 year shelf life study is currently ongoing (this is the reason why the current report for the shelf-life is an interim report). Its technical characteristics are acceptable for a suspension concentrate (SC) formulation type. The intended concentration of use is 0.25% to 1% v/v.

2.3 DATA ON APPLICATION AND EFFICACY

2.3.1 Summary of effectiveness

For efficacy related elements, only limited information is provided to address the requirements of Article 4(3) of Regulation (EC) No 1107/2009. Detailed consideration of efficacy will occur in the subsequent product authorisation process when a full biological assessment dossier will be required. Therefore, only limited efficacy information is required in the present section.

Level of control will depend on the weed species, stage of growth and environmental conditions. The weed spectrum presented below for Lenacil 500 g/L SC is only informative and is divided by administrative zone. It has not been reviewed by the RMS.

Weed species are classified on the base of their susceptibility to lenacil:

Very susceptible (VS)	Susceptible (S)	Moderate susceptible (MS)	Partial Susceptible or Suppression (PS)	Not susceptible (NS)

95-100%	85-94.9%	75-84.9%	60-74.9%	<60%

Latin name (EPPO CODE)	Central Zone	South Zone
Aethusa cynapium (AETCY)	NS	-
Amaranthus retroflexus (AMARE)	VS	VS
Ammi majus (AMIMA)	NS	-
Brassica napus (BRSNN)	VS	VS
Capsella bursa-pastoris (CAPBP)	VS	VS
Chenopodium album (CHEAL)	MS	MS
Fumaria officinalis (FUMOF)	VS	VS
Galium aparine (GALAP)	S	S
Geranium pusillum (GERPU)	MS	-
Lamium purpureum (LAMPU)	VS	-
Matricaria chamomilla (MATCH)	MS	S
Myosotis arvensis (MYOAR)	VS	S
Polygonum aviculare (POLAV)	VS	S
Fallopia convolvulus (POLCO)	VS	S
Sinapis arvensis (SINAR)	S	S
Stellaria media (STEME)	S	S
Thlaspi arvense (THLAR)	VS	VS
Veronica persica (VERPE)	VS	VS
Viola arvensis (VIOAR)	VS	VS

Products containing the active substance lenacil have been evaluated in EU Member States under uniform principles (see Document D2 for the list of currently authorised uses in the EU and extent of use). Data packages submitted in compliance with data requirements at the time of evaluation satisfied the Member States authorities.

The RMS agrees with the previous paragraph. For completeness, a brief overview of data evaluated by EU Member States and their conclusions is presented in Vol.3 CP-B3.9.2 and B3.9.3.

2.3.2 Summary of information on the development of resistance

When applying the modifiers (resistance management) proposed by the applicant, the risk is reduced and the use of lenacil can be considered as presenting a low risk of resistance. The proposed modifiers are part of the good agricultural practice and can be easily applied by end-users. Moreover, the good agricultural practice in sugar beet recommends the application of the Repeated Low Dose Program (RLDP) which consists in the application of a tank mix of several herbicides with different modes of action at a rate which is adapted to the development stage of each weed flush. This system is by itself a good resistance management strategy. On top of resistance management proposed by the applicant here above, promotion of the use of a RLDP is necessary. For details please refer to section B.3.7 of Vol.3 CA-B.3.

2.3.3 Summary of adverse effects on treated crops

Extract from the evaluation in the Southern Zone:
According to the trials submitted Lenacil had no negative effect in the yield, the sugar content and the content of Na, K and N of sugar beets.
According to the trials submitted Lenacil had no negative effect in the yield of fodder beets.

Use of the product alone

Phytotoxicity symptoms were detected in several trials after one application of Lenacil, however these symptoms were softer than with the tested standards and they were not important at the end of the crop, even with an application of 1000 g a.s./ha (5 trials in maritime EPPO climatic zone).

Use of the product in herbicide programs

Phytotoxicity symptoms were detected in several trials, however these symptoms were similar to the program without Lenacil and they were not important at the end of the crop.

Extract from the evaluation in the Central Zone:
It is noticed that not all the possible scenarios of dose splitting were tested. However, as lenacil is root acting and as the applications are very close from each other, only the cumulative amount of active substance (= the maximum applied rate) has to be taken into account for crop safety. Based on both selectivity and efficacy trials that have been performed in the Maritime and North-East EPPO zone with formulations containing lenacil used alone or in combination with partners in a Repeated Low Dose Program, it can be concluded according to the Uniform Principles, that lenacil formulations are not expected to have detrimental effect on sugar beet crop when applied according to the proposed GAP. The same conclusions applies to yield and quality of the harvested product. However, it is known that on light (sand and sandy-loam) soils, phytotoxicity can be observed temporarily after lenacil application as mentioned in the Belgian registration certificate of Lenacil 500 g/L SC. These positive conclusions can be extrapolated to fodder beet. Member States may consider the possibility to grant a registration to that crop according to its possible minor crop status.

Due to the fact that nearly no phytotoxicity was recorded in the selectivity trials, and always temporarily, and that the product is used since many years without any effect on sugar beet seeds crops, no detrimental effect is to be expected on parts of plant used for propagating purpose.

2.3.4 Summary of observations on other undesirable or unintended side-effects

Extract from the evaluation in the Southern Zone:
Regarding the adverse effects on beneficial organisms, the final conclusion is based on the risk assessment outcome of the Ecotoxicology Section.

No adverse effects on other plants including adjacent crops are expected provided that Lenacil 500 g/L SC is used in compliance with Good Agricultural Practice.

Extract from the evaluation in the Central Zone:
Adverse effects on beneficial organisms: See ecotoxicological evaluation.

The impact on adjacent crops was addressed according to EPPO Guideline 257. No detrimental effect is to be expected on adjacent crops.

2.4 FURTHER INFORMATION

2.4.1 Summary of methods and precautions concerning handling, storage, transport or fire

Information was provided from the MSDS of the active substance and formulation, respectively.

Active substance:

Handling and Storage:

Handling

Avoid contact with skin, eyes and clothing. Do not breathe dust or spray mist. Wear personal protective equipment. Prepare the working solution as given on the label(s) and/or the user instructions. Use prepared working solution as soon as possible - Do not store. Provide appropriate exhaust ventilation at places where dust is formed.

Use only according to our recommendations. Use only clean equipment. Keep away from heat and sources of ignition. During processing, dust may form explosive mixture in air.

Storage

Store in a place accessible by authorized persons only. Store in original container. Keep containers tightly closed in a dry, cool and well-ventilated place. Keep out of the reach of children. Keep away from food, drink and animal feedingstuffs.

Transport:	ADR	
	Transport hazard class:	9
	Packaging group:	III
	UN Number :	3077
	Proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (Lenacil)
	IATA_C	
	Class:	9
	Packaging group:	III
	UN Number :	3077
	Proper shipping name:	Environmentally hazardous substance, solid, n.o.s. (Lenacil)
	IMDG	
	Class:	9
	Packaging group:	III
	UN-No.:	3077
	Proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (Lenacil)
	Marine pollutant:	Marine pollutant

Fire Fighting Measures:

Flash Point:	Not applicable
Hazardous Products of Combustion:	None known.
Extinguishing Media:	Does not sustain combustion. In case of fire, use water spray, dry chemical, foam or carbon dioxide (CO_2).
Extinguishing media which shall not be used for safety reason:	High volume water jet (contamination risk).
Unusual fire and Explosion Hazards:	None known. Not explosive
Fire Fighting Equipment:	Fire fighters and others exposed to products of combustion should wear self-contained breathing apparatus. Equipment should be thoroughly decontaminated after use.
Further information:	(on small fires) if the area is heavily exposed to fire and if conditions permit, let the fire burn itself out since water may increase the area contaminated. Cool containers/tanks with spray water. Prevent fire extinguishing water from contaminating surface water or the ground water system. Collect contaminated fire extinguishing water separately. This must not be discharged into drains. Fire residues and contaminated fire extinguishing water must be disposed of in accordance with local regulations.

Personal protection:

Personal precautions:	Avoid breathing dust. Use personal protective equipment.

Eye protection:	Safety glasses with side-shields conforming to EN166
Hand protection:	Material : nitrile rubber Glove thickness: 0.3 mm Glove length: standard glove type Protection index: Class 6 Wearing time:8 h
Skin and body protection:	Manufacturing and processing work. Full protective clothing Type 5 (EN 13982-2).
Respiratory protection:	Manufacturing and processing work. Half mask with a particle filter FFP1 (EN149).
Hygiene measures:	Handle in accordance with good industrial hygiene and safety practice. Regular cleaning of equipment, work area and clothing. Keep working clothes separately. Contaminated work clothing should not be allowed out of the workplace. For environmental protection remove and wash all contaminated protective equipment before re-use. Remove clothing/PPE immediately if material gets inside. Wash thoroughly and put on clean clothing. Dispose of rinse water in accordance with local and national regulations. Wash hands before breaks and at the end of the workday.

Plant protection product:

Handling
Use only according to our recommendations. Wear personal protective equipment. Use only clean equipment. Provide adequate ventilation. Do not breathe vapours or spray mist. When opening containers, avoid breathing vapours that may be emanating. Prepare the working solution as given on the label(s) and/or the user instructions. Use prepared working solution as soon as possible - Do not store. To avoid spills during handling keep bottle on a metal tray. Wash hands before breaks and immediately after handling the product. Remove and wash contaminated clothing before re-use. Never return unused material to storage receptacle. Avoid exceeding of the given occupational exposure limits.

Warehouse storage
Store in a dry place in accordance with relevant specific regulations.

User storage
Store in original container. Keep containers tightly closed in a dry, cool and well ventilated place. Store in a place accessible by authorized persons only. Keep out of the reach of children. Keep away from food, drink and animal feeding stuffs.
No special restrictions on storage with other products. Keep away from bases.

Transport

ADR	
Class:	9
Packaging group:	III
Classification Code:	M6
HI No.:	90
UN-No.:	3082
Labelling No.:	9
Proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, LIQUID, N.O.S. (Lenacil)
IMDG	
Class:	9
Packaging group:	III
UN-No.:	3082
Labelling No.:	9
Proper shipping name:	ENVIRONMENTALLY HAZARDOUS SUBSTANCE, SOLID, N.O.S. (Lenacil)

Firefighting measures

Suitable extinguishing media are water spray, dry chemical and carbon dioxide (CO2). Extinguishing media which shall not be used for safety reasons: High volume water jet (contamination risk).
Specific hazards during firefighting: Hazardous decomposition products formed under fire conditions are carbon dioxide (CO2) and nitrogen oxides.
Prevent fire extinguishing water from contaminating surface water or the ground water system. Collect contaminated fire extinguishing water separately. This must not be discharged into drains. Fire residues and contaminated fire extinguishing water must be disposed of in accordance with local regulations. (on small fires)
If area is heavily exposed to fire and if conditions permit, let fire burn itself out since water may increase the area contaminated. Cool containers /tanks with water spray.

Protective clothing and equipment proposed-nature

On the basis of the toxicological profile of Lenacil 500 g/L SC (the a.s. lenacil has been classified as Carc. Cat. 2 (H351)) it is appropriate to recommend the use of specific protective clothing or equipment, and in addition, on the basis of good agricultural practice when handling pesticides, details are provided below under the next point.

Protective clothing and equipment proposed-characteristics

Respiratory protection

- Manufacturing and processing work: Half mask with a particle filter FFP2 (EN149)
- Field and greenhouse application: Not required; except in case of aerosol formation. Half mask with combination filter A2/P2 (EN 141)

Hand protection

- Material: Nitrile rubber
- Glove thickness: 0,4 - 0,7 mm
- Wearing time: 480 min

Please observe the instructions regarding permeability and breakthrough time which are provided by the supplier of the gloves. Also take into consideration the specific local conditions under which the product is used, such as the danger of cuts, abrasion, and the contact time., The suitability for a specific workplace should be discussed with the producers of the protective gloves.

Eye protection

Safety glasses with side-shields conforming to EN166

Skin and body protection

- Manufacturing and processing work: Full protective clothing Type 5 + 6 (EN ISO 13982-2 / EN 13034)
- Field and greenhouse application: Full protective clothing Type 3 (EN 14605)

Hygiene measures

Handle in accordance with good industrial hygiene and safety practice. Regular cleaning of equipment, work area and clothing. Contaminated work clothing should not be allowed out of the workplace. Wash hands before breaks and immediately after handling the product. When using do not eat, drink or smoke. Keep away from food, drink and animal feeding stuffs. For environmental protection remove and wash all contaminated protective equipment before reuse. Dispose of rinse water in accordance with local and national regulations.

Protective measures
All chemical protective clothing should be visually inspected prior to use. Clothing and gloves should be replaced in case of chemical or physical damage or if contaminated.

Sufficient data to evaluate suitability and effectiveness of protective clothing and equipment under realistic conditions of use
On the basis of the toxicological profile of Lenacil 500 g/L SC it is not appropriate to recommend the use of specific protective clothing or equipment, however the following are recommended on the basis of good agricultural practice when handling pesticides.
Glove thickness: 0.4 – 0.7 mm.
Wearing time: 480 min.
Please observe the instructions regarding permeability and breakthrough time which are provided by the supplier of the gloves. Also take into consideration the specific local conditions under which the product is used, such as the danger of cuts, abrasion, and the contact time.
Protect eyes with safety glasses with side-shields.
In the case of dust or aerosol formation use a respirator with an approved filter. Suitable mask with particle filter P3 (European standard EN 143).

No information is provided on the suitability of such clothing as its use is recommended on the basis of general advice for all plant protection products.

Procedures to minimise the generation of waste
Only purchase and store quantities of product required in the short term. Do not open larger containers than is necessary for immediate use. Do not prepare spray mixture volumes greater than is necessary for immediate use.

Information on combustion products likely to be generated in the event of fire
Lenacil 500 g/L SC is non-flammable, non-explosive and not an oxidizer. None of the components contain halogens. In the event of a fire Lenacil 500 g/L SC is likely to produce normal products of combustion, i.e. hazardous decomposition products formed under fire conditions. Carbon dioxide (CO2) nitrogen oxides

2.4.2 Summary of procedures for destruction or decontamination

Active substance:

No new information has been provided. The information below is taken from the original EU review.
A specific study on the thermal decomposition has not been carried out. Current practice is to incinerate at a temperature greater than 900°C with a residence time of 24 seconds in the chamber. Oxygen supply should be adjusted to generate <100 ppm CO in the stack.

Consideration of content of halogens is not relevant.

Package product wastes: Waste should be disposed of in accordance with local and national regulations. Packaging should be incinerated at a suitable, licensed plant.

Plant protection product:

1. Neutralisation procedure

Details of proposed procedure for small quantities
Take up spilled product with absorbing agent and store in a tightly closed container. Dispose of it according to section 13 of the MSDS.

Evaluation of products of neutralization (small quantities)
Evaluation of products of neutralisation is not possible. Lenacil 500 g/L SC undergoes decomposition when treated with alkaline solutions.

Procedure for disposal of neutralized waste (small quantities)
Not applicable. See above "Details of proposed procedure for small quantities"

Details of proposed procedure for large quantities
Take up spilled product with absorbing agent and store in a tightly closed container. Dispose of it according to section 13 of the MSDS.

Evaluation of products of neutralization (large quantities)
Evaluation of products of neutralization is not possible. Lenacil 500 g/L SC undergoes decomposition when treated with alkaline solutions

Procedure for disposal of neutralized waste (large quantities)
Not applicable. See above "Evaluation of products of neutralization (large quantities)"

2. Controlled incineration

Pyrolytic behaviour of the active substance under controlled conditions at 800°C and the content of polyhalogenated dibenzo-p-dioxins in the products of hydrolysis
A specific study on the thermal decomposition has not been carried out. Current practice used for experimental quantities of lenacil is to incinerate at a temperature greater than 900°C with a residence time of 24 seconds in the chamber. Oxygen supply should be adjusted to generate <100 ppm carbon monoxide in the stack. The consideration of content of halogens is not relevant.

Detailed instructions for safe disposal of the plant protection product and its packaging
Package product wastes: Triple rinse empty containers and place the rinsate into the spray tank. Close and label the waste receptacles. Dispose of them at a suitable waste incineration plant holding a permit delivered by the competent authorities. Do not contaminate ground water. Disposal should be carried out in accordance with the official local regulations. Where large quantities are concerned consult the supplier.

Methods other than controlled incineration for disposal
No other methods are currently available.

2.4.3 Summary of emergency measures in case of an accident

Active substance:

Containment of spillage and cleaning-up
Clean-up methods - small spillage Sweep up or vacuum up spillage and collect in suitable container for disposal.
Clean-up methods - small spillage Contain spillage, pick up with an electrically protected vacuum cleaner or by wet-brushing and transfer to a container for disposal according to local regulations.
Other information: Never return spills in original containers for re-use. Dispose of in accordance with local regulations.

Environmental precautions
Prevent further leakage or spillage if safe to do so. Use appropriate container to avoid environmental contamination. Do not flush into surface water or sanitary sewer system. Do not allow material to contaminate ground water system. Local authorities should be advised if significant spillages cannot be contained. If the product contaminates rivers and lakes or drains inform respective authorities.
Prevent material from entering sewers, waterways, or low areas.

Personal precautions

See above (personal precautions: 2.4.1).

First aid measures

Inhalation:	Move to fresh air. Consult a physician after significant exposure. Artificial respiration and/or oxygen may be necessary.

Skin contact:	Take off contaminated clothing and shoes immediately. Wash off immediately with soap and plenty of water. In case of skin irritation or allergic reactions see a physician. Wash contaminated clothing before re-use.
Eye contact:	If easy to do, remove contact lens, if worn. Hold eye open and rinse slowly and gently with water for 15-20 minutes. If eye irritation persists, consult a specialist.
Ingestion:	Obtain medical attention. DO NOT induce vomiting unless directed to do so by a physician or poison control center. If victim is conscious: rinse mouth with water.

Waste treatment methods
In accordance with local and national regulations, the product must be incinerated in a suitable incineration plant holding a permit delivered by the competent authorities. The product should not be allowed to enter drains, water courses or the soil. Do not re-use empty containers.

Plant protection product:

Containment of spillages
Clean-up methods - small spillage: Soak up with inert absorbent material. Sweep up or vacuum up spillage and collect in suitable container for disposal.
Clean-up methods - large spillage: Contain spillage, soak up with non-combustible absorbent material, (e.g. sand, earth, diatomaceous earth, vermiculite) and transfer to a container for disposal according to local / national regulations. Large spills should be collected mechanically (remove by pumping) for disposal. Collect leaking liquid in sealable (metal/plastic) containers.
Never return spills in original containers for re-use. Dispose of in accordance with local regulations.

Decontamination of areas, vehicles and buildings
Clean-up methods - small spillage: Soak up with inert absorbent material. Sweep up or vacuum up spillage and collect in suitable container for disposal.
Clean-up methods - large spillage: Contain spillage, soak up with non-combustible absorbent material, (e.g. sand, earth, diatomaceous earth, vermiculite) and transfer to a container for disposal according to local / national regulations (see appendix 1). Large spills should be collected mechanically (remove by pumping) for disposal. Collect leaking liquid in sealable (metal/plastic) containers.

Disposal of damaged packaging, absorbents and other materials
Dispose in accordance with local and national regulations. Must be incinerated in a suitable incineration plant holding a permit delivered by the competent authorities.
Do not re-use empty containers. Dispose of as unused product. Do not contaminate ponds, waterways or ditches with chemical or used container.

Protection of emergency workers and bystanders
Use protective clothing as proposed (B4.2 in this document). Keep bystanders away from the affected area.

First aid measures

General advice:	Never give anything by mouth to an unconscious person.
Inhalation:	Move to fresh air. Oxygen or artificial respiration if needed. Consult a physician after significant exposure.
Skin contact:	Take off contaminated clothing and shoes immediately. Wash off immediately with soap and plenty of water. In the case of skin irritation or allergic reactions see a physician. Wash contaminated clothing before re-use.
Eye contact:	Hold eye open and rinse slowly and gently with water for 15-20 minutes. If eye irritation persists, consult a specialist.
Ingestion :	Obtain medical attention. Do not induce vomiting without medical advice. If victim is conscious: Rinse mouth with water.

2.5 METHODS OF ANALYSIS

2.5.1 Methods used for the generation of pre-authorisation data

2.5.1.1. Methods for the determination of the active substance in the technical material and formulation

The lenacil content in technical material is determined by the validated method B0634.220.03.ES using reversed-phase HPLC-UV or UPLC-UV.

Validated methods for determining impurities are also available (see Vol. 4).

A validated method using reversed-phase HPLC-UV is also available to determine lenacil content in the formulation.

Pending the decision regarding the impurities (potentially) relevant, then validated analytical methods should be available to determine the impurities in the formulation **(potential data gap).**

2.5.1.2. Analytical methods for the determination of residues in the different matrices

Methods of analysis have been submitted to support new studies in relation to the risk assessment and have been assessed in accordance with the guidance document SANCO/3029/99 rev.4.

The validation of the method according to SANCO/3029/99 rev. 4 is mainly discussed in Volume 3 B5-CA. The acceptability of the overall study is discussed in volume of the respective expertise area. The table below summarizes all the pre-registration methods used in support of residues, fate, (eco)toxicology and physico-chemical studies considered for risk assessment and reports if the methods are considered as fully or sufficiently validated according to SANCO/3029/99 and/or if they are considered “fit for purposes”.

Dossier part	Residue (matrix)	Analytical method	Reference	Validated according to SANCO/3029/99 rev. 4
Methods in water, buffer solutions, organic solvents and any additional matrices used in the physical and chemical properties tests	Lenacil (surface, tap and ground water) Method used in support of the determination of the water solubility of lenacil (Vol.3 CA-B2.5/02)	**HPLC-UV:** (LOQ = 0.1 µg/L) Working range: 0.1 – 1.0 µg/L 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).	CA 4.1.2(g)/01 (IIA 4.2.3.1-01) – Wittig A. (2002) – Report PR02/001 Vol. 3 CA-B.5.1.2.7 – Study No. 1.	The method is validated and considered "fit for purpose".
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 residues studies	**Plant commodities**			
	Lenacil (sugar beet leaves [high water] and roots [high starch]) Method used in support of: - Vol.3 CA-B.7.3.1 Storage stability (Hamberger 2002); B.7.3 Magnitude of residues (Pollmann, 2002)	**Modified DFG S19**: **HPLC-MS/MS** (LOQ = 0.02 mg/kg) Working range: 0.02 – 0.2 mg/kg (in leaves the level of 4 mg/kg has also been tested but with n = 2) Extraction with acetone/water (2/1), followed by liq./liq. partition with ethyl acetate/cyclohexane 1/1.	CA 4.1.2(e)/01 (IIA 4.2.1.1-01 and IIA 6.3-02b) – Mende P. (2002) (primary method validation) CA 4.1.2(e)/02 (IIA 4.2.1.1-02) – Turnbull G. (2003) (ILV) Vol. 3 CA-B.5.1.2.5.1 – Studies No. 1 - 2	The method is sufficiently validated taken into account the overall data (primary validation, ILV and procedural recoveries from study of Hamberger 2002). Method is considered "fit for purpose".
	Lenacil (sugar beet leaves [high water] and roots [high starch]) Method used in support of: - Vol.3 CA-B.7.3 Magnitude of residue	**Modified DFG S19:** **GC-MSD** (LOQ = 0.01 mg/kg, not sufficiently validated) Working range:	CA 4.1.2(e)/04 (IIA 4.2.1.1-03) – Tillkes M. (1998) Vol. 3 CA-B.5.1.2.5.1 – Study No. 4	Method is not sufficiently validated. The number of replicates at each fortification level was insufficient for a primary method. The validation data are insufficient to allow to

	(Tillkes, 1998)	0.01 – 0.1 mg/kg (not sufficiently validated) Extraction with acetone/water (2/1), followed by liq./liq. Partition with ethyl acetate/cyclohexane 1/1.		consider the method as "fit for purpose".
	Lenacil (sugar beet leaves [high wate] and roots [high starch]) Method used in support of: - Vol.3 CA-B.7.3 Magnitude of residue (Anderson & Kakkonen, 2006)	**Modified version of method of Fillion et al. 2000:** HPLC-MS/MS (LOQ = 0.02 mg/kg) Working range: 0.02 – 0.2 mg/kg (root) 0.02 – 20 mg/kg (plant) Extraction with acetonitrile/water (50/50), followed by liq./liq. Partition with ethyl acetate/cyclohexane 1/1.	CA 4.1.2(e)/05 (IIA 4.2.1.1-04 and IIA 6.3-04) – Witte (2006) for the analytical part in the study by Anderson I., Kakkonen J.E. (2006) Vol. 3 CA-B.5.1.2.5.1 – Study No. 5	Method is sufficiently validated and considered "fit for purpose".
	Animal commodities			
	/	/	/	/
Methods In soil, water, sediment, air and any additional matrices used in support of environmental fate studies	Metabolite IN-KE121 (Soil and aq. calcium chloride solutions) Method used in support of the adsorption/desorption study of metabolites, breakdown and reaction products from the initial DAR: - Vol. 3-B.8.1.2.1.2 - Kane T. (2004).	**HPLC-MS/MS** (LOQ = 2.5 ng/g for soil analysis and 0.005 mg/L for calcium chloride extracts analysis) Working range: Soil : 2.5 – 10000 ng/g Aq. calcium chloride solution: 0.005 – 5 mg/L) Soil: Sequential extraction twice with acetonitrile and once with acetonitrile/water: 3/1.	CA 4.1.2(a)/01 – Kane T. (2004) – Report No. 063/042264 Vol. 3 CA-B.5.1.2.1 – Study No. 1	Method is validated and considered "fit for purpose".

		Aq.calcium chloride solution: direct analysis.		
	Metabolite IN-KF313 (Soil and aq. calcium chloride solutions) Method used in support of the adsorption/desorption study: - Wright D., Gilbert J. and Heslop D . (2011) cited in Vol.3 CA B.8.1.2.2 (study report however not available, noting in Monograph and addendum 2009).	**UPLC-MS/MS** (LOQ = 2.5 µg/g for soil analysis and 0.005 mg/L for calcium chloride extracts analysis) Working range: Soil : 2.5 – 8 µg/g Aq. calcium chloride solution: 0.005 – 10 mg/L) Soil: CLE 8224952-01V.S: Sequential extraction twice with acetonitrile and once with acetonitrile/water: 3/1 (v/v) CLE 8224952-02V.S: Sequential extraction twice with acetonitrile and once with acetonitrile/water: 3/1 (v/v) followed by further extraction with acetonitrile:0.1 M ammonium acetate (1:1, v/v) Aq. calcium chloride solution: direct analysis after dilution with water (CLE 8224952-01V.A)	CA 4.1.2(a)/02 – Wright D., Gilbert J. and Heslop D. (2011) – Report No. 8224952 Vol. 3 CA-B.5.1.2.1 – Study No. 2	Method is considered sufficiently validated considering the different soils combined and "fit for purpose".
	Metabolite IN-KF313 (surface water)	**HPLC-MS/MS** (LOQ = 0.1 µg/L) Working range: 0.1 – 1.0 µg/L Direct analysis	CA 4.1.2(a)/03 – Jooβ S. (2015) – P3463G Vol. 3 CA-B.5.1.2.1 – Study No. 3	Method is validated. The method was not specifically used in any risk assessment studies and was developed as a water enforcement method for the metabolite.

	Lenacil (soil) Fate does not identify a risk assessment study where the method was used.	**GC-MS** (LOQ = 0.05 mg/kg) Working range: 0.05 – 2.55 mg/kg Extraction with acetone/dichloromethane (twice) and redissolution in acetone	CA 4.2(b)/01 – Brodsky J. and Zietz E. (1990) – Report No. BE-A-11-90-10-BF Vol. 3 CA-B.5.2.3 – Study No. 1	Method is sufficiently validated considering the different soils combined. The method is proposed as the monitoring method in soil by the applicant.
	Lenacil and metabolite IN-KF313 (Soil) Method used in support of soil dissipation study: - Vol.3 CA-B.8.1.1.2.2.1 (Pollmann, B.; 2003)	**HPLC-MS/MS:** (LOQ = 0.02 mg/kg for each analyte) Working range: 0.02 – 0.2 mg/kg for both analytes (+ limited recoveries at 0.5 mg/kg) Extraction (twice) with acetone/dichloromethane	CA 4.2(b)/02 - Mende (2003) – Study 20011048/E1-FSD Vol. 3 CA-B.5.2.3 – Study No. 2	Method is validated and considered "fit for purpose".
Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies	Lenacil (Diet feed) Method used in support of toxicity studies: -IIA 5.3.1.1-01 (dietary administration to Han Wistar rats – 4w) -IIA 5.3.2.2-01 (90d in mice) - IIA 5.5.2-01 (oncogenicity in mice) The method is also used in the following studies: - CA 5.3.2 (13-w in Han Wistar rats) - CA 5.3.2 (13-w beagle dogs)	**HPLC-UV:** (LOQ = 100mg/kg) Working range: 100 – 20000 mg/kg Procedural recoveries occurred in several studies in the 50 – 50000 mg/kg range and further validation occurred in the 100 – 50000 mg/kg range in two studies. Extraction with methanol	CA 4.1.2(c)/01 – [redacted] (2002) – Report No. ACD 001/010098 CA 4.1.2(c)/02 – [redacted] (1991) – Report No. HLR293-91 CA 4.1.2(c)/03 – [redacted] (1994) – Report No. HLR336-93 Vol. 3 CA-B.5.1.2.3 – Studies No. 1, 2 and 3	Method is validated and considered "fit for purpose".

	- CA 5.5 (chronic tox. and carcino.- han wistar rats, 104-w) - CA 5.6.1 (reproductive effects in Han Wistar Rats) - CA 5.8.2 (potential effects on thyroid function after 20-w in Han Wistar rats)			
	Lenacil (Diet feed) Method used in support of: - toxicity study IIA 5.6.1.2-01 (reproductive study on rats – 2 successive generation)	**HPLC-UV:** (LOQ = 50mg/kg) Working range: 50 – 50000 mg/kg Extraction with methanol	CA 4.1.2(c)/04 – [redacted] (2003a) – Report No. ACD 020/023865 Vol. 3 CA-B.5.1.2.3 – Study No. 4	Method is validated and considered "fit for purpose".
	Lenacil (0.5% w/v methylcellulose formulation) Method used in support of : - toxicity study IIA 5.6.2.1-04 on effects on embryo-fetal development in rats (continuous gavage) - CA 5.6.2 (effects on embryo-fetal development in CD rats – oral gavage administration)	**HPLC-UV:** (LOQ=1mg/mL) Working range: 1-100 mg/mL Extraction with methanol	CA 4.1.2(c)/05 – [redacted] (2003b) – Report No. ACD 058/032316 Vol. 3 CA-B.5.1.2.3 – Study No. 5	Method is validated and considered "fit for purpose".
Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies	Lenacil (1% w/v methylcellulose formulation)	**HPLC-UV** (LOQ = 50 mg/mL) Working range: 50 – 400 mg/mL Dilution with the mobile phase	CA 4.1.2(f)/01 (CA8.1.1.1/01 ~~IIA 8.1.1-01~~) - [redacted] (2002a) – ACD 048/022425 CA 4.1.2(f)/02 (CA8.1.1.1/02 ~~IIA 8.1.1-02~~) – [redacted] (2002b) – ACD 049/022426	Method is sufficiently validated and considered "fit for purpose".

	Method used in support of the ecotoxicity study: **CA8.1.1.1/01 (critical) and CA8.1.1.1/02 (critical) (acute oral toxicity bird)**		Vol. 3 CA-B.5.1.2.6 – Studies No 1 and 2.	
	Lenacil (aq. media: diatom, OECD, dechlorinated tap water, lemna media) Method used in support of the ecotox. studies: CA8.2.1/01 (fish) CA8.2.6.1/01 (algal) CA8.2.6.2/01(algal) CA8.2.7/01 (Lemna)	**HPLC-UV** (lowest expected achievable LOQ from the different water types = 0.2 µg/L but the following LOQs are set for the different water types based on the available validation data : LOQ (diatom) = 0.2 µg/L LOQ (OECD) = 2.5 µg/L LOQ (dechlorinated tap water) = 1 mg/L LOQ (lemna) = 1.011 µg/L None of these LOQs can be considered as fully validated (n = 2) Working range (diatom) = 0.2 – 75.3 µg/L Working range (OECD) = 2.5 – 625 µg/L Working range (dechlorinated tap water) = 1 – 6.3 mg/L Working range (Lemna) = 1.011 – 1011 µg/L None of these working ranges can be considered as fully validated (n = 2 at each level) Extraction by liq/liq partition into dichloromethane followed by addition of acetonitrile/water 20/80, v/v	CA 4.1.2(f)/03 (CA8.2.6.1/01 ~~IIA 8.2.6-02~~)– Flatman, D. (2003c) – Report No. ACD 034/022511 CA 4.1.2(f)/04 (CA8.2.1/01 ~~IIA 8.2.1-03~~) – [redacted] (2003a) – Report No.ACD 035/022512 CA 4.1.2(f)/05 (CA8.2.6.2/01 ~~IIA 8.2.6-01~~) – Flatman, D. (2003) – Report No. ACD 036/024694 CA 4.1.2(f)/06 (IIA 8.2.8-01) – Flatman, D. (2003) – Report No. ACD 039/023827 Vol. 3 CA-B.5.1.2.6 – Studies No 3 to 6.	Method is not fully validated but the overall available data indicates that the method can be considered "fit for purpose".
	IN-KE121 metabolite (algal medium : OECD)	**HPLC-UV** (LOQ = 0.8 mg/L)	CA 4.1.2(f)/07 (CA8.2.6.1/03 ~~IIA 8.2.6-04~~)– Jenkins C.A. (2004a) – ACD 064/042730	Method is not fully validated but the overall available data indicates that the method can be

	Method used in support of the ecotox. study: **CA8.2.6.1/03 (critical) (algal)**	LOQ cannot be considered as fully validated (n = 2); the level of 1 mg/L is more reliable by the available data Working range: 0.8 – 129 mg/L The working range cannot be considered as fully validated (n = 1 or 2 at each fortification level) Dilution with acetonitrile/water (3/7, v/v) or acetonitrile	Vol. 3 CA-B.5.1.2.6 – Study No 7.	considered "fit for purpose".
	IN-KF313 metabolite (algal medium : OECD) Method used in support of the ecotox. study: **CA8.2.6.1/04 (critical) (algal)**	**HPLC-UV** (LOQ = 0.26 mg/L) LOQ cannot be considered as fully validated (n = 2) Working range: 0.26 – 13 mg/L The working range cannot be considered as fully validated (n = 1 or 2 at each fortification level) Dilution with acetonitrile/water (3/7, v/v) or acetonitrile	CA 4.1.2(f)/08 (CA8.2.6.1/04 ~~IIA 8.2.6-05~~) – Jenkins C.A. (2004b) – ACD 066/042848 Vol. 3 CA-B.5.1.2.6 – Study No.8	Method is not fully validated but the overall available data indicates that the method can be considered "fit for purpose".
	Lenacil (stock solution) Method used in support of the ecotox. study: KCP 10.6.2/01	**HPLC-UV** (LOQ proposed at 2.4 mg PPP/L after dilution by a factor 40 but not substantiated by data) Working range: 2.4 – 24 g PPP/L Dilution with methanol	CA 4.1.2(f)/08.01 (KCP 10.6.2/01) - Gossman a. and Meinerling M. (2006) - Project 26803086 Vol. 3 CA-B.5.1.2.6 – Study No.8.01	Method cannot be stated fully validated but seems to be "fit for purpose". However, based on the available validation data, this cannot be confirmed with certainty. However, the study is not taken into consideration.

	Lenacil (formulation) Lenacil, IN-KE121 and IN-KF313 (water and sediment) Method used in support of the ecotox. study: **KCP 10.2.3/01 (critical) (microcosm)**	**Analysis of lenacil in Venzar 80 WP : HPLC-UV** No validation data. Reference is made to ACD 013/033850 **Analysis in water: LC-MS/MS** (LOQ = 0.1 µg/L) Working range: 0.1 – 200 µg/L Extraction using C18 SPE. Cartridge eluted with methanol prior to reconstitution in water/methanol 50/50 v/v. **Analysis in sediment: LC-MS/MS** (LOQ = 0.1 µg/L) Working range: 0.1 – 200 µg/L Extraction with acetonitrile, followed by evaporation and reconstitution in water/methanol 50/50 v/v.	**CA 4.1.2(f)/08.02 (KCP 10.2.3/01)** Jenkins C.A. (2005) - Report ACD 072/043691 Vol. 3 CA-B.5.1.2.6 – Study No.8.02	HPLC-UV for the determination of lenacil in Venzar 80 WP: no validation data. **Reference is made to ACD 013/033850 which should be provided if containing validation data** and for the sake of completeness. Methods however exists to determine lenacil in Venzar 80 WP and were accepted in the initial monograph or at zonal level. LC-MS/MS (water): Method is validated for the three analytes in water and is considered "fit for purpose". LC-MS/MS (sediment): The method is validated for the three analytes. The endpoints are however not expressed in sediment.
	Lenacil (water) Method used in support of the ecotox. studies: -CA8.2.1/02 (acute, fish) -CA8.2.1/03 (acute, fish) -CA8.2.2.1/01 (chronic, fish)	**HPLC-UV (ODS-Hypersil 60 × 4.6 mm except in CA8.2.2.1/02: Zorbax RX-C8 150 × 2.1 mm)** LOQ stated at 0.1 mg/L in all studies except in CA8.2.4.1/01 for which LOQ was proposed at 25 mg/L and in CA8.2.2.1/02 where the LOQ was proposed at 7.6 µg/L No validation data	**CA 4.1.2(f)/08.03 to CA 4.1.2(f)/08.08** Hutton D.G. (1991) – Report 198-91 Hutton D.G. (1991) – Report 199-91 Hutton D.G. (1991) – Report 200-91 Hutton D.G. (1989) – Report 86-89 Hutton D.G. (1989) – Report 130-89 Kreamer G.C. (1996) – Report 235-96 Vol. 3 CA-B.5.1.2.6 – Studies	**Not validated (no validation data in the report). Validation data should be provided. However, it is noted that the results are not critical for the risk assessment.**

	-CA8.2.2.1/02 (chronic, fish) (critical) -CA8.2.4.1/01 (acute, daphnia) -CA8.2.5.1/01 (chronic, daphnia)		No.8.03 to 8.08	
	Lenacil (20 × AAP medium) The exact ecotoxicological studies in which the method has been used cannot be clearly identified. This should be clarified by the notifier.	**HPLC-MS/MS** (LOQ = 0.2 µg/L set in the report but proposed by RMS to be fixed at 4 µg/L supported by the available validation data) Working range: 4 – 75 µg/L Dilution with the algal medium.	CA 4.1.2.(f)/09 [KCA 4.1.2.16/01] – Nixon W. B., Kendall T. Z (2012) – Study Dupont-35031 (Wildlife No. 112C-192) Vol. 3 CA-B.5.1.2.6 – Study No. 9	Method is validated. The method has however not been used and/or referenced fully and exactly as such in any ecotox study. The method is regarded as supportive data.
	Lenacil (M4 medium) Method used in support of ecotox. study: **CA 8.2.4.1/02 (critical) (Daphnia)**	**HPLC-MS/MS** (LOQ = 50.43 µg/L taking into account the dilution factor of 1000). Working range: 50.43 – 100.87 µg/L after dilution (corresponding to 50.43 – 100.87 mg/L before dilution) Dilution with M4-medium	CA 4.1.2(f)/10 [KCA 4.1.2.16/02] – Renner P., (2016a) – Study No. 15 10 48 031 W Vol. 3 CA-B.5.1.2.6 – Study No. 10	Method is validated and considered "fit for purpose".
	Lenacil (M4 medium) Method used in support of ecotox. study: **CA 8.2.5.1/02 (critical) (Daphnia)**	**HPLC-MS/MS** (LOQ = 7.02 µg/L) Working range: 7.02 – 120.6 µg/L after dilution (corresponding to 7.02 – 1206 µg/L before dilution) Dilution with M4-medium	CA 4.1.2(f)/11 [KCA 4.1.2.16/03] – Renner P., (2016b) – Study No. 15 10 48 032 W Vol. 3 CA-B.5.1.2.6 – Study No. 11	Method is validated and considered "fit for purpose".

	Lenacil (OECD medium) Method used in support of the ecotox. study (Algae): **CA 8.2.6.2/02 (critical)** CA 8.2.6.2/03 CA 8.2.6.2/04 CA 8.2.6.2/05 CA 8.2.6.2/06	**HPLC-MS/MS** (LOQ = 0.5 µg/L) Working range: 0.5 – 4.97 µg/L Direct injection or after dilution if necessary.	CA 4.1.2(f)/12 to CA 4.1.2(f)/17 [KCA 4.1.2.16/04 to CA 4.1.2.16/09] – Wenzel A., (2014 a -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 Vol. 3 CA-B.5.1.2.6 – Study No. 12 to 17	**Method is validated in the mentioned working range. 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 with the calibration range.**
	Lenacil (plant growth medium) Method used in support of the ecotox. studies: CA 8.2.7/02 **CA 8.2.7/03 (critical)**	**HPLC-MS/MS** (LOQ = 0.1 µg/L) Working range: 0.1 – 1 µg/L Dilution with methanol and mixing with the IS	CA 4.1.2(f)/18 [KCA 4.1.2.16/10] – Wenzel A. (2012a) – DPT 001/4 80/C CA 4.1.2(f)/19 [KCA 4.1.2.16/11] – Wenzel A. 2012b) – DPT 001/4 80/D Vol. 3 CA-B.5.1.2.6 – Studies No. 18 to 19	**Method is validated in the mentioned working range. 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 0.1 – 1 µg/L but remain with the calibration range.**
	Lenacil (aqueous test solutions or sugar feeding solutions) Method used in support of the ecotox. studies : **CP10.6.2/02 (non-target plants) (critical)** **CP10.3.1.2/01 (chronic oral tox. on honey bee) (critical)**	**HPLC-UV (US ES Pharm RP18, 150 × 3.0 mm, 5 µm)** **Aqueous test solutions (Stürtz):** (LOQ = 9 g PPP/L [4.1g a.s./L] corresponding to 90 mg PPP/L [41 mg a.s./L] after dilution) Working range: 9 – 13 g PPP/L (4.1 – 6 g a.s./L) corresponding to 90 – 130 mg PPP/L (41 – 60 mg a.s./L) after dilution.	CA 4.1.2(f)/20 [KCA 4.1.2.16/12] - Stürz S., Knebel N. (2016) – Study 95231087 CA 4.1.2(f)/23 [KCA 4.1.2.17/01] – Haupt S., Knebel N. (2016a) – Study 95231136 Vol. 3 CA-B.5.1.2.6 – Studies No. 20 and 23	Method is validated and considered "fit for purpose".

		Sugar feeding solutions (Haupt S.): (LOQ = 1 g PPP/L [0.45 g a.s./L] corresponding to 20 mg PPP/L [9 mg a.s./L] after dilution Working range: 1 – 20 g PPP/L [0.45 – 9.1 g a.s./L] corresponding to 20 – 100 mg PPP/L [9 – 50 mg a.s./L] after dilution. Dilution with acetonitrile/pure water (50/50, v/v).		
	Lenacil (algal medium – reconstituted water and 20 × AAP medium) Method used in support of the ecotox. study: **- CP 10.2.1/02 (plants)** **- CP 10.2.1/01 (pseudokirchneriella subcapitata)**	**LC-MS/MS :** (LOQ = 1 µg PPP/L in CP 10.2.1/01 and 10 µg PPP/L in CP 10.2.1/02) Working range: 1 – 100 µg PPP/L in CP 10.2.1/01 and 10 – 100 µg PPP/L in CP 10.2.1/02 Enrichment by SPE, elution with acetonitrile/water: 50/50	CA 4.1.2(f)/20.01 and CA 4.1.2(f)/20.02 Pawlowski S. and Wydra V. (2006) – reports 26801210 and 26802240 Vol. 3 CA-B.5.1.2.6 – Studies No. 20.01 and 20.02	Method is sufficiently validated and considered "fit for purpose".
	Lenacil (avian diet formulations) Method used in support of the old ecotox. study: CA8.1.1.2/01 (dietary toxicity in bird)	**HPLC-UV** (LOQ = 156 ppm) Working range: 156 – 5000 ppm Extraction with methanol	CA 4.1.2(f)/21 [CA8.1.1.2/01 ~~IIA 8.1.2.01~~] – Rodgers M.H. (2004a) – DPT 637/033931 Vol. 3 CA-B.5.1.2.6 – Study No. 21.	Method is validated and considered "fit for purpose".
	Lenacil (avian diet formulations)	**HPLC-UV**	CA 4.1.2(f)/22 [CA8.1.1.3/01 ~~IIA 8.1.3.01~~] – [redacted] (1996) – AMR 3419-95	Method is not fully validated but it is considered that there is sufficient indication to

	Method used in support of the old ecotox study: **CA8.1.1.3/01 (critical) (reproduction study in bird)**	(LOQ not stated. Based on the overall data an LOQ of 100 ppm seems reasonable and is proposed by RMS) Working range: 98.5 – 1024 ppm The working range cannot be considered as fully validated during the recovery experiments (n = 1) but the overall data (recovery experiments, homogeneity, stability, dose verifications) seems to indicate that the method works correctly within this range. Extraction with methanol	Vol. 3 CA-B.5.1.2.6 – Study No. 22.	consider the method "fit for purpose".
	Lenacil (aqueous test solutions or sugar feeding solutions) Method used in support of the ecotox. studies : **CP10.3.1.2/02 (Chronic oral toxicity test on bumble bee) (critical)** CP10.3.1.3/01 (honey bees larval tox. Test)	**HPLC-UV (column US ES RP18, 250 × 4 mm, 5 µm):** **Aqueous test solutions:** (LOQ = 25 g PPP/L [11.4 g a.s./L] corresponding to 0.05 g PPP/L [0.02 g a.s./L] after dilution) Working range: 25 – 35 g PPP/L (11.4 – 15.96 g a.s./L) corresponding to 0.05 – 0.07 g PPP/L (0.02 – 0.03 g a.s./L) after dilution. **Sugar feeding solutions:** (LOQ = 0.04 g PPP/L [18.2 mg a.s./L] corresponding to 2 mg PPP/L [0.9 mg a.s./L] after dilution Working range: 0.04 – 0.9 g PPP/L [18.2 – 410 mg a.s./L] corresponding to 2 – 18 mg PPP/L [0.9 – 8.2 mg a.s./L] after dilution.	CA 4.1.2(f)/24 [KCA 4.1.2.17/02] – Haupt S., Knebel N. (2016b) – Study 95231107 CA 4.1.2(f)/25 [KCA 4.1.2.17/03] – Haupt S., Knebel N. (2016c) – Study 95231032 Vol. 3 CA-B.5.1.2.6 – Studies No. 24 and 25.	Method is validated and considered "fit for purpose".

		Dilution with acetonitrile/pure water (50/50, v/v).		

2.5.2 Methods for post control and monitoring purposes

For the assessment of the different analytical methods proposed for monitoring purposes, the current criteria of SANCO/825/00 rev.8.1 were used.

According to these criteria, the analytical methods listed here below are considered adequate for monitoring and enforcement purposes.

2.5.2.1. Analytical methods for the determination of residues in plant

For the residue definition for monitoring in plant matrices, please refer to Vol. 1 – level 2- B.2.7 and Vol. 1 – level 2 – B.2.13. Based on that residue definition, residue analytical methods are required for the parent molecule lenacil in food/feed of plant origin.

For plant commodities, the fully validated method DFG S19 using LC-MS/MS enabling the determination of lenacil is available for enforcement/monitoring purposes in high water, acidic, oily and dry matrices (see Table below) with a LOQ of 0.01 mg/kg.

Matrix	Method	analyte	Confirmatory method	ILV	LOQ	Reference
Food/feed of plant origin (high water, acidic, oily, dry [high starch])	Based on DFG S19 (LC-MS/MS, 2 transitions)	Lenacil	Confirmation simultaneous to the primary method (LC-MS/MS, 2 transitions)	Yes (in high water, oily and dry matrices)*	0.01 mg/kg	CA 4.2(a)/01 (KCA 4.2.1/01) – Witte A.. (2011a) Vol.3 CA-B.5.2.1 – study No.1 CA 4.2(a)/02 – (KCA 4.2.1/02) – Witte A. (2015) Vol.3 CA-B.5.2.1 – study No.2 CA 4.2(a)/03 – (KCA 4.2.1/03) – Witte A.. (2011b) Vol.3 CA-B.5.2.1 – study No.3 CA 4.2(a)/04 – (KCA 4.2.1/04) – Mende P. (2011) Vol.3 CA-B.5.2.1 – study No.4 (ILV) CA 4.2(a)/05 – (KCA 4.2.1/05) – Richter S. and Stanislowski, T. (2015) Vol.3 CA-B.5.2.1 – study No.5 (ILV)

* 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.

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 were performed and instead the applicant provides a statement. Acetonitrile/water was shown to be a good solvent for extraction in the metabolism studies (> 70% of TRR extracted with lenacil representing more than 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 Analytcial 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.**

Extraction efficiency has not been demonstrated in the other matrices. This should be addressed later at national level in case where the use in new crops with new residues studies will be envisaged at national levels.

2.5.2.2. Analytical methods for the determination of residues in products of animal origin

For the residue definition for monitoring in animal matrices, please refer to Vol. 1 – level 2- B.2.7 and Vol. 1 – level 2 – B.2.13.

For animal commodities, a fully validated method using LC-MS/MS based on QuEChERS and enabling the determination of lenacil is available for enforcement/monitoring purposes in meat, milk, fat, liver and eggs (see Table below) with a LOQ of 0.01 mg/kg.

Matrix	Method	analyte	Confirmatory method	ILV	LOQ	Reference
Food/feed of animal origin: milk, meat, fat, liver and egg	Multi-residue LC-MS/MS based on QuEChERS (2 transitions)	Lenacil	Confirmation simultaneous to the primary method (LC-MS/MS, 2 transitions)	Yes in all matrices	0.01 mg/kg	CA 4.2(a)/06 (KCA 4.2.2/01) – Wagner B. (2016) Vol.3 CA-B.5.2.2 – study No.1 CA 4.2(a)/07 (KCA 4.2.2/02) – Bodsch, J. (2016) (ILV) Vol.3 CA-B.5.2.2 – study No.2

Extraction efficiency has not been demonstrated. Nevertheless, the extraction efficiency cannot be demonstrated and addressed at this stage since no samples from radiolabeled animal metabolism or samples with incurred residues from feeding studies are not existing (livestock metabolism or livestock feeding studies are not available).

2.5.2.3. Analytical methods for the determination of residues in soil

For residue definition for monitoring in soil (metconazole), please refer to Vol. 1 B.2.13.

For the purpose of renewal, no new monitoring methods are proposed. Reference is made to both methods (GC-MS and HPLC-MS/MS) considered in the initial monograph. In the initial monograph, the GC-MS was proposed as the monitoring method and the HPLC-MS/MS was considered as a confirmatory method of the GC-MS.

For the purpose of the renewal, the notifier recommended the HPLC-MS/MS (more specific than GC-MS) as the monitoring method with the GC-MS being considered as the confirmatory method. However, RMS could not fully agree with that proposal since the LOQ level of the HPLC-MS/MS set at 0.02 mg/kg was not confirmed by the GC-MS (0.05 mg/kg). Consequently, the notifier proposed again to consider the GC-MS as the proposed monitoring method in soils with the HPLC-MS/MS as confirmatory method. RMS however noted that although, it can be reasonably expected that the HPLC-MS/MS works accurately and precisely at 0.05 mg/kg (LOQ level of the GC-MS) based on data generated in the 0.02 - 0.5 mg/kg with full validation at 0.02 and 0.2 mg/kg, the validation results at 0.05 mg/kg (as well as at a ten times higher level) are very limited (only one replicate) and the recovery result is quite limit since of 71% (no % RSD). Moreover, it the LOQ of 0.05 mg/kg for the GC-MS comply with the LC50 for the most sensitive non-target organism (40 mg as./kg), the LOQ seems to not fully comply with the ErC10 value of the most sensitive crop (73.4 g a.s./ha, corresponding to a concentration of ~ 0.0489 mg/kg).

Therefore, RMS thinks that in order to have a fully "state of the art" method and in full agreement with the SANCO/825/00 regarding validation, **a full re-validation of the method HPLC-MS/MS should preferably occur by monitoring and validation of two mass transitions (data gap).**

The notifier informed the RMS in the course of the assessment that a re-validation of the soil method (HPLC-M/MS) will be conducted immediately to satisfy new requirements (two mass transitions will be monitored).

Matrix	Method	analytes	Confirmatory method	ILV	LOQ	Reference
Soil	HPLC-MS/MS (one mass transition)	Lenacil and metabolite IN-KF313	Method is in principle self-confirmatory but validation data were only generated for one mass transition. GC-MS is proposed as confirmatory method (see below)	Not required	0.02 mg/kg for each analyte	CA 4.2(b)/02 (IIA 4.2.2.1-02) – Mende P. (2003) – 20011048/E1-FSD Vol.3 CA-B.5.2.3 – Study No. 2
Soil	GC-MS (one mass)	Lenacil	Proposed as the confirmatory method of the HPLC-MS/MS	Not required	0.05 mg/kg	CA 4.2(b)/01 – Brodsky J. and Zietz E. (1990) – BE-A-11-90-10-BF Vol.3 CA-B.5.2.3 – Study No. 1

2.5.2.4. Analytical methods for the determination of residues in water

For residue definition for monitoring in surface and drinking water, please refer to Vol. 1 B.2.13.

For the purpose of renewal, a new method is available for the determination of lenacil in surface and drinking water, and supersedes the previous one submitted in the frame of the first Annex I inclusion. The method has been fully validated according to SANCO/825/00 rev.8.1 and successfully independently validated and can therefore be recommended for enforcement/monitoring purposes with a validated LOQ of 0.1 µg/L.

Matrix	Method	analytes	Confirmatory method	ILV	LOQ	Reference
Drinking and surface water	LC-MS/MS (2 mass transitions)	Lenacil	Confirmation simultaneous to the primary method (monitoring of 2 mass transitions)	Yes	0.1 µg/L*	CA 4.2(b)/03 [KCA 4.2.4/01] – Witte A (2009) – Study 09D2203-01-VMWA CA 4.2(b)/04 [KCA 4.2.4/02] – Link Th. (2016) – Study No. IF-15/03444276 Vol.3 CA B.5.2.4 – Studies No. 1 and 2

* 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 for *Elodea canadensis*).

2.5.2.5. Analytical methods for the determination of residues in air

For residue definition for monitoring in air (metconazole), please refer to Vol. 1 B.2.13.

For the purpose of renewal, no new monitoring methods to determine lenacil in air are proposed. Reference is made to the original monitoring method presented in the initial monograph.
The method is fully validated according to SANCO/825/00 rev. 8.1 and can therefore be recommended for enforcement/monitoring purposes. The validated LOQ of the method (0.1 mg/m^3) complies with the concentration C (0.12 mg/m^3) calculated based on the AOEL value of 0.4 mg/kg body weight/day according to SANCO/825/00 rev. 8.1. The AOEL value proposed for renewal is kept as initially for the first approval of the active substance.

Matrix	Method	analytes	Confirmatory method	ILV	LOQ	Reference
Air	HPLC-MS/MS (one mass transition)	Lenacil	Method is in principle highly specific if two mass transitions are followed. However, here only one mass transition was followed and the method has not been confirmed.	Not required	0.1 mg/m^3	CA 4.2(c)/01 (IIA 4.2.4.1-01) – Rawle (2005) – Study CEMS-2788 Vol.3 CA B.5.2.5 – Study No. 1

The method has not been confirmed since although HPLC-MS/MS is known to be a self-confirmatory method by monitoring two mass transitions, this has not been achieved during the study. Nevertheless, a confirmatory method is not required in this case since there is a sufficient confirmatory method for the determination of lenacil in water.

2.5.2.6. Analytical methods for the determination of residues in body fluids and tissues

For the residue definition in body fluids and tissues monitoring, please refer to Vol. 1 B.2.13.

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)**.

2.6 EFFECTS ON HUMAN AND ANIMAL HEALTH

2.6.1 Summary of absorption, distribution, metabolism and excretion in mammals *[equivalent to section 9 of the CLH report template]*

Table 1: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Absorption, distribution, metabolism and excretion of lenacil in the rat. Study is in compliance with Dir EEC 87/302/EEC, equivalent to OECD test guideline n° 417 (1984).	Absorption Based on total urine excretion: single LD 59% - repeated LD 72% (10 mg/kg bw), and taking into account the levels in cage wash, tissue and carcass, as well as the biliary excretion (6-17%), lenacil absorption is 83-100% after repeated low dose. Distribution Widely distributed, with high concentrations are also identified in excretory organs, and GIT. Metabolism Metabolic reactions: oxidation, dehydrogenation and glutathione conjugation/ glucuronidation. The recovered and identified metabolites in-vitro are in line with those found in-vivo in the rat. Excretion Rapid and complete	Absorption considered complete	[redacted] 1996 (HLR 62-94)
Interspecies comparison of in vitro metabolism of]Lenacil in mouse, rat, dog and human hepatocytes.	High metabolic stability in human and dog hepatocytes (96% and 103%, respectively, remaining after 120 min. incubation) but significant metabolism in rodents. Extent of conversion was 50.0% in mouse hepatocytes and 95.7% in rat hepatocytes after 120 minutes of incubation. The calculated *in vitro* $t_{1/2}$ values were 115 min. for mouse hepatocytes, 26 min. for rat hepatocytes and were above 120 min. for dog and human hepatocytes: rat < mouse < dog ~ human. No human-specific metabolite was detected..		Piñeiro Costas, 2016 (Project 512721)

2.6.1.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Not relevant. For C&L.

Absorption

Based upon the urinary excreted radioactivity after a single dose (10 mg/kg b.w.), oral absorption represents 59% of the administered low dose. Taking into account the metabolite identification in urine, it appears clearly that oral absorption is more important as also suggested by the results of repeated dosing (7 doses) where urine radio-activity amounts to 72 (♂)-86(♀)% at 144h. In contrast, at high dose (1000 mg/kg b.w.), urinary excretion is limited to about 8%, indicating a saturation effect.

In the repeated dose toxicity studies in rats and dogs and in the carcinogenicity study in rodents, the liver was identified to be at target. For this reason, the excretion via the bile (7% in ♂ and 17% in ♀ which was determined at 12 and 24 hours after oral administration could potentially be taken into account in the determination of the total absorption.

Therefore, the total gastrointestinal absorption, after repeated low-dose administration (the most relevant), taking into account urine excretion, tissues and residual carcass levels, would amount to 77+6 (bile)=**83%** in the ♂, and 89 (17% bile)~**100%** in the ♀.

Distribution

Maximal lenacil-related tissue concentration is observed in the gastrointestinal tract as well as in its content. High concentrations are also identified in excretory organs (liver, kidneys). Six days after oral administration, radioactivity is still detected in a large part of tissues at a very low concentration, with a higher concentration in excretory organs. At that time, at 10 mg/kg bw, ♂ rat carcass retained the highest concentration representing 0.78% of the administered dose. The increase of total residue concentration between the low and high dose groups was less than the 100-fold increase in the dose. There was no evidence of tissue accumulation after single dose administration.

Metabolism

The *in-vivo* metabolism of the absorbed lenacil is extensive. Up to about 58% of the administered dose was characterised in urine after a single oral low dose, and up to 81% after repeated dosing. The major biotransformation pathway is hydroxylation of either the cyclohexyl or cyclopentenyl ring, or both rings. The major component in urine was a hydroxylated metabolite of lenacil with the hydroxyl group on C5 or C6. No glucuronide or sulfate conjugates is released by glucuronidase or sulfatase.

An *in-vitro* experiment was conducted in order to investigate the comparative metabolism in rat, mouse, dog and human hepatocytes. The findings suggest that the metabolism is much more extensive in rodents as compared to that in the human and the dog. All of the major metabolites (≥5% of total radioactivity) could be identified and structures are proposed. Metabolic reactions observed included oxidation, dehydrogenation and combinations with glutathione conjugation and glucuronidation. The recovered and identified metabolites *in-vitro* are in line with those found *in-vivo* in the rat. No human-specific metabolite was detected.

Excretion

Radioactivity is mainly excreted into urine within 12-24h. Urine represents the main excretion route after low single dose reaching about 60% of the dose (as compared to about 32% excreted in the faeces). There is no important quantitative differences between ♂ and ♀ rats.

When the oral dose is repeatedly administered, urinary excretion is increased to 72-86% (as compared to about 12-19% faecal excretion) and a slight delay in excretion occurs as well, suggesting an increase in oral absorption and/or an induction of biotransformation of lenacil.

After oral high dose administration, urinary excretion is strongly reduced to 5-8% of the dose (while faecal excretion amounts to 81-87% of the dose), suggesting saturation of intestinal absorption.

Recovery of radioactivity ranges between 92 and 100%.

The metabolic profile identified in bile, was similar to that seen in faeces and urine. These results could suggest that enterohepatic circulation may occur.

2.6.2 Summary of acute toxicity

2.6.2.1 Acute toxicity - oral route [equivalent to section 10.1 of the CLH report template]

Table 2: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral toxicity, Dir 96/54/EEC Method B.1 tris, equivalent to OECD 423 (1996),	Rat, Sprague-Dawley, 5 animals/sex	Lenacil technical grade Batch n° 141712003, Purity 98,6%	5000 mg/kg, 14 days	>5000 mg/kg	[redacted] 2001 (ACD 004/013224/AC)

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
No deviation Study accepted					

Table 3: Summary table of human data on acute oral toxicity : not relevant (no data)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference

Table 4: Summary table of other studies relevant for acute oral toxicity : not relevant (only 1 animal study)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

2.6.2.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

2.6.2.1.2 Comparison with the CLP criteria regarding acute oral toxicity

LD_{50} >5000 mg/kg, above the criteria of 2000 mg/kg below which classification applies, according to CLP. Lenacil classification is thus not justified.

2.6.2.1.3 Conclusion on classification and labelling for acute oral toxicity

Lenacil is not classified for oral acute toxicity, according to CLP criteria.

2.6.2.2 Acute toxicity - dermal route [equivalent to section 10.2 of the CLH report template]

Table 5: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute dermal toxicity, EEC Directive 92/69/EEC Method B.3, equivalent to OECD 402 (1987), No deviation **Study accepted**	Rat, Hsd:Sprague-Dawley, 5 animals/sex	Lenacil technical grade Batch n° 141712003, Purity 98,6%	5000 mg/kg, 14 days	>5000 mg/kg	[redacted] 2001 (ACD 005/013220/AC)

Table 6: Summary table of human data on acute dermal toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference

Table 7: Summary table of other studies relevant for acute dermal toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

2.6.2.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

2.6.2.2.2 Comparison with the CLP criteria regarding acute dermal toxicity

LD_{50} >5000 mg/kg, above the criteria of 2000 mg/kg below which classification applies, according to CLP. Lenacil classification is thus not justified.

2.6.2.2.3 Conclusion on classification and labelling for acute dermal toxicity

Lenacil is not classified for acute dermal toxicity, according to CLP criteria

2.6.2.3 Acute toxicity - inhalation route [equivalent to section 10.3 of the CLH report template]

Table 8: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Acute inhalation toxicity, Directive 92/96/EEC Method B.2, equivalent to OECD 403 (1987), No deviation **Study accepted**	Rat, Crl: CD (SD) IGS BR, Sprague-Dawley in origin, Five animals/sex	Lenacil technical grade, Batch No. 141712003, Purity 98.6%	5.12 mg/L particulate aerosol, 4 hours	> 5.12 mg/l in air	[redacted] 2001 (ACD 021/013229)

Table 9: Summary table of human data on acute inhalation toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference

Table 10: Summary table of other studies relevant for acute inhalation toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

2.6.2.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

2.6.2.3.2 Comparison with the CLP criteria regarding acute inhalation toxicity

The available study provided no evidence that the LC_{50} of lenacil in rats is below the criteria of 5 mg/l triggering classification, according to CLP. Lenacil classification is thus not justified.

2.6.2.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Lenacil is not classified for acute inhalation toxicity, according to CLP criteria.

2.6.2.4 Skin corrosion/irritation [equivalent to section 10.4 of the CLH report template]

Table 11: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results - Observations and time point of onset[2] - Mean scores/animal - Reversibility	Reference
Skin irritation Directive 92/69/EEC Method B.4, equivalent to OECD 404 (1992), No deviation **Study accepted**	Rabbit, New Zealand, Three females	Lenacil technical grade, Batch No. 141712003, Purity 98.6%	0.5 g of Lenacil technical was applied under a 2-ply 25 mm x 25 mm porous gauze pad, which had been moistened with 0.5 ml distilled water, Four hours	No dermal irritation Animal 1 <Score erythema>24+48+72h=0 <Score oedema >24+48+72h=0 Animal 2 <Score erythema>24+48+72h=0 <Score oedema >24+48+72h=0 Animal 3 <Score erythema>24+48+72h=0 <Score oedema >24+48+72h=0	[redacted] 2001 (ACD 006/013201/SE)

Table 12: Summary table of human data on skin corrosion/irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference

Table 13: Summary table of other studies relevant for skin corrosion/irritation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

2.6.2.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

2.6.2.4.2 Comparison with the CLP criteria regarding skin corrosion/irritation

In the absence of any irritation sign, lenacil did not fulfil the criteria for skin irritation under CLP either in terms of severity of scores or in terms of irreversibility. Lenacil classification is thus not justified.

2.6.2.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Lenacil is not classified for skin corrosion/irritation, according to CLP criteria

2.6.2.5 Serious eye damage/eye irritation [equivalent to section 10.5 of the CLH report template]

Table 14: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results - Observations and time point of onset[2] - Mean scores/animal - Reversibility	Reference
Eye irritation Directive 92/69/EEC Method B.5, equivalent to OECD 405 (1987), No deviation **Study accepted**	Rabbit, New Zealand, Two females, one male	Lenacil technical grade, Batch No. 141712003, Purity 98.6%	0.1 ml of lenacil technical (Mean weight 70 mg)/1 eye/animal	No eye irritation Animal 1 (female) <Score cornea opacity>24+48+72h = 0 <Score iris>24+48+72h = 0 <Score erythema>24+48+72h = 0.3 >Score chemosis>24+48+72h = 0 Animal 2 (male) <Score cornea opacity>24+48+72h = 0 <Score iris>24+48+72h = 0 <Score erythema>24+48+72h = 0.3 >Score chemosis>24+48+72h = 0 Animal 3 (female) <Score cornea opacity>24+48+72h = 0 <Score iris>24+48+72h = 0 <Score erythema>24+48+72h = 0 >Score chemosis>24+48+72h = 0 When present, erythema was observed at 24h only, complete resolution was seen at 48h.	[redacted] 2001 (ACD 007/013273/SE)

Table 15: Summary table of human data on serious eye damage/eye irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference

Table 16: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

2.6.2.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

2.6.2.5.2 Comparison with the CLP criteria regarding serious eye damage/eye irritation

Signs of irritation were limited to slight conjunctival redness and was reversible after 24h. Mean 24+48+72 hours value for this parameter was not higher than 0.3, below the threshold of classification of 1 according to CLP. Lenacil classification is thus not justified.

2.6.2.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Lenacil is not classified for eye irritation, according to CLP criteria.

2.6.2.6 Respiratory sensitisation [equivalent to section 10.6 of the CLH report template]

Table 17: Summary table of animal studies on respiratory sensitisation

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference

Table 18: Summary table of human data on respiratory sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference

Table 19: Summary table of other studies relevant for respiratory sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

2.6.2.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

No human or experimental data was available to assess respiratory sensitisation potential, but neither the structure of lenacil (compared to overt respiratory sensitisers known thus far) nor the existing guideline inhalation study indicates any concern as regards respiratory sensitisation. No classification is proposed.

2.6.2.6.2 Comparison with the CLP criteria regarding respiratory sensitisation

2.6.2.6.3 Conclusion on classification and labelling for respiratory sensitisation

2.6.2.7 Skin sensitisation [equivalent to section 10.7 of the CLH report template]

Table 20: Summary table of animal studies on skin sensitisation

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Closed-patch repeated insult dermal sensitization study (maximisation method)	Guinea pig, Duncan Hartley albino, Twenty adult male	Lenacil technical grade, Batch No. 9038, Purity	Main study: Intradermal Induction phase: injection of 0.1 mL of a 1.5% (w/v) suspension of lenacil technical with or without Freunds Complete Adjuvant, for 7 days Topical induction phase: patches with 0.3 mL of control, test article or	No skin sensitisation	[redacted] 1992 (HLO 34-92)

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Not fully in compliance with Dir EEC 96/54/EEC, Annex IV C or 92/69-84/449 or OECD test guideline n° 406 (1981-92), But deviations acceptable. **Study accepted**	and female animals	98.2% (reanalysed 98.5%)	positive control article were applied for two weeks Topical challenge		

Table 21: Summary table of human data on skin sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference

Table 22: Summary table of other studies relevant for skin sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

2.6.2.7.1 Short summary and overall relevance of the provided information on skin sensitisation

2.6.2.7.2 Comparison with the CLP criteria regarding skin sensitisation

Although it was noted that the intradermal induction in the GPMT test was performed at too low a concentration, the result of this test did not fulfil the criteria of 30% of animals with a positive reaction that would indicate a skin sensitisation potential at the doses tested. Lenacil classification is thus not justified.

2.6.2.7.3 Conclusion on classification and labelling for skin sensitisation

Lenacil is not classified for skin sensitization, according to CLP criteria

2.6.2.8 Phototoxicity

Table 23: Summary table of studies on phototoxicity

Method, guideline, deviations[1] if any	Test substance	Dose levels duration of exposure	Results	Reference
***In vitro* phototoxicity Neutral Red Uptake (NRU)**	Lenacil technical grade, Batch FEB12HE004,	Eight lenacil concentrations (0.01 to 31.62 µg/ml) in 200	No phototoxicity	Westerink WMA, 2016 (Project 511052)

assay Compliant with Regulation (EC) No. 440/2008, Part B.41 (31 May 2008) and OECD TG432 as of 13 April 2004.. **Study accepted**	purity: see Certificate of Analysis	µL EBSS medium tested in 8 independent replicates		

Table 24: Summary table of human data on phototoxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference

Table 25: Summary table of other studies relevant for phototoxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

2.6.2.9 Aspiration hazard [equivalent to section 10.13 of the CLH report template]

Table 26: Summary table of evidence for aspiration hazard

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

2.6.2.9.1 Short summary and overall relevance of the provided information on aspiration hazard

Not relevant (physico-chemical properties do not trigger classification (solid a.s.)

2.6.2.9.2 Comparison with the CLP criteria regarding aspiration hazard

2.6.2.9.3 Conclusion on classification and labelling for aspiration hazard

2.6.2.10 Specific target organ toxicity-single exposure (STOT SE) [equivalent to section 10.11 of the CLH report template]

Table 27: Summary table of animal studies on STOT SE (specific target organ toxicity-single exposure)

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference

Table 28: Summary table of human data on STOT SE (specific target organ toxicity-single exposure)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference

Table 29: Summary table of other studies relevant for STOT SE (specific target organ toxicity-single exposure)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

2.6.2.10.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure (STOT SE)

No findings were reported indicating a concern for toxicity following a single exposure by the oral, dermal and inhalation administration routes. In the acute inhalation study, exaggerated breathing was reported in all rats; however, it was considered insufficient by RMS to regard the substance as a respiratory irritant. In addition, the necropsy did not reveal any adverse findings and breathing was not affected in repeated oral administration studies.

2.6.2.10.2 Comparison with the CLP criteria regarding STOT SE (specific target organ toxicity-single exposure)

No acute human data were reported and experimental data did not indicate target organ toxicity following acute exposure. Without any findings indicative of a histological alteration of the respiratory tract, the observation of transient breathing pattern did not justify classifying Lenacil for respiratory tract irritation.

2.6.2.10.3 Conclusion on classification and labelling for STOT SE (specific target organ toxicity-single exposure)

Lenacil is not classified as STOT-SE, according to CLP criteria.

2.6.3 Summary of repeated dose toxicity (short-term and long-term toxicity) [section 10.12 of the CLH report]

2.6.3.1 Specific target organ toxicity-repeated exposure (STOT RE) [equivalent to section 10.12 of the CLH report template]

Table 30: Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure)

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
28-day oral, diet Study not fully in compliance with Dir. EEC 96/54/EEC Annex IV D or 92/69-84/449 or OECD test guideline n° 407 Deviations: *no blood tests were performed. Tissues were stored but not examined for histopathology.* Rat, Wistar, 5 animals/sex/group **Study accepted**	Lenacil Batch No. 141712003 Purity 98.6% *(0, 5000, 10000 (wk 1-2)/ 30000 (wk 3-4), 20000 (wk 1-2)/ 50000 (wk 3-4) ppm),* equivalent to: ♂: 0, 571, 1269/2978, 2545/5029 mg/kg bw/d ♀: 0. 631, 1288/3576, 2643/5913 mg/kg bw/d	**NOAEL** *5000 ppm* = **571 mg/kg bw/d** LOAEL *10000/30000 ppm* = 1269/2978 mg/kg bw/d, based on: ↑uterine fluid distention. At top-dose (*20000/50000 ppm* =2545/5029 mg/kg bw/d): ↑liver w:	[redacted] 2002a (ACD 001/010098)
28-day oral, diet Study partially meets the current OECD Test guideline 407 Deviations: *tissues for histopathology were prepared from only 1 animal/sex/dose but were not examined* Dog, Beagle, 1 animal/sex/dose **Study accepted**	Lenacil Batch No. 141712003 Purity 98.6% *(0, 5000, 20000, 50000 ppm),* equivalent to ♂: 0, 219, 807, 1941 mg/kg bw/d ♀: 0, 242, 967, 2331 mg/kg bw/d	**NOAEL** *5000 ppm* = **219 mg/kg bw/d** LOAEL *20000 ppm* = 807 mg/kg bw/d, based on: ↓b.w. change, ↑liver w, ↓kidneys w ↓thymus w, ↓WBC, ↓neutrophils	[redacted], 2001 (ACD 003/013230)
90-day oral, diet EEC Directive 88/302/EEC, EEC Directive 92/69/EEC, EEC Directive 96/54/EEC equivalent to OECD 408. No deviation Rat, Wistar, 10 animals/sex/group **Study accepted**	Lenacil Batch No. 141712003 Purity 98.6% *(0, 500, 5000, 50000 ppm),* equivalent to ♂: 0, 41, 412, 4357 mg/kg bw/d ♀: 0, 45, 468, 4893 mg/kg bw/d	**NOAEL** *500 ppm* = **41 mg/kg bw/d** LOAEL *5000 ppm* = 412 mg/kg bw/d, based on : ↓b.w. change, clinical signs, leukopenia, ↑proteinuria (♂),↑liver w, ↑Schmorl's^{+} pigment in thyroid cells At top-dose: ↑SG, ↑liver w, ↑thyroid w, hepatocyte hypertrophy, lymph node hypercellularity	[redacted] 2002b (ACD 002/013903) [redacted] 2004 (ACD 055/024499)
90-day, oral, diet Study not fully in compliance with Dir 2001/59/EC or 87/302 or OECD test guideline n° 408 (1998-81). Deviations: *the coagulation time was not measured; epididimydes, thymus, uterus and ovaries were not weighed; salivary gland, stomach and urinary bladder were not examined for histopathology; blood chemistry was limited to proteins; and duration of treatment and sacrifice time was not clearly reported.* Mouse, CD-1, 10 animals/sex/dose	Lenacil Batch No. 9038, purity 98.2% *(0, 100, 1000, 5000, 10000 ppm),* equivalent to: ♂: 0, 16, 157, 787, 1616 mg/kg bw/d ♀: 0, 20, 207, 1127, 2150 mg/kg bw/d	**NOAEL** *1000 ppm* = **157 mg/kg bw/d** LOAEL *5000 ppm* = 787 mg/kg bw/d, based on: ↑liver w Disregarding blood toxicity at the next-lower dose of 16 mg/kg bw/d At top-dose: ↑proteinuria, ↑liver w, ↑spleen w, ↑LN hyperplasia, ↑liver + spleen extramedullary haematopoiesis	[redacted] 1991 (HLR 293-91)

90-day, oral, diet EEC Directive 96/54/EEC Method B.27, equivalent to OECD 409 No deviation Dog, Beagle, 4 animals/sex/dose **Study accepted**	Lenacil Batch No. 141712003 Purity 98.6% *(0, 1000, 5000, 25000 ppm),* equivalent to: ♂: 0, 44, 221, 1121 mg/kg bw/d ♀: 0, 46, 225, 1102 mg/kg bw/d	**NOAEL** *1000 ppm* = **44 mg/kg bw/d** LOAEL *5000 ppm* = 221 mg/kg bw/d, based on: ↓reticulocytes, ↑γGT, ↑cholesterol, ↓phosphorus, ↑adrenal w, ↓spleen w, ↓thymus w, ↑liver w, ↑thyroid w, small thymus, ↑hepatocyte hypertrophy, ↑skin folliculitis. At top-dose: ↓body w, ↑APTT, ↑AP, ↑thymus involution, ↓epididymides sperm, ↑pituitary cysts, ↑pneumonitis.	[redacted] 2002 (ACD 022/014297)

Table 31: Summary table of human data on repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference

Table 32: Summary table of other studies relevant for repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

2.6.3.1.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure (short-term and long-term toxicity)

Lenacil was administered for a 13-week period in the diet of rats, mice and dogs at doses of approximately 15 mg/kg bw/d up to 4400 mg/kg bw/d.
In rat and mice, at doses of 100-400mg/kg bw/d, WBC count was decreased, without evidence of inflammatory change in any tissue, or any effect in lymphoid tissues.
In rats, at dose levels ranging from 400 to 4000 mg/kg bw/d, some blood electrolytes were altered and proteins were increased in urine suggesting a loss of the kidney ability to filter adequately blood. However, there were no effects upon kidney weight and kidney microscopy appeared normal. At these dose levels, liver weight was increased and hepatocyte centrilobular hypertrophy was noted at top dose. Some other organ weights were altered at top dose in rats without histological findings to support an adverse effect in these organs excepting for thyroid where thyroid follicular epithelium staining indicative of lipofuscin was observed at 5000 ppm onwards without any evidence of organ atrophy. After a 4 week rest, animals showed some recovery. Additional histopathological examinations of the thyroid were performed.
In mice at top doses of 1600-2500 mg/kg bw/d, white blood cell toxicity was observed and extramedullary haematopoiesis was increased in liver and spleen.
In dogs, at dose of 220 mg/kg bw/d onwards, liver weight was increased and centrilobular / midzonal hepatocyte hypertrophy was observed. At top dose, some dogs had thymus involution/atrophy.

The lowest NOAEL was agreed to be in the 13-week rat/dog study, around 41-44 mg/kg b.w./d, taking into account findings at >200 mg/kg b.w./d. and disregarding non-dose dependent effects of the a.s. on the WBC compartment in the mouse at a lower dose.
Overall, non-dose responsive blood toxicity was observed, further supported by extramedullary haematopoiesis at high doses, and occasional signs of cellularity changes in lymph nodes or thymus involution. The MoA remained unexplained.

Based on the available data on short-term toxicity and taking into account the type of effects observed, a classification of lenacil for repeated dose toxicity is not required according to the criteria laid down in Regulation (EU) No. 1272/2008 (CLP).

Table 33: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days [if adequate, otherwise please delete]

Study reference	Effective dose (mg/kg/day)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study

2.6.3.1.2 Comparison with the CLP criteria regarding STOT RE (specific target organ toxicity-repeated exposure)

Based on the available data on short-term toxicity and taking into account the type of effects observed, a classification of lenacil for repeated dose toxicity is not required according to the criteria laid down in Regulation (EU) No. 1272/2008 (CLP).

2.6.3.1.3 Conclusion on classification and labelling for STOT RE (specific target organ toxicity-repeated exposure)

Lenacil is not classified as STOT-RE, according to CLP criteria

2.6.4 Summary of genotoxicity / germ cell mutagenicity [equivalent to section 10.8 of the CLH report template]

Table 34: Summary table of genotoxicity/germ cell mutagenicity tests *in vitro*

Method, guideline, deviations[1] if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations/ Results	Reference
***In vitro* bacterial mutagenicity (Ames)** Directive 2000/32/EC Method B.13/14, OECD 471 No deviation **Study accepted**	Lenacil, Batch No: 141712003, Purity 98.6%	*S. typhimurium* TA1535, TA1537, TA98 and TA100 and *E. coli* strain WP2uvrA/pKM101 (CM891) 5-5000 µg/plate (±S-9)	Negative	May K., 2001 (ACD 016/013217)
***In vitro* bacterial mutagenicity (Ames)** Study not fully in compliance with Dir EEC 2000/32/EEC Annex 4D 92/69 or 84/449 or OECD test guideline n° 471 (1997-83). Deviations: *strain TA 102 and E.coli WP2uvrA were not included. Strains were not tested for their quality criteria. Experiment was not repeated. Pre-test was not performed. Limited experimental information.* **Study providing complementary information**	Lenacil, Batch No: INB-634-50, Purity not specified	*S. typhimurium* TA1535, TA1537, TA1538T, TA98, and TA100 up to 500 µg/plate	Negative	Russell J.F., 1977 (HLR 601-77)

Method, guideline, deviations[1] if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations/ Results	Reference
***In vitro* bacterial mutagenicity (Ames)** Study not fully in compliance with Dir EEC 2000/32/EEC Annex 4D, 92/69 or 84/449 or OECD test guideline n° 471 (1997-83). Deviations: *strain TA 102 and E.coli WP2uvrA were not included in the study. Experimental protocol not described.* **Study providing complementary information**	DPX-B634-107, Purity: not specified	*S. typhimurium* TA1535, TA97, TA98, and TA100 up to 5000 µg/plate	Negative	D'Amico S.W., 1994 (HLR 413-94)
***In vitro* chromosome aberration (clastogenicity)** Study in compliance with Directive 2000/32/EC Method B.10 (2000), equivalent to OECD 473 (1997). **Study accepted**	Lenacil, Batch No. 141712003, Purity 98.6%	Cultured human peripheral blood lymphocytes 625-5000 µg/mL (±S-9)	Positive without S9 mix/negative with S9 mix	Allais L., 2001 (ACD 017/013707)
***In vitro* mammalian chromosomal aberration.** Study partly in compliance with Directive 440/2008/EC Method B.10 (2017), equivalent to OECD 473 (2016). Deviation: *lenacil was diluted in DMSO, which seemed to be suboptimal* **Study considered to provide complementary information**	Lenacil, Batch/No. 047303003, Purity 99.33%	Cultured human peripheral blood lymphocytes 25-100 µg/mL (±S-9)	Negative	Kellum S.N., 2017 (49348)
***In vitro* mammalian cell mutagenicity (CHO/HGPRT)** Study not fully in compliance with Dir EEC 2000/32/EEC Annex 4E or 87/302 or OECD test guideline n° 476 (1997-84). Deviation: *diameter of colonies was not measured for control cells* (OK for 87/302) **Study accepted**	Lenacil technical Batch No. 141712003, Purity 98.6%)	Mouse lymphoma L5178Y cells 39-5000 µg/mL (with and without S-9)	Negative	Clare, G., 2003 (ACD 053/023530)
In vitro unscheduled DNA synthesis. Guidelines were not reported. The study does not meet the current OECD Test Guideline 486. **Study accepted**	Lenacil (Batch no. 8903, purity not stated in report)	Rat primary hepatocytes 0.078-10 µg/mL^{-1}	Negative	Riach & Mohammed 1990 (IRI 6135)

Table 35: Summary table of genotoxicity/mutagenicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations[1] if any	Test substance	Relevant information about the study (as applicable)	Observations/ Results	Reference
***In vivo* micronucleus** The study meets the current OECD Test Guideline 473 with a deviation. Deviation: *the results were reported without SD*. However, reconduct is unlikely to yield a significantly different result because of the fact that study was conducted according to current guidelines and under GLP conditions **Study accepted**	Lenacil, Batch No. 141712003, Purity 98.6%	Mouse bone marrow 500, 1000, 2000 mg/kg bw	Negative	[redacted] 2001 (ACD 018/013472)

Table 36: Summary table of human data relevant for genotoxicity / germ cell mutagenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference

2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity / germ cell mutagenicity

Lenacil technical showed no evidence of mutagenic activity *in vitro*, in the *Salmonella typhimurium* bacterial system.

No mutagenic potential was exhibited in the *in vitro* mouse lymphoma cell mutation assay and the substance did not induce unscheduled DNA synthesis in cultures of primary rat hepatocytes when tested at concentrations extending into the toxic range.

However, lenacil technical did show evidence of clastogenic activity in human lymphocytes in *in vitro* cytogenetic test system in the absence of S9 mix only, and in the presence of slight precipitation (2500-5000 µg/mL). The positive result was not replicated in a more recent study. However, although the top concentration in this recent assay (not higher than 100 µg/mL) exhibited some precipitation, no cytotoxicity was demonstrated. It was therefore questioned if the second assay was tested at sufficiently high concentration since chromosome aberrations were noted in the first assay, tested at >12× higher but at acceptable cytotoxicity (not <45% MI) compared with the more recent assay.

No clastogenic activity was observed in the presence of S9 mix in either test.

Lenacil technical did not show any evidence of causing chromosome damage or bone marrow cell toxicity *in vivo* when administered orally to mice. The results of the ADME study (Ghantous, 1996) suggested that lenacil becomes quantitatively bioavailable in the bone marrow after oral dosing in the rat. Repeated dosing in the mouse also also indicates some haemotoxicity in the WBC, providing limited evidence that the test substance reached the bone marrow in the *in vivo* micronucleus assay.

Based on the battery of genetic toxicology studies that have been conducted with lenacil, it can be overall concluded that lenacil does not cause genetic damage and, therefore, does not pose a mutagenic risk, and classification of lenacil with respect to mutagenicity/genotoxicity is not required according to the criteria laid down in Regulation (EU) No. 1272/2008 (CLP).

2.6.4.2 Comparison with the CLP criteria regarding genotoxicity / germ cell mutagenicity

Considering the negative outcome of the available *in vivo* bone marrow micronucleus test that was performed up to the limit dose of 2000 mg/kg, Lenacil is considered to be non-mutagenic *in vivo*.

2.6.4.3 Conclusion on classification and labelling for genotoxicity / germ cell mutagenicity

A classification of lenacil for mutagenicity is not justified, according to CLP criteria.

2.6.5 Summary of long-term toxicity and carcinogenicity [equivalent to section 10.9 of the CLH report template]

Table 37: Summary table of animal studies on long-term toxicity and carcinogenicity

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL**	**Reference**
Two-year (24-month) oral, diet, chronic toxicity part. Study in compliance with Dir EEC 87/302/EEC Annex V B or OECD test guideline n° 453 (1981) Rat, Wistar, 20 animals/sex/dose **Study accepted**	Lenacil, Batch No. 141712003, Purity 98.6% *0, 250, 2500, 25000 ppm,* equivalent to ♂: 0, 12, 118, 1223 mg/kg bw/d ♀: 0, 16, 160, 1699 mg/kg bw/d	**NOAEL**: *250 ppm* = **12 mg/kg bw/d** LOAEL *2500 ppm* = 118 mg/kg bw/d, based on: Clinical signs (perigenital staining, eye discolouration°), ↓motility°, ↓lymphocyte count, ↑triglyceride level, ↑adrenal w, ↑thyroid weight°, enlarged thyroid, fluid distended/ hyperplastic uterus. Top-dose (25000 ppm =1223 mg/kg bw/d): clinical signs (skin/subcutis exfoliation/scabs, ventral swollen/firm areas, exophthalmos°), thin appearance, ↓body w (gain) °, proteinuria°, haematology (↓platelet count, ↓APTT,) ↓A/G ratio, ↑TSH, ↑organ weight° (spleen, kidney, liver), thyroids black°/luminal concretions°, enlarged/swollen spleen, small testes, adrenal ceroid accumulation and accessory tissue, liver CL hypertrophy°	[redacted] 2003 (ACD 045/024288)
Two-year (24-month) oral, diet, carcinogenicity part. Study in compliance with Dir EEC 87/302/EEC Annex V B or OECD test guideline n° 453 (1981) Rat, Wistar, 50 animals/sex/dose **Study accepted**	Lenacil, Batch No. 141712003, Purity 98.6% *0, 250, 2500, 25000 ppm,* equivalent to ♂: 0, 12, 118, 1223 mg/kg bw/d ♀: 0, 16, 160, 1699 mg/kg bw/d	**NOAEL**: *250 ppm* = **12 mg/kg bw/d** **LOAEL** *2500 ppm* = 118 mg/kg bw/d, based on: ↑mammary adenocarcinoma and thyroid C-cell adenoma Top-dose (25000 ppm =1223 mg/kg bw/d): ↑adrenal cortical adenoma and mammary adenoma	[redacted] 2003 (ACD 045/042214)
Eighteen (18)-month oral, diet toxicity and carcinogenicity Study not fully in compliance with Dir EEC 87/302/EEC Annex V B or OECD test guideline n° 451 (1981). A review of this study indicates that it partially meets the current OECD Test Guideline 453. Deviations: *-Differential blood counts were obtained from only ten mice in the control and high dose groups* *- The ovaries were not weighed* These deviations are not considered to have had a severe impact on the reliability and the interpretation of the study results. The study is considered valid Mouse, CRL-CD®-1(ICR)BR, 80 animals/sex/dose. **Study accepted**	Lenacil, Batch No. No. 9038, Purity 98.2%-98.5% *0, 100, 2500, 7000 ppm,* equivalent to ♂: 0, 14, 332, 977 mg/kg bw/d ♀: 0, 20, 482, 1358 mg/kg bw/d	Chronic toxicity **NOAEL** = 100 ppm = **14 mg/kg bw/d** LOAEL= 2500 ppm = 332 mg/kg bw/d, based on: eye discolouration, ↓kidney w, ↓spleen w, ↑hepatocellular karyomegaly Top-dose (7000 ppm =977 mg/kg bw/d): Exophthalmos, ↑liver w, ↑alveolar histiocytosis, ↑hepatocellular CL hypertrophy, ↑pituitary cysts, ↑kidney tubular cysts Carcinogenicity **NOAEL** = 2500 ppm = **332 mg/kg bw/d** LOAEL = 7000 ppm = 977 mg/kg bw/d, based on: ↑lung alveolar carcinoma° (♂), liver adenoma° (♂). Limited ↑Leydig cell hyperplasia and Harderian gland adenoma	[redacted] 1994 (HLR 336-93)

Table 38: Summary table of human data on long-term toxicity and carcinogenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference

Table 39: Summary table of other studies relevant for long-term toxicity and carcinogenicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

2.6.5.1 Short summary and overall relevance of the provided information on long-term toxicity and carcinogenicity

-In **rats**, the systemic NOAEL was determined to be 12 mg/kg b.w.d, based on clinical signs, decreased motility, decreased WBC counts, increased adrenal and thyroid weights (confimed by macroscopically enlarged thyroid) and fluid distended and hyperplastic uterus at 118 mg/kg b.w./d. Clinical chemistry findings at LOAEL were limited to increased cholesterol levels.
At top dose, further clinical signs (skin, eye) were detected. Top-dose animals exhibited haematological modifications (decreased platelet count, APTT), decreased A/G ratio and increased TSH level (however without effect on T_3-T_4). Increased weights were observed in spleen, kidney and liver.
The relevance of the liver, thyroid and spleen findings was corroborated by hypertrophy, discolouration of thyroids, and enlarged spleens respectively. Other notorious top-dose findings included increased incidences of alveolar histiocytosis, pituitary and kidney tubular cysts.

-In **mice**, the systemic NOAEL was determined to be 14 mg/kg b.w./d, based on spleen weight (exhibiting a dose-responsive decrease at the two highest doses). Major findings at the higher doses (mid and/or top-dose) included ocular effects, and increased liver weight associated with centrilobular hypertrophy. Other top-dose findings included increased incidences of alveolar histiocytosis, pituitary and kidney tubular cysts.
In concordance with observations in subchronic studies in rodents and dogs, the relevance of effects on the white blood cell compartment was questioned. Treated animals showed consistently alterations of WBC counts, however, often without a proper dose- or time-response. In the absence of any study investigating the immunotoxic potential of lenacil, the findings remain without explanation.

Thyroid and mammary gland tumours were observed in ♀ **rats**. Thyroid follicular cell adenomas were borderline within laboratory historical control data and C-cell tumours were considered as age and gender-dependent.
Therefore, thyroid tumours were not considered toxicologically relevant in terms of human exposure. Based on the incidences of mammary adenocarcinomas, the carcinogenic NOAEL in rats was established at 12 mg/kg b.w./d, and lenacil was considered a Cat.2 carcinogenic substance.

In the oncogenicity study in **mice**, liver and lung tumours were observed in ♂treated at the top-dose. The incidence of multiple liver adenomas was outside the laboratory historical control range but was covered by historical control data of [redacted] for Crl:CD-1 BR mice. The incidence of lung single alveolar adenoma was above the laboratory historical control range, but the incidence of lung single alveolar carcinoma was within the laboratory historical control data. When taken together, the combined lung adenoma and carcinoma incidence was outside the laboratory historical control data but it is presumably because of the adenoma incidence. However, because the increase was small and did not demonstrate a decreased latency period. Lung and liver tumours were considered of equivocal relevance for humans. The carcinogenic NOAEL in mice was established at 332 mg/kg bw/day.

The incidence of malignant mammary adenocarcinoma was above the historical background range of the laboratory but well within the historical control range of the [redacted] database. For this reason, the RMS considered the increased incidence of mammary adenocarcinoma as being an equivocal finding. Since an increase in mammary adenocarcinoma is usually associated with increases in mammary fibroadenomas and acinar hyperplasia and as these associated increases were not observed, the increased incidences in mammary adenocarcinoma were regarded to be unrelated to lenacil treatment by the notifier and for this reason, a classification with respect to carcinogenicity is not triggered.

In the framework of the assessment of the CLH report on the harmonised classification and labelling of lenacil by the RAC Committee of ECHA, the increased incidences of mammary adenocarcinoma were considered relevant

for humans by the RAC Committee. As a consequence, lenacil was classified as a category 2 carcinogen (Carc. 2; H351). This harmonised classification proposal has meanwhile been adopted (see Regulation (EC) No. 2015/1221) and the substance is included into Annex VI of the CLP regulation (Regulation (EC) No. 1272/2008).

2.6.5.2 Comparison with the CLP criteria regarding carcinogenicity

Table 40: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans

2.6.5.3 Conclusion on classification and labelling for carcinogenicity

At this stage, the assessment indicates that there is no need to revise the existing harmonised classification of the a.s. lenacil

2.6.6 Summary of reproductive toxicity [equivalent to section 10.10 of the CLH report template]

2.6.6.1 Adverse effects on sexual function and fertility – generational studies [equivalent to section 10.10.1 of the CLH report template]

Table 41: Summary table of animal studies on adverse effects on sexual function and fertility – generational studies

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL	Reference
2G oral, pilot study, diet Guidelines used in this preliminary study were not reported. A review of this publication indicates that it does not meet the current guideline OECD Test Guideline 416 and has been superseded with ACD 020/023865. Rat, Hsd Brl Han Wistar, F0: 8 animals/sex/group F1: 12 animals/sex/group **Study providing complementary information**	Lenacil Batch No. 141712003, Purity 98.6% *0, 10000, 25000, 50000 ppm,* equivalent to ♂: 0, 749, 1952, 3840 mg/kg bw/d ♀: 0, 755, 2003, 4014 mg/kg bw/d	<u>Maternal</u> **NOAEL** *25000 ppm* = **1952 mg/kg bw/d** LOAEL *50000 ppm* = 3840 mg/kg bw/d based on ↓body w, ↑clinical signs (alopecia) <u>Offspring</u> NOAEL *50000 ppm* = 3840 mg/kg bw/d <u>Reproductive</u> NOAEL *50000 ppm* = 3840 mg/kg bw/d	[redacted] 2002 (ACD 019/010186)
2G oral, main study, diet, EC test method B.35 (1999) equivalent to OECD 416 (1999), OPPTS 870.3800 (1998), JMAFF 12 Nohsan No. 8147 (2000). Rat, Hsd Brl Han Wistar, F0: 28 animals/sex/group F1: 24 animals/sex/group	Lenacil Batch No. 141712003, Purity 98.6% *0, 1000, 10000, 50000 ppm* ♂: 0, 82, 817, 4279 mg/kg bw/d ♀: 0, 93, 935, 4787 mg/kg bw/d	<u>Maternal</u> **NOAEL** *1000 ppm* = **82 mg/kg bw/d** LOAEL 10000 = 817 mg/kg bw/d, based on: ↓body w, ↑clinical signs, ↑liver w, ↑thyroid w, ↑pituitary w, ↓spleen w, ↓thymus w, ↑dark thyroids, ↑thyroid cell necrosis <u>Offspring</u> **NOAEL** *1000 ppm* = **82 mg/kg bw/d**	[redacted] 2003 (ACD 020/023865)

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL	Reference
Study accepted		LOAEL *10000 ppm* = 817 mg/kg bw/d, based on: ↓body weight (lactation), ↓spleen w, ↓thymus w <u>Reproductive</u> **NOAEL** 50000 ppm = **4279 mg/kg bw/d** LOAEL >50000 ppm = >4279 mg/kg bw/d.	

Table 42: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference

Table 43: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

2.6.6.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility – generational studies

In a ***preliminary reproduction study***, dietary administration of lenacil to rats at concentrations of 10000, 25000 or 50000 ppm was generally well-tolerated. Effects consisted of slightly low bodyweight gain prior to pairing for F_0 ♀ at top-dose and for all treated ♀during mid-lactation, and clinical signs. Mating performance, fertility and development of subsequent F_1 progeny, up to physical sexual maturation, showed no adverse effects of treatment. Dietary concentrations up to 50000 ppm were therefore considered suitable for use in the main two-generation study in this strain of rat.

In the ***main 2-generation reproduction study***, dietary administration of lenacil was assessed in rats at concentrations of 1000, 10000 or 50000 ppm. At 10000 ppm (817 mg/kg b.w./d) and 50000 ppm (4279 mg/kg b.w./d), maternal body weight was slightly altered, dams exhibited alopecia, and there was evidence of thyroid toxicity: increased weight, altered metabolism and histopathology. At these doses, dose-dependent decreases of spleen and thymus weight , and increased liver and pituitary weights were observed.
There were no effect on reproductive organs or reproductive performance at any of the dietary concentrations and offspring survival was not affected by treatment. There was no effect upon the physical and sexual development of the offspring.
At 10000 ppm and above, body weight gain of offsprings were reduced during lactation from d7 of age for the F_1 offspring and from d4 of age for the F_2 offspring. Whether treatment caused a reduction in milk production or quality, or whether the offspring was exposed to lenacil via milk could not be ascertained in this study.
Overall, both reproduction and development studies are not indicative of fertility or developmental adverse findings, suggesting that, at least based on the investigated parameters, lenacil is unlikely to be an endocrine disrupting substance.

2.6.6.1.2 Comparison with the CLP criteria regarding adverse effects on sexual function and fertility

2.6.6.2 Adverse effects on development [equivalent to section 10.10.4 of the CLH report template]

Table 44: Summary table of animal studies on adverse effects on development

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Developmental oral, pilot study, gavage. The study does not meet OECD 414, and is superceded by the (ACD 058/032316) Patten R. 2003c study. Rat, CD Sprague Dawley, 11 ♀/group. **The study is considered to provide complementary information**	Lenacil Batch No. DPX-B634091, Purity 98.5% 0, 500, 1000, 4000 mg/kg bw/d, over days 7-16 of gestation.	Maternal **NOAEL = 1000 mg/kg bw/d** LOAEL = 4000 mg/kg bw/d, based on ↓body w, ↑clinical signs (alopecia) Developmental (n.a.) (n.a.) investigation not available	[redacted] 1996 (HLR 996-96)
Developmental oral, pilot study, gavage. The study does not meet OECD 414, and is superseded by ACD 058/032316 (Patten R. 2003c study) Rat, CD Sprague Dawley, 6 ♀/group **The study is considered to provide complementary information**	Lenacil Batch No. 141712003, Purity 98.6% 0, 100, 300, 1000 mg/kg bw/d, from gestation days 1 to 19.	Maternal **NOAEL = 300 mg/kg bw/d** LOAEL = 1000 mg/kg bw/d, based on ↑clinical signs (staining) Developmental **NOAEL = 1000 mg/kg bw/d** LOAEL = >1000 mg/kg bw/d	[redacted] 2003b (ACD 057/030001)
Developmental oral, main study, gavage. EU Guideline B.31, equivalent to OECD 414. Rat, CD Sprague Dawley, 22 ♀/group **Study accepted**	Lenacil Batch No. 141712003 Purity 98.6% 0, 100, 300, 1000 mg/kg bw/d, from gestation days 1 to 19.	Maternal **NOAEL = 100 mg/kg bw/d** LOAEL = 300 mg/kg bw/d, based on ↑clinical signs (alopecia, staining) Developmental **NOAEL = 300 mg/kg bw/d** LOAEL = 1000 mg/kg bw/d, based on ↑skeletal variants (thickened rib, ossification delay vertebrae)	[redacted] 2003c (ACD 058/032316)
Developmental oral, main study, gavage Dir EEC 87/302/EEC or OECD test guideline n° 417 (1984). Rabbit, Hra:NZW, 20 ♀/group **Study accepted**	Lenacil Batch No. 9038 Purity 98.5% 0, 50; 200, 1000, 4000 mg/kg bw/d, on gestation days 7 to 19.	Maternal **NOAEL = 1000 mg/kg bw/d** LOAEL = 4000 mg/kg bw/d, based on ↑clinical signs (alopecia, staining) Developmental **NOAEL = 4000 mg/kg bw/d** LOAEL = > 4000 mg/kg bw/d	[redacted] 1991 (HLR 626-91)

Table 45: Summary table of human data on adverse effects on development

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**

Table 46: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

2.6.6.2.1 Short summary and overall relevance of the provided information on adverse effects on development

Oral administration of lenacil in a ***developmental rat study*** at 100, 300 or 1000 mg/kg b.w./d did weakly affect maternal or foetal parameters at the mid- and top-doses tested. The lowest dose was identified as a NOAEL on the basis of clinical signs *i.e.* fore limb alopecia and yellow ventral staining in dams, and variations in ossification performance in the foetuses) at 300 mg/kg b.w./d and above.

Oral administration of lenacil in a ***developmental rabbit study*** at doses of 50, 200, 1000, or 4000 mg/kg b.w./d did not affect foetal parameters at any of the doses tested in the main study (although there were indications of slightly increased resorptions/litter in the preliminary rabbit development study, to be confirmed by detailed assessment of this study). Maternal toxicity (decreased body weight gain) was evident at a daily dose of 4000 mg/kg b.w./d. Therefore, the NOAEL was 1000 mg/kg/day for the dam and greater than 4000 mg/kg/day for the conceptus.

Overall, both reproduction and development studies are not indicative of fertility or developmental adverse findings, suggesting that, at least based on the investigated parameters, lenacil is unlikely to be an endocrine disrupting substance.

2.6.6.2.2 Comparison with the CLP criteria regarding adverse effects on development

Adverse effects on development of the offspring were not observed, thus lenacil does not trigger a classification for developmental toxicity, according to CLP criteria.

2.6.6.3 Adverse effects on or via lactation [equivalent to section 10.10.7 of the CLH report template]

Table 47: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference

Table 48: Summary table of human data on effects on or via lactation

Type o data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference

Table 49: Summary table of other studies relevant for effects on or via lactation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

2.6.6.3.1 Short summary and overall relevance of the provided information on effects on or via lactation

The initial birth weight of the F_1 and F_2 offspring was unaffected by maternal treatment but there was a reduction of weight gain at 50000ppm that occurred from day 7 of age for the F_1 offspring and from day 4 of age for the F_2 offspring. This effect occurred before that offspring begin to consume solid food suggesting an effect via lactation. Whether treatment caused a reduction in milk production or quality or whether the offspring were exposed to lenacil via the milk cannot be ascertained in this study.
The parental NOAEL in rats was determined to be 82 mg/kg bw/day, based on evidence of reduced body weights, clinical signs, increased pituitary, liver and thyroid weight, decreased spleen and thymus weight, dark thyroids, and thyroid cell necrosis at 817 mg/kg bw/day onwards. The offspring NOAELs was determined to be 82 mg/kg bw/day, on the basis of decreased body weight during lactation and decreased spleen and thymus weights.

Due to the very high dose level applied in the study (RMS: >800 mg/kg b.w./d) the decrease in offspring weight gain (not higher than 10%) during lactation was considered to reflect offspring rather than reproductive toxicity and that a classification for lactational effects is therefore not required.

2.6.6.3.2 Comparison with the CLP criteria regarding effects on or via lactation

2.6.6.4 Conclusion on classification and labelling for reproductive toxicity

The reproduction and development studies are not indicative of fertility or developmental adverse findings. A classification of lenacil for reproductive toxicity is not justified, according to CLP criteria.

2.6.7 Summary of neurotoxicity

Table 50: Summary table of animal studies on neurotoxicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results: - NOAEL/LOAEL - target tissue/organ -critical effect at LOAEL	Reference

A waiver was provided, justifying to dismiss acute, subchronic or developmental neurotoxicity studies. The waiver was accepted.

2.6.8 Summary of other toxicological studies

2.6.8.1 Toxicity studies of metabolites and impurities

No studies on metabolites were performed. Studies and hazard assessments on impurities: see confidential DRAR (Vol.4).

The outcome (alerts and possible MoA relevant for genotoxicity, carcinogenicity or ED potential) for the various lenacil groundwater metabolites were evaluated using QSAR models.
A general conclusion regarding the relevance of the potential groundwater metabolites of Lenacil, using QSAR expert systems provided by the notifier was possible.
A final evidence, excluding the relevance of the environmental metabolites of lenacil, classified Carc. Cat.2, has not been provided. However, in the meanwhile it has been demonstrated that the non-polar metabolites, having alerts like these of lenacil itself, do not pose a risk for the groundwater. Polar metabolites may theoretically leach into the groundwater when taken together, but it appeared that the pool of polar metabolites were constituted by about 30 different breakdown products, for which no single structure could be fully elucidated, but which are most probably completely mineralised in the end. The multitude of different polar metabolites indicate that each of them do not breach the trigger of concern of 0.1 µg/L.

However, a tentative evaluation was made, on the basis of a number of potentially representative polar metabolites.
As regards the genotoxicity, PM1-PM4 are considered overall negative. For PM5, although alerts were flagged based on aminobenzene moieties, PM5 itself has no aromatic amine part, but a substituted cyclopentene ring, rendering any comparison with aromatic amines rather void.
Metabolite IPM1 has a mixed genotoxicological outcome. Although unlikely genotoxic, the final predictions of IPM1 and PM5 is undecided.
As regards the carcinogenicity, the models predict the metabolites being negative in almost all cases, except for IPM1 showing an equivocal result (+ for ISS but – for OnoLogic). Therefore, the outcome of IPM1 is equivocal for carcinogenicity.
However, it was stated that the molecular structure of IPM1 was indicative of high instability, while synthesis, and thus testing of this metabolite was demonstrated to be impossible.
It may thus be concluded that further investigation on potential effects of IPM1 is not necessary.

As regards the endocrine activity, the models provide in general a negative prediction for most metabolites, but contradictory results for PM4 and PM5. Therefore, the outcome of these 2 metabolites is also considered equivocal. However, since also lenacil was further assessed for endocrine effects, and the outcome was negative, further concern about any metabolite is no longer present.

2.6.8.2 Supplementary studies on the active substance

In a study investigating **potential effects on thyroid function** after 20 weeks of treatment in ♀Han Wistar rats using the "perchlorate discharge test" the ability of the thyroid to take-up and organify iodide was unaffected. Measurements of T_3 made during the study indicated that the test substance was not acting as an inhibitor of the deiodinase which convert T_4 into T_3, but a definite MoA remains unknown. **A further *in vitro*-test was announced** (see below).

No **immunotoxicity** assay was submitted. Whereas no overt signs of immunotoxic action could be identified in any repeated toxicity assay, there is still some uncertainty, since the lymphoid blood line and related organs such as spleen and/or thymus are consistently altered, albeit not always a non-dose-dependent way. The current studies do not highlight an clear immunotoxic effect, but some uncertainty remains as regards the interpretation of the observed findings throughout the repeated toxicity studies.

Therefore, RMS requests the conduct of this assay.

2.6.8.3 Endocrine disrupting properties

Since lenacil exhibited effects on the thyroid, uterus and mammary tissue in a number of guideline studies, notifier conducted 7 level 2 *in-vitro* studies and one level-3 *in-vivo* study, according to the OECD Conceptual Framework for testing and assessment of endocrine disrupters. Specific measurements of circulating thyroid hormones (T_3, T_4, TSH) were performed in 2 repeated toxicity studies (10-20 wk and 52 wk), and another non-guideline assay included the assessment of iodine organification with a perchlorate discharge test.
The results were as followed *in-vitro*:

- Lenacil did not competitively bind to the oestrogen receptor in rat uterine cytosol when tested up to a maximum concentration of 10^{-4} M, and was thus considered a non-inhibitor in the oestrogen receptor binding assay.
- Lenacil did neither show oestrogenic agonist nor antagonist activity in the hERα-HeLa-9903 cell line when tested up to a maximum concentration of 10^{-5} M.
- Lenacil did not competitively bind to the androgen receptor when tested up to a maximum concentration of 10^{-4} M, and was thus considered a non-inhibitor in the androgen receptor binding assay.
- Lenacil did not show any androgenic agonist or antagonist activity in a stable transfected CHO-K1 cell line when tested up to a maximum concentration of 10^{-4} M.
- Lenacil did not show any aromatase inhibiting activity in human recombinant microsomes, when tested up to a maximum concentration of 3.16×10^{-5} M.
- Lenacil did not did not alter oestradiol or testosterone synthesis in a steroidogenesis assay in the adrenocorcital H295R cell line, when tested up to a maximum concentration of 3.16×10^{-6} M.
- Lenacil did not inhibit peroxidase in porcine thyrocytes when tested up to a maximum concentration of 5×10^{-4} M.

Further results *in-vivo*:

- Lenacil did not induce changes in uterine parameters associated with oestrogen receptor agonism in ovariectomised adult ♀SD-rats administered by gavage up to 1000 mg/kg b.w./d for 5 consecutive days.
- Lenacil did not inhibit the thyroidal deiodinase/peroxidase in a perchlorate discharge test in adult ♀Wistar-rats administered in the diet up to 4421 mg/kg b.w./d for 20 consecutive weeks.
- Lenacil did not meaningfully alter the circulating thyroid hormone (T_3, T_4) levels in the interim (1yr) sacrificed rats assayed in a 2yr-dietary study up to 1200/1700 mg/kg b.w./d. A weak n.s.s. increase (28-33%) of TSH was observed, which was not considered of high concern in the view of the no-effect on the thyroidal hormones.
- Lenacil did significantly decrease the T3- and TSH-levels in ♂SD rats treated during 2-week in a dietary assay, but the effect was not reproduced in the 10wk- or 52 wk dietary study. T4 levels remained unaltered, while slight modifications of uncertain toxicological significance were found in the 10wk-20wk- and 52 wk-phases. The thyroid hormone measurements were inconsistent, and also difficult to interpret given the variabel experimental set-up.

As complementary information:

- Lenacil did strongly induced the hepatic CYPB1 isoform, and moderately induces UDPGT in ♂SD rats treated in the abovementioned 2-week dietary assay . While this type of substances may cause induction of certain metabolic enzymes in the liver, this normally results in increased clearance of T_4 by induction of T4-UDPGT, which is suggestive of increased clearance of THs. Subsequently, this may result in an increase of TSH that, in turn, would stimulate thyroid growth manifested by follicular cell hypertrophy/hyperplasia/neoplasia. While some features are in line with this hypothesis, the hormonal measurements are not completely in line with it, leaving the possibility that some other mechanism may play a role, currently incompletely revealed for lenacil.

It could thus be concluded that lenacil was devoid of any oestrogenic, anti-oestrogenic, androgenic or anti-androgenic activity, tested at appropriate levels as recommended. Inconsistent effects were noted on the thyroid hormone-levels, but from the guideline studies is became clear that lenacil may be thyrotoxic, however at doses associated with relatively high systemic toxicity, and mainly at doses exceeding the accepted limit dose of 1000 mg/kg b.w./d.

While no reprotoxic effects were reported, it could be discussed whether a DNT study would be desirable, in the case that developing young animals would possibly be more sensitive for neurodevelopmental endpoints

2.6.9 Summary of medical data and information

There have been no incidents or accidents involving lenacil that have been reported at the formulations sites of the finished product.
Staff involved in the production of lenacil have been supervised since 1993 and examined clinically at regular intervals The medical care at the production site includes medical history, eye test, listening test, functional check of the lungs, blood and urine tests, ECGs, measurement of blood pressure, neurological status, clinical checks as well as consultation on the use of personal protective equipment. There were no findings attributable to the involvement in lenacil production. No consequential damages of the staff involved in the production of lenacil are known.
Lenacil-based products have been commercially available in Europe since 1993 and no cases of accidental poisoning or incidents related to the agricultural use of lenacil were identified in the literature search that was conducted in conjunction with this EU Renewal submission. .
There is no relevant exposure of the general population which would allow epidemiological investigations to be conducted. Hence no epidemiological studies have been conducted with lenacil.

2.6.10 Toxicological end points for risk assessment (reference values)

Table 51: Overview of relevant studies for derivation of reference values for risk assessment

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
Rat	2 yr- oral, diet, Wistar rat toxicity and carcinogenicity study	Lenacil, Batch No. 141712003, purity 98.6%	Clinical signs, ↓lymphocyte count, ↑triglyceride level, ↑adrenal w, ↑thyroid weight, enlarged thyroid, fluid distended/ hyperplastic uterus ↑mammary adenocarcinoma and thyroid C-cell adenoma.	12 mg/kg bw/d ***(relevant for ADI)***	118 mg/kg bw/d	
Rat	Developmental oral, gavage, main study	Lenacil Batch No 141712003, Purity 98.6%	↑skeletal variants (thickened rib, ossification delay vertebrae)	300 mg/kg bw/d ***(relevant for ARfD and AAOEL)***	1000 mg/kg bw/d	
Rat	90-day oral, diet study	Lenacil Batch No 141712003, Purity 98.6%	↓b.w. change, clinical signs, leukopenia, ↑proteinuria (♂),↑liver w, ↑Schmorl's+ pigment in thyroid cells	41 mg/kg bw/d ***(relevant for AOEL)***	412 mg/kg bw/d	

2.6.10.1 Toxicological end point for assessment of risk following long-term dietary exposure – ADI (acceptable daily intake)

The EU Commission has established the ADI of lenacil at 0.12 mg/kg bw/day. This is based on the long-term/carcinogenicity study in the rats (ACD 045/042214) where the carcinogenic NOAEL in rats was finally established at 12 mg/kg bw/day although mammary adenocarcinomas were only seen in females and for which a NOAEL of about 16 mg/kg bw/day can be derived.

Hence, the NOAEL from the long-term/carcinogenicity rat study was used for setting of the ADI, with a NOAEL = 12 (12-16) mg/kg bw/d = 250 ppm. An assessment factor of 100 for inter- and intra-species extrapolation is sufficient. The ADI of lenacil was derived at:

ADI = 0.12 mg/kg b.w./d

2.6.10.2 Toxicological end point for assessment of risk following acute dietary exposure - ARfD (acute reference dose)

Since during renewal, a developmental NOAEL was established (lowest most relevant developmental NOAEL = 300 mg/kg b.w./d in the main rat developmental study, based upon skeletal ossification delays at 1000 mg/kg b.w./d), it may be discussed whether the establishment of an ARfD would be necessary.
If yes, then an assessment factor of 100 for inter- and intra-species extrapolation is sufficient.

The ARfD of lenacil could be derived at:
ARfD = 300 mg/kg bw/d ÷100 = **3 mg/kg b.w./d**

If accepted, the AAOEL could likewise be established at the same level.

2.6.10.3 Toxicological end point for assessment of occupational, bystander and residents risks – AOEL (acceptable operator exposure level)

The results of the lenacil 90-day feeding study in rats, supported by the 90-day study in dogs, are considered appropriate for the derivation of the AOEL of lenacil. The rat feeding study reveals a slightly lower NOAEL than the dog study (40.6 vs. 44 mg/kg/day). Using the most conservative NOAEL of ~40 mg/kg bw/day and applying a safety factor of 100 to account for differences in inter- and intraspecies sensitivity, the AOEL of lenacil, in line with the former EFSA conclusion, is derived at:
AOEL = 0.4 mg/kg bw/d

2.6.10.4 Toxicological end point for assessment of occupational, bystander and residents risks – AAOEL (acute acceptable operator exposure level)

AAOEL = 3 mg/kg bw/d

2.6.11 Summary of product exposure and risk assessment

Dermal absorption of the representative formulation (Lenacil 500 g/L SC)

Concentrate: 25% (default)
Spray dilution:75 % (default)

Summary of exposure assessment (Lenacil 500 g/L SC)

Operator exposure

Application method Crop	Model	PPE	Active ingredient	Total systemic exposure[1] (mg/kg bw/day)[2]	AOEL covered[3]
Tractor-mounted boom, hydraulic nozzles, sugar and fodder beet	EFSA guidance model	NO PPE	lenacil	0.2968	**74.21%**

[1] Systemic exposure based on dermal absorption of 25% (concentrated product) and 75% (diluted product) for lenacil.
[2] Body weight: 60 kg/person.
[3] Total systemic exposure x 100 / systemic AOEL (0.4 mg/kg bw/day)

According to the model calculations, it can be concluded that the risk for the operator using Lenacil 500 g/L SC on sugar and fodder beet is acceptable, even where protective clothing and equipment used in normal agricultural practice are not used.

Bystander and resident exposure

Application method Crop	Model		Active ingredient	Estimated bystander exposure (mg/kg bw/day)[1]	AOEL covered[2]
Tractor-mounted boom sprayer application outdoors to low crops	German model (Martin)	Bystander (adults)	lenacil	0.0018148	**0.45%**
		Bystander (children)	lenacil	0.0014190	**0.35%**
	EFSA guidance model	Resident (adults)	lenacil	0.0416	**10.39%**

		Resident (children)	lenacil	0.1113	**27.81%**
	German model (Martin)	Resident (adults)	lenacil	0.0001323	**0.03%**
		Resident (children)	lenacil	0.0001975	**0.05%**

[1] Body weight: 60 kg for adults, 10 kg for children (EFSA guidance model); 60 kg for adults, 16.15 kg for children (German model)
[2] Total systemic exposure x 100 / systemic AOEL (0.4 mg/kg bw/day)

According to the model calculations, it can be concluded that the risk for the bystander and the resident is acceptable, following exposure from the intended agricultural use of Lenacil 500 g/L SC in sugar an fodder beet according to the proposed GAP and label recommendations.

Worker exposure

Model	**PPE**	**Active ingredient**	**Total systemic exposure (mg/kg bw/day)[1]**	**AOEL covered[2]**
EFSA Guidance Model	No PPE	lenacil	0,0525	**13.13%**

[1] Body weight: 60 kg/person.
[2] Total systemic exposure x 100 / systemic AOEL (0.4 mg/kg bw/day)

According to the calculations it can be concluded that the risk for workers performing crop inspection in sugar and fodder beet treated with Lenacil 500 g/L SC is safe for the worker and no unacceptable health risk can be identified.

2.7 RESIDUE

2.7.1 Summary of storage stability of residues

In the framework of the original EU peer review, a freezer storage stability study with lenacil in sugar beet was evaluated. Lenacil residues can be considered as stable in sugar beet leaves and roots for at least 254 days (i.e. ca. 8.5 months) following storage at -18˚C (BE, 2007-2009; EFSA, 2009).
In the framework of renewal of a.s. approval, no new data on storage stability were provided.

2.7.2 Summary of metabolism, distribution and expression of residues in plants, poultry, lactating ruminants, pigs and fish

Plants

In the framework of the original EU peer review, a metabolism study on **sugar beet** (Zhang & Glunt, 1997) was evaluated and accepted to support the post-emergence use of lenacil on sugar and fodder beet (BE, 2007-2009; EFSA, 2009).

Lenacil radio-labelled in the pyrimidine ring was applied twice with a total application rate of 525 g a.s./ha, which is more or less in line with the notified critical GAP (*cf.* ***1.5.1***: restriction of maximum application rate to 500 g a.s./ha per season). The growth stages at the time of applications of BBCH 14 and 16 were earlier than the critical notified growth stage of BBCH 31. However, the study was considered acceptable, as a change of the metabolic pattern is not expected for an application at a later crop growth stage. This would result in higher levels of TRR, but a less extensive metabolism.
The TRR in root samples was maximum 0.03 mg/kg and therefore too low for identification of metabolites. Moderate metabolism was observed in samples of foliage. **Lenacil**, which was the main component of the TRR in all samples, declined from 96% of TRR (7 mg/kg) at the day of the first application to 28 % of TRR (0.04 mg/kg) 115 days after the second application. The only identified metabolites were **IN-KC943** formed by hydroxylation in position 7 of lenacil (max. 3.1% of TRR; <0.01 mg/kg) and its **glucosides** (max. 11% of TRR; <0.02 mg/kg). The polar fraction of metabolites, some of which could be hydrolysed by *β*-glucosidase, accounted for max. 38% of TRR (0.06 mg/kg). As no single polar metabolite exceeded 10% of TRR or 0.05 mg eq./kg, no attempts were made to further characterise or identify them.

In the framework of the assessment of confirmatory data following first a.s. approval (BE, 2013), it was mentioned that primary crop metabolism of lenacil had also been investigated in **spinach** and **strawberries** (Chrzanowski, 1978). However, as a detailed assessment of these two pre-GLP studies had not yet been reported previously (BE, 2013), it has been included in the DRAR (BE, 2019), in order to enable consideration of all available metabolism studies during EU peer review. However, the RMS concludes that the metabolism studies on spinach and strawberries are not acceptable as guideline-compliant studies and are merely to be considered as supportive/supplementary information (with limited reliability).

Although the reliability of the reported results could not be fully verified on the basis of the limited information presented in the study reports, the results seem to indicate that, in the case of pre-emergence soil treatment (at 0.6 kg/ha) immediately after sowing of spinach, or in the case of post-emergence (soil) treatment on strawberries (at 1-2x 1.6 kg/ha), the parent compound lenacil is only a minor/negligible fraction of the total residues (<1%TRR) in mature spinach leaves and strawberry fruits, respectively. At conditions similar to the design of the studies, no significant levels of parent lenacil in spinach leaves or strawberry fruit are to be expected (<0.01 mg/kg). The limited characterization of metabolites suggested the presence of several ring-hydroxylated homologs of lenacil, which are at least partially conjugated. Relatively large polar fractions could not be identified.

RMS notes that the confined rotational crop (CRC) study with **application to bare soil** at 485 g a.s./ha *(vide infra – **2.7.7.1**)* showed an overall similar metabolic profile in spinach sown after soil treatment, i.e. lenacil at max. 1%TRR, a large polar metabolite fraction and several metabolites tentatively identified/characterized as hydroxylated homologs of lenacil and (glucose) conjugates. There were indications of hydroxylation on the cyclopentapyrimidine ring (e.g. IN-KQ961) and/or on the cyclohexyl ring (e.g. IN-KD304). Thus, the confined rotational crop (CRC) study also provides some useful indications (complementary to the pre-GLP study on spinach mentioned further above) on the nature of residues in spinach to be expected following a pre-emergence application (i.e. a use that has been authorised in some EU MSs).

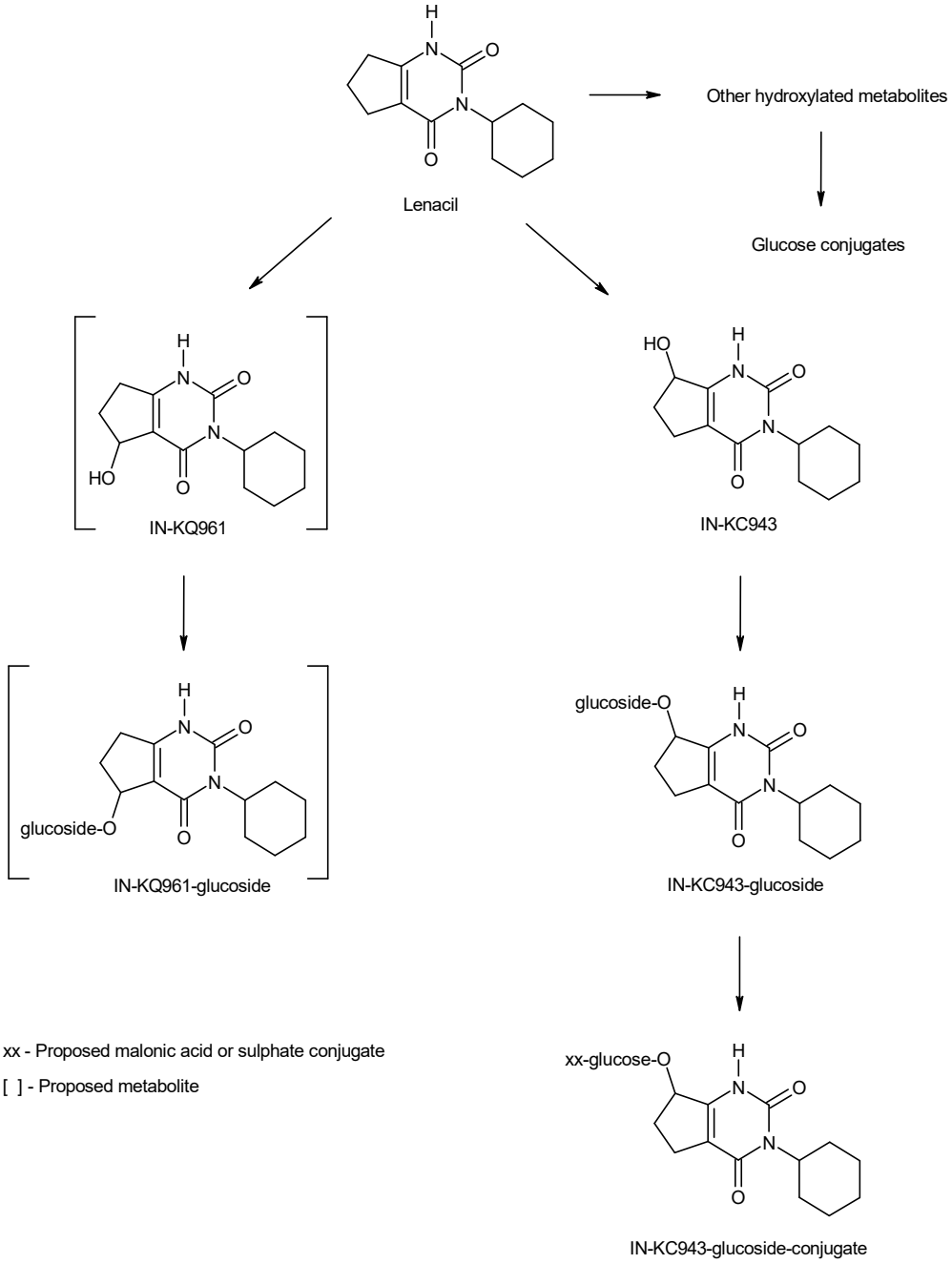
In this regard, it is acknowledged that there is a difference in application timing between the CRC study (spinach sown after bare soil treatment at the earliest plant-back interval of 68 DAT) and a pre-emergence application where the soil is treated practically just after spinach has been sown. It is presumed that in the case of soil spraying immediately after sowing, there would be less time allowing for metabolism/degradation of lenacil in the soil before uptake by the plant, which would imply relatively higher amounts of lenacil available for uptake and possible transfer to edible crop parts, while relatively lower amounts of metabolites would be expected to be available in the soil for uptake by the plant. In addition, the soil ageing of the residues would be *nihil* or at least much less, meaning that residues would be not or less bound to the soil and hence to a higher extent available for uptake by the plants. As a consequence, higher total residues and higher lenacil levels – compared to the results of the CRC study – in the spinach crop sown in soil that is treated immediately after sowing cannot be excluded. Thus, from a quantitative point of view, the representativeness of the CRC study to support a pre-emergence application is limited. Nevertheless, from a qualitative point of view, the metabolic pattern in the spinach crop is not expected to be significantly different for an application immediately after sowing, i.e. also a complex mixture of (conjugated) ring hydroxylated (and oxidised) metabolites, possibly together with some unmetabolised parent lenacil, is to be expected.

In **summary**, a GLP-compliant metabolism study on sugar beet (post-emergence treatment) is available, though it only provides metabolite identification and elucidation of the metabolic pathway in foliage. In sugar beet, lenacil is metabolised primarily via hydroxylation of the cyclopentyl ring to form IN-KC943 (7-hydroxy-lenacil). Secondary metabolism occurs via sugar conjugation of the hydroxyl metabolite(s). See **Figure 2.7.1-1**. Further metabolism studies in spinach (pre-emergence soil treatment) and strawberries (post-emergence soil treatment) suggest a similar metabolic pattern in these crops, but those studies have a limited reliability. Nevertheless, the available confined rotational metabolism study (*vide infra – **2.7.7.1***) provides useful complementary and consistent information in support of a possible pre-emergence use on spinach (i.e. beyond the scope of the representative uses).

In general, the results of the confined rotational crop study indicate that the **nature of residues in rotational crops is similar to that in primary crops** *(vide infra – **2.7.7.1**)*.

Figure 2.7.1-1: Proposed metabolic pathway of [2-^{14}C]-Lenacil in sugar beet leaves

[Ref.: Zhang & Glunt, 1997]



Animal

Ruminants, pigs, poultry

In the framework of the original EU peer review, no livestock or fish metabolism studies were provided and these were not required as the calculated dietary burden was either below or borderline (and worst-case) to the trigger value (cf. EFSA, 2009).

However, considering also possible residues in rotational crops that can be used for livestock feeding, the dietary burden trigger is significantly exceeded, particularly for ruminants (*vide infra* – ***2.7.5.2***). Therefore, without any mitigation measures in place to avoid residues in rotational crops, livestock metabolism studies would be required (**data gap**). The co-RMS (AT) fully agreed with this data gap.

Regarding metabolites in (rotational) crop parts used as feed items, no data addressing the metabolism in livestock animals (and transfer to animal commodities) were provided. In the absence of any livestock metabolism study (even not on the parent compound) and taken the limited identification of metabolites in primary and rotational crops into account, a significant livestock dietary exposure to metabolites cannot be excluded, looking at the high TRR levels in some livestock feed items (e.g. 6.1 ppm, parent eq., in wheat straw). Thus, the **potential transfer of metabolite residues from livestock feed items into animal commodities could not be addressed and this remains an uncertainty in the assessment**.

Fish

A fish metabolism study was not provided.

However, investigation of the potential occurrence of residues of lenacil in fish feed and consequently in fish matrices in accordance with the published EU Commission Working document SANCO/11187/2013 (31 January 2013 – rev.3) (EC, 2013)[1] is not deemed necessary, because lenacil is not fat-soluble (log Pow < 3 – *vide supra* – ***2.2.1***) and because sugar beet and fodder beet (i.e. crops covered by the representative uses) or commodities derived thereof are not significantly used as ingredients for fish feed.

[1] An officially agreed guidance document on the metabolism in fish was not yet available at EU level. Nevertheless, EU Commission Working document SANCO/11187/2013 (31 January 2013 – rev.3) is available and contains recommendations with regard to testing criteria, testing protocols and dietary burden calculations. The working document applies to applications as from 1/1/2014.

2.7.3 Definition of the residue

A comparative summary of the residue definitions for lenacil is presented in **Table 2.7.3-1**, showing the current proposals (in the framework of renewal of a.s. approval), the previous proposals (in the framework of the original EU peer review) and the current legal definitions under the MRL regulation.

Table 2.7.3-1: Overview of residue definition(s) (proposals) for lenacil in different EU evaluation frameworks

	DAR (2007-2009) / EFSA (2009) ***Dir. 91/414/EEC*** (*)	***Reg. (EC) 396/2005*** (**) → *Reg. (EC) No 149/2008*	**DRAR (2019)** ***Reg. (EC) 1107/2009*** (***)
Plant			
Monitoring	Lenacil [foliar application on root crops]	Lenacil	Lenacil
Risk assessment		*Not applicable*	Lenacil [post-emergence use on root crops & pre-emergence use on leafy crops]
Animal (tissues, poultry eggs, milk)			
Monitoring	*Not required*	Lenacil	Inconclusive (**data gap**)
Risk assessment		*Not applicable*	

Plants:

(*)

In the framework of the original EU peer review pursuant to Council Directive 91/414/EEC (cf. EFSA, 2009), the residue definition for plant was discussed during the experts' meeting, taking the following into consideration:

- 1/3 of the identified total residue in sugar beet leaves (0.01 – 0.02 mg/kg) was metabolite IN-KC943 (7-OH-lenacil) and its conjugates. The metabolite plus conjugates thus accounted for approx. 50% of the levels of parent lenacil (28%TRR; 0.04 mg/kg) in leaves. Furthermore, it was noted that the sugar beet metabolism study was performed at earlier crop growth stage (BBCH 14-16) compared to the critical notified GAP (BBCH 31) and hence, at a later time of application according to GAP criteria, it was expected that parent would be more prevalent in the crops.
- The metabolite IN-KC943 (with hydroxylation of the parent compound on the 7-position) was not as such detected or identified in the rat metabolism study.
- The classification of the parent lenacil as carcinogenic (cat. 2).

The PRAPeR experts' meeting 69 on Mammalian Toxicology (May 2009) concluded that IN-KC943 is structurally closely related to the major metabolite (P5) of lenacil in the rat, identified as a hydroxylated metabolite of lenacil with the OH group on the C5- or C6- position (found in urine and faeces in rat) and therefore is covered by the toxicological studies of the parent compound. See metabolic pathway scheme in **Figure 2.7.3-1**. If the metabolite were included in the residue definition, the reference values of lenacil could be applied.

The PRAPeR experts' meeting 70 on Residues (May 2009) discussed if the metabolite IN-KC943 and its conjugates should be included in the residue definition. For the notified use, parent lenacil was the most prevalent residue in leaves. It was acknowledged that the nature of residues in roots had not been elucidated, but the TRR in roots was <0.01 mg/kg at harvest and the metabolic pathway in roots was not expected to be very different from metabolism in leaves. Therefore, the following residue definition for monitoring and risk assessment for **root crops** (foliar application) was proposed: **lenacil** only.
However, it was emphasized that the residue definition was **to be re-discussed for further uses** including uses on other root crops (e.g. smaller root crops, later applications or higher application rates where residues might be expected in the roots) or spinach. For such further uses, it was deemed useful to clarify the toxicological relevance of IN-KC943 (7-OH-lenacil) in order to be able to consider the metabolism study on sugar beet as representative for all root crops.

(**)

The review of existing EU MRLs according to art. 12 of Reg. (EC) No 396/2005 has not yet been finalised. The current residue definition for MRL enforcement is lenacil, which is consistent with the proposal made under the original EU peer review under Council Directive 91/414/EEC (see above).

(***)

In the framework of renewal of a.s. approval, the applicant again proposed lenacil as the residue definition for risk assessment and monitoring. Upon request of the RMS, the applicant provided following statement supporting the non-relevance of plant metabolites observed in the primary and/or rotational crop metabolism studies:

<<
With regards to the toxicological relevance of plant metabolites, the major metabolites detected in the primary and the rotational crop metabolism studies consisted of hydroxylated lenacil species and their (glucose-) conjugated forms. The proposed metabolic pathway of lenacil, which was observed in the rat (see Section 6, CA 6.2.6) was found to be qualitatively similar to the proposed metabolic pathway in rotational crops. In the rat, lenacil was extensively metabolized, mainly by hydroxylation at different positions on either the cyclohexyl or cyclopentyl ring, or both rings. In Section 5 (Mammalian Toxicology), CA 5.8., the toxicological potential of lenacil metabolites was extensively discussed. The metabolites identified in the rat metabolism study were found to be readily excretable based on polarity considerations and would therefore not present any toxicity not previously observed for the parent lenacil. Therefore, lenacil metabolites are not expected to be of higher toxicity due to their structural similarity to lenacil.

With regards to the magnitude of residues of metabolites in rotational crop metabolism studies in Section CA 6.6, at the 182 DAT plant back interval residues of metabolites in crops relevant for human consumption are expected to be ≤ 0.03 mg/kg and in crops relevant for animal feeding ≤ 0.2 mg/kg. The 182 DAT plant back interval is considered the most relevant plant back interval in terms of agronomic practice for sugar and fodder beet. However, considering the confined nature of the study and the single-dose application instead of multiple applications spread over time, residues in rotational crops are not considered significant.
>>

Indeed, hydroxylation of lenacil on the cyclopentapyrimidine ring (cf. metabolite IN-KC943) and subsequent conjugation was observed in the primary crop metabolism study (on sugar beet; foliar treatment). The detection in rotational crops of metabolites tentatively identified/characterized as hydroxylated lenacil metabolites – on the cyclopentapyrimidine ring (e.g. IN-KQ961) and/or on the cyclohexyl ring (e.g. IN-KD304) – and (glucose) conjugates is consistent with the pathway of metabolism in treated sugar beet. Therefore, the **setting of a separate residue definition for rotational crops is considered not necessary**.

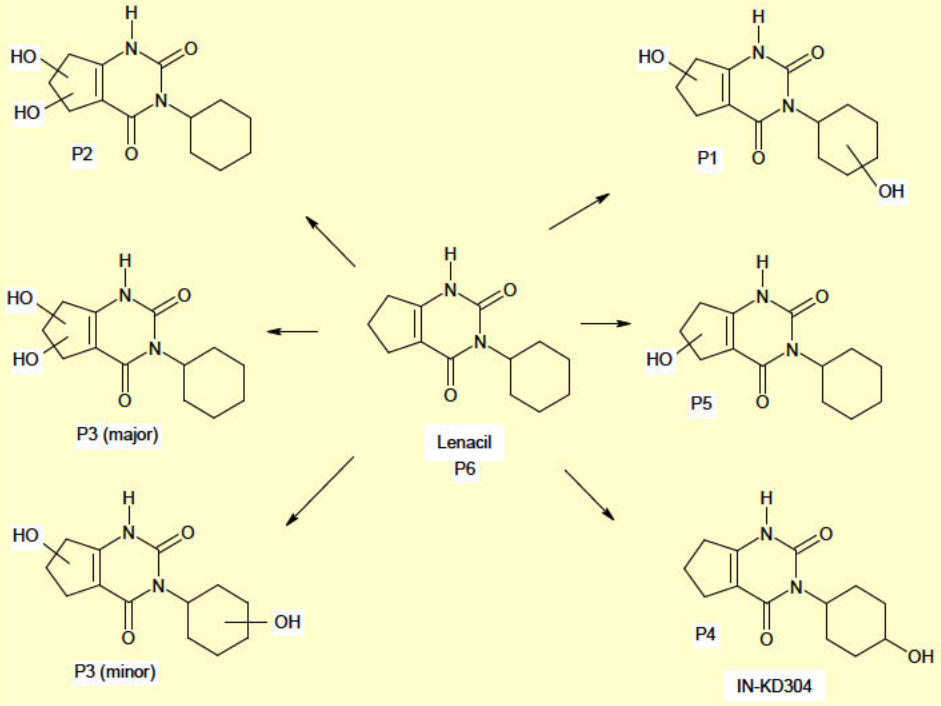
RMS note: During the previous commenting round on the confirmatory data on rotational crops, the point was raised by EFSA and MSs that significant residue levels of compounds structurally related to lenacil were found in food and feed even at 182 d plant back interval and that it is likely they may contribute significantly to the overall risk assessment (since in absence of targeted tox data for the different hydroxy compounds, the same toxicological properties as the parent could be attributed to all metabolites). Therefore, the RMS has further investigated the possible occurrence and magnitude of metabolite residues in rotational crops, as well as their possible contribution to the overall consumer dietary risk: see further below in section ***2.7.7.2***.

The nature of residues in **leafy crops** grown in soil that is treated pre-emergence (post-sowing) with lenacil is expected to be similar to the nature of residues in rotational spinach as revealed by the CRC study, although a relatively higher portion of lenacil cannot be fully excluded. The presumption of a similar qualitative nature of residues is supported by the indicative results derived from the old pre-GLP metabolism study in spinach with pre-emergence treatment post-sowing (*vide supra* – ***2.7.2***). **It is therefore concluded that no additional metabolism study on leafy vegetables would be required to cover a pre-emergence use on leafy crops (e.g. spinach).** The co-RMS (AT) agreed with this.

The tentatively identified/characterised metabolites in primary and rotational crops are similar to the **metabolic profile** observed in **rat**.

Rat metabolism studies evaluated during first EU review of lenacil (BE, 2007; EFSA, 2009) ***(see DRAR Vol.3, B.6.1.1.1 –*** [redacted] ***s, 1996)*** showed that orally administered lenacil is extensively metabolised by the rat, mainly by hydroxylation at different positions on either the cyclohexyl or cyclopentyl ring, or on both rings. The major metabolite (peak P5) observed in rat urine (3-31% of administered dose) was characterized as a hydroxylated metabolite of lenacil with the OH group either on the C5-position (i.e. IN-KQ961) or on the C6-position. Another metabolite peak observed in rat urine (peak P4 – 0.4-20% of administered dose) was identified as IN-KD304. No glucuronide or sulfate conjugates were released by glucuronidase or sulfatase. See **Figure 2.7.3-1** below.

Figure 2.7.3-1: Proposed metabolic pathway of [2-^{14}C]-Lenacil in rat *[Ref.:* [redacted] *, 1996]*



Therefore, from the toxicological point of view, it is considered that the many hydroxylated metabolites recovered in the primary crop study and rotational crop study were probably the same as those found in the rat metabolism study. Moreover, the hydroxylated metabolites are unlikely to be of higher toxicity than the parent compound. Therefore, it is considered that the toxicity of the hydroxylated metabolites is covered by the toxicological data package.

Further toxicological considerations on metabolites: see ***2.6.8.1 (and Vol.3, B.6.8.1.2)***.

Animals:

No residue definition can be proposed for animal matrices, as no metabolism studies were provided (*vide supra* – ***2.7.2***).

Fat solubility

Lenacil can be considered non fat-soluble (log Pow 1.3-1.7 at 25°C; *vide supra* – ***2.2.1***).

2.7.4 Summary of residue trials in plants and identification of critical GAP

In the framework of the original EU peer review of lenacil, the representative use was the post-emergence use (BBCH 10-31) on sugar beet and fodder beet with 1-4 applications (interval 7-14 days) and a maximum application rate of 0.5 kg a.s./ha per season (cf. EFSA, 2009). The representative formulation was VENZAR 80 WP (Wettable Powder; 800 g/kg lenacil).

In the framework of renewal of a.s. approval, essentially the same representative use was supported by the applicant, though for a different representative formulation (Lenacil 500 g/L SC; Suspension Concentrate): see **Table 2.7.4-1**.

Table 2.7.4-1: Proposed critical GAP for the use of Lenacil 500 g/L SC on sugar beet and fodder beet

Crop	Outdoor/ Protected	Growth stage	Number of Applications	Application Interval (days)	Application rate per treatment		Min. PHI (days)
					water L/ha	g a.s./ha	
Sugar Beet and Fodder Beet	Outdoor	BBCH 10-31	**1**	7-14	100-400	**500**	None*
Sugar Beet and Fodder Beet	Outdoor	BBCH 10-31	4	7-14	100-400	125	None*

* PHI is covered by conditions of use and/or growing period between application and harvest

No pre-harvest interval (PHI) has been proposed for lenacil, as it is used early in the season according to crop growth stage. Sugar and fodder beet can be sown from mid-March to early April and the time needed to reach maturity is about 6 to 8 months. Based on the last application to sugar beet/fodder beet at growth stage BBCH 31 (beginning of crop cover – leaves cover 10 % of ground), the interval between the last application and harvest will normally be 90 to 120 days.

Equivalency of formulations

The GAP for Lenacil 500 g/L **SC** is – from a residue perspective – identical to the GAP for the representative formulation considered during the original EU peer review (Venzar 80 **WP**). The change of formulation type (from WP to SC) is not expected to have an impact on the magnitude of residues, taking into account that both formulations are diluted in water before application (and do not contain any oils or organic solvents) and that it concerns a use with application at early crop growth stage (with a pre-harvest interval of approximately 90-120 days in practice).

Supervised residue trials

A summary of the results from the supervised residue trials on sugar beet is presented in **Table 2.7.4-2**. According to SANCO 7525/VI/95-rev. 10.3 (EC, 2017), the residue database on sugar beet can be extrapolated to fodder beet.

Northern Europe

A total of 7 residue trials carried out in Northern Europe in the years 1995 and 2001 were submitted on sugar beet. Three of the trials[2] were performed at growth stage BBCH 14-19, which is within the notified GAP, but earlier than the cGAP (BBCH 31). They were regarded as not acceptable, as the samples were analysed 26 months after the harvest and therefore are not supported by storage stability data (cf. ***2.7.1***). Furthermore, the analytical method used was insufficiently validated.
Four of the trials were regarded as acceptable. The application was carried out at BBCH 37, which is later than the notified cGAP (BBCH 31). However, residues were below the LOQ (<0.02 mg/kg) in all root samples at mature harvest (70-90 DAT). The trials are supported by storage stability studies and the analytical method used is fully validated. Residue levels found in tops ranged from < LOQ (<0.02 mg/kg) to 0.04 mg/kg.

[2] Study report no F-95-001-RES (Tillkes, 1998)

Southern Europe

Three trials in sugar beet were carried out in the years 2002 and 2005 in Southern Europe with application at BBCH 31-32 and BBCH 38, respectively. They were regarded as acceptable. Residues were below LOQ (<0.02 mg/kg) in all root samples at mature harvest (60-120 DAT). The trials are supported by storage stability data and the analytical method used was fully validated. Residue levels in sugar beet tops were below LOQ (<0.02 mg/kg) after application at BBCH 31 and 0.03 mg/kg in the trial with application at BBCH 38.
As residues below the LOQ were found in all samples of sugar beet roots, the submitted residue trials were regarded as sufficient to support the notified representative use in Northern and Southern Europe. Moreover, the <LOQ results in roots are consistent with what is expected on the basis of the representative metabolism study on sugar beet, which showed that the TRR in roots was max. 0.03 mg/kg (parent eq.) 99 days after treatment and <0.01 mg/kg (parent eq.) 130 days after treatment (*vide supra* – ***2.7.2***).

Influence of adjuvants

In a study by Kucharski *et al.* (2011), of which the results were published, the influence of adjuvant addition (oil, surfactant, and multicomponent) on lenacil residue levels in soil and roots of sugar beet was investigated. The addition of adjuvants caused an apparent increase of the active substance residue levels in soil and roots of sugar beet in comparison with the treatments where lenacil was used without adjuvant. Beside the addition of adjuvants, also the weather conditions (especially rainfall) appeared to have an influence on the residue levels in the top soil layer and roots of sugar beet.

Table 2.7.4-2: Summary of residues data from the supervised residue trials

Commodity	Region/ Indoor (a)	Residue levels observed in the supervised residue trials (mg/kg)	Comments/Source	Calculated MRL (mg/kg)	HR (b) (mg/kg)	STMR (c) (mg/kg)	CF (d)
Representative uses							
Sugar beet	NEU	Roots: <0.02 (4x) Tops: <0.02 (3x), 0.04	Residue trials with application at growth stage BBCH 37 (cf. cGAP: BBCH 31); Extrapolation to fodder beet possible	0.02* (roots)	Roots: <0.02 Tops: **0.04**	Roots: <0.02 Tops: <0.02	n.a.
	SEU	Roots: <0.02 (3x) Tops: <0.02 (2x), 0.03	Residue trials with application at cGAP-compliant growth stage (BBCH 31) or later (BBCH 38); Extrapolation to fodder beet possible		Roots: <0.02 Tops: 0.03	Roots: <0.02 Tops: <0.02	n.a.

* Indicates that the MRL is proposed at the limit of quantification.

Mo: residue levels expressed according to the monitoring residue definition; RA: residue levels expressed according to risk assessment residue definition.

(a): NEU: Outdoor trials conducted in northern Europe, SEU: Outdoor trials conducted in southern Europe, Indoor: indoor EU trials or Country code: if non-EU trials.

(b): Highest residue. The highest residue for risk assessment (RA) refers to the whole commodity and not to the edible portion.

(c): Supervised trials median residue. The median residue for risk assessment (RA) refers to the whole commodity and not to the edible portion.

(d): Conversion factor to recalculate residues according to the residue definition for monitoring to the residue definition for risk assessment.

2.7.5 Summary of feeding studies in poultry, ruminants, pigs and fish

2.7.5.1 Re-entry period for livestock (to area to be grazed) or withholding period for animal feeding stuffs

Lenacil is not intended for use in areas where livestock animals may be grazed. Therefore, no re-entry period has been proposed by the applicant.

Residues of lenacil in sugar beet decline rapidly after application to very low levels at harvest. Sugar beet and fodder beet are not used for feeding purposes prior to harvest. Therefore, an additional withholding period after normal harvest for animal feeding stuffs has not been proposed by the applicant.

Note: The need for a re-entry period and/or the prohibition of the feeding of sugar beet tops after thinning and crop failure was discussed during the original peer review, taking into account the practices in different countries. It was agreed during the experts' meeting (PRAPeR 70 – May 2009) that livestock animals are not supposed to graze on such an area and that thinning out the sugar beet crop is not relevant anymore nowadays (seeds selection). Furthermore, the experts were of the opinion that the crop is not fed to livestock in the case of crop failure, but remains on the field and is ploughed. Therefore, a re-entry period and/or the prohibition of the feeding of sugar beet tops was not deemed necessary for the representative uses.

2.7.5.2 Livestock dietary burden calculation

Sugar beet and fodder beet (roots and leaves/tops) are potential feed items for livestock.

In the framework of the original EU peer review (BE, 2007-2009), intake calculations for livestock had been performed according to EU guideline 7031/VI/95 rev.4 (EC working document Appendix G on Livestock feeding studies) considering lenacil residue levels of 0.02 mg/kg (LOQ) in sugar/fodder beet roots and 0.04 mg/kg (HR) in sugar/fodder beet leaves and tops. Former maximum intake calculations for poultry did not indicate an exceedance of the trigger value, but the estimated potential intake of lenacil in cattle and pigs slightly was in the range of 0.105 – 0.135 mg/kg diet (dry weight basis)/day and thus exceeded the formerly relevant legal trigger value of 0.1 mg/kg diet/day.
However, the need for livestock metabolism studies was discussed during the original peer review and it was agreed during the experts' meeting (PRAPeR 70 – May 2009) that livestock metabolism studies were not required (cf. EFSA, 2009) because of the following:

- The estimated dietary burden for dairy cattle and beef cattle was borderline (0.105–0.135 mg/kg diet dry matter).
- The calculated intake was probably an overestimation of the actual potential intake, as it was based on a residue input value for beet tops/leaves (0.04 mg/kg) derived from field trials performed at a more critical growth stage (BBCH 37/38) compared to the notified critical growth stage (BBCH 31) and on a residue input value of 0.02* mg/kg (*LOQ) for roots, although residues of lenacil in roots are likely to be much lower than the LOQ of 0.02 mg/kg. The theoretical residue level in roots contributed significantly (50%) to the calculated total livestock dietary burden.
- As indicated by the metabolism data in rats (which shows a metabolism towards compounds of (more) polar nature), accumulation of lenacil residues is not expected in livestock. Therefore significant residues in animal matrices are not very likely.

In the framework of renewal of a.s. approval, the livestock dietary burden was recalculated considering the OECD feedstuff table (EU diets for 9 different animal species) and the OECD approaches presented in the OECD guidance document No 73 on residue in livestock (Sept. 2013). The calculation tool developed by EFSA (*Animal model 2017.xls* – EFSA, 2017)[3] was used by the RMS.
Furthermore, in addition to potential residues of lenacil in primary (sugar/fodder beet) crops, also the impact of potential residues of lenacil in rotational crops on the livestock intake of lenacil residues has been investigated, taking the results from the CRC study into account (*vide infra* – ***2.7.7***).

Regarding only lenacil residues in **primary crops** (sugar beet and fodder beet), the calculation according to new (OECD) methodology shows findings similar to those discussed during the original peer review (see above), i.e. the estimated maximum dietary intake only slightly exceeds the trigger of 0.004 mg/kg bw/day for (dairy) cattle: see **Table 2.7.5.2-2**. Thus, the conclusions of the experts' meeting (PRAPeR 70 – see above) remain valid.

Regarding lenacil residues in both **primary crops and rotational crops**, the following was considered by the RMS:

- The CRC study provided only information for a leafy crop (spinach), a root crop (turnip) and a cereal crop (wheat).

- Spinach is not a livestock feed item but the results in rotational grown spinach could be used to represent potential residues in other green forage crops which are consumed by livestock (e.g. kale leaves). Turnips (tops and roots) and wheat (grain/straw and forage/silage/hay) are livestock feed items. The results in turnips are also applicable to swedes and carrots[4] and the results in wheat are applicable to all small grain cereal crops. In the absence of more specific data (e.g. from field trials), results in wheat were also extrapolated to other crops of the 'cereal/grass group', namely maize (cultivated for grain/stover production or for forage/silage) and grass (forage/silage/hay).

- Crops of the fruits and fruiting vegetables group are very likely irrelevant for rotation with sugar or fodder beet according to agronomic practice (though this may be relevant for other uses beyond the representative uses).

- The residue situation in feed items derived from crops belonging to the group of pulses and oilseeds remains very unpredictable on the basis of the limited data available from the CRC study. As no appropriate data are available, potential residues in crops of this crop group are **not covered by the calculations**.

- While the RMS agrees with the applicant that the results of the 182 DAT plant-back interval are the most relevant for the representative use on sugar and fodder beets (mainly due to the long cultivation period) (*vide infra* – ***2.7.7.3***), RMS has in first instance considered the results of the 30/68-days plant back interval, in order to cover also the scenario of original crop failure. Furthermore, the shorter plant-black intervals may also be relevant for further uses beyond the representative uses, e.g. on crops with shorter cultivation period (e.g. spinach) and taking into account that higher levels of lenacil were observed in rotational crops sown after a shorter PBI.

- For detailed results from the 30/68 days PBI, the reader is referred to ***DRAR Vol.3, B.7 (Table B.7.6.1-7)***. The residue levels of lenacil in rotational crops sown 30 DAT (68 DAT for spinach) were well below 0.01 mg/kg (ND – 0.003 mg/kg) in spinach leaves and wheat grain. Therefore, no further consideration was given to the feed items derived from rotational crops represented by those crop parts (i.e. green forage and cereal grain). In contrast, significant levels of lenacil (≥0.01 mg/kg) were measured in turnip roots (0.011 mg/kg), turnip leaves (0.045 mg/kg), wheat straw (0.075 mg/kg), wheat forage (0.22 mg/kg) an wheat hay (0.28 mg/kg), i.e. mainly crop parts representative for commodities exclusively relevant for livestock feeding (root crop leaves and cereal straw/forage/hay). All input values used are summarized in **Table 2.7.5.2-1**.

Results of the livestock intake estimation regarding primary crop and rotational crop residues (30-days PBI) are presented in **Table 2.7.5.2-3**. The impact from cereal forage (extrapolated to grass forage) and/or cereal hay/silage is clearly significant, resulting in a maximum dietary burden far above the trigger value of 0.004 mg/kg bw/d for ruminants (up to 0.028 mg/kg bw/d) and slightly exceeding the trigger value for the pig and poultry diets (up to 0.007 mg/kg bw/d).

[3] Cugier, Jean-Pierre, & Ferreira, Lucien. (2017). EU Animal burden calculator - animals. https://zenodo.org/record/827275#.Wco5TU8Uncs

[4] No extrapolation to potatoes was made, as it was anyway deemed unusual to grow potatoes as replacement/succeeding crop of beet crops.

Table 2.7.5.2-1: Input values used for animal burden calculations (lenacil) [a]

Feed commodity	Median dietary burden		Maximum dietary burden	
	(mg/kg)	Comment	(mg/kg)	Comment
Risk assessment residue definition: lenacil				
Forages				
Barley, forage	0.22	Rot. crop (wheat forage)	0.22	Rot. crop (wheat forage)
Barley, straw	0.075	Rot. crop (wheat straw)	0.075	Rot. crop (wheat straw)
Barley, silage	0.29	Rot. crop (wheat forage) x $PF_{default}$ (1.3)	0.29	Rot. crop (wheat forage) x $PF_{default}$ (1.3)
Mangel beet, fodder	0.02	STMR (sugar beet tops)	0.04	HR (sugar beet tops) [b]
Sugar beet, tops	0.02	STMR (=LOQ)	0.04	HR (NEU) [b]
Corn (field), forage/silage	0.22	Rot. crop (wheat forage)	0.22	Rot. crop (wheat forage)
Corn (field/pop), stover	0.075	Rot. crop (wheat straw)	0.075	Rot. crop (wheat straw)
Grass, forage (fresh)	0.22	Rot. crop (wheat forage)	0.22	Rot. crop (wheat forage)
Grass, hay	0.28	Rot. crop (wheat hay)	0.28	Rot. crop (wheat hay)
Grass, silage	0.35	Rot. crop (wheat forage) x $PF_{default}$ (1.6)	0.35	Rot. crop (wheat forage) x $PF_{default}$ (1.6)
Millet, forage	0.22	Rot. crop (wheat forage)	0.22	Rot. crop (wheat forage)
Millet, straw	0.075	Rot. crop (wheat straw)	0.075	Rot. crop (wheat straw)
Oat, forage	0.22	Rot. crop (wheat forage)	0.22	Rot. crop (wheat forage)
Oat, straw	0.075	Rot. crop (wheat straw)	0.075	Rot. crop (wheat straw)
Rye, forage	0.22	Rot. crop (wheat forage)	0.22	Rot. crop (wheat forage)
Rye, straw	0.075	Rot. crop (wheat straw)	0.075	Rot. crop (wheat straw)
Sorghum, forage	0.22	Rot. crop (wheat forage)	0.22	Rot. crop (wheat forage)
Sorghum, stover	0.075	Rot. crop (wheat straw)	0.075	Rot. crop (wheat straw)
Sorghum, silage	0.13	Rot. crop (wheat forage) x $PF_{default}$ (0.6)	0.13	Rot. crop (wheat forage) x $PF_{default}$ (0.6)
Triticale, forage	0.22	Rot. crop (wheat forage)	0.22	Rot. crop (wheat forage)
Triticale, hay	0.28	Rot. crop (wheat hay)	0.28	Rot. crop (wheat hay)
Triticale, straw	0.075	Rot. crop (wheat straw)	0.075	Rot. crop (wheat straw)
Wheat, forage	0.22	Rot. crop (wheat forage)	0.22	Rot. crop (wheat forage)
Wheat, hay	0.28	Rot. crop (wheat hay)	0.28	Rot. crop (wheat hay)
Wheat, straw	0.075	Rot. crop (wheat straw)	0.075	Rot. crop (wheat straw)
Roots and tubers				
Carrot, culls	0.011	Rot. crop (turnip root)	0.011	Rot. crop (turnip root)
Swede, roots	0.011	Rot. crop (turnip root)	0.011	Rot. crop (turnip root)

Feed commodity	Median dietary burden		Maximum dietary burden	
	(mg/kg)	Comment	(mg/kg)	Comment
Turnip, roots	0.011	Rot. crop (turnip root)	0.011	Rot. crop (turnip root)
By-products				
Sugar beet, dried pulp	0.02	STMR (=LOQ)	0.02	STMR (=LOQ)
Sugar beet, ensiled pulp	0.02	STMR (=LOQ)	0.02	STMR (=LOQ)
Sugar beet, molasses	0.02	STMR (=LOQ)	0.02	STMR (=LOQ)

[a] input values derived for the rotational crops were based on the results for the 30-days plant-back interval in the CRC study

[b] derived from field trials performed at a more critical growth stage (BBCH 37/38) compared to the notified critical growth stage (BBCH 31); residues in sugar beet tops following treatment at BBCH 31 were <0.02 mg/kg.

Table 2.7.5.2-2: Results livestock dietary burden calculation (lenacil) – primary crop (sugar/fodder beet) residues

Relevant groups	Dietary burden expressed in		Most critical diet (a)	Most critical commodity (b)		Trigger exceeded (Yes/No)
	mg/kg bw per day					0.004 mg/kg bw/day
	Median	Maximum				
Cattle (all diets)	0,003	0,005	Dairy cattle	Beet, mangel	fodder	**Yes**
Cattle (dairy only)	0,003	0,005	Dairy cattle	Beet, mangel	fodder	**Yes**
Sheep (all diets)	0,001	0,002	Lamb	Beet, sugar	tops	No
Sheep (ewe only)	0,001	0,001	Ram/Ewe	Beet, sugar	tops	No
Swine (all diets)	0,001	0,001	Swine (breeding)	Beet, mangel	fodder	No
Poultry (all diets)	0,000	0,001	Poultry layer	Beet, sugar	tops	No
Poultry (layer only)	0,000	0,001	Poultry layer	Beet, sugar	tops	No

(a): When several diets are relevant (e.g. cattle, sheep and poultry "all diets"), the most critical diet is identified from the maximum dietary burdens expressed as "mg/kg bw per day"

(b): The most critical commodity is the major contributor identified from the maximum dietary burden expressed as "mg/kg bw per day".

Table 2.7.5.2-3: Results livestock dietary burden calculation (lenacil) – primary and rotational crop (30d PBI) residues

Relevant groups	Dietary burden expressed in		Most critical diet (a)	Most critical commodity (b)		Trigger exceeded (Yes/No)
	mg/kg bw per day					0.004 mg/kg bw/day
	Median	Maximum				
Cattle (all diets)	0,022	0,022	Dairy cattle	Grass	forage (fresh)	**Yes**
Cattle (dairy only)	0,022	0,022	Dairy cattle	Grass	forage (fresh)	**Yes**
Sheep (all diets)	0,028	0,028	Ram/Ewe	Grass	forage (fresh)	**Yes**
Sheep (ewe only)	0,028	0,028	Ram/Ewe	Grass	forage (fresh)	**Yes**
Swine (all diets)	0,005	0,005	Swine (breeding)	Grass	forage (fresh)	**Yes**
Poultry (all diets)	0,007	0,007	Poultry layer	Wheat	forage	**Yes**
Poultry (layer only)	0,007	0,007	Poultry layer	Wheat	forage	**Yes**

(a): When several diets are relevant (e.g. cattle, sheep and poultry "all diets"), the most critical diet is identified from the maximum dietary burdens expressed as "mg/kg bw per day"
(b): The most critical commodity is the major contributor identified from the maximum dietary burden expressed as "mg/kg bw per day".

Due to the obvious exceedance of the livestock dietary burden trigger (see **Table 2.7.5.2-3**), normally metabolism studies would be required (at least in ruminants) and, depending on the results, also a (ruminant) feeding study could be necessary. No such data were provided (**data gap** – see also ***2.7.2***).

Considering that the available data package does not cover a significant intake by livestock animals, significant levels of lenacil in some rotational crops that can be used as feed items, should be avoided. In this respect, RMS notes that cereal forage (and silage/hay) is not relevant in the case of cultivation of cereals for grain production. The other major contributor to the livestock dietary intake is grass forage. Therefore, **mitigation measures would be possible**, e.g. by applying the following **restriction with regard to rotational/succeeding crops (in case of crop failure)**:

<<
In order to avoid residues in livestock feed items, possible succeeding/rotational crops in case of original crop failure are restricted to root vegetables[5], spinach[6] and cereals – i.e. small grain cereals and other crops of the 'cereal group' such as maize – for grain production only (i.e. use of forage, silage or hay for livestock feeding is not allowed).
>>

To demonstrate the effectiveness of such a restriction, the RMS has performed an alternative calculation, by excluding residues in forage/hay/silage of cereal and grass crop sown as succeeding crop 30 DAT (following original crop failure). The results of this alternative livestock dietary burden calculation (reflecting the proposed mitigation measures) are presented in **Table 2.7.5.2-4**. These results are very similar to those obtained when considering primary crops only (*vide supra* – ***Table 2.7.5.2-2***), i.e. a slight exceedance of the trigger of 0.004 mg/kg bw/day for the (dairy) cattle diet. This demonstrates that the contribution of potential residues in straw and tops of rotational cereal and root crops, respectively, to the maximum livestock dietary intake is negligible. Thus, the conclusions of the experts' meeting (PRAPeR 70 – see above) remain valid for the scenario with mitigation measures.

[5] A more narrow restriction within the root crop group (to sugar/fodder beets) might be necessary for other reasons (risk for MRL exceedance); see further below; see also ***2.7.7.4***.
[6] As phytotoxicity for lettuce was observed in the CRC study 30 DAT, lettuce is not recommended as replacement crop (in case of failure of the original crop) and therefore a restriction to spinach (investigated crop) is justified. See also ***2.7.7.3***.

Table 2.7.5.2-4: Results livestock dietary burden calculation (lenacil) – primary and rotational crop (30d PBI) residues with mitigation measure (restriction of rotational crops)

Relevant groups	Dietary burden expressed in		Most critical diet (a)	Most critical commodity (b)		Trigger exceeded (Yes/No)
	mg/kg bw per day					0.004 mg/kg bw/day
	Median	Maximum				
Cattle (all diets)	0,004	0,005	Dairy cattle	Beet, mangel	fodder	**Yes**
Cattle (dairy only)	0,004	0,005	Dairy cattle	Beet, mangel	fodder	**Yes**
Sheep (all diets)	0,004	0,004	Lamb	Swede	roots	No
Sheep (ewe only)	0,003	0,003	Ram/Ewe	Swede	roots	No
Swine (all diets)	0,002	0,002	Swine (breeding)	Beet, mangel	fodder	No
Poultry (all diets)	0,001	0,001	Poultry layer	Swede	roots	No
Poultry (layer only)	0,001	0,001	Poultry layer	Swede	roots	No

(a): When several diets are relevant (e.g. cattle, sheep and poultry "all diets"), the most critical diet is identified from the maximum dietary burdens expressed as "mg/kg bw per day"
(b): The most critical commodity is the major contributor identified from the maximum dietary burden expressed as "mg/kg bw per day".

An analogous livestock intake estimation has also been performed combining primary crop residues with residues in rotational crops associated with a plant-back interval of approximately 6 months, assuming lenacil residue levels of 0.078 mg/kg in cereal/grass forage (extrapolated from wheat forage 182-days PBI), 0.03 mg/kg in cereal/grass hay (extrapolated from wheat hay 182-days PBI) and 0.01 mg/kg in cereal straw (cf. 0.007 mg/kg in wheat straw 182-days PBI). It is recalled that at the 182-days PBI, lenacil residues were well below 0.01 mg/kg in spinach leaves, turnip roots/leaves and wheat grain (cf. ***DRAR Vol.3, B.7, Table B.7.6.1-8***) and therefore, residues in corresponding rotational crop feed items have not been included in the calculation.
Even considering a waiting period of 6 months between last application and sowing or planting succeeding crops, the maximum livestock dietary intake for ruminants might theoretically still exceed the trigger value, with estimated maximum intakes up to 0.010 mg/kg bw/day (see **Table 2.7.5.2-5**), which would trigger further investigation of metabolism in ruminants.
The main contributors to the exceedance of the trigger are potential residues of lenacil in forage from grass, maize and barley grown as rotational crops. Only if those rotational crops were excluded (possible **mitigation measures**), livestock dietary intake estimations would be comparable to those in ***Table 2.7.5.2-2*** and ***Table 2.7.5.2-4*** *(vide supra)*, i.e. with max. 0.005 mg/kg bw/day for dairy cattle (due to residues in fodder beet tops).

Table 2.7.5.2-5: Results livestock dietary burden calculation (lenacil) – primary and rotational crop (182d PBI) residues

Relevant groups	Dietary burden expressed in		Most critical diet (a)	Most critical commodity (b)		Trigger exceeded (Yes/No)
	mg/kg bw per day					0.004 mg/kg bw/day
	Median	Maximum				
Cattle (all diets)	0,009	0,009	Dairy cattle	Grass	forage (fresh)	**Yes**
Cattle (dairy only)	0,009	0,009	Dairy cattle	Grass	forage (fresh)	**Yes**
Sheep (all diets)	0,010	0,010	Ram/Ewe	Grass	forage (fresh)	**Yes**
Sheep (ewe only)	0,010	0,010	Ram/Ewe	Grass	forage (fresh)	**Yes**
Swine (all diets)	0,001	0,001	Swine (breeding)	Grass	forage (fresh)	No
Poultry (all diets)	0,002	0,002	Poultry layer	Wheat	forage	No
Poultry (layer only)	0,002	0,002	Poultry layer	Wheat	forage	No

(a): When several diets are relevant (e.g. cattle, sheep and poultry "all diets"), the most critical diet is identified from the maximum dietary burdens expressed as "mg/kg bw per day"
(b): The most critical commodity is the major contributor identified from the maximum dietary burden expressed as "mg/kg bw per day".

In summary, the livestock dietary burden estimates for lenacil significantly exceed the trigger value (particularly in ruminants) if residues in rotational crops are taken into account. Since the available data package does not cover a significant intake by livestock animals (i.e. no metabolism studies are available), **mitigation measures are necessary** to avoid significant levels of lenacil in some rotational crops that may be used as (major) feed items.

A possible mitigation measure would be a **restriction with regard to rotational/succeeding crops as follows:**

- A general **waiting period of at least 6 months** (from application to drilling/planting of the rotational/succeeding crop) should be respected and **possible succeeding/rotational crops are restricted** to root/tuber vegetables, leafy vegetables and cereals – i.e. small grain cereals and other crops of the 'cereal group' such as maize – for grain production only (i.e. use of forage, silage or hay for livestock feeding is not allowed). *Note: For the representative use of Lenacil 500 g/L SC on sugar beets and fodder beets, the waiting period will in practice be covered by the cultivation period (if no crop failure occurs).*

- If lenacil is applied and **crop failure** occurs for any reason, it is recommended to **wait at least 30 days** after application to plant/drill a replacement crop. **Possible replacement crops are restricted** to sugar and fodder beet[7], spinach[8] or a cereal crop (incl. maize) for grain production only (i.e. use of forage, silage or hay for livestock feeding is not allowed)."

Note: The possible mitigation measures outlined above have been elaborated by the RMS; they were not part of the representative uses as notified by the applicant (see also ***2.7.7.3***). Nevertheless, during the finalisation of the DRAR, the applicant expressed its agreement with the recommended restrictions proposed by the RMS.

2.7.5.3 Livestock feeding studies

No livestock feeding studies were provided.

[7] The reason for restricting to sugar and fodder beet (and thus excluding other root/tuber crops) is to avoid quantifiable residues of lenacil at or above 0.01 mg/kg in root/tuber vegetables with shorter cultivation period (as compared to sugar/fodder beets). Thus, such a restriction is deemed appropriate, to avoid MRL exceedance, assuming that MRLs will be reduced to the default level / new LOQ of 0.01 mg/kg. See also ***2.7.7.4***.
[8] As phytotoxicity for lettuce was observed in the CRC study 30 DAT, lettuce is not recommended as replacement crop (in case of failure of the original crop) and therefore a restriction to spinach (investigated crop) is justified. See also ***2.7.7.3***.

2.7.5.4 Fish feed burden calculation

Sugar beet and fodder beet (i.e. crops covered by the representative uses) or commodities derived thereof are not significantly used as ingredients for fish feed. Furthermore, the log Pow for lenacil is in the range of 1.3 – 1.7 (*vide supra* – ***2.2.1***), which is below the trigger value of 3 mentioned in the published EU Commission Working document SANCO/11187/2013 (31 January 2013 – rev.3) (EC, 2013)[9]. Therefore, no further consideration is necessary in the context of this assessment. The representative uses on sugar beet and fodder beet are not expected to result in residues of lenacil (or its more polar metabolites) in fish feed and consequently in edible fish matrices.

No fish feeding studies were provided, but this is considered adequately justified.

[9] An officially agreed guidance document on the metabolism in fish was not yet available at EU level. Nevertheless, EU Commission Working document SANCO/11187/2013 (31 January 2013 – rev.3) is available and contains recommendations with regard to testing criteria, testing protocols and dietary burden calculations. The working document applies to applications as from 1/1/2014.

2.7.6 Summary of effects of processing

Regarding the representative uses, sugar beet roots are processed into sugar.

In the framework of the original EU peer review, data on the effects of processing on the nature of the residues or on residue levels were not deemed required, since no significant residues (i.e. no residues above > 0.1 mg/kg) were found in sugar beet roots (BE, 2007-2009; EFSA, 2009).

In the framework of renewal of a.s. approval, no new information with regard to the effect of processing was provided; the need for such studies was waived by the applicant (see ***Vol.3, B.7.5***).

The RMS is of the opinion that a standard hydrolysis study simulating processing conditions is not required, since the occurrence of residue levels of lenacil at or above 0.01 mg/kg in sugar beet roots is unlikely. Furthermore, studies investigating the magnitude of lenacil residues in processed commodities (e.g. sugar) are not required, because residue levels in sugar beet root are clearly expected to be below 0.1 mg/kg and there is no concern with regard to dietary intake (TMDI well below 10% ADI – see ***2.7.9***).

2.7.7 Summary of residues in rotational crops

2.7.7.1 Metabolism in rotational crops

In the framework of the original EU peer review, a data gap was set to provide confirmatory data on rotational crops, including possible phytotoxicity effects (cf. EFSA, 2009):

> *"Confined rotational crop studies are not available. According to the RMS the notifier recommended succeeding crops should not be planted or drilled until at least 120 days after application of lenacil because of its phytotoxicity. If crop failure occurred during this period only sugar beet, red beet (beetroot) or spinach could be planted. However, no data on phytotoxicity tests have been submitted by the notifier.*
> *DT_{90} values of 61 to 291 days were found for the degradation of lenacil in soil in field studies carried out in Germany, France and Spain. The study with a DT_{90} of 291 days was carried out under rather extreme climatic conditions in Spain. However, these conditions were regarded as a possible scenario by the PRAPeR experts' meeting 67 on fate and behaviour. Therefore, the PRAPeR 70 meeting concluded that a metabolism study on rotational crops taking into account possible phytotoxicity problems is necessary."*

In the framework of renewal of a.s. approval, the review in the environmental fate section (*vide infra* – ***2.8.1***) concluded that the $DT_{90,\,soil}$ values for the parent compound lenacil derived from laboratory (aerobic soil degradation) studies were in the range of 21-96 days. The $DT_{90,\,soil}$ observed for parent lenacil in the field study (4 soils) ranged from 61 days to 291 days, as previously mentioned (see above). For the two major soil metabolites, IN-KF313 and IN-KE121, $DT_{90,lab}$ values from the aerobic soil degradation studies were in the range of 13-108 days and 4-61 days, respectively. While the slight exceedance of the trigger value (DT_{90} > 100 days) for metabolite IN-KF313 is acknowledged, it is to be noted that IN-KF313 was also identified in soil in the confined rotational crop study conducted with lenacil (see further below). Therefore, potential uptake and metabolism of this metabolite is deemed covered by that study; a particular study for IN-KF313 is not deemed necessary.

In the framework of the assessment of confirmatory data following first a.s. approval (BE, 2013), the nature of residues of lenacil in rotational crops was investigated in a leafy vegetable (spinach), a root crop (turnip) and cereal (wheat) grain, sown 30/68, 180 and 365 days after treatment (DAT) at an application rate of 485 g a.i./ha, which corresponds to 0.97 N of the maximum seasonal application rate (i.e. 500 g a.i./ha) for the supported representative uses on sugar and fodder beet. A summary of the study characteristics is given in **Table 2.7.7.1-1** below.

Note: The confirmatory data regarding residues had not been previously covered by a specific EFSA conclusion; only a reporting table collating all MS/EFSA comments and RMS's responses had been drawn up. Therefore, the confined rotational crop study (Hurst, 2013) has been highlighted as "new" assessment in the DRAR (BE, 2019): see also ***Vol.3, B.7.6.1***.

Table 2.7.7.1-1: Summary of confined rotational crop (CRC) study with [^{14}C]-lenacil *(Hurst, 2013)*

Crop group	crop	Label position	Application and sampling details			
			Method	Rate (kg a.s./ ha)	Plant-back interval (DAT)	Plant fraction sampled (growth stage at harvest)
Leafy vegetables	Spinach	[^{14}C]-lenacil (radiolabel at 2-position of pyrimidine ring)	Outdoor application to bare soil (sandy loam soil) + mixing top 5 cm soil	0.485	68 182 365	Immature (BBCH 32-39) and mature leaves (BBCH 49)
Root and tuber vegetables	Turnip				30 182 365	Root and leaves (BBCH 45-49)
Cereals	Spring wheat				30 182 365	Forage (BBCH 21-30); hay (BBCH 41-49); straw and grain (BBCH 89)

Overall, the CRC study shows that lenacil related soil residues are taken up by succeeding crops (cereal, leafy vegetable and root crop) and that there is significant translocation of residues from roots into upper plant parts. However, the level of the resulting residue is dependent on the level of available residue in the soil: TRR levels in almost all crop fractions declined over time, which is consistent with increase of soil bound residues (not available for uptake by plants) over time as demonstrated by soil extraction/analysis.

The amount of parent lenacil detected in rotational crops was lower with increased soil ageing (i.e. with longer plant-back intervals), which is consistent with the fact that lenacil is readily metabolised in most soils *(cf. **2.8.1**)*. In all crop fractions representative of commodities relevant for human consumption, lenacil residues were below 0.02 mg/kg (for all plant-back intervals), with a maximum level of 0.011 mg/kg in turnip roots (sown 30 DAT).

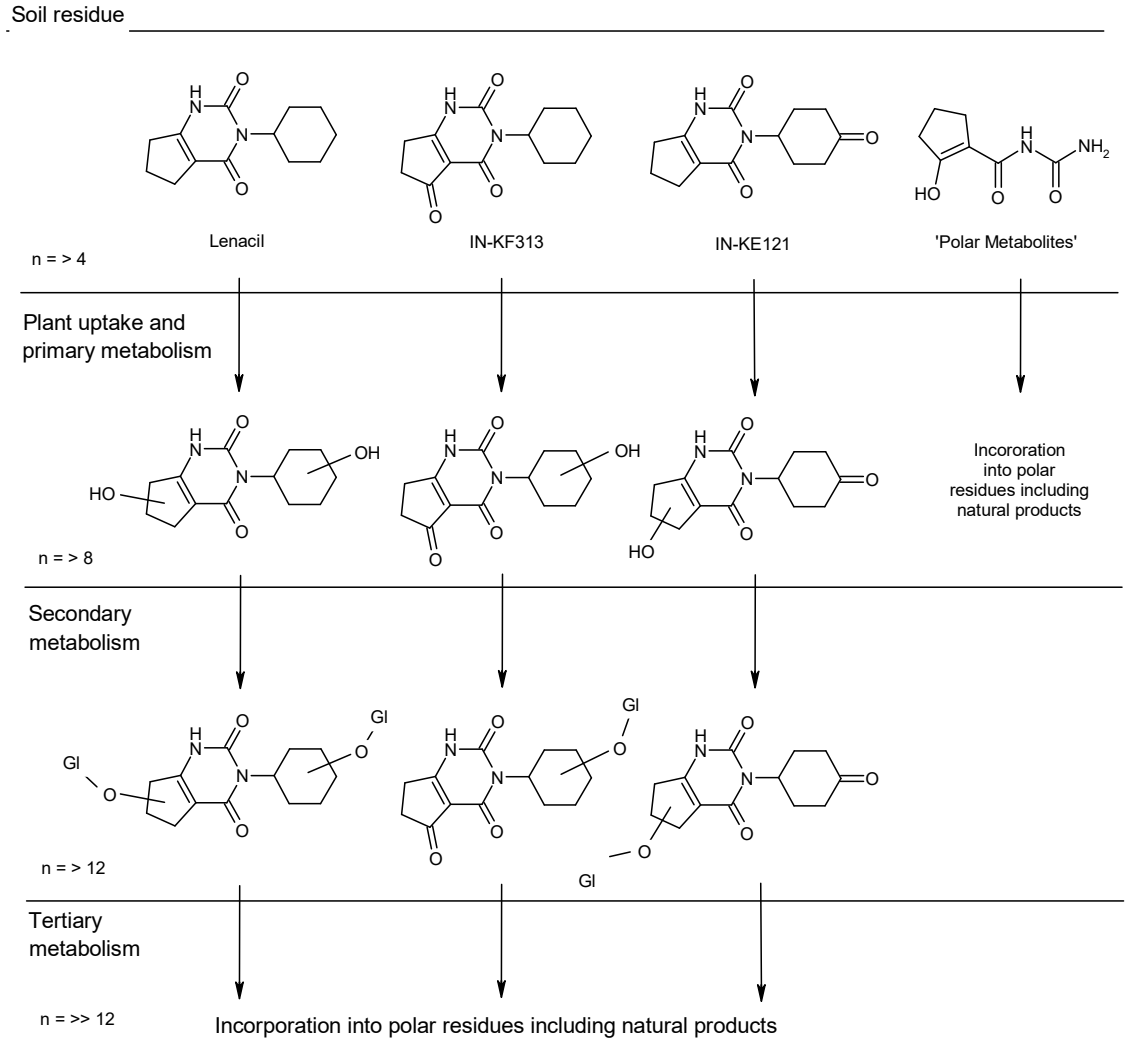
The degradation of lenacil in soil, combined with metabolism in the plant, produces a complex metabolite profile in rotational crops, including a considerable amount of polar residues. Characterisation and tentative identification of metabolite regions indicated that lenacil and its hydroxylated (and oxidized) metabolites are taken up from the soil and further transformation in the plant occurs via further hydroxylation in a range of positions and via (glucose) conjugation. There were indications of hydroxylation on the cyclopentyl ring (e.g. IN-KQ961) and/or on the cyclohexyl ring (e.g. IN-KD304). This proposed metabolic pathway/pattern (see **Figure 2.7.7.1-1**) is consistent with the pathway/pattern seen in primary crop (sugar beet) (cf. ***2.7.2***), as well as in rat (cf. ***2.7.3***). In wheat grain and wheat straw, there were indications of tight binding to and/or incorporation of residues in natural plant constituents.

Note: Soil analysis in the CRC study revealed the presence of metabolites tentatively identified as **IN-KE121**, **IN-KF313** and **IN-KQ957**. These findings are in agreement with the general conclusions on degradation of lenacil in soil (*vide infra – **2.8.1***), i.e. that degradation proceeds initially via hydroxylation (and subsequent oxidation) of the parent molecule to form the metabolites IN-KE121 and IN-KF313. Furthermore, it is noted that in the aerobic soil degradation study by Theis (2003), besides IN-KE121 (4'-oxo-lenacil) also another breakdown product was characterized as a lenacil derivative with the oxo-function (at an unconfirmed position) on the cyclohexane ring [see Vol.3 (CA), B.8.1.1.1.1 – peaks M14.0/M15.0]. This could have been IN-KQ957 (2'-oxo-lenacil), as tentatively identified in the CRC study.

Phytotoxic effects on lettuce (sown 30 DAT) were observed in the CRC study. Phytotoxic effects were not observed in wheat or turnip sown at the same interval (30 DAT) and were not reported in spinach sown 68 DAT.

Based on the results of the confined rotational crop study, the **setting of a separate residue definition for rotational crops is considered not necessary.** A consideration on the potential occurrence and magnitude of metabolites in rotational crops is presented under ***2.7.7.2** (vide infra)*.

Figure 2.7.7.1-1: Proposed uptake of lenacil residues and metabolic pathway of lenacil in rotational crops



2.7.7.2 Magnitude of residues in rotational crops

No limited field tests or field residue trials in rotational crops have been conducted; the need for such studies was waived by the applicant, who referred to the results of the confined rotational crop (CRC) study. On the basis of the results of that CRC study, the potential magnitude of residues (and relevance of metabolites) in rotational crops has been judged by the RMS: see ***Vol.3, B.7.6.2***.

Regarding only residues of the **parent compound lenacil**, the CRC study indicates that lenacil may theoretically occur at levels > 0.01 mg/kg in (small) root crops that are sown as succeeding crop relatively fast after treatment, e.g. in the case of crop failure (cf. 0.011 mg/kg in turnip root sown 30 DAT). In such a scenario, significant levels of lenacil (>0.01 mg/kg) may also occur in parts of root crops or cereal crops that are used exclusively as livestock feed items (cf. 0.045 mg/kg in turnip leaves, 0.075 mg/kg in wheat straw, 0.22 mg/kg in wheat forage and 0.28 mg/kg in wheat hay. This has been considered in the estimation of the livestock dietary burden (*vide supra – **2.7.5.2***).

In rotational or succeeding crops sown at least 6 months after treatment, residues of parent lenacil at levels > 0.01 mg/kg are not expected, expect maybe in cereal forage or hay (cf. 0.078 mg/kg in forage and 0.030 in hay of wheat sown 182 DAT).

However, according to the applicant, the results of the 30/68-days plant-back interval are not applicable to the supported use on sugar and fodder beet and the results of the 182-days plant-back interval are the most relevant to **agronomic practice of crop rotation**. Indeed, sugar and fodder beet can be sown from mid-March to early April and the time needed to reach maturity is about 6 to 8 months. Thus, sugar/fodder beets treated post-emergence at BBCH 10-31 (normally during May) will be harvested in the autumn (normally September to November) and will therefore not be planted with a rotational crop such as winter wheat until late autumn (e.g. November) or with other types of crop until the following spring (March/April), i.e. at least 6 months after last application. Therefore, RMS agrees that the **results of the 182 DAT plant-back interval are the most relevant for the supported use on sugar and fodder beet.** Nevertheless, also **crop failure** needs to be considered as a **possible scenario**. According to the supported GAP on sugar/fodder beets, it is possible to carry out a single application at a rate of 0.5 kg a.s./ha. Thus, crop failure could in principle occur after a single application at maximum seasonal dose (0.5 kg/ha) and in such a case, the residue levels observed in the CRC study could be regarded as more or less representative. However, it should be noted that in the 30/68 PBI crop samples relevant for human consumption, the individual metabolite levels were relatively low (max. 0.038 mg/kg). In a (possibly more realistic) situation where the maximum seasonal application dose of 0.5 kg a.s./ha would be split in several applications (max. 4x 0.125 kg a.s./ha), it is unlikely that the maximum number of applications (4) would already have been made before crop failure occurs. Hence, the overall amount of residues available in soil for uptake by the succeeding crop sown as replacement would be expected to be lower than the amount resulting from a seasonal maximum application dose of 500 g a.i./ha (4x125 g a.i./ha). As a consequence, it could be presumed that residue levels of parent lenacil and individual metabolite levels in commodities relevant for human consumption would be lower (<0.02 mg/kg) than those observed in the 30/68 DAT rotational crop fractions (up to 0.038 mg/kg).

Particularly with regard to the occurrence and magnitude of **metabolites** in rotational crops (occurring via uptake from the soil and/or via breakdown in the plant), the following is considered:

It is acknowledged that significant residue levels of metabolites (>0.01 mg/kg, parent equivalents) were found in food and feed even at 182-days plant back interval (particularly in spinach). Taken into account that several individual metabolite regions probably represent multiple components, individual metabolite levels are expected to be ≤ 0.03 mg/kg in rotational crop commodities for human consumption and ≤ 0.2 mg/kg in those used for animal feeding.

However, the majority of the (tentatively) identified metabolites are likely to be structurally related to lenacil (e.g. hydroxylated lenacil homologs). In absence of targeted toxicological data for the different hydroxylated compounds, the same toxicological properties could be attributed to all these metabolites and the toxicological reference values for lenacil could be used for structurally related metabolites (*vide supra – **2.7.3***). Due to this presumed toxicological equivalence, it could be deemed reasonable to sum up all (tentative) hydroxylated metabolites, even if present at levels <10% TRR.

To investigate whether or not the residues of metabolites in rotational crops would contribute significantly to the overall risk assessment, the RMS has conducted an indicative worst-case risk assessment. The intention of this screening exercise was to get some reassurance that the levels of the different metabolites in rotational crops – of which the majority are likely to be structurally related to lenacil – are not of any concern.

As a first screening, the RMS considered the sum of all individual residue compounds, by using the rotational crop TRR levels for the 30/68-days plant-back interval (see **Table 2.7.7.2-1** below, based on ***Table B.7.6.1-1*** in DRAR Vol.3, B.7) as input values in PRIMo rev.2 for the corresponding target crop groups (i.e. root & tuber vegetables, sugar beet root, leafy vegetables and cereals).
This calculation results in a chronic intake (TMDI) contribution of max. 3.1% of the ADI (of parent lenacil; 0.12 mg/kg bw/day). Taking into account that probably (a part of) the (hydroxylated) metabolites are less toxic than the parent (see toxicological considerations in ***Vol.1, 2.6.8.1/2.7.3*** and ***Vol.3, B.6.8.1.2***), the calculated intake is likely to be a worst-case (over)estimation. Therefore, it is concluded that the contribution of metabolite residues to the consumer risk is negligible and the presence of these metabolites in rotational crops is not of any concern for the consumer. As a consequence, the **setting of a separate residue definition for rotational crops is considered not necessary**.

Table 2.7.7.2-1: Input values (TRR) used for screening of contribution of rotational crop TRR in target crop groups (commodities for human consumption) to overall consumer risk

Matrix	Plant-back interval (days)	TRR (mg/kg)	Extrapolated to target crop group(s)/commodities
Mature spinach	68 DAT	0.259	Leaf vegetables & fresh herbs
Turnip roots	30 DAT	0.115	Root and tuber vegetables; Sugar beet root
Wheat grain	30 DAT	0.143	Cereals (grain)

2.7.7.3 Waiting period between last application and sowing or planting succeeding crops and restrictions in crop rotation

In the framework of the original EU peer review, the applicant had mentioned following general recommendation:

"*Succeeding crops should not be planted or drilled until at least 120 days after application of lenacil because of its phytotoxicity. If crop failure occurred during this period only sugar beet, red beet (beetroot) or spinach could be planted."*

However, following assessment of the confirmatory data (CRC study) (cf. BE, 2013), the RMS concluded that this statement was not fully supported by the available data on phytotoxicity and residues in rotational crops.

In the framework of the renewal of a.s. approval (admissibility check), the following had been initially stated by the applicant concerning the waiting period between last application and sowing/planting of succeeding crops:

"However, when lenacil 500 g/L SC is applied and crop failure occurs for any reason only sugar and fodder beet, red beet, mangels, spinach or strawberries may be planted within four months of application. […] *Only winter cereals should be planted in the same calendar year after a beet crop have been treated with Lenacil 500 g/L SC.* […] *Eggplant or melon should not be sown or planted after less than 12 months after lenacil 500 g/L SC application."*

The rationale behind these recommendations from the applicant was unclear (e.g. recommendations for mangels, strawberries, eggplant or melon were not supported by any verifiable data). The applicant could not address the RMS's request to clarify the suggested restrictions and to explain on which data (residues or phytotoxicity) they were based. The recommendations mentioned above were withdrawn by the applicant and the only restriction proposed by the applicant in its submitted dossier was to exclude lettuce as a replacement crop in case of original crop failure (see (1) below). Based on the overall assessment of the data, the **RMS considers additional restrictions necessary: see (2) and (3) below.**

(1) For reasons of **phytotoxicity**, lettuce is not recommended as replacement crop in case of failure of the original crop (cf. phytotoxicity observed for lettuce sown 30 DAT in the confined rotational crop study).

Note: On the basis of the CRC study, further rotational restrictions or a waiting period (beyond 30 days) for reasons of phytotoxicity do not seem necessary, since no relevant phytotoxic effects were observed in wheat, turnip (sown 30 DAT or later) or spinach (sown 68 DAT[10] or later) in the CRC study. Nevertheless, the necessity of a waiting period for succeeding crops must be considered at MS level for each other use (beyond the representative use on sugar/fodder beet), e.g. on other crops with shorter cultivation period or uses with higher application doses, in order to avoid significant phytotoxic effects. In that regard, it remains to be verified whether complementary or additional recommendations are justified on the basis of data available in the efficacy part of the dossiers. However, broader recommendations/restrictions might be proposed by the applicants at national level for reasons of precaution (cf. product stewardship) and/or based on experiences in agronomic practice.

(2) In order to avoid significant residue levels of lenacil in some rotational crops that may be used as (major) **livestock feed** items, following restriction is deemed necessary (*vide supra – **2.7.5.2***):

A **waiting period of at least 6 months** (from application to drilling/planting of the rotational/succeeding crop) should be respected and **possible succeeding/rotational crops are restricted** to root/tuber vegetables, leafy vegetables and cereals – i.e. small grain cereals and other crops of the 'cereal group' such as maize – for grain production only (i.e. use of forage, silage or hay for livestock feeding is not allowed).

Note: For the representative use of Lenacil 500 g/L SC on sugar beets and fodder beets, the waiting period will in practice be covered by the cultivation period (if no crop failure occurs).

[10] Even if 68 DAT was the shortest plant-back interval tested for phytotoxic effect on spinach, RMS considers it unlikely that there would be effects on spinach sown 30 DAT, taking into account that pre-emergence uses on spinach with application doses up to 2000 g lenacil/ha are authorized in some EU MS (cf. notified GAPs in the framework of the initial launch of the MRL review; *Evaluation Report on review of existing MRLs for lenacil – Belgium, 7 July 2010*).

(3) In order to avoid significant residue levels of lenacil in succeeding crops (in the case of original crop failure) – which may be used for **human consumption** (e.g. oilseeds and pulses, fruits and fruiting vegetables, root crops with short cultivation period) or as (major) **livestock feed** items – following restriction is necessary (*vide supra* – ***2.7.5.2***):

If lenacil is applied and **crop failure** occurs for any reason, it is recommended to **wait at least 30 days** after application to plant/drill a replacement crop. **Possible replacement crops are restricted** to sugar and fodder beet[11], spinach[12] or a cereal crop (incl. maize) for grain production only (i.e. use of forage, silage or hay for livestock feeding is not allowed)."

Note: The possible mitigation measures outlined above have been elaborated by the RMS; they were not part of the representative uses as notified by the applicant. Nevertheless, during the finalisation of the DRAR, the applicant expressed its agreement with the recommended restrictions proposed by the RMS.

2.7.7.4 Necessity of MRLs for rotational crops:

For all plant-back intervals, residue levels of lenacil – which is the sole compound included in the residue definition for monitoring – were <0.02 mg/kg (LOQ of former analytical method for enforcement) in all rotational crop parts representative for commodities exclusively used for human consumption. Residue levels of lenacil were also <0.01 mg/kg (LOQ of new analytical method for enforcement – see ***2.5.2***) in all rotational crop parts representative for commodities for human consumption, except in turnip root sown 30 DAT (0.011 mg/kg).

The finding in turnip root may be regarded as representative for (small) root crops that are sown as succeeding/replacement crop relatively fast after treatment, e.g. in the case of original crop failure. Furthermore, no data were available for crops of the oilseeds/pulses group and fruits and fruiting vegetables group. Therefore, lenacil residues at or above 0.01 mg/kg in those crops cannot be fully excluded.

However, **if the restrictions as proposed by the RMS above (see *2.7.7.3*) are respected, it is not deemed necessary to set specific MRLs** (above the default/LOQ MRL of 0.01 mg/kg) for succeeding/rotational crops.

[11] The reason for restricting to sugar and fodder beet (and thus excluding other root/tuber crops) is to avoid quantifiable residues of lenacil at or above 0.01 mg/kg in root/tuber vegetables with shorter cultivation period (as compared to sugar/fodder beets). Thus, such a restriction is deemed appropriate, to avoid MRL exceedance, assuming that MRLs will be reduced to the default level / new LOQ of 0.01 mg/kg. See also ***2.7.7.4***.

[12] As phytotoxicity for lettuce was observed in the CRC study 30 DAT, lettuce is not recommended as replacement crop (in case of failure of the original crop) and therefore a restriction to spinach (investigated crop) is justified. See also ***2.7.7.1***.

2.7.8 Summary of other studies

2.7.8.1 Effect on the residue level in pollen and bee products

No data were provided; the applicant provided arguments to waive the need for studies (see ***Vol.3, B.7.5***).

However, the representative uses supported in the framework of renewal of a.s. approval are on sugar beets and fodder beets, which are harvested before flowering according to good agricultural practice. As a consequence, honey bees will not be exposed to residues in the primary sugar/fodder beet.

Note: In case sugar beet were grown for seed production, significant residues in nectar and pollen of the flowering sugar beet plant (in the second vegetation year) would not be expected, considering the very low residue levels of lenacil expected in sugar beet root (<0.02 mg/kg) at harvest in the first vegetation year. Therefore, the risk of significant residues being transferred from the sugar beet to bee products is considered negligible.

As lenacil is applied on sugar/fodder beet during spring, the possibility of residues on flowering **non-target plants** visited by foraging honey bees may also need further consideration.

- However, flowering in-field weeds can be reasonably excluded, since lenacil is used as herbicide at early crop growth stage.
- With regard to possible adjacent non-target plants (i.e. at the outer borders of the field), it is difficult to predict the residue levels on non-target plants, particularly in their pollen/nectar potentially collected by honey bees, because 'non-target plants' cover a non-homogenous group of plants and because no specific residue data are available. In one supervised residue trial (G01N005R – see ***Vol.3, B.7.3, Table B.7.3.1-1***), residues of lenacil on sugar beet leaves were measured just after spray application, as well as 15 and 28 days after treatment (DAT): 3.8, 1.4 and 0.09 mg/kg, respectively.
    - It can be reasonably expected that the order of magnitude of residues on the adjacent plants – which do not receive a direct spray treatment – will be considerably lower than on the treated primary crop (sugar/fodder beet). According to SANCO/10329/2002 (rev.2 final – 17 October 2002)[13], the spray drift to adjacent terrestrial plants (at 1 meter distance of the field edge) is estimated to represent 2.77% of the dose applied to a field crop. Considering the values measured in sugar beet leaves (see above), this corresponds to expected residue levels in adjacent non-target plants of around 0.11, 0.038 and 0.0025 mg/kg (0, 15, 28 DAT, respectively).
    - Potential exposure of honey bees to initially relatively high residue levels (and transfer of residues to honey comb) is likely to be weighed out by exposure to lower residue levels at later foraging occasions.
    - Furthermore, the acreage of potentially contaminated adjacent plants is expected to be limited and consequently, the possible contribution to contamination of honey will also be limited.

    Although it is acknowledged that the residue data in aerial parts are very limited, the available results of the sugar beet residue trial indicate that residue levels of lenacil on aerial parts rapidly decline after application and anticipated residue levels in adjacent non-target crops are relatively low.

Considering the low residue levels of lenacil observed in aerial parts of rotational crops (*vide supra* – ***2.7.7.2***), it is deemed unlikely that significant contamination of honey with lenacil residues would occur via exposure of foraging honey bees to rotational crops after application of lenacil.

Overall, the RMS is of the opinion that the potential exposure of honey bees to residues of lenacil is limited, and consequently, a significant transfer of residues into bee products such as honey, is unlikely. No further data are deemed necessary.

[13] Draft Working Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (European Commission, 2002)

2.7.9 Estimation of the potential and actual exposure through diet and other sources

Chronic dietary intake

In the framework of the original EU peer review (BE, 2007-2009), chronic consumer intake calculations had been performed using the consumption data from the WHO standard European diet for adults (WHO/FSF/FOS/98.3, GEMS/Food Regional Diets, rev. Sept. 2003), German 3-5 years old children's diet (Chronic VELS-Modell, BfR, Stand 10. April 2006, v1.6) and the diets for adults, school children, infants, toddlers, vegetarians and elderly from UK (PSD Ten Consumer exposure model 1999 – extreme consumers). The LOQ of 0.02 mg/kg was used as input value for lenacil residues in sugar beet (root). Theoretical maximum daily intakes (TMDI) were very low (<0.1% of ADI of 0.12 mg/kg bw/d).
In addition, a calculation was carried out with the EFSA PRIMo model (rev.2) and no chronic intake concerns were identified during the original EU review for the representative uses, taking into account the toxicological reference values that were agreed in the framework of the review under Dir. 91/414/EEC (ADI of 0.12 mg/kg bw/d – *see review report SANCO/833/08 – 11 May 2010*), i.e. theoretical maximum daily intake (TMDI) was at or below 0.4% of the ADI for all considered consumer groups (cf. EFSA, 2009).

In the framework of renewal of a.s. approval (DRAR – BE, 2019), no change to the Acceptable Daily Intake (ADI) of lenacil is proposed (*vide supra – **2.6.10.1***), i.e.

ADI = 0.12 mg/kg b.w./day (i.e. no change compared to ADI established in original EU review framework)

The chronic consumer dietary risk resulting from the representative uses on sugar beets and fodder beets was calculated according to the internationally agreed methodology using the consumption data included in revision 2 of the EFSA Pesticide Residue Intake Model (PRIMo[14]). Input values are clarified in **Table 2.7.9.1**.

Acute dietary intake

In the framework of the original EU peer review (BE, 2007-2009), an acute dietary risk assessment was not performed, as an Acute Reference Dose (ARfD) had not been allocated to lenacil (and deemed not necessary – *see review report SANCO/833/08 – 11 May 2010*).

However, in the framework of renewal of a.s. approval (DRAR – BE, 2019), an ARfD is proposed for lenacil (*vide supra – **2.6.10.2***):

ARfD = 3 mg/kg b.w. (i.e. **change** compared to original EU review)

The acute consumer dietary risk resulting from the representative uses on sugar beets and fodder beets was calculated according to the internationally agreed methodology using the consumption data included in revision 2 of the EFSA Pesticide Residue Intake Model (PRIMo[15]). Input values are clarified in **Table 2.7.9.1**.

Table 2.7.9-1: Input values for the consumer risk assessment

Commodity	Chronic risk assessment		Acute risk assessment	
	Input (mg/kg)	Comment	Input (mg/kg)	Comment
Risk assessment residue definition: lenacil				
Sugar beet (root)	0.02	MRL (=LOQ in supervised residue trials)	0.02	HR (=LOQ in supervised residue trials)

[14] EFSA (2007). PRIMo rev.2 – Pesticide Residue Intake Model. https://www.efsa.europa.eu/en/applications/pesticides/tools. See also EFSA (2007). Reasoned opinion on the potential chronic and acute risk to consumers health arising from proposed temporary EU MRLs. 15 March 2007. *Available online: www.efsa.europa.eu*

[15] EFSA (2007). PRIMo rev.2 – Pesticide Residue Intake Model. https://www.efsa.europa.eu/en/applications/pesticides/tools. See also EFSA (2007). Reasoned opinion on the potential chronic and acute risk to consumers health arising from proposed temporary EU MRLs. 15 March 2007. *Available online: www.efsa.europa.eu*

With an estimated TMDI of max. 0.4% of the ADI (UK toddler) and an IESTI (International Estimated Short-Term Intake) of well below 0.1% (IESTI = max. 0.0013 mg/kg bw), it is concluded that the short-term or long-term intake of sugar from sugar beets treated according to the representative uses does not present any concern for the consumer. A copy of the EFSA PRIMo report sheet is presented in **Figure 2.7.9-1**.

Remark: The consumer dietary risk assessment is to be considered as provisional, since potential transfer of residues from livestock feed items (derived from rotational crops) into animal commodities was not addressed: see data gap identified in section ***2.7.2.***

Impact of new ARfD on EU MRLs

An ARfD for lenacil is proposed in the framework of renewal, whereas this had not been considered necessary in the framework of the original EU peer review. The RMS has therefore checked whether this may have a significant impact on the safety of the existing EU MRLs (Reg. (EC) No 149/2008).

Assuming lenacil residues were present in raw commodities of plant or animal origin at the corresponding, current EU MRL, no acute dietary risk would be expected for consumers, because for each of the current EU MRLs, the associated maximum short-term dietary intake is well below the ARfD (<1% ARfD), considering the consumption data in EFSA PRIMo rev.2a. However, the EU MRLs for lenacil remain to be reviewed, according to art.12 of Reg. (EC) No 396/2005 (see also ***2.7.10***), but on the basis of the screening performed, there are no indications that a prioritisation of that MRL review is needed.

Additional consumer exposure through drinking water resulting from groundwater metabolites

Neither parent nor identified metabolites in ground water had a PECgw above the trigger value of 0.75 µg/L. Therefore, no consumer risk assessment for residues potentially occurring in drinking water was conducted.

Figure 2.7.9-1: Outcome of the chronic consumer risk assessment (EFSA PRIMo rev.2) (repr. uses only)

lenacil

Status of the active substance:		Code no.	
LOQ (mg/kg bw):	0,02	proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0,12**	ARfD (mg/kg bw):	**3**
Source of ADI:	**RAR**	Source of ARfD:	**RAR**
Year of evaluation:	**2018**	Year of evaluation:	**2018**

Prepare workbook for refined calculations

Undo refined calculations

ADI proposal (RMS) unchanged compared to ADI established under original EU review (EFSA, 2009; EC, 2010); **New ARfD** proposed by RMS in RAR (2018) (previously no ARfD allocated to lenacil)

The risk assessment has been performed on the basis of the MRL calculated for sugar beet root (0,02 mg/kg).

Chronic risk assessment

TMDI (range) in % of ADI
minimum - maximum

No of diets exceeding ADI: ---

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
0,4	UK Toddler	0,4	Sugar beet (root)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	0,4
0,2	UK Infant	0,2	Sugar beet (root)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	0,2
0,1	UK Adult	0,1	Sugar beet (root)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	0,1
0,1	UK vegetarian	0,1	Sugar beet (root)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	0,1
0,0	WHO Cluster diet B	0,0	Sugar beet (root)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	0,0
0,0	WHO cluster diet E	0,0	Sugar beet (root)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	0,0
0,0	WHO regional European diet	0,0	Sugar beet (root)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	0,0
0,0	WHO cluster diet D	0,0	Sugar beet (root)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	0,0
0,0	WHO cluster diet D	0,0	Sugar beet (root)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	0,0
	DK adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	DK adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	DK adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	DK adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	DK adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	DK adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	DK adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	DK adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	DK adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	DK adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	DK adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	DK adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	DK adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	DK adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	DK adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	DK adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	DK adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	
	DK adult		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)	

Conclusion:
The estimated Theoretical Maximum Daily Intakes (TMDI), based on pTMRLs were below the ADI.
A long-term intake of residues of lenacil is unlikely to present a public health concern.

Acute risk assessment /children						Acute risk assessment / adults / general population					
The acute risk assessment is based on the ARfD.											
For each commodity the calculation is based on the highest reported MS consumption per kg bw and the corresponding unit weight from the MS with the critical consumption. If no data on the unit weight was available from that MS an average European unit weight was used for the IESTI calculation.											
In the **IESTI 1** calculation, the variability factors were 10, 7 or 5 (according to JMPR manual 2002), for lettuce a variability factor of 5 was used. In the **IESTI 2** calculations, the variability factors of 10 and 7 were replaced by 5. For lettuce the calculation was performed with a variabilty factor of 3.											
Threshold MRL is the calculated residue level which would leads to an exposure equivalent to 100 % of the ARfD.											
Unprocessed commodities	**No of commodities for which ARfD/ADI is exceeded (IESTI 1):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 1):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2):**		---
	IESTI 1	*)	**)	**IESTI 2**	*)	**)	**IESTI 1**	*)	**)	**IESTI 2**	*)	**)
	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)
	0,0	Sugar beet (root)	0,02 / -	0,0	Sugar beet (root)	0,02 / -	0,0	Sugar beet (root)	0,02 / -	0,0	Sugar beet (root)	0,02 / -

2.7.10 Proposed MRLs and compliance with existing MRLs

A temporary EU MRL of 0.1 mg/kg has been established for lenacil in sugar beet under Commission Regulation (EC) No 149/2008 of 29 January 2008 amending Regulation (EC) No 396/2005, awaiting the review of all existing EU MRLs for lenacil in accordance with art. 12(1) of Regulation (EC) No 396/2005. The MRL review has been put on hold; (re)launch and finalisation of the MRL review is foreseen for after renewal of a.s. approval of lenacil.

As summarised in section **2.7.4** (***vide supra***), residues of lenacil in sugar beet roots following applications of Lenacil 500 g/L SC according to the critical GAP are <0.02 mg/kg. It is noted that an analytical method for monitoring and enforcement purposes is available with a (lower) validated LOQ of 0.01 mg/kg (*vide supra – **2.5.2***).

An overview of existing and proposed EU MRLs for lenacil for sugar beet root (i.e. commodity relevant to the representative uses) is given in **Table 2.7.10-1**. The need for MRLs to cover possible residues of lenacil in rotational crops depends on possible restrictions that may be imposed at Member State level (*vide supra – **2.7.7.4***).

Table 2.7.10-1: Existing and proposed MRLs s for lenacil in sugar beet root

Commodity (code)	Existing EU MRL (mg/kg)*	Proposed EU MRL (mg/kg)	Comment
Sugar beet root (0900010)	0.1	0.02	The submitted data are sufficient to derive an MRL proposal for the representative use (NEU+SEU). Risk for consumers unlikely. The currently existing (higher) EU MRL (0.1 mg/kg) does not pose any concern for the consumer either. An official MRL application according to art. 6-10 of Reg. (EC) No 396/2005 – in view of reducing the current EU MRL in sugar beet root – was not introduced in the framework of renewal of a.s. approval. However, this was not deemed required at this stage, as the reduction of the EU MRLs (not only for sugar beet, but for nearly all commodities) will be dealt with in the framework of the MRL review according to art.12 of Reg. (EC) No 396/2005, which is foreseen after renewal of approval of lenacil.
Other commodities of plant origin (as rotational crops)	0.1 (0.5 for tropical root and tuber vegetables)	Further risk management considerations required	Whether it is necessary to set specific MRLs (above 0.01 mg/kg) for rotational crops, depends on possible rotational crop restrictions (*vide supra – **2.7.7.4***). Without restrictions, lenacil residues at or above 0.01 mg/kg cannot be excluded in (small) root crops, in oilseed/pulse crops, fruits and fruiting vegetables.
Products of animal origin (1000000)	0.1	No MRL proposal; Further risk management considerations required	The submitted data are insufficient to derive an MRL proposal for animal products. The need to set MRLs (above 0.01 mg/kg) depends on possible rotational crop restrictions.

* Reg. (EC) No 149/2008

Note: According to the applicant's dossier supporting renewal of a.s. approval (Doc. E-2), MRL values above 0.01 mg/kg for lenacil are established in several non-EU countries (Argentina, Canada, Egypt, Iceland, Japan, Kazakhstan, Malaysia, Morocco, New Zealand, Norway, Russia, South Africa, Switzerland, Turkey), for garden beet root/tops, sugar beet root/tops, spinaches, strawberry, Swiss chard and/or potatoes. However, this statement could not be verified by the RMS.

2.7.11 Proposed import tolerances and compliance with existing import tolerances

Not applicable

2.8 FATE AND BEHAVIOUR IN THE ENVIRONMENT

The environmental fate properties assessment for lenacil is based on the Draft Assessment Report (2007-2009), the Addendum to the Draft Assessment Report (2012-2013), the EFSA Conclusion on the peer review of confirmatory data submitted for the active substance lenacil (2013) and the studies submitted for the renewal of the active substance (DRAR 2019).

All the studies on the fate and behaviour of lenacil in the environment were performed under GLP and according to EPA, OECD or equivalent guidelines.

The Notifier performed a literature survey on lenacil (Criollo, 2016) and its metabolites IN-KF313 and IN-KE121 (Anonymous, 2018) The results of the literature search, following the principles of the EFSA Guidance Document entitled "Submission of scientific peer reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009" (EFSA Journal 2011;9(2):2092), are presented in detail in the Vol. 3 CA B.8, Section B.8.6. All details about the protocol used, the selection of databases and the list of keywords are given in Vol. 3 CA B.8. No relevant public literature articles were identified for lenacil or the metabolites considered for the risk assessment in the Fate and behavior section.

2.8.1 Summary of fate and behaviour in soil

Aerobic soil metabolism studies

Three soil experiments treated with 4,7a-$^{14}C_2$- or 2-^{14}C-lenacil were carried out under aerobic conditions in the laboratory (20°C, 40% maximum water holding capacity (MWHC)) in the dark (Theis, 2003; Millais, 2003; Hurst, 2012). The formation of residues not extracted were a sink for the applied lenacil (19.4-25.8% AR and 14.5-16.9% AR for the 4,7a-$^{14}C_2$- and the 2-^{14}C-labels, respectively, after 120 days). Volatile compounds including presumably mainly carbon dioxide, accounted for 47.6-61.1% AR for the 4,7a-$^{14}C_2$-label and 74.1-77% AR for the 2-^{14}C-label after 120 days. The major (>10% AR or >5% AR on 2 consecutive sampling dates) extractable breakdown products presented were metabolite IN-KE 121 (maximum occurrence 9.7-13.9% AR at 14-30 days), metabolite IN-KF 313 (maximum occurrence 14.7-21.7% AR at 14-30 days) and the unidentified metabolite „Polar B" (maximum occurrence 6.8-14.6% AR at 91-120 days). Furthermore in one soil there was also a minor non-transient unidentified breakdown product denoted „M15.0" that accounted for more than 5%AR at two consecutive sampling times. Based on the attempts made by the Notifier to identify this metabolite, this product was characterised as an oxo-isomer of lenacil, which is formed by the oxidation of the cyclohexyl ring. The identified metabolite IN-KE 121 is also an oxo-isomer of lenacil (7-oxo-lenacil), but from the available information the conformity of these transformation products could not be fully confirmed. The available information on the identity and the further use of the degradation data of the metabolite M15.0 was discussed at the PRAPeR 67 meeting. The experts agreed that M15.0 is either identical to IN-KE 121 or is a positional isomer of IN-KE 121 with the keto-function on the cyclohexane ring, and agreed moreover that the exposure assessment for IN-KE 121 would probably cover the assessment for M15.0 even with respect to degradation.

On the basis of the submitted studies, the major route of degradation for lenacil in soil is due to microbial decomposition via hydroxylation and oxidation of either the cyclopentyl ring or the cyclohexyl ring to form the major soil metabolites IN-KF313 and IN-KE121, which are further microbially degraded to a series of minor, chemically instable polar metabolites and then further to CO_2 and bound residues. The link between the primary metabolic pathway and the terminal lenacil degradation product CO_2 is the pyrimidine ring opening mechanism. The polar soil degradation products which are identified (IPM1, also named Unknown Polar 3) and partly proposed (PM1-PM5), are multicomponent, comprising upwards of 33 individual polar metabolites, each accounting for less than 5% of applied lenacil. No photodegradation product except CO_2 was observed. Therefore, the proposed degradation pathway of lenacil in soil is as shown in Figure 2.8.1-1.

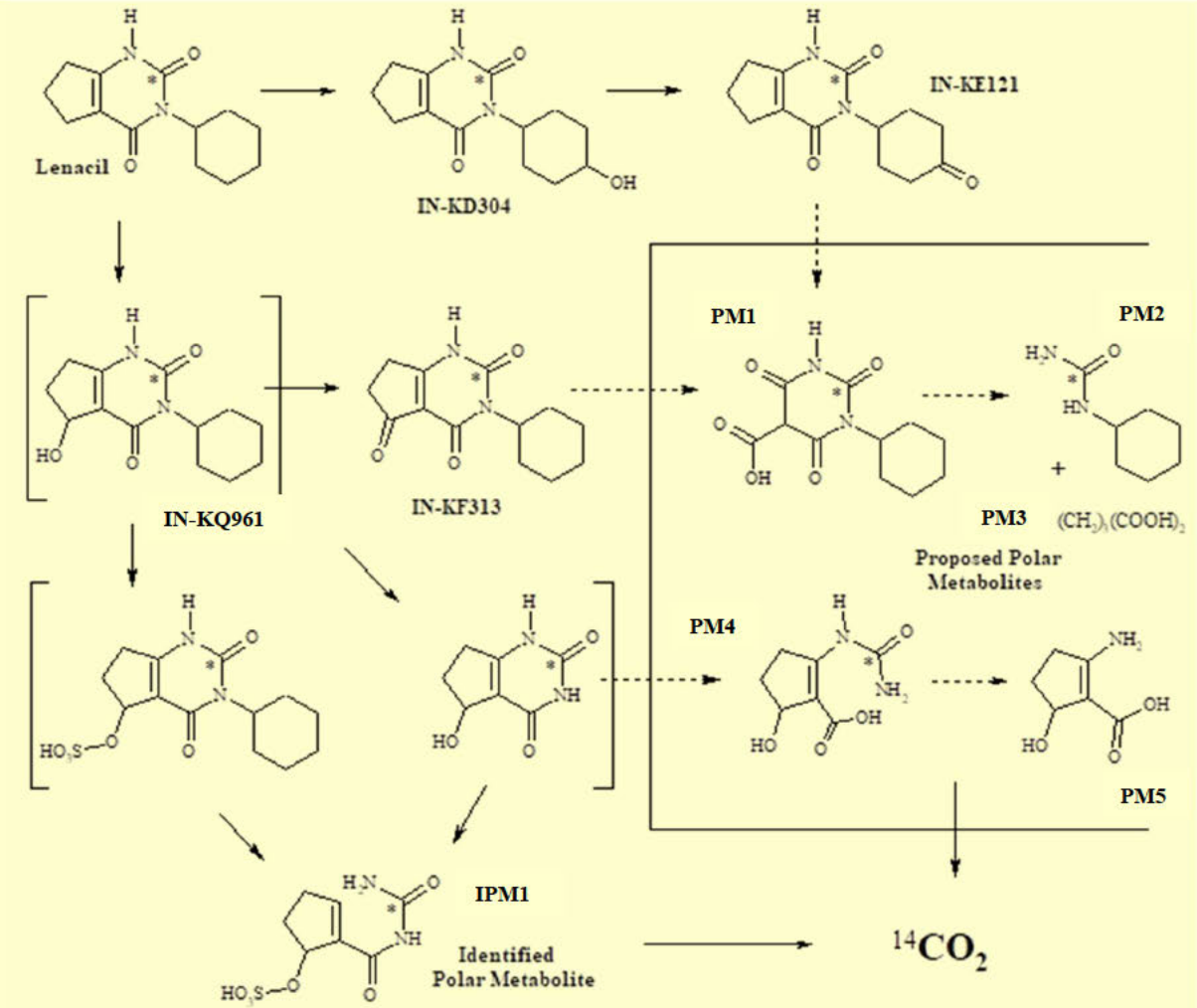


Figure 2.8.1-1: degradation pathway of lenacil in soil
* shows position of radiolabelled carbon.
solid arrow indicates confirmed pathway of degradation
broken arrow indicates proposed or analogous pathway of degradation
structures in brackets were not identified.

One experiment with 4,7a-$^{14}C_2$-lenacil (Millais, 2003) was repeated at 10 °C in which metabolite IN-KE 121 reached 7.8% AR (on day 30), metabolite IN-KF 313 reached 9.4% AR (on day 60) and the amount of the breakdown product denoted „Polars" was observed above 10% AR (maximum occurrence 12.5% AR at 120 days). Unextractable residue amounted up to 20.9% AR and volatiles (presumably consisting of mainly carbon dioxide) reached a maximum of 24.3% AR after 120 d (end of the experiment).

Soil persistence endpoints (DT_{50}) values for lenacil under aerobic conditions were calculated to be 5.3-20.9 days (at 20°C and 40% MWHC or pF2 soil moisture content, n=8). After normalization of these values to FOCUS reference conditions (20°C and pF2 soil moisture content), the range became 5.6-15.4 days, with a geometric mean of 10.5 days.

Soil persistence endpoints (DT_{50}) values were also calculated for the metabolite IN-KF 313. The soil DT_{50} were calculated to be between 1.7-16.1 days (at 20°C and 40% MWHC or pF2 soil moisture content, n=8). After normalisation to FOCUS reference conditions (20°C and pF2 soil moisture content) this range became 2.2-17.7 days, with a geometric mean of 10.7 days.

Degradation parameters for the metabolite IN-KE 121 in soil under aerobic conditions were also estimated from the results of the studies with the parent compound. Soil persistence endpoints (DT_{50}) values at 20°C were calculated

to be 1.3-18.3 days (at 20°C and 40% MWHC or pF2 soil moisture content, n=8). After normalization of these values to FOCUS reference conditions (20°C and pF2 soil moisture content), the range became 1.3-30.2 days, with a geometric mean of 7.6 days.

Based on the available data sets including some information from the physical-chemical section, it is considered that the degradation of lenacil and its identified metabolites is not dependent on the soil pH.

Anaerobic soil metabolism studies
No anaerobic soil degradation study was available.

Adsorption/desorption
The adsorption/desorption of lenacil was investigated in 7 soils at 20°C or 25°C in satisfactory batch adsorption experiments. KFoc values varied from 75 to 254 mL/g (geomean 114.8 mL/g), indicating that lenacil is rather slightly mobile in soil (according to Mensink et al., 1995). Freundlich coefficients ranged from 0.86 – 0.94 (median 0.89).

The adsorption/desorption of the metabolites IN-KF313 and IN-KE121 was investigated in 6 and 3 soils respectively. The adsorption of the metabolites IN-KF313 and IN-KE121 was pH dependent. For soils with pH ≤ 7, calculated adsorption KFoc for IN-KF313 varied from 79.0-823.8 mL/g (geomean 368.5 mL/g) and the 1/n values ranged from 0.69 – 1.00 (mean 0.89). For soils with pH > 7, calculated adsorption KFoc for IN-KF313 varied from 55-76 mL/g (geomean 63.1 mL/g) and the 1/n values ranged from 0.91 – 0.96 (mean 0.93).
For soils with pH ≤ 7, calculated adsorption KFoc for IN-KE121 varied from 40.4-43.5 mL/g (geomean 41.9 mL/g) and the 1/n values ranged from 0.92 – 0.96 (mean 0.94). For the soil with pH > 7, calculated adsorption KFoc for IN-KE121 was 30.5 mL/g and the 1/n value was 0.96.

Soil photolysis
The photodegradation rate of Lenacil on soil at 20°C is equivalent to 67.6 days assuming summer sunlight equivalents (12 hour days) at latitude 40°N. For irradiated soil treated with 14C-Lenacil, total mean recoveries of radioactivity were in the range of 95.7 to 105.3% AR and for the controls 99.9 to 104.5% AR.

Volatile radioactivity accounted for 15.7% AR at 15 days for the irradiated soil samples of which most (15.6% AR) was carbon dioxide. No significant volatile radioactivity (<0.1% AR) was found in the control samples. No major degradates were detected in soil extracts, although H1 reached a maximum of 7.6%AR. TLC indicated that this radioactivity was associated with more than one component.

Mobility in soil
Column leaching studies with lenacil were not conducted since reliable adsorption coefficient values have been obtained in adsorption/desorption studies reported (Volume 3 CA B.8 Section B.8.1.2.1.1). However, a lysimeter study was performed (Schnöder, 2004a). In this lysimeter study, Lenacil was applied as a split application to sugar beets grown on two lysimeters in spring 1995. The first application was performed at a rate of 200 g a.s./ha (at growth stage 12-14) and the second application at a rate of 300 g a.s./ha (at growth stage 16 to 18).

Neither lenacil nor the main soil metabolite, IN-KF313, were detected in the leachate (LOD 0.050 µg/L as active ingredient equivalent) during four years of monitoring (IN-KE121 was not monitored). An unknown component (M3) more polar than lenacil or IN-KF313 was isolated but could not be identified by LC/MS. This unknown component was not the metabolite IN-KE121. Other polar components were also observed. MS analysis indicated that M1 would be a ring open structure with the loss of one nitrogen.
In the mean of the first year of monitoring, the two polar components (M1 and M2) and the less polar M3 exceeded 0.10 µg/L, while only the mean concentration of M1 (and M3 in one of the lysimeters with 0.104 µg/L) was found to be above 0.10 µg/L in the second year of monitoring. No individual component exceeded 0.10 µg/L in the third and fourth monitoring years.
The total radioactivity recovered in the soil was 13.17% (lysimeter 1/1) to 11.76% (lysimeter 1/2) of the applied radioactivity. Extractable residues in the upper three soil layers represent in total 0.33% AR and 0.31% AR, respectively, in lysimeters 1/2 and 2/2. These fractions could not be further investigated. The majority of radioactivity in the soil was found to be non-extractable (bound) residues (12.86% AR and 10.84% AR, respectively, in lysimeters 1/2 and 2/2).

Field soil dissipation studies
Field soil dissipation studies were provided from 4 sites in Europe (2 in Germany, 1 each in France and Spain) where spray applications of lenacil (one for each site) were made in June or July. Using the residue levels of parent lenacil determined over the top 10 cm (no residues were detected below 10 cm soil layer), single first order DT_{50} were

between 18-88 days. Small residues (< LOQ) of the major soil metabolite IN-KF 313 were detected only in a few cases in the top 10 cm layer, therefore no decline kinetics were calculated for this metabolite.

2.8.2 Summary of fate and behaviour in water and sediment [equivalent to section 11.1 of the CLH report template]

Water/sediment systems

A study describing the biodegradation of Lenacil in water/sediment system is available (Theis, 2002). The study was carried out with two independent water/sediment systems. The 1st test system was taken from a pond near 'Schaephysen' (Germany) and the 2nd system was taken from the Rückhaltebecken (Germany).

In both sediment types there was movement of Lenacil from the water to the sediment. Evolution of $^{14}CO_2$ was up to 3.8% AR in the Rückhaltebecken system after 120 days. In the Schaephysen system the $^{14}CO_2$ was slightly greater at 4.8% AR after 120 days. The level of bound residue was 16.5% and 10.6% AR after 120 days, respectively in the Rückhaltebecken system and the Schaephysen system.

Lenacil accounted for 49.8% AR and 46.4% AR in the whole system after 120 days, respectively in the Rückhaltebecken system and in the Schaephysen system.

Distribution of lenacil in water and sediment phases in both systems accounted for as following. In the Rückhaltebecken system, lenacil accounted for 92.8% AR at day 0 in the water phase, declining to 24.5% AR after 120 days. In the sediment phase, a maximum of 30.6% AR was accounted for after 58 days, and accounted for 25.2% AR at day 120. In the Schaephysen system, lenacil accounted for 90.6% AR at day 0 in the water phase, declining to 5.5% AR after 120 days. In the sediment phase, a maximum of 51.8% AR was accounted for after 30 days, and accounted for 41.9% AR at day 120.

In both systems there was only one significant metabolite which accounted for > 10% AR or > 5% AR in 2 successive samplings, M20.5 (5-oxo-Lenacil, also known as IN-KF313). IN-KF313 peaked in the sediment phase on day 88 or 120 reaching the maximum levels of 10.7% AR in the sediment phase of Schaephysen system. In the water phase, IN-KF313 reached the maximum of 7.5-7.8% AR during the study. The metabolite M15.0 which occurred at maximum 5.2% AR was partially identified as oxo-Lenacil. The terminal metabolite, CO_2, was a minimal sink in the material balance, accounting for only 3.8-4.8% AR in these systems by the study end. Residues not extracted from sediment accounted for 10.6-16.5% AR at study end. Lenacil degradation was minimal in the sterile water/sediment systems.

A kinetic evaluation in compliance with current FOCUS guidance (2006 and 2014) was conducted for this study (Pietsch, K., 2016).The result obtained gave Lenacil whole system DT_{50} values of 122.9 days in the Rückhaltebecken system and 93.8 days in the Schaephysen system. Corresponding DT_{90} values were 712.6 and 793.7 days. For the water compartment, the results obtained gave Lenacil water DT_{50} values of 32.3 days in the Rückhaltebecken system and 11.6 days in the Schaephysen system. Corresponding DT_{90} values were 241.4 and 77.9 days. Insufficient data were available to calculate separate degradation rates for the metabolites IN-KF313 and IN-KE121. A default value of 1000 days is considered as appropriate endpoint for both metabolites.

2.8.2.1 Rapid degradability of organic substances

Table 2.8.2.1-1: Summary of relevant information on degradation

Property	Method	Results*	Key or Supportive study[1]	Remarks	Reference
Stability					
Hydrolysis	SETAC, OPPTS 835.2110 (1998), EEC Directive 91/414/EEC as amended by Directive 95/36/EC GLP	pH 4: stable pH 7: stable pH 9: stable	acceptable	Purity > 97%	ACD 046/013764 Caldwell, E, 2002
Dissociation constant	See 1.3 Physico-chemical properties	See 1.3 Physico-chemical properties	-	See 1.3 Physico-chemical properties	See 1.3 Physico-chemical properties
Water Photolysis	SETAC, EEC Directive 91/414/EEC as	pH 5: stable	acceptable	Purity > 97%	ACD 047/022138 Millais, A., 2002

Property	Method	Results*	Key or Supportive study[1]	Remarks	Reference
	amended by Directive 95/36/EC GLP				
	U.S. EPA Pesticide Assessment Guidelines Subdivision N, 161-2 for conduct of aqueous photolysis studies GLP	pH 5: stable pH 7: stable pH 9: DT_{50} = 49 days, IN-KQ961 formed up to 13.8%	Supportive information	Purity > 98%	AMR 2431-92 Boucher, C.R., 1994
Soil photolysis	EEC Directive 91/414/EEC as amended by Directive 95/36/EC, SETAC (1995) GLP	DT_{50} = 67.6 days	Supportive information	Purity > 97%	ACD 041/023429 Millais, A., 2002
Biodegradation					
Ready biodegradability	EC Directive 92/69, C.4-C, 'Determination of Ready Biodegradability, CO2 Evolution Test' (formerly method C5 of EC Directive 84/449). OECD test guideline 301B, 'Ready Biodegradability, CO2 Evolution Test.' OPPTS Method 835.3110 (m), "Carbon Dioxide Evolution Test." (adopted January 1998). GLP	Not biodegradable according to the criteria of OECD 301 B	acceptable	Purity > 98.6%	ACD037/013644 Barnes, S.P., 2001
Water/sediment system	Richtlinen für die Prüfung von Pflanzenschutzmitteln im Zulassungsverfahren' part IV, 5-1, of the 'Biologische Bundesanstalt für Land- und Forstwirtschaft', Germany and 91/414/EWG GLP	Persistence endpoints at level PI: DT_{50} whole system = 93.8 days – 122.9 days	acceptable	Purity ≥ 98.5%	A&M 00-078 Theis, M., 2002
Aerobic soil degradation in laboratory conditions	Richtlinien für die Prüfung von Pflanzenschutzmitteln im Zulassungsverfahren' part IV, 4-1, of the 'Biologische Bundesanstalt für Land- und Forstwirtschaft', Germany and 91/414/EWG	DT_{50} = 11.9 days (persistence endpoint)	acceptable	Purity > 97%	A&M00-077 Theis, M., 2003

Property	Method	Results*	Key or Supportive study[1]	Remarks	Reference
	GLP				
	EEC Directive 91/414/EEC as amended by Directive 95/36/EC, SETAC 'Procedures for Assessing the Environmental Fate and Ecotoxicology of Pesticides', March 1995 GLP	DT_{50} = 10.4, 14.6, 10.0 and 20.9 days	acceptable	Purity > 97%	ACD 042/023664 Millais, A., 2003
	OECD 307 (2002) GLP	DT_{50} = 6.4, 13.3 and 5.3 (persistence endpoints)	acceptable	Purity > 98%	ACD 8247509 Hurst, L., 2012
Field soil dissipation	IVA guideline for residue trials (Beutel *et al.*, 1992), the BBA guidelines part IV, 4-1 (Schinkel *et al.*, 1986), the SETAC guideline (Lynch, 1995). GLP	DT_{50} = 25, 28, 18 and 88 days	Supportive information	Purity : VENZAR 80% WP product containing 816 g/kg lenacil	20011048/E1-FSD Pollmann, B., 2003

** data on full mineralization should be reported*

2.8.2.1.1 Ready biodegradability

The Assessment of Ready Biodegradability in a Modified Sturm Test (Barnes, 2001) has shown that Lenacil is not ready biodegradable since mean cumulative CO_2 production by mixtures containing lenacil technical was negligible and had achieved, at most, 2% of the theoretical value by the end of the test on Day 29.

2.8.2.1.2 BOD5/COD

No data available. A ready degradability study was submitted (Barnes, 2001) and demonstrates that lenacil is not ready biodegradable.

2.8.2.2 Other convincing scientific evidence

Not relevant.

2.8.2.2.1 Aquatic simulation tests

Please refer to 2.8.2.

2.8.2.2.2 Field investigations and monitoring data (if relevant for C&L)

Please refer to 2.8.1 and 2.8.4.

2.8.2.2.3 Inherent and enhanced ready biodegradability tests

No data available.

2.8.2.2.4 Soil and sediment degradation data

Stability
Lenacil is a weak acid with a pKa of 10.7. Hydrolysis and photolysis are of minor importance for its degradation in the environment.

Aerobic Soil degradation
The main degradation pathways in soil involved oxidation of the cyclopentapyrimidine moiety to IN-KF313 (3-cyclohexyl-6,7-dihydro-7-1H-cyclopentapyrimidine-2,4,5(3H)-trione) and oxidation of the cyclohexane moiety to IN-KE121 followed by oxidation of both degradates to carbon dioxide. Both metabolites were formed under aerobic conditions at levels >10% AR. Soil persistence endpoints (DT_{50}) values for lenacil under aerobic conditions were calculated to be 5.3-20.9 days (at 20°C and 40% MWHC or pF2 soil moisture content, n=8). After normalization of these values to FOCUS reference conditions (20°C and pF2 soil moisture content), the range became 5.6-15.4 days, with a geometric mean of 10.5 days.

Surface Water and Sediment
In a water sediment study, using Lenacil, IN-KF313 was the only major metabolite (>10% AR) detected reaching a maximum of 17.8% in the total system (water compartment maximum 7.8%). DT_{50} values of lenacil for the whole system were calculated to be 93.8–122.9 days.

As conclusion concerning the classification of the substance, the results of the ready biodegradability test and the results of the water/sediment study need to be checked for the compliance with the rapid degradability criteria of the CLP Regulation (Annex I pt. 4.1.2.9.). In the ready biodegradability test, CO_2 production by mixtures containing lenacil technical was negligible (at most, 2% of the theoretical value on Day 29). In the water/sediment study, lenacil remained at 49.3% AR in the water phase at day 30 in one of the water/sediment system. As conclusion, from these results, it can be concluded that lenacil is not rapidly degradable according to the CLP criteria.

2.8.2.2.5 Hydrolysis

The 'preliminary test' at 50°C (Caldwell, 2002) demonstrates that Lenacil is hydrolytically stable within the pH range of 4 to 9. No further tests are required and the hydrolytical DT_{50} at 25°C can be estimated to be greater than 1 year.

2.8.2.2.6 Photochemical degradation

Please refer to 2.8.1.

2.8.2.2.7 Other / Weight of evidence

Not relevant.

2.8.3 Summary of fate and behaviour in air

The low vapour pressure of 1.7×10^{-9} Pa at 25°C indicates little potential for volatilisation of the active substance and thus it would not be expected to be found in any significant concentration in the air. The Henry's law constant ($H = 1.3 \times 10^{-7}$ Pa $m^3.mol^{-1}$) calculated from the water solubility value of 3 mg/L and vapour pressure 1.7×10^{-9} Pa at 25 °C indicates that Lenacil is very slightly volatile from water.

The potential persistence of the compound in air has been calculated according to the models developed by Atkinson which estimate the atmospheric oxidative DT_{50} is 2.8 hours. Therefore Lenacil is not expected to be found in the atmosphere.

2.8.3.1 Hazardous to the ozone layer

Table 2.8.3-1: Summary table of studies on hazards to the ozone layer

Method	Results	Remarks	Reference
Data lacking			

2.8.3.1.1 Short summary and overall relevance of the provided information on hazards to the ozone layer

Data lacking.

2.8.3.1.2 Comparison with the CLP criteria

Data lacking.

2.8.3.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Data lacking.

2.8.4 Summary of monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products

Martinez *et al* (2000) analysed surface water and groundwater samples in 1998 from the Guarena and Almar river basins in Spain. No lenacil was found (detection limit <0.025 µg/L) in the 18 surface water and 23 groundwater samples analysed. Beernaerts *et al* (2003) carried out a 2 year (1998-1999) monitoring study of the Dyle river in Belgium which is representative of a large part of the country. River water samples were taken each month from 8 sites. Peak concentrations of lenacil were less than 2 µg/L immediately after application and declined to undetectable within the next few sampling occasions. The peak values may have been caused by point source contamination associated with the applications.
For the renewal of approval, the Notifier submitted an updated literature search. A few publications were found dealing with environmental monitoring of various pesticides in surface water (Poland, USA), groundwater and/or sediment (USA) mentioning also lenacil. These papers are discussed in the literature survey in Volume 3 B.8 CA Section B.8.6. Literature shows that lenacil was not detected in significant quantities in any of the groundwater and surface water sampling exercises. Lenacil was found only once at concentration <0.01 µg/L in surface water (USA) (Battaglin et al., 2011) in periods around occurrence of soybean rust, it was not detected in any other publication.

2.8.5 Definition of the residues in the environment requiring further assessment

Residue definition in soil for risk assessment: lenacil, IN-KF313 and IN-KE121.
Residue definition in ground water for risk assessment: lenacil, IN-KF313 and IN-KE121.
Residue definition in surface water for risk assessment: lenacil, IN-KF313 and IN-KE121
Residue definition in air for risk assessment: lenacil

2.8.6 Summary of exposure calculations and product assessment

The critical uses for the risk assessment of lenacil in this section are summarised in Table 2.8.6-1.

Table 2.8.6-1: Critical use pattern of lenacil 500 g/L SC

Crops	FOCUS Crop	Earliest representative application timing	Target BBCH growth stage	Application rate (g a.s./ha)	No. of applications (-)	Min. interval between applications (-)
Sugar and folder beets	Sugar beets	Emergence + 7 days	10-31	500	1	-
			10-31	125	4	7

The impact of formulants is limited to short-term effects such as formation of stable spray dispersions or to facilitate uptake by target organisms, while their influence on long-term processes, such as degradation and distribution is negligible. Therefore, for the purposes of this risk assessment it is assumed that formulants do not influence the fate and behaviour of the active substance in the environment and are not considered further.

The kinetic endpoints proposed for soil exposure assessment are given below in the Table 2.8.6-2. The degradation of lenacil and its metabolites is not pH-dependent.

Table 2.8.6-2: Kinetic endpoints for lenacil and its metabolites recommended to be used in the soil exposure assessment.

Type of assessment	Compound	Recommended kinetic endpoints		
		Soil DT_{50} [days]	Type of value[1]	Kinetic model
Soil exposure assessment (PEC_{SOIL})	Lenacil	20.9	Worst-case lab value	SFO
	IN-KF313	16.1	Worst-case lab value	SFO
	IN-KE121	18.3	Worst-case lab value	SFO
Groundwater exposure assessment (PEC_{GW})	Lenacil	10.5	Geomean of lab values (n = 8)	SFO
	IN-KF313	10.7	Geomean of lab values (n = 8)	SFO
	IN-KE121	7.6	Geomean of lab values (n = 8)	SFO
Surface water exposure assessment (PEC_{SW}/PEC_{SED})	Lenacil	10.5	Geomean of lab values (n = 8)	SFO
	IN-KF313	10.7	Geomean of lab values (n = 8)	SFO
	IN-KE121	7.6	Geomean of lab values (n = 8)	SFO

[1]DT_{50} values are normalized to 20°C and pF 2

For the metabolites, the maximum occurrence in soil and the formation fractions from parent recommended to be used in soil exposure assessment are given in Table 2.8.6-3.

Table 2.8.6-3: Maximum occurrence in soil and formation fractions from parent recommended for metabolites to be used in exposure assessment calculations.

Compound	Maximum occurrence in soil [%][1]	Formation fraction from parent [-][2]
IN-KF313	21.7	0.52
IN-KE121	13.9	0.36

[1]To be used in Soil and Surface Water exposure assessment
[2]To be used in Groundwater exposure assessment

Soil

PEC_{SOIL} calculations for lenacil were performed using worst-case DT_{50} values from the laboratory studies. As a worst-case approach a single application of 500 g a.s./ha (the total maximum use rate) considering an interception of 20% (corresponding to the recommended BBCH stages from FOCUS, 2014b[16]) was taken into account for the calculations. Key inputs and assumptions are summarized in Tables 2.8.6-2 and 2.8.6-3.

Due to DT_{50} values <100 days for lenacil and all its relevant soil metabolites only the maximum (initial) values are relevant. A plateau concentration expressed as background concentration resulting from long-term use was not calculated.

PEC_{SOIL} values in the top 5 cm of soil were calculated following a single application of 500 g a.s./ha to allow comparison with the appropriate ecotoxicological studies on soil-dwelling organisms, such as earthworms.

Initial PEC_S values

Active substance

[16] FOCUS (2014b): Generic Guidance for Tier 1 FOCUS Ground Water Assessments, version 2.2. May 2014. 66 pp.

A soil bulk density of 1.5 g/cm^3 and a soil depth of 5 cm was assumed for calculating $PEC_{s,ini}$. Using these assumptions, the concentration of a test substance in soil immediately after a single application is calculated as follows:

$$PEC_{s,ini} = \frac{(A-(A \times p))}{d \times bd \times 100} \quad \text{[Equation 1]}$$

Where:
- $PEC_{s,ini}$ = Predicted initial environmental concentration in soil after first appl. (mg/kg)
- A = Application rate (g/ha)
- p = Plant interception (fraction)
- d = Depth of soil layer (cm) (5 cm for $PEC_{s,ini}$; 20 cm for $PEC_{s,max.plateau}$)
- bd = Bulk density (g/cm^3)

Metabolites

The initial PEC_{SOIL} values for the metabolites IN-KF313 and IN-KE121 were generated by assuming the maximum conversion of lenacil to these metabolites that was observed in the aerobic metabolism or field soil dissipation studies using the following equation:

$$PEC_{s,ini,metabolite} = PEC_{s,ini} \times AR_{max} \times \left(MW_{metabolite}/MW_{parent}\right) \quad \text{[Equation 2]}$$

Where:
- $PEC_{s,ini,metabolite}$ = Initial PEC for the metabolite (mg/kg)
- $PEC_{s,ini}$ = Initial PEC for the parent (mg/kg)
- AR_{max} = Maximum percent radioactivity represented by the metabolite (fraction)
- $MW_{metabolite}$ = Molecular weight of metabolite (g/mol)
- MW_{parent} = Molecular weight of parent (g/mol)

The maximum levels of the degradation products IN-KF313 and IN-KE121 in laboratory soil degradation studies were found to be 21.7 and 13.9% of applied radiolabel, respectively.

Short and long term PECs values

The actual concentration at a day t is simulated as given in equation (3):

$$PEC_{s,actual,t} = PEC_{s,ini} \times e^{-kx} \quad \text{[Equation 3]}$$

Where:
- $PEC_{s,actual,t}$ = Actual predicted environmental concentration at time t after $PEC_{s,ini}$ for single appl. (mg/kg)
- k = Degradation rate (1/d)
- t = Time after $PEC_{s,ini}$ (d)

The time weighted averages are calculated as given in equation (4):

$$TWA_t = \frac{PEC_{s,ini} \times (1-e^{-kxt})}{k \times t} \quad \text{[Equation 4]}$$

Actual and time-weighted average PECs values for lenacil and its metabolites IN-KF313 and IN-KE121 are given in Table 2.8.6-4, Table 2.8.6-5 and Table 2.8.6-6 respectively.

Table 2.8.6-4: Predicted Environmental Concentrations of lenacil in soil (PEC_{SOIL}) after application of LENACIL 500 G/L SC (500 g a.s./ha) to sugar beets.

Time (days)	Lenacil	
	Actual PEC_{SOIL} (mg/kg)	TWA PEC_{SOIL} (mg/kg)
0	0.533	-
1	0.516	0.525
2	0.499	0.516
4	0.467	0.499
7	0.423	0.476
14	0.335	0.427
28	0.211	0.347
50	0.102	0.260
100	0.019	0.155

Table 2.8.6-5: Predicted Environmental Concentrations of the metabolite IN-KF313 in soil (PEC_{SOIL}) after application of LENACIL 500 G/L SC (500 g a.s./ha) to sugar beets.

Time (days)	IN-KF313	
	Actual PEC_{SOIL} (mg/kg)	TWA PEC_{SOIL} (mg/kg)
0	0.123	-
1	0.117	0.120
2	0.113	0.118
4	0.103	0.113
7	0.091	0.106
14	0.067	0.092
28	0.037	0.071
50	0.014	0.050
100	0.002	0.028

Table 2.8.6-6: Predicted Environmental Concentrations of the metabolite IN-KE121 in soil (PEC_{SOIL}) after application of LENACIL 500 G/L SC (500 g a.s./ha) to sugar beets.

Time (days)	IN-KE121	
	Actual PEC_{SOIL} (mg/kg)	TWA PEC_{SOIL} (mg/kg)
0	0.079	-
1	0.076	0.077
2	0.073	0.076
4	0.068	0.073
7	0.060	0.069
14	0.046	0.061
28	0.027	0.048
50	0.012	0.035
100	0.002	0.020

The initial PEC_{SOIL} values are summarized in Table 2.8.6-7.

Table 2.8.6-7: Maximum Predicted Environmental Concentration of the compounds in soil after application of 1L/ha LENACIL 500 G/L SC (20% interception)

Compound	Initial PEC_{SOIL} [mg/kg]
Lenacil	0.533
IN-KF313	0.123
IN-KE121	0.079

Groundwater

The predicted groundwater concentrations (PEC_{GW}) of lenacil and its soil major metabolites IN-KF313 and IN-KE121 were determined following recommendations of the FOCUS workgroup (FOCUS, 2000, 2014b, European Commission, 2014). The most current versions of the FOCUS models, i.e. FOCUS PEARL 4.4.4, FOCUS PELMO 5.5.3 and FOCUS MACRO 5.5.4, were used to generate PEC_{GW} values in order to assess potentially safe European use regions in the context of Annex I positive listing consideration. The FOCUS crop 'sugar beets' was chosen for the simulations. All scenarios which are parameterized for sugar beets were considered. Each scenario involves a fixed combination of crop, soil, and climatic parameters to represent the range of conditions across Europe. For FOCUS MACRO 5.5.4, only the Châteaudun scenario was considered.

Single and multiple applications as well as different application rates to sugar beets were simulated in consideration of the recommended use patterns and in order to cover the worst case application. The sorption behavior of the metabolites IN-KF313 and IN-KE121 proved to be pH dependent. Therefore calculations under acidic and alkaline conditions were carried out.

Substance parameters

Key input parameters for lenacil and its significant soil degradation products are summarized in the Table 2.8.6-8 and are further described in the modelling report (Núñez García-Cuerva, 2016).

Table 2.8.6-8: Key input parameters used in PEC_{GW} simulations for lenacil and its soil degradation products

Parameter	Compound	Reference

	Lenacil	IN-KF313	IN-KE121	
Molecular weight [g/mol]	234.3	248.3	248.3	
Water solubility (pH 7) [mg/L]	3.0 (20°C)	261.8 (25°C)	1020.0 (20°C)	
Vapour pressure (25°C) [Pa]	1.70×10^{-9}	1.51×10^{-7}	1.51×10^{-7}	
Half-life in soil [days]	10.5	10.7	7.6	Vol. 3 CA B.8.1.1.1.1. Geomean of lab values
Freunlich K_{FOC}/K_{FOM} [mL/g]	114.8/66.6	pH >7: 63.1/36.6 pH<7: 368.5/213.7	pH >7: 30.5/17.7 pH<7: 41.9/24.3	Vol. 3 CA B.8.1.2.1. Geometric means
Average Freundlich 1/n [-]	0.89	pH >7: 0.93 pH<7: 0.89	pH >7: 0.96 pH<7: 0.94	Vol. 3 CA B.8.1.2.1. Arithmetic means
Formation fraction (from parent)	-	0.52	0.36	Vol. 3 CA B.8.1.1.1.1. Arithmetic means
Plant uptake	0	0	0	Default value, worst case
Q_{10}	2.58	2.58	2.58	Default value

The PEC_{GW} calculations for lenacil and its degradation products were based on the geometric mean $DegT_{50}$ values determined from the laboratory degradation studies. The $DegT_{50}$ values from the laboratory studies were normalized for a temperature of 20 °C and a moisture content of pF = 2 before calculating the geometric mean value. Geometric mean organic carbon normalized sorption coefficients were taken forward where possible.

Calculation of concentrations in groundwater

The PEC_{GW} values were calculated for the active substance lenacil and its major soil metabolites IN-KE121 and IN-KF313. The polar soil degradation products which are partly identified are proposed as multicomponent, comprising upwards of 7 individual polar metabolites, each accounting for less than 5% of applied lenacil. The polar fractions (M1, M2 and M3) found above 0.1 µg/L in the lysimeter leachates were presumed to consist of a high number of polar individual compounds. Finally up to 33 polar sub-fractions are separated in the leachates of the microlysimeter study, suggesting a non-relevance of the polar fractions in leachates for exposure assessment. Therefore, the polar metabolites were not considered any further in the PEC_{GW} assessment.

Results obtained by FOCUS PEARL, FOCUS PELMO and FOCUS MACRO are summarized in Table 2.8.6-9, Table 2.8.6-10 and Table 2.8.6-11 respectively.

Table 2.8.6-9: The 80th percentile PEC_{GW} at 1 m depth for lenacil and its soil metabolites following application to sugar beets under alkaline (pH > 7) and acidic (pH ≤ 7) soil conditions with FOCUS PEARL 4.4.4

80th percentile PEC_{GW} (µg/L)			
Alkaline/Acidic conditions			
1 x 500 g a.s./ha			
Scenario	**Lenacil**	**IN-KF313**	**IN-KE121**
Châteaudun	<0.001	0.011 / <0.001	0.035 / 0.007
Hamburg	<0.001	0.005 / <0.001	0.030 / 0.005
Jokioinen	<0.001	0.001 / <0.001	0.024 / 0.001
Kremsmünster	<0.001	0.002 / <0.001	0.013 / 0.003
Okehampton	<0.001	0.006 / <0.001	0.021 / 0.004
Piacenza	<0.001	0.002 / <0.001	0.003 / 0.001
Porto	<0.001	0.001 / <0.001	0.002 / <0.001
Sevilla	<0.001	<0.001 / <0.001	0.024 / 0.002
Thiva	<0.001	<0.001 / <0.001	0.001 / <0.001
4 x 125 g a.s./ha			
Scenario	**Lenacil**	**IN-KF313**	**IN-KE121**
Châteaudun	<0.001	0.012 / <0.001	0.038 / 0.008
Hamburg	<0.001	0.005 / <0.001	0.039 / 0.007
Jokioinen	<0.001	0.001 / <0.001	0.032 / 0.002
Kremsmünster	<0.001	0.002 / <0.001	0.014 / 0.003
Okehampton	<0.001	0.006 / <0.001	0.024 / 0.005
Piacenza	<0.001	0.002 / <0.001	0.002 / <0.001
Porto	<0.001	<0.001 / <0.001	0.002 / <0.001
Sevilla	<0.001	<0.001 / <0.001	0.013 / 0.001
Thiva	<0.001	<0.001 / <0.001	0.001 / <0.001

Table 2.8.6-10: The 80th percentile PEC_{GW} at 1 m depth for lenacil and its soil metabolites following application to sugar beets under alkaline (pH > 7) and acidic (pH ≤ 7) soil conditions with FOCUS PELMO 5.5.3

80th percentile PEC_{GW} (µg/L)			
Alkaline / Acidic conditions			
1 x 500 g a.s./ha			
Scenario	**Lenacil**	**IN-KF313**	**IN-KE121**
Châteaudun	<0.001	0.003 / <0.001	0.010 / 0.002
Hamburg	<0.001	0.004 / <0.001	0.030 / 0.005
Jokioinen	<0.001	0.001 / <0.001	0.032 / 0.002
Kremsmünster	<0.001	0.003 / <0.001	0.017 / 0.003
Okehampton	<0.001	0.013 / <0.001	0.031 / 0.008
Piacenza	<0.001	0.006 / <0.001	0.007 / 0.002
Porto	<0.001	0.006 / <0.001	0.010 / 0.002
Sevilla	<0.001	0.001 / <0.001	0.087 / 0.010
Thiva	<0.001	<0.001 / <0.001	0.001 / <0.001
4 x 125 g a.s./ha			
Scenario	**Lenacil**	**IN-KF313**	**IN-KE121**
Châteaudun	<0.001	0.003 / <0.001	0.011 / 0.002
Hamburg	<0.001	0.005 / <0.001	0.037 / 0.007
Jokioinen	<0.001	0.001 / <0.001	0.041 / 0.003
Kremsmünster	<0.001	0.003 / <0.001	0.019 / 0.003
Okehampton	<0.001	0.013 / <0.001	0.035 / 0.010
Piacenza	<0.001	0.007 / <0.001	0.005 / 0.002
Porto	<0.001	0.004 / <0.001	0.009 / 0.002
Sevilla	<0.001	0.001 / <0.001	0.077 / 0.009
Thiva	<0.001	<0.001 / <0.001	0.001 / <0.001

Table 2.8.6-11: The 80th percentile PEC_{GW} at 1 m depth for lenacil and its soil metabolites following application to sugar beets under alkaline (pH > 7) and acidic (pH ≤ 7) soil conditions with FOCUS MACRO 5.5.4

80th percentile PEC_{GW} (µg/L)			
Alkaline / Acidic conditions			
1 x 500 g a.s./ha			
Scenario	**Lenacil**	**IN-KF313**	**IN-KE121**
Châteaudun	<0.001	0.005 / <0.001	0.009 / 0.003
4 x 125 g a.s./ha			
Scenario	**Lenacil**	**IN-KF313**	**IN-KE121**
Châteaudun	<0.001	0.007 / <0.001	0.012 / 0.004

The simulation runs resulted in concentrations below 0.1 µg/L for lenacil and its metabolites IN-KF313 and IN-KE121 in all scenario / model combinations.

Surface water and sediment

The predicted surface water and sediment concentrations (PEC_{SW} and PEC_{SED}) of lenacil and its major soil and aqueous degradates, IN-KF313 and IN-KE121, were generated in a stepwise approach. Step 1 and 2 calculations were performed for lenacil and its metabolites, while Step 3 and 4 calculations were performed for the parent substance lenacil only. All simulations at Steps 1-4 were conducted with the tools Steps 1-2 in FOCUS 3.2, FOCUS SWASH 5.3, FOCUS MACRO 5.5.4, FOCUS PRZM 4.3.1, FOCUS TOXSWA 4.4.3, and SWAN 4.0.1 and were based on the recommendations of EFSA and the FOCUS surface water workgroup.

Single and multiple applications as well as different application rates to sugar beets were simulated in consideration of the recommended use patterns and in order to cover the worst case application (Table 2.8.6-12). The sorption behavior of the metabolites IN-KF313 and IN-KE121 proved to be pH dependent. Therefore calculations under acidic and alkaline conditions were carried out.

The FOCUS crop 'sugar beets' was chosen for the simulations. All scenarios which are parameterized for sugar beets were considered.

Table 2.8.6-12: Overview of the simulated application regime of lenacil

Crop/Use	FOCUS crop	Number of applications	Application rate (g a.s./ha)	Interval (days)	BBCH
Sugar and fodder beet	Sugar beets	1	500	-	10-31
		4	125	7	

Substance parameters

For all substances, geometric mean aerobic soil laboratory half-lives corrected to standard conditions (20 °C and pF 2 moisture) were used to represent degradation in soil. Simulations for the degradation products also considered the maximum observed occurrence in soil. Geometric means of organic carbon normalized sorption coefficients (K_{FOC}) and arithmetic means of Freundlich sorption exponents (1/n) were taken forward. The sorption behavior of the metabolites IN-KF313 and IN-KE121 proved to be pH dependent, therefore alkaline and acid conditions were tested.

The geometric mean total system half-live from the water/sediment study was used to describe dissipation in water and sediment over time at Step 1-2 level for lenacil.

Due to the K_{FOC} of lenacil (2000 mL/g > K_{FOC} > ca 100 mL/g), two approaches need to be considered for Step 3 and Step 4 calculations as recommended by FOCUS (2006, 2014, 2015) assuming either phase (water or sediment) as the main compartment for degradation.

- Option 1: The geometric total system DT_{50} of 109.2 days was used to model degradation in the water phase and the default value of 1000 days was assigned to the sediment phase.

- Option 2: The DT_{50} in water was set to the default value of 1000 days while the geometric total system DT_{50} of 109.2 days was assigned to the sediment compartment.

For IN-KF313 and IN-KE121, DT_{50} values for water and sediment could not be determined. Hence, the FOCUS default of 1000 days was assigned to the water and the sediment phase.

Key substance input parameters for lenacil and its metabolites are summarized in Table 2.8.6-13.

Table 2.8.6-13: Chemical parameters for lenacil and metabolites

Parameter	Compound			Reference
	Lenacil	IN-KF313	IN-KE121	
Molecular weight [g/mol]	234.3	248.3	248.3	
Water solubility (pH 7) [mg/L]	3.0 (20°C)	261.8 (25°C)	1020.0 (20°C)	
Vapor pressure (25°C) [Pa]	1.7×10^{-9}	-	-	
Laboratory soil DT_{50} [days]	10.5	10.7	7.6	Vol. 3 CA B.8.1.1.1.1. Geomean of lab values
Freundlich K_{FOC} [mL/g]	114.8	pH>7: 61.3 pH<7: 368.5	pH>7: 30.5 pH<7: 41.9	Vol. 3 CA B.8.1.2.1. Geometric means
Average Freundlich 1/n	0.89	-	-	Vol. 3 CA B.8.1.2.1. Arithmetic means
Water-sediment system DT_{50} [days]	109.2 [a]	1000 [b]	1000 [b]	Vol. 3 CA B.8.2.2.3. (Pietsch, 2016)
Water phase DT_{50} [days]	Step 2: 109.2 [a] Step 3: 109.2 [a]/1000 *	1000 [b]	1000 [b]	Vol. 3 CA B.8.2.2.3. (Pietsch, 2016)
Sediment phase DT_{50} [days]	Step 2: 109.2 [a] Step 3: 1000/109.2 *[a]	1000 [b]	1000 [b]	Vol. 3 CA B.8.2.2.3. (Pietsch, 2016)
Maximum occurrence in soil [%]	-	21.7	13.9	Vol. 3 CA B.1.1.2.1.1.
Maximum occurrence in water/sediment [%]	-	17.8	5.2	Vol. 3 CA B.8.2.2.3. (Pietsch, 2016)
Plant uptake	0	-	-	Default value
Q_{10}	2.58	2.58	2.58	Default value
Wash-off	MACRO: 0.05 1/mm PRZM: 0.50 1/cm	-	-	Default values

*At step 3, as K_{FOC} of lenacil is between 100-2000 mL/g, two different calculations were performed:

- Option 1: DT_{50} water = 109.2 d; DT_{50} sed = 1000 d (default)
- Option 2: DT_{50} water = 1000 d (default); DT_{50} sed = 109.2 d

[a] Geometric mean, n = 2, total system
[b] Default value

Results
Step 1-4 calculations were performed for lenacil whereas for the metabolites only Steps1/2 simulations were required.

FOCUS Step 1 and 2 results for lenacil and its metabolites are summarized in Table B.2.8.6-14 through Table 2.8.6-16.
FOCUS Step 3 results for lenacil are shown in Table 2.8.6-17 through Table 2.8.6-22.

Using the FOCUS Step 3 tools, maximum PEC_{SW} and PEC_{SED} values at Step 3 are 3.672 µg/L and 1.111 µg/kg (sugar beets, 1 x 500 g a.s. /ha, R3 stream scenario) and 5.597 µg/L and 1.683 µg/kg (sugar beets, 4 x 125 g a.s. /ha, R3 stream scenario).

Further refinements were introduced at Step 4. Refinement was only conducted for scenarios with PEC_{SW} > 2.019 µg/L (RAC value proposed by the Notifier, different from the one proposed in the Ecotoxicological Section of the DRAR) and the main entry resulting from drift or runoff at Step 3. For 1 x 500 g a.s./ha the scenarios where drift was the main entry pathway a 5 m no-spray zone was introduced. Furthermore, a 10 m vegetated filter strip was simulated for run-off scenarios when it was needed. Regarding the application 4 x 125 g a.s./ha, a 20 m vegetated filter strip was simulated for run-off scenarios with PEC_{SW} > 2.019 µg/L at Step 3. Summary results can be found in Table 2.8.6-17 through Table 2.8.6-22.

In cases where drift is the main entry route, the introduction of a 5 m drift buffer reduces the maximum PEC_{SW} values to ≤ 0.905 µg/L (sugar beet, 1 x 500 g a.s./ha).

Considering the run-off scenarios, employing 10 m or 20 m vegetated filter strip reduces the maximum initial PEC_{SW} values to ≤ 1.812 µg/L (sugar beets, 1 x 500 g a.s./ha) or to ≤ 1.339 µg/L (sugar beets, 4 x 125 g a.s./ha), respectively. Additionally, the combination of 5 m drift buffer and 10 m vegetated filter strip reduces the maximum initial PEC_{SW} to ≤ 1.675 µg/L (sugar beets, 1 x 500 g a.s./ha).

Table 2.8.6-14: Step 1 calculations: Summary of maximum PEC_{SW} and PEC_{SED} values for lenacil and metabolites relevant to sugar beets

	Sugar beets, 1 x 500 g a.s./ha		Sugar beets, 1 x 125 g a.s./ha		Sugar beets, 4 x 125 g a.s./ha	
	PEC_{SW} (µg/L)	PEC_{SED} (µg/kg)	PEC_{SW} (µg/L)	PEC_{SED} (µg/kg)	PEC_{SW} (µg/L)	PEC_{SED} (µg/kg)
Lenacil	149.140	169.434	37.285	42.358	149.140	169.434
IN-KF313 (alkaline)	65.220	41.083	16.305	10.271	65.220	41.083
IN-KF313 (acidic)	47.649	174.413	11.912	43.603	47.649	174.413
IN-KE121 (alkaline)	32.671	9.955	8.168	2.489	32.671	9.955
IN-KE121 (acidic)	32.204	13.479	8.051	3.370	32.204	13.479

Table 2.8.6-15: Step 2 calculations: Summary of maximum PEC_{SW} and PEC_{SED} values for lenacil and metabolites relevant to sugar beets (1 x 500 g a.s./ha)

	March – May			
	North Europe		South Europe	
	PEC_{SW} (µg/L)	PEC_{SED} (µg/kg)	PEC_{SW} (µg/L)	PEC_{SED} (µg/kg)
Lenacil	21.828	24.694	39.587	44.953
IN-KF313 (alkaline)	8.748	5.503	16.676	10.502
IN-KF313 (acidic)	6.415	23.360	12.179	44.585
IN-KE121 (alkaline)	3.951	1.203	7.657	2.333
IN-KE121 (acidic)	3.896	1.629	7.547	3.158

Table 2.8.6-16: Step 2 calculations: Summary of maximum PEC_{SW} and PEC_{SED} values for lenacil and metabolites relevant to sugar beets (4 x 125 g a.s./ha)

	March – May							
	Single application				Multiple application			
	North Europe		South Europe		North Europe		South Europe	
	PEC_{SW} (µg/L)	PEC_{SED} (µg/kg)	PEC_{SW} (µg/L)	PEC_{SED} (µg/kg)	PEC_{SW} (µg/L)	PEC_{SED} (µg/kg)	PEC_{SW} (µg/L)	PEC_{SED} (µg/kg)
Lenacil	5.457	6.174	9.897	11.238	12.680	14.335	22.789	25.867
IN-KF313 (alkaline)	2.187	1.376	4.169	2.626	5.082	3.196	9.616	6.055
IN-KF313 (acidic)	1.604	5.840	3.045	11.146	3.732	13.567	7.028	25.704
IN-KE121 (alkaline)	0.988	0.301	1.914	0.583	2.063	0.628	3.960	1.206
IN-KE121 (acidic)	0.974	0.407	1.887	0.790	2.034	0.850	3.904	1.634

Table 2.8.6-17: Step 3 and step 4 calculations: Summary of maximum PEC_{SW} and PEC_{SED} values for lenacil relevant to sugar beets, 1 x 500 g a.s./ha, Option 1 [a]

Step	Scenario	Step 4 refinement	Maximum PEC_{SW} (µg/L)	7 days TWA PEC_{SW} (µg/L)	Max. PEC_{SW} caused by	Maximum PEC_{SED} (µg/kg)
3	D3 ditch		**2.624**	0.451	Drift	0.706
	D4 pond		0.111	0.105	Drift	0.251
	D4 stream		**2.143**	0.020	Drift	0.098
	R1 pond		0.174	0.167	Runoff	0.421
	R1 stream		**2.067**	0.177	Runoff	0.505
	R3 stream		**3.672**	0.519	Runoff	1.111
4	D3 ditch	5 m DB [b]	0.860	0.148	Drift	0.244
	D4 stream		0.905	0.011	Drift	0.053
4	R1 stream	10 m VFS [c]	**1.812**	0.080	Drift	0.243
	R3 stream	5 m DB [b] + 10 m VFS [c]	**1.675**	0.239	Runoff	0.521

[a] Two options regarding degradation in water and sediment were considered due to its K_{FOC} value at Step 3 and 4:
Option 1: DT_{50} in water = 109.2 d and DT_{50} in sediment = 1000 d
Option 2: DT_{50} in water = 1000 d and DT_{50} in sediment = 109.2 d.
[b] DB = Drift buffer
[c] VFS = Vegetated filter strip

Table 2.8.6-18: Step 3 and step 4 calculations: Summary of maximum PEC_{SW} and PEC_{SED} values for lenacil relevant to sugar beets, 1 x 500 g a.s./ha, Option 2 [a]

Step	Scenario	Step 4 refinement	Maximum PEC_{SW} (µg/L)	7 days TWA PEC_{SW} (µg/L)	Max. PEC_{SW} caused by	Maximum PEC_{SED} (µg/kg)
3	D3 ditch		**2.624**	0.452	Drift	0.706
	D4 pond		0.111	0.106	Drift	0.254
	D4 stream		**2.143**	0.020	Drift	0.097
	R1 pond		0.178	0.172	Runoff	0.426
	R1 stream		**2.067**	0.177	Runoff	0.503
	R3 stream		**3.672**	0.519	Runoff	1.111
4	D3 ditch	5 m DB [b]	0.860	0.148	Drift	0.245
	D4 stream		0.905	0.011	Drift	0.052
4	R1 stream	10 m VFS [c]	**1.812**	0.080	Drift	0.242
	R3 stream	5 m DB [b] + 10 m VFS [c]	**1.675**	0.239	Runoff	0.520

[a] Two options regarding degradation in water and sediment were considered due to its K_{FOC} value at Step 3 and 4:
Option 1: DT_{50} in water = 109.2 d and DT_{50} in sediment = 1000 d
Option 2: DT_{50} in water = 1000 d and DT_{50} in sediment = 109.2 d.
[b] DB = Drift buffer

[c] VFS = Vegetated filter strip

Table 2.8.6-19: Step 3 and step 4 calculations: Summary of maximum PEC_{SW} and PEC_{SED} values for lenacil relevant to sugar beets, 1 x 125 g a.s./ha, Option 1 [a]

Step	Scenario	Step 4 refinement	Maximum PEC_{SW} (µg/L)	7 days TWA PEC_{SW} (µg/L)	Max. PEC_{SW} caused by	Maximum PEC_{SED} (µg/kg)
3 [b]	D3 ditch	-	0.656	0.113	Drift	0.189
	D4 pond		0.027	0.026	Drift	0.066
	D4 stream		0.536	0.005	Drift	0.024
	R1 pond		0.047	0.045	Runoff	0.122
	R1 stream		0.591	0.052	Runoff	0.150
	R3 stream		0.963	0.136	Runoff	0.310

[a] Two options regarding degradation in water and sediment were considered due to its KFOC value at Step 3 and 4:
Option 1: DT_{50} in water = 109.2 d and DT_{50} in sediment = 1000 d
Option 2: DT_{50} in water = 1000 d and DT_{50} in sediment = 109.2 d.
[b] Safe at Step 3, Step 4 calculations were not needed

Table 2.8.6-20: Step 3 and step 4 calculations: Summary of maximum PEC_{SW} and PEC_{SED} values for lenacil relevant to sugar beets, 1 x 125 g a.s./ha, Option 2 [a]

Step	Scenario	Step 4 refinement	Maximum PEC_{SW} (µg/L)	7 days TWA PEC_{SW} (µg/L)	Max. PEC_{SW} caused by	Maximum PEC_{SED} (µg/kg)
3[b]	D3 ditch	-	0.656	0.113	Drift	0.189
	D4 pond		0.028	0.026	Drift	0.067
	D4 stream		0.536	0.005	Drift	0.024
	R1 pond		0.048	0.046	Runoff	0.123
	R1 stream		0.591	0.052	Runoff	0.149
	R3 stream		0.963	0.136	Runoff	0.309

[a] Two options regarding degradation in water and sediment were considered due to its KFOC value at Step 3 and 4:
Option 1: DT_{50} in water = 109.2 d and DT_{50} in sediment = 1000 d
Option 2: DT_{50} in water = 1000 d and DT_{50} in sediment = 109.2 d.
[b] Safe at Step 3, Step 4 calculations were not needed

Table 2.8.6-21: Step 3 and step 4 calculations: Summary of maximum PEC_{SW} and PEC_{SED} values for lenacil relevant to sugar beets, 4 x 125 g a.s./ha, Option 1 [a]

Step	Scenario	Step 4 refinement	Maximum PEC_{SW} (µg/L)	7 days TWA PEC_{SW} (µg/L)	Max. PEC_{SW} caused by	Maximum PEC_{SED} (µg/kg)
3	D3 ditch	-	0.441	0.080	Drift	0.192
	D4 pond		0.061	0.059	Drift	0.183
	D4 stream		0.373	0.008	Drift	0.045
	R1 pond		0.314	0.304	Drift	0.827
	R1 stream		**5.544**	0.500	Runoff	1.228
	R3 stream		**5.597**	0.813	Runoff	1.683
4	R1 stream	20 m VFS[b]	**1.315**	0.118	Drift	0.310
	R3 stream		**1.339**	0.213	Drift	0.436

[a] Two options regarding degradation in water and sediment were considered due to its K_{FOC} value at Step 3 and 4:
Option 1: DT_{50} in water = 109.2 d and DT_{50} in sediment = 1000 d
Option 2: DT_{50} in water = 1000 d and DT_{50} in sediment = 109.2 d.
[b] VFS = Vegetated filter strip

Table 2.8.6-22: Step 3 and step 4 calculations: Summary of maximum PEC_{SW} and PEC_{SED} values for lenacil relevant to sugar beets, 4 x 125 g a.s./ha, Option 2 [a]

Step	Scenario	Step 4 refinement	Maximum PEC_{SW} (µg/L)	7 days TWA PEC_{SW} (µg/L)	Max. PEC_{SW} caused by	Maximum PEC_{SED} (µg/kg)
3	D3 ditch	-	0.441	0.080	Drift	0.189
	D4 pond		0.065	0.063	Drift	0.185
	D4 stream		0.373	0.008	Drift	0.043

	R1 pond		0.337	0.327	Runoff	0.845
	R1 stream		**5.544**	0.500	Runoff	1.227
	R3 stream		**5.597**	0.813	Runoff	1.681
4	R1 stream	20 m VFS [b]	**1.315**	0.118	Drift	0.309
	R3 stream		**1.339**	0.213	Drift	0.435

[a] Two options regarding degradation in water and sediment were considered due to its K_{FOC} value at Step 3 and 4:
Option 1: DT_{50} in water = 109.2 d and DT_{50} in sediment = 1000 d
Option 2: DT_{50} in water = 1000 d and DT_{50} in sediment = 109.2 d.
[b] VFS = Vegetated filter strip

Modelling results for the metabolites IN-KF313 and IN-KE121 at Step1/2 were considered acceptable for subsequent ecotoxicological risk assessment. At Step 2 simulations, the maximum PEC_{SW} and PEC_{SED} for IN-KF313 are 16.676 µg/L and 44.585 µg/kg, respectively (1 x 500 g a.s./ha, South Europe, minimal crop cover) and for IN-KE121, 7.657 µg/L and 3.158 µg/kg, respectively (1 x 500 g a.s./ha, South Europe, minimal crop cover) were obtained.

According to the Ecotoxicology section a RAC value of 1.06 µg/L (cf. Vol.3 CA B.9, Section B.9.4.3.1) has to be considered in the risk assessment in surface water. This value differs from the one proposed by the Notifier (2.019 µg/L).

At Step 3, the overall maximum PEC_{SW} values of lenacil for different scenarios were still above the RAC. Maximum PEC_{SW} of 5.597 µg/L for the use pattern of 4 x 125 g a.s./ha (R3 scenario, stream, multiple application) and 3.672 µg/L for the use pattern of 1 x 500 g a.s./ha (R3 scenario, stream) were obtained, and further refinement was needed. These scenarios were simulated at Step 4.

All the other scenarios stay below the trigger of 1.06 µg/L at Step 3. Thus, despite of the change of RAC, Step 4 calculations for other scenarios are not required.

But at Step 4, the PEC_{SW} for some scenarios are still above the value of 1.06 µg/L (in bold in Table 2.8.6-16 through Table 2.8.6-22).

For the use pattern of 1 x 500 g a.s./ha, the PEC_{SW} values for the scenarios R1 stream (1.812 µg/L) and R3 stream (1.675 µg/L) remain above the RAC despite the use of 10 m VFS for R1 stream and a combination of 5m DB and 10m VFS for R3 stream.

For the use pattern of 4 x 125 g a.s./ha, the PEC_{SW} values for the scenarios R1 stream (1.315 µg/L) and R3 stream (1.339 µg/L) remain above the RAC despite the use of 20 m VFS for both scenarios.

The risk mitigation measures applied for the Step 4 calculations are not sufficient. A risk to the aquatic organisms cannot be excluded.

Air

The low vapor pressure and Henry's law constant of the active substance lenacil indicate a low potential for volatilization from soil under practical conditions of use. The vapor pressure of lenacil is 1.7×10^{-9} Pa at 25°C. Furthermore, the Henry's law constant of lenacil is 1.3×10^{-7} Pa m^3/mol suggesting little potential for volatilization in the environment.

The calculation of the second-order rate constant and associated half-life for the reaction of the active substance in the gas phase in the troposphere was made using the method of Atkinson and resulted in a DT_{50} of 2.8 hours. Given that lenacil is non-volatile by virtue of its very low vapor pressure and calculated Henry's Law constant, only negligible quantities would be transferred to the troposphere. These negligible quantities would then undergo rapid photochemical oxidative degradation, and therefore, lenacil does not have the potential for long range transport in air.

The type of formulation and inert substances used in Lenacil 500 g/L SC are not expected to affect the volatilization of the active substance. Therefore, the fate and behavior of Lenacil 500 g/L SC in air was not specifically tested.

Other routes of exposure

No other routes of exposure are expected after application of Lenacil 500 g/L SC and, thus no additional estimations of concentrations are required.

2.9 EFFECTS ON NON-TARGET SPECIES

2.9.1 Summary of effects on birds and other terrestrial vertebrates

2.9.1.1 Birds

Avian acute oral, short-term dietary and long-term reproduction studies have been carried out with lenacil. The toxicity endpoints for lenacil are summarized in Table 2.9.1.1-1.

Table 2.9.1.1-1: Summary of endpoints for birds for the active substance lenacil

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Mallard duck (*Anas platyrhynchos)*	Lenacil technical	Single dose (Acute oral toxicity)	LD_{50}	**> 2000 mg a.s./kg bw**	CA8.1.1.1/01 [redacted] 2002a
Bobwhite quail (*Colinus virginianus*)	Lenacil technical	Single dose (Acute oral toxicity)	LD_{50}	**> 2000 mg a.s./kg bw**	CA8.1.1.1/02 [redacted] 2002b
Bobwhite quail (*Colinus virginianus*)	Lenacil technical	5 days (Short-term dietary toxicity)	LC_{50}	> 5000 mg a.s./kg diet (> 1088 mg a.s./kg bw/d)	CA8.1.1.2/01 [redacted] 2004a
Bobwhite quail (*Colinus virginianus*)	Lenacil technical	21 weeks (Sub chronic and reproduction)	NOEL EC_{10} EC_{20}	1024 mg a.s./kg diet **(= 100.4 mg a.s./kg bw/d)** NE NE	CA8.1.1.3/01 [redacted] *et al.*, 1996

*Note: ND – could not be determined; NE – not estimated; **bold** – endpoint used for the current risk assessment*

2.9.1.2 Mammals

Mammalian acute oral, short-term dietary and long-term reproduction studies have been carried out with lenacil. The available acute and reprotoxicity endpoints, considered relevant for the ecotoxicological risk assessment, are summarized in Table 2.9.1.2-1 and Table 2.9.1.2-2, respectively.

Table 2.9.1.2-1: Summary of acute oral toxicity endpoints for mammals.

Organism	Test substance	Timescale (Test type)	Vehicle	Endpoint	Toxicity value	Reference
Rat	Lenacil technical	Single dose by gavage (Acute oral)	Formulated in 1% w/v aqueous methyl-cellulose	LD_{50}	**> 5000 mg a.s./kg bw**	[redacted] 2001
Rat	Lenacil 500 g/L SC	Single dose by gavage (Acute oral)	water	LD_{50}	**> 2000 mg form/kg bw**	[redacted] 2005

Note: endpoints in bold are considered in the risk assessment

Table 2.9.1.2-2: Summary of short-term dietary and long-term reproduction endpoints for mammals.

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Rat	Lenacil technical	28-day dynamic exposure	NOAEL	571 mg a.s./kg bw/day	[redacted] 2002a
Dog	Lenacil technical	28-day dynamic exposure	NOAEL	219 mg a.s./kg bw/day	[redacted] 2001
Rat	Lenacil technical	90-day dynamic exposure	NOAEL	41 mg a.s./kg bw/day	[redacted] 2002b [redacted] 2004
Mouse	Lenacil technical	90-day dynamic exposure	NOAEL	157 mg a.s./kg bw/day	[redacted] 1991
Dog	Lenacil technical	90-day dynamic exposure	NOAEL	44 mg a.s./kg bw/day	[redacted] 2002
Rat	Lenacil technical	2-generation reproduction pilot study	NOAEL[1]	1952 mg a.s./kg bw/day	[redacted] 2002
Rat	Lenacil technical	2-generation reproduction main study	NOAEL[1] NOAEL[2]	1952 mg a.s./kg bw/day **82 mg a.s./kg bw/day**	[redacted] 2003a
Rat	Lenacil technical	Developmental toxicity pilot study	NOAEL[1]	1000 mg a.s./kg bw/day	[redacted] 2003b
Rat	Lenacil technical	Developmental toxicity main study	NOAEL[1]	100 mg a.s./kg bw/day	[redacted] 2003c
Rabbit	Lenacil technical	Developmental toxicity main study	NOAEL[1]	4000 mg a.s./kg bw/day	[redacted] 1991

Note: endpoints in bold are considered in the risk assessment; [1] endpoint for reproduction parameters only (offspring and reproductive for 2-generation reproductive toxicity study and developmental for developmental toxicity study); [2] endpoint for maternal toxicity

Following the EFSA Guidance Document (2009), all available short-term dietary and long-term reproduction studies were considered to determine the ecotoxicologically relevant endpoint for **reproductive/developmental toxicity** to mammals. Based on an overall assessment of the ecotoxicologically relevant effects in the available studies, the NOAEL of 82 mg a.s./kg bw/day, obtained from the 2-generation studies with rats, was considered as the ecotoxicologically relevant endpoint for use in the long-term risk assessment for mammals.

2.9.2 Summary of effects on aquatic organisms [section 11.5 of the CLH report]

Aquatic toxicity studies were conducted with fish, aquatic invertebrates, algae and aquatic plants for either lenacil or the representative formulation Lenacil 500 g/L SC. A summary of the toxicity endpoints is shown in Table 2.9.2-1.

Studies with IN-KE 121 and IN-KF 313, the major metabolites of lenacil in surface water, have also been conducted. The endpoints for these metabolites are summarized in Table 2.9.2-2.

Further information on the available studies and a discussion of the results in relation to the CLP classification is provided in the sections below.

Table 2.9.2-1: Summary of aquatic toxicity data on lenacil, tested as technical active substance or in the representative formulation Lenacil 500 g/L SC.

Species	Test substance	Timescale (test type)	Endpoint	Toxicity value[M]	Reference
Fish					
Common carp (*Cyprinus carpio*)	lenacil technical	96 hours, acute (semi-static)	LC_{50}	> 3100 µg a.s./L	CA8.2.1/01 [redacted] 2003a
Rainbow trout (*Oncorhynchus mykiss*)	lenacil technical	96 hours, acute (static)	LC_{50}	**> 2000 µg a.s./L**	CA8.2.1/02 [redacted] 1991a
Fathead minnow (*Pimephales promelas*)	lenacil technical	96 hours, acute (static)	LC_{50}	> 2000 µg a.s./L	CA8.2.1/03 [redacted] 1991b
Rainbow trout (*Oncorhynchus mykiss*)	lenacil technical	21 days, chronic (flow-through)	NOEC EC_{20} EC_{10}	2300 µg a.s./L ND ND	CA8.2.2.1/01 [redacted] 1991c
Rainbow trout (*Oncorhynchus mykiss*)	lenacil technical	90 days, chronic (flow-through)	NOEC EC_{20} EC_{10}	**160 µg a.s./L** ND ND	CA8.2.2.1/02 [redacted] 1996
Aquatic invertebrates					
Water flea (*Daphnia magna*)	lenacil technical	48 hours, acute (static)	EC_{50}	> 8400 µg a.s./L	CA8.2.4.1/01 Hutton D.G., 1989a
Water flea (*Daphnia magna*)	lenacil technical	48 hours, acute (static)	EC_{50}	**> 3110 µg a.s./L**	CA8.2.4.1/02 Renner P., 2016a
Water flea (*Daphnia magna*)	lenacil technical	21 days, chronic (semi-static)	NOEC EC_{20} EC_{10}	19 µg a.s./L NE **17 µg a.s./L**	CA8.2.5.1/02 Renner P., 2016b
Algae					
Green microalgae (*Pseudo-kirchneriella subcapitata*)	lenacil technical	96 hours, chronic (static)	96h E_bC_{50} 96h E_bC_{20} 96h E_bC_{10} 96h ErC_{50} 96h E_rC_{20} 96h E_rC_{10} NOEC	6.5 µg a.s./L 4.32 µg a.s./L 3.45 µg a.s./L 15 µg a.s./L 9.02 µg a.s./L 5.87 µg a.s./L 3.4 µg a.s./L	CA8.2.6.1/01 Flatman D., 2003c

Species	Test substance	Timescale (test type)	Endpoint	Toxicity value[M]	Reference
Green microalgae (*Pseudo-kirchneriella subcapitata*)	Lenacil 500 g/L SC	72 hours, chronic (static)	72h E_bC_{50}	7.12 µg form./L [N] (= 2.97 µg a.s./L)	CP10.2.1/01 Pawlowski S. and Wydra V., 2006
			72h E_bC_{20}	NE	
			72h E_bC_{10}	3.21 µg form./L [N] (= 1.34 µg a.s./L)	
			$NOEC_b$	1.00 µg form./L [N] (= 0.42 µg a.s./L)	
			72h E_rC_{50}	**22.04 µg form./L [N] (9.18 µg a.s./L)**	
			72h E_rC_{20}	NE	
			72h E_rC_{10}	6.91 µg form./L [N] (= 2.88 µg a.s./L)	
			$NOEC_r$	3.10 µg form./L [N] (= 1.29 µg a.s./L)	
Fresh water diatom (*Naviculla pelliculosa*)	lenacil technical	72 hours, chronic (static)	72h E_bC_{50}	36 µg a.s./L	CA8.2.6.2/01 Flatman D., 2003b
			72h E_bC_{20}	16.5 µg a.s./L	
			72h E_bC_{10}	13.1 µg a.s./L	
			72h E_rC_{50}	96 µg a.s./L	
			72h E_rC_{20}	31.3 µg a.s./L	
			72h E_rC_{10}	20.4 µg a.s./L	
			NOEC	11 µg a.s./L	
unicellular freshwater green algae (*Ankistrodesmus falcatus*)	lenacil technical	72 hours, chronic (static)	72h E_yC_{50}	8.56 µg a.s./L[I]	CA8.2.6.2/02 Wenzel A., 2014a
			72h E_yC_{20}	5.68 µg a.s./L[I]	
			72h E_yC_{10}	4.59 µg a.s./L[I]	
			72h E_rC_{50}	**13.3 µg a.s./L[I]**	
			72h E_rC_{20}	8.62 µg a.s./L[I]	
			72h E_rC_{10}	6.85 µg a.s./L[I]	
			NOEC	2.59 µg a.s./L[I]	

Species	Test substance	Timescale (test type)	Endpoint	Toxicity value[M]	Reference
unicellular cyanobacteria (*Synechococcus leopoliensis*)	lenacil technical	72 hours, chronic (static)	72h E_yC_{50} 72h E_yC_{20} 72h E_yC_{10} 72h E_rC_{50} 72h E_rC_{20} 72h E_rC_{10} NOEC	66.5 µg a.s./L[I] 42.0 µg a.s./L[I] 33.1 µg a.s./L[I] 209 µg a.s./L[I] 69.7 µg a.s./L[I] 39.3 µg a.s./L[I] 29.6 µg a.s./L[I]	CA8.2.6.2/03 Wenzel A., 2014b
filamentous cyanobacteria (*Anabaena flos-aquae*)	lenacil technical	72 hours, chronic (static)	72h E_yC_{50} 72h E_yC_{20} 72h E_yC_{10} 72h E_rC_{50} 72h E_rC_{20} 72h E_rC_{10} NOEC	345 µg a.s./L[I] 150 µg a.s./L[I] 97.0 µg a.s./L[I] > 643 µg a.s./L[I] 325 µg a.s./L[I] 186 µg a.s./L[I] 113 µg a.s./L[I]	CA8.2.6.2/04 Wenzel A., 2014c
unicellular alga (*Closterium cornu*)	lenacil technical	96 hours, chronic (static)	72h E_yC_{50} 72h E_yC_{20} 72h E_yC_{10} $NOEC_y$ 72h E_rC_{50} 72h E_rC_{20} 72h E_rC_{10} $NOEC_r$	35.3 µg a.s./L 20.0 µg a.s./L 14.9 µg a.s./L 7.34 µg a.s./L 70.6 µg a.s./L 35.3 µg a.s./L 24.6 µg a.s./L 21.6 µg a.s./L	CA8.2.6.2/05 Wenzel A., 2014d
unicellular yellow-green (*Xanthonema debile*)	lenacil technical	72 hours, chronic (static)	72h E_yC_{50} 72h E_yC_{20} 72h E_yC_{10} 72h E_rC_{50} 72h E_rC_{20} 72h E_rC_{10} NOEC	29.8 µg a.s./L[I] 15.6 µg a.s./L[I] 11.2 µg a.s./L[I] 132 µg a.s./L[I] 19.2 µg a.s./L[I] 7.03 µg a.s./L[I] 10.9 µg a.s./L[I]	CA8.2.6.2/06 Wenzel A., 2014e

Species	Test substance	Timescale (test type)	Endpoint	Toxicity value[M]	Reference
Aquatic plants					
Lemna gibba	lenacil technical	7 days, chronic (semi-static)	E_bC_{50} E_bC_{20} E_bC_{10} E_rC_{50} E_rC_{20} E_rC_{10} NOEC	19.2 µg a.s./L 10.6 µg a.s./L 7.5 µg a.s./L 28.6 µg a.s./L 15.9 µg a.s./L 11.3 µg a.s./L 8.8 µg a.s./L	CA8.2.7/01 Flatman D., 2003d
Lemna gibba	Lenacil 500 g/L SC	7 days, chronic (semi-static)	72h E_yC_{50} 72h E_yC_{20} 72h E_yC_{10} 72h E_rC_{50} 72h E_rC_{20} 72h E_rC_{10} NOEC	18 µg form./L[N] (= 7.5 µg a.s./L) ND ND **48 µg form./L[N] (= 20.0 µg a.s./L)** NE 12 µg form./L[N] (= 5.0 µg a.s./L) 3 µg form./L[N] (= 1.2 µg a.s./L)	CP10.2.1/02 Pawlowski S., 2006
Chara globularis	lenacil technical	7 days, chronic (static)	E_yC_{50} E_yC_{20} E_yC_{10} E_rC_{50} E_rC_{20} E_rC_{10} NOEC	13.54 µg a.s./L 11.29 µg a.s./L 10.46 µg a.s./L 13.57 µg a.s./L 11.31 µg a.s./L 10.46 µg a.s./L 7.53 µg a.s./L	CA8.2.7/02 Wenzel A., 2012a

Species	Test substance	Timescale (test type)	Endpoint	Toxicity value[M]	Reference
Elodea canadensis	lenacil technical	7 days, chronic (static)	E_yC_{50}	3.78 µg a.s./L[I]	CA8.2.7/03 Wenzel A., 2012b
			E_yC_{20}	3.59 µg a.s./L[I]	
			E_yC_{10}	3.49 µg a.s./L[I]	
			E_rC_{50}	**3.78 µg a.s./L[I]**	
			E_rC_{20}	3.59 µg a.s./L[I]	
			E_rC_{10}	3.49 µg a.s./L[I]	
			NOEC	**3.18 µg a.s./L[I]**	

Notes: ***bold*** *– values used for risk assessment*
M – based on mean measured concentrations unless otherwise stated
N – based on nominal concentration(s)
I – based on initial measured concentration(s)
ND – could not be determined
NE – not estimated

Table 2.9.2-2: Summary of aquatic toxicity data on the major metabolites of lenacil (IN-KE121 and IN-KF313).

Species	Test substance	Timescale (test type)	Endpoint	Toxicity value M	Reference
Algae					
Green microalgae *(Pseudo-kirchneriella subcapitata)*	IK-KE121	72 hours, chronic (static)	72h E_bC_{50}	10.7 mg/L	CA 8.2.6.1/03 Jenkins C.A, 2004a
			72h E_bC_{20}	7.48 mg/L	
			72h E_bC_{10}	5.99 mg/L	
			$NOEC_b$	1.36 mg/L	
			72h ErC_{50}	27.8 mg/L	
			72h E_rC_{20}	14.77 mg/L	
			72h E_rC_{10}	8.81 mg/L	
			$NOEC_r$	4.26 mg/L	
Green microalgae *(Pseudo-kirchneriella subcapitata)*	IN-KF313	72 hours, chronic (static)	72h E_bC_{50}	2.10 mg/L	CA 8.2.6.1/04 Jenkins C.A, 2004b
			72h E_bC_{20}	1.44 mg/L	
			72h E_bC_{10}	1.13 mg/L	
			$NOEC_b$	0.601 mg/L	
			72h ErC_{50}	4.27 mg/L	
			72h E_rC_{20}	2.54 mg/L	
			72h E_rC_{10}	1.80 mg/L	
			$NOEC_r$	1.26 mg/L	

Notes: ***bold*** *– values used for risk assessment*
M – based on mean measured concentrations unless otherwise stated
N – based on nominal concentration(s)

2.9.2.1 Bioaccumulation [equivalent to section 11.4 of the CLH report template]

The estimation of bioaccumulation potential in fish is based on the partition coefficient n-octanol/water (log P_{OW}) of the active substance. In the section on physico-chemical properties different values for the log P_{OW} pending on the pH were measured.

The log P_{OW} values are then compared with the threshold values for bioaccumulation (threshold CLP ≥ 4). The log P_{OW} of lenacil is lower than both threshold values. Therefore, no experimental bioaccumulation data are required. The potential risk for bioaccumulation in tissues of aquatic organisms is low.

Table 2.9.2.1-1 Summary of relevant information on bioaccumulation

Method	Species	Results	Key or Supportive study	Remarks	Reference
EEC-method A8 GLP (Partition coefficient n-octanol/water)	-	pH 4: Log Pow = 1.70 pH 7: Log Pow = 1.70 pH 9 : Log Pow = 1.25	Key study	99 % pure. All at 25°C	ACD 025/014039 Comb, A.L. 2002a

2.9.2.1.1 Estimated bioaccumulation

No data available and not required (see 2.9.2.1).

2.9.2.1.2 Measured partition coefficient and bioaccumulation test data

No data available and not required (see 2.9.2.1).

2.9.2.2 Acute aquatic hazard [equivalent to section 11.5 of the CLH report template]

The relevant studies on the acute aquatic toxicity of lenacil are shown in the table below. This table contains the the information included in the previous CLH report for lenacil (Dossier Submitted by Belgium in 2011), and was updated with the studies that were newly submitted for the current Annexs I renewal application for lenacil.

Table 2.9.2.2-1 Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Key or Supportive study	Remarks	Reference
acute fish study based on OECD 203 and US EPA 72-1 GLP	*Oncorrhynchus mykiss*	lenacil, purity: 98.2%, batch n°: 9038	**LC_{50}> 2.0 mg a.s./L** (mean measured)	Acceptable. Key study	96 h static fingerlings 10 fish/replicate 1 replicate/treatment	CA8.2.1/ 02 [redacted] 1991a
acute fish study based on OECD 203 and US EPA 72-1 GLP	*Pimephales promelas*	lenacil, purity: 98.2%, batch n°: 9038	**LC_{50}> 2.0 mg a.s./L** (mean measured)	Acceptable. Key study	96 h static juveniles 10 fish/replicate 1 replicate/treatment	CA8.2.1/ 03 [redacted] 1991b
acute fish study based on OECD 203, 92/69/EEC method C.1 and draft US	*Cyprinus carpio*	lenacil, purity: 98.6%, batch n°: 141712003	LC_{50}> 3.1 mg a.s./L (mean measured)	Acceptable. Supportive study	96 h semi-static mean weight: 1.26 g mean standard length: 4.3 cm 10 fish/replicate 3 replicates/treatment	CA8.2.1/ 01 [redacted] 2003a

EPA OPPTS 850.1075 GLP						
acute daphnia study based on OECD 202 and US EPA 72-2 GLP	*Daphnia magna*	lenacil, purity: 95.1%, blended batch n°s: 8802 and 8805	EC_{50}> 8.4 mg a.s./L (measured after 48 h)	Acceptable. Supportive study	48 h static 5 daphnids /replicate 4 replicates /treatment	CA8.2.4.1/ 01 Hutton D.G., 1989a
acute daphnia study based on OECD 202 GLP	*Daphnia magna*	lenacil, purity: 98.82%, batch n°: JUL14HE 010	**EC_{50}> 3.11 mg a.s./L** (mean measured)	Acceptable. Key study	48 h static 5 daphnids /replicate 4 replicates /treatment	CA8.2.4.1/ 02 Renner P., 2016a
algal growth inhibition study based on OECD 201, 92/69/EEC method C.3 and draft US EPA OPPTS 850.5400 GLP	*Pseudokircherniella subcapitata*	lenacil, purity: 98.6%, batch n°: 141712003	E_bC_{50} (72 h) = 0.0077 mg a.s./L E_bC_{50} (96 h) = 0.0065 mg a.s./L E_rC_{50} (72 h) = 0.016 mg a.s./L E_rC_{50} (96 h) = 0.015 mg a.s./L NOEC (96 h) = 0.0034 mg a.s./L (mean measured)	Acceptable. Supportive study	96 h static initial cell count: 1 x 10^4/mL 6 replicates for control and solvent control 3 replicates /treatment	CA8.2.6.1/ 01 Flatman D., 2003c
algal growth inhibition study based on OECD 201 and 92/69/EEC method C.3 GLP	*Navicula pelliculosa*	lenacil, purity: 98.6%, batch n°: 141712003	E_bC_{50} = 0.036 mg a.s./L E_rC_{50} = 0.096 mg a.s./L NOEC = 0.011 mg a.s./L (mean measured)	Acceptable. Supportive study	72 h static initial cell count: 1 x 10^4/mL 6 replicates for control 3 replicates/treatment	CA8.2.6.2/ 01 Flatman D., 2003b
algal growth inhibition study based on OECD 201 GLP	*Ankistrodesmus falcatus*	lenacil, purity: 99.3%, batch n°: 0190813	E_yC_{50} = 0.00856 mg a.s./L **E_rC_{50} = 0.0133 mg a.s./L NOEC = 0.00259 mg a.s./L** (initial measured)	Acceptable. Key study	72 h static initial cell count: 2500/mL 8 replicates for control and solvent control 4 replicates /treatment	CA8.2.6.2/ 02 Wenzel A., 2014a

algal growth inhibition study based on OECD 201 GLP	*Synechococcus leopoliensis*	lenacil, purity: 99.3%, batch n°: 0190813	E_yC_{50} = 0.0665 mg a.s./L E_rC_{50} = 0.209 mg a.s./L NOEC = 0.0296 mg a.s./L (initial measured)	Acceptable. Supportive study	72 h static initial cell count: 1 x 10^4/mL 6 replicates for control and solvent control 4 replicates /treatment	CA8.2.6.2/ 03 Wenzel A., 2014b
algal growth inhibition study based on OECD 201 GLP	*Anabaena flos-aquae*	lenacil, purity: 99.3%, batch n°: 0190813	E_yC_{50} = 0.345 mg a.s./L E_rC_{50} > 0.643 mg a.s./L NOEC = 0.113 mg a.s./L (initial measured)	Acceptable. Supportive study	72 h static initial cell count: 1 x 10^4/mL 6 replicates for control and solvent control 4 replicates /treatment	CA8.2.6.2/ 04 Wenzel A., 2014c
algal growth inhibition study based on OECD 201 GLP	*Closterium cornu*	lenacil, purity: 99.3%, batch n°: 0190813	E_yC_{50} = 0.0353 mg a.s./L E_rC_{50} = 0.0706 mg a.s./L NOEC = 0.0216 mg a.s./L (initial measured)	Acceptable. Supportive study	72 h static initial cell count: 2500/mL 8 replicates for control and solvent control 4 replicates /treatment	CA8.2.6.2/ 05 Wenzel A., 2014d
algal growth inhibition study based on OECD 201 GLP	*Xanthonema debile*	lenacil, purity: 99.3%, batch n°: 0190813	E_yC_{50} = 0.0298 mg a.s./L E_rC_{50} = 0.132 mg a.s./L NOEC = 0.0109 mg a.s./L (initial measured)	Acceptable. Supportive study	72 h static initial cell count: 1 x 10^4/mL 8 replicates for control and solvent control 4 replicates /treatment	CA8.2.6.2/ 06 Wenzel A., 2014e
Lemna growth inhibition study based on OECD draft and US EPA draft OPPTS 850.4400 GLP	*Lemna gibba*	lenacil, purity: 98.6%, batch n°: 141712003	E_bC_{50} = 0.019 mg a.s./L E_rC_{50} = 0.029 mg a.s./L NOEC = 0.0088 mg a.s./L (mean measured)	Acceptable. Supportive study	7 d semi-static inoculation with 4 plants bearing 3 fronds 3 replicates for control, solvent control and per treatment	CA8.2.7/ 01 Flatman D., 2003d
Maltby *et al.* (2010) (draft version of	*Chara globularis*	lenacil, purity: 99.1%,	E_yC_{50} = 0.01354 mg a.s./L	Acceptable. Supportive study	7 d static A single pot containing 5 plants in each test vessel	CA8.2.7/ 02 Wenzel A., 2012a

OECD 239)		batch n°: 200010003	E_rC_{50} = 0.01357 mg a.s./L NOEC = 0.00753 mg a.s./L (mean measured)		6 replicates for control 3 replicates /treatment	
Maltby *et al.* (2010) (draft version of OECD 239)	*Elodea canadensis*	lenacil, purity: 99.1%, batch n°: 200010003	E_vC_{50} = 0.00378 mg a.s./L **E_rC_{50} = 0.00378 mg a.s./L NOEC = 0.00318 mg a.s./L** (mean measured)	Acceptable. Key study	7 d static A single pot containing 5 plants in each test vessel 6 replicates for control 3 replicates /treatment	CA8.2.7/ 03 Wenzel A., 2012b

2.9.2.2.1 Acute (short-term) toxicity to fish

The information below was extracted from Volume 3 (CA), Section B.9.2 'Effect on aquatic organisms'. Three acute (short-term) toxicity studies with fish are available for lenacil.

Report:	**CA8.2.1/01. [redacted] (2003a)** **Lenacil technical acute toxicity to fish (*Cyprinus carpio*).**

Report No.: ACD 035/022512

Guidelines: EC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC, Part C, Method 1 "Acute Toxicity for Fish"
OECD Guideline 203: Fish, Acute Toxicity Test
US EPA (OPPTS) Method 850.1075 (Fish, Acute Toxicity Test, Freshwater and Marine)
Japanese Ministry of Agriculture, Forestry and Fisheries, test data for Registration of Agricultural Chemicals, 12 Nohsan No. 8147, Agricultural Production Bureau, November 24 2000

GLP: Yes

Previous evaluation: In DAR (November 2007); relevant for renewal application

Materials and methods:

Test substance: lenacil, batch no: 141712003, chemical purity: 98.6 %

Test species: common carp (*Cyprinus carpio*)

Number of organisms, weight, length, loading: 3 replicates with 10 fish for the control and per treatment group, mean weight: 1.26 g (SD = 0.24 g), mean standard length: 4.3 cm (SD = 0.2 cm), loading of 0.63 g fish/L (static volume)

Type of test: semi-static (daily batchwise renewal) acute toxicity test (96 hours), limit test

Applied and measured concentrations:

Nominal concentrations: control; solvent control (dimethylformamide); 100 % saturation

Mean measured concentrations: 0; 0; 3.0 - 3.1 mg a.s./L (100 – 103 % of nominal concentration)

Test conditions: temperature: 23 °C
pH: 7.4 – 7.6
oxygen content: 7.3 – 7.5 mg/L O_2 (84 – 86 % O_2 saturation)
total hardness: 152 - 170 mg/L $CaCO_3$
photoperiod: 16 hours light : 8 hours dark cycle

light intensity: 503 - 615 lux

Analytical methods: high performance liquid chromatography (HPLC) with UV detection (absorbance at 270 nm)

Test procedure:

Lenacil technical has a water solubility of 6 mg/L at 25 °C and is stable to hydrolytic degradation. Based on this information and the expected low solubility of the compound, a method of solution preparation was employed to obtain a saturated solution under the proposed test conditions.

The test substance, 1000 mg, was dissolved in 10 mLs of dimethylformamide (DMF) to give a solvent stock solution of 100 mg/mL. An aliquot, 2.2 mL, of this solvent stock solution was spiked into 22 L of diluent medium, in triplicate, to give nominal test concentrations of 10 mg/L (containing 100 µL auxiliary solvent per litre dilution water). These were allowed to stir overnight to give 100 % saturated solutions and allowed to settle for 10 minutes, then the mid-section was siphoned off. All three solutions were mixed together and distributed between the three test vessels.

Test concentrations were verified by chemical analysis. Duplicate samples, 50 mL, were taken from the solvent control and test concentration (samples from replicate vessels pooled) at 0 and 72 hours (fresh media), 24 and 96 hours (expired media) for analysis.

One test concentration was prepared plus one control and one solvent control (100 µL auxiliary solvent per litre), each in triplicate. Ten fish were added to each vessel.

Fish were placed without conscious bias into glass aquaria containing prepared test medium or diluent water plus 100 µL auxiliary solvent per litre, as appropriate. Each vessel contained 20 litres of medium to a depth of 19 cm (approximate dimensions of vessel: 25 x 46 x 25 cm). This provided a loading of 0.63 g body weight/litre (static volume).

A preliminary range finding study was conducted with test concentrations ranging from 0.10 to 100 % saturated solutions. Results of range finding indicated that no lethal or sub-lethal effects had occurred at any test level.

Based on the results of this range finding study, a definitive test was conducted as a limit study at the maximum achievable concentration:

Nominal concentration: 100 % saturation

Mean measured concentration: 3.1 mg/L

The nominal exposure concentration quoted in this report refers to the test material as received; no allowance has been made for a purity of less than 100 %.

Fish were exposed to the test or control conditions for a period of 96 hours with daily batchwise renewal of test medium to ensure the maintenance of satisfactory environmental conditions and near nominal exposure levels.

The criteria of death employed in this study were (i) absence of respiratory movement and (ii) absence of response to physical stimulation of the caudal peduncle.

In addition to observations on mortality at 15 minutes and 3, 6, 24, 48, 72 and 96 hours, subjective assessments were made on the type and incidence of sub-lethal effects compared with control fish.

Findings:

Analytical results:

Measured concentrations of lenacil in test solutions are presented in Table 2.9.2.2.1-1.

All results, in terms of test concentration, are expressed as mean measured. Measured concentrations ranged from 2.6 to 3.4 mg a.s./L.

Table 2.9.2.2.1-1: Summary of chemical analysis results: Lenacil technical: measured concentrations

Occasion	Nominal concentration (% sat)	Measured concentration (mg a.s./L)
0 hours	Solvent control R1	ND
	Solvent control R2	ND
	Solvent control R3	ND
	100 R1	3.398
	100 R2	3.173
	100 R3	3.316
24 hours	Solvent control R1	ND
	Solvent control R2	ND

	Solvent control R3	ND
	100 R1	3.247
	100 R2	2.662
	100 R3	3.196
72 hours	Solvent control R1	ND
	Solvent control R2	ND
	Solvent control R3	ND
	100 R1	2.883
	100 R2	3.164
	100 R3	3.120
96 hours	Solvent control R1	ND
	Solvent control R2	ND
	Solvent control R3	ND
	100 R1	2.677
	100 R2	2.924
	100 R3	2.593

ND none detected (limit of detection: 0.04 mg/L)

Mortality:

The cumulative mortality during the test is presented in Table B.9.2.2.1-2Table . One mortality in one replicate of the control died after 24 hours. No mortalities occurred in the solvent controls or at the treatment level of 3.1 mg a.s./L.

Table 2.9.2.2.1-2: Mortality (%) of common carp (*Cyprinus carpio*) exposed to lenacil for 96 hours in a semi-static, unaerated test

Mean measured concentration (mg/L)	Number of fish	Cumulative mortality			
		24 h	48 h	72 h	96 h
0 (control)	30	1	1	1	1
0 (solvent control)	30	0	0	0	0
3.1	30	0	0	0	0

Behavioural observations:

There were no marked sub-lethal reactions to exposure throughout the 96 hour exposure period of this study.

Conclusions of the study:

The 96-hour LC_{50} value for lenacil technical with common carp was > 3.1 mg a.s./L. The "no-observed effect concentration" for lenacil technical with common carp was 3.1 mg a.s./L. The "lowest-observed effect concentration" for lenacil technical with common carp was > 3.1 mg a.s./L.

RMS comments and conclusions:

The test design was in line with the OECD Test Guideline 203 and the validity criteria were met:

- The mortality in the control did not exceed 10 % (measured: 0.034 %)
- The dissolved oxygen concentration was at least 60 % of the air saturation value throughout the test (measured: 7.3 – 7.5 mg/L O_2, which corresponds to approximately 100 – 103 % of the air saturation value at 23 °C)
- The concentration of the substance being tested has been satisfactorily maintained (measured: > 100 % of nominal concentrations)

Therefore, the study is still considered acceptable and the endpoints reliable for use in the risk assessment.

The analytical method used could not be fully validated according to the EU Guidance SANCO/3029/99 rev. 4. However, the available validation data from all studies where this method was used (CA8.2.1/02, CA8.2.6.1/01, CA8.2.6.2/01 and CA8.2.7/01) seem to indicate that the method works well and can be considered "fit for purpose".

The LOQ for dechlorinated tap water was set at 1 mg/L (please refer to Vol. 3 (CA), Section B.5.1.2.6 – studies no. 3 to 6, for further details). The working range covers the test concentrations.

LC_{50} (*Cyprinus carpio*, 96 h) > 3.1 mg a.s./L (mean measured)
NOEC (*Cyprinus carpio,* 96 h) = 3.1 mg a.s./L (mean measured)

Report:	**CA8.2.1/02. [redacted] (1991a)** **Static, acute, 96-hour LC_{50} of DPX-B634-91 (lenacil) to rainbow trout (*Oncorhynchus mykiss*).**

Report No.:	HLR 199-91
Guidelines:	OECD Guideline 203: Fish, Acute Toxicity Test US EPA Pesticide Assessment Guidelines Subdivision E, 72-1
GLP:	Yes
Previous evaluation:	In DAR (November 2007); relevant for renewal application

Materials and methods:

Test substance:	lenacil, batch no: 9038, chemical purity: 98.2 %
Test species:	rainbow trout (*Oncorhynchus mykiss*)
Number of organisms, weight, length, loading:	1 replicate with 10 fish for the control and per treatment group, mean weight: 0.50 g (range: 0.20 – 0.99 g), mean standard length: 3.3 cm (range: 2.4 – 4.0 cm), mean total length: 3.8 cm (2.9 – 4.7 cm), loading of 0.33 g fish/L
Type of test:	static acute toxicity test (96 hours)
Applied and measured concentrations:	
Nominal concentrations:	control; solvent control (dimethylformamide); 0.26, 0.44, 0.72, 1.2 and 2.0 mg a.s./L
Mean measured concentrations:	0; 0; 0.48, 0.51, 0.80, 1.3 and 2.0 mg a.s./L (100 – 185 % of nominal concentration)
Test conditions:	temperature: 12.3 °C (12.0 – 12.6 °C) pH: 6.6 – 7.8 oxygen content: 8.1 – 9.2 mg/L O_2 (75 – 85 % O_2 saturation) total hardness: 75 mg/L $CaCO_3$ photoperiod: 16 hours light : 8 hours dark cycle light intensity: 247 lux
Analytical methods:	high performance liquid chromatography (HPLC) with UV detection (absorbance at 254 nm)

Test procedure:
Attempts to dissolve the test substance were made by adding various amounts up to 20 mg/L of lenacil to 15 liters of dilution water in a 20 L aquarium, along with approximately 0.1 mL/L of DMF. The mixture was stirred for 6 hours and samples were taken periodically and analysed for lenacil. Lenacil maximum measured concentrations were approximately 2 mg/L at the end of 6 hours. A stock solution was prepared by adding 15 mL of DMF and 1.5 g of lenacil to 150 liters of well water (10 m/L) and mixing for approximately 6 hours. Again, samples were taken with time, and again the maximum concentration of lenacil found was approximately 2 mg/L. Precipitate was present in the stock solution. Five test concentrations, a water control, and a DMF control were used. Nominal concentrations were 13, 22, 36, 60 and 100 % of saturation obtained in the 150 L stock tank, or 0.26, 0.44, 0.72, 1.2 and 2.0 mg/L, based on dilutions of the 2.0 mg/L measured concentration.
The fingerling rainbow trout were held at the laboratory for 42 days of age at test start. Glass aquaria (20 L) containing 15 liters of test solution (approximately 18-cm liquid depth) were employed. One replicate of each test concentration with ten fish was used. Fish were added to the test concentrations using random numbers. The rainbow trout ranged from 2.4 to 4.0 cm in standard length (mean 3.3 cm), 2.9 to 4.7 cm in total length (mean 3.8 cm), and 0.20 to 0.99 g in weight (mean 0.50 g). The loading was 0.33 g fish/L. Test solutions were held between 12.0 to 12.6 °C (mean 12.3 °C) and were unaerated. Fish were not fed 48 hours prior to nor during the test. A photoperiod of 16 hours light (247 lux) versus 8 hours darkness was employed. Observations were made every 24 hours.

Dissolved oxygen and pH were measured in the water control and in all test concentrations before fish were added at the beginning of the test and approximately every 24 hours thereafter. Solutions for quantitative analysis were taken from controls and selected test solutions at the beginning and end of the study.

Findings:

Analytical results:
Measured concentrations of lenacil in test solutions are presented in Table 2.9.2.2.1-3Table .
Nominal lenacil concentrations were 13, 22, 36, 60 and 100 % of saturation, or 0.26, 0.44, 0.72, 1.2 and 2.0 mg a.s./L based on the 2.0 mg a.s./L measured concentration. Mean, measured concentrations were 0.48, 0.51, 0.80, 1.3 and 2.0 mg a.s./L.

Table 2.9.2.2.1-3: Measured concentrations of lenacil in test solutions

Nominal concentration (% of saturation, mg a.s./L)	Measured test concentration (mg a.s./L)		
	Day 0	Day 4	Average[1]
Control	0.00	0.00	0.00
Solvent control	0.00	0.00	0.00
13, 0.26	0.40	0.56	0.48
22, 0.44	0.51	0.51	0.51
36, 0.72	0.72	0.87	0.80
60, 1.2	1.25	1.40	1.30
100, 2.0	2.00	2.03	2.00

[1] *Average concentrations are reported to two significant figures*

Mortality:
The cumulative mortality during the test is presented in Table 2.9.2.2.1-4. No mortalities occurred in the controls or at any treatment level.

Table 2.9.9.2.1-4: Mortality (%) of rainbow trout (*Oncorhynchus mykiss*) exposed to lenacil for 96 hours in a static, unaerated test

Mean measured concentration (mg/L)	Number of fish	Cumulative mortality			
		24 h	48 h	72 h	96 h
0 (control)	10	0	0	0	0
0 (solvent control)	10	0	0	0	0
0.48	10	0	0	0	0
0.51	10	0	0	0	0
0.80	10	0	0	0	0
1.2	10	0	0	0	0
2.0	10	0	0	0	0

Behavioural observations:
No unusual behaviour or signs of intoxication were observed at any treatment level.

Conclusions of the study:
Lenacil was not toxic at measured test concentrations up to 2.0 mg/L (apparent solubility limit in the dilution water) in a 96-hour, unaerated, static test using fingerling rainbow trout (*Oncorhynchus mykiss*).

RMS comments and conclusions:
The test design was in line with the OECD Test Guideline 203 and the validity criteria were met:

- The mortality in the control did not exceed 10 % (measured: 0 %)
- The dissolved oxygen concentration was at least 60 % of the air saturation value throughout the test (measured: 8.1 – 9.2 mg/L O_2, which corresponds to approximately 75 – 85 % of the air saturation value at 12 °C)
- The concentration of the substance being tested has been satisfactorily maintained (measured: > 100 % of nominal concentrations)

Therefore, the study is still considered acceptable and the endpoints reliable for use in the risk assessment.

The analytical method used could not be fully validated according to the EU Guidance SANCO/3029/99 rev. 4. No validation of the HPLC-UV method occurred in the study reports. The analytical phase only reports the results of the measured concentrations with some data on linearity and chromatograms. It is therefore difficult to state if the method was "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – studies no. 8.03, for further details). However, other HPLC-UV methods were validated with a LOQ that would cover the concentrations tested in this study. Further, the results for this study are not critical for the risk assessment. Therefore, the submission of additional validation data is not considered necessary.

LC_{50} (*Oncorhynchus mykiss*, 96 h) > 2.0 mg a.s./L (mean measured)
NOEC (*Oncorhynchus mykiss,* 96 h) = 2.0 mg a.s./L (mean measured)

Report:	**CA8.2.1/03. [redacted] (1991b)** **Static, acute, 96-hour LC_{50} of DPX-B634-91 (lenacil) to fathead minnows (*Pimephales promelas*).**

Report No.:	HLR 198-91
Guidelines:	OECD Guideline 203: Fish, Acute Toxicity Test US EPA Pesticide Assessment Guidelines Subdivision E, 72-1
GLP:	Yes
Previous evaluation:	In DAR (November 2007); relevant for renewal application

Materials and methods:

Test substance:	lenacil, batch no: 9038, chemical purity: 98.2 %
Test species:	fathead minnow (*Pimephales promelas*)
Number of organisms, weight, length, loading:	1 replicate with 10 fish for the control and per treatment group, mean weight: 0.63 g (range: 0.34 – 0.74 g), mean standard length: 3.7 cm (range: 3.2 – 3.9 cm), mean total length: 4.4 cm (3.8 – 4.7 cm), loading of 0.42 g fish/L
Type of test:	static acute toxicity test (96 hours)
Applied and measured concentrations:	
Nominal concentrations:	control; solvent control (dimethylformamide); 0.26, 0.44, 0.72, 1.2 and 2.0 mg a.s./L
Mean measured concentrations:	0; 0; 0.38, 0.48, 0.80, 1.2 and 2.0 mg a.s./L (100 – 146 % of nominal concentration)
Test conditions:	temperature: 22.3 °C (22.2 – 22.5 °C) pH: 7.0 – 7.6 oxygen content: 6.0 – 8.6 mg/L O_2 (68 – 98 % O_2 saturation) total hardness: 72 mg/L $CaCO_3$ photoperiod: 16 hours light : 8 hours dark cycle light intensity: 387 lux
Analytical methods:	high performance liquid chromatography (HPLC) with UV detection (absorbance at 254 nm)

Test procedure:
Attempts to dissolve the test substance were made by adding various amounts up to 20 mg/L of lenacil to 15 liters of dilution water in a 20 L aquarium, along with approximately 0.1 mL/L of DMF. The mixture was stirred for 6 hours and samples were taken periodically and analysed for lenacil. Lenacil maximum measured concentrations were approximately 2 mg/L at the end of 6 hours. A stock solution was prepared by adding 15 mL of DMF and 1.5 g of lenacil to 150 liters of well water (10 mg/L) and mixing for approximately 6 hours. Again, samples were taken with time, and again the maximum concentration of lenacil found was approximately 2 mg/L. Precipitate was present in the stock solution. Five test concentrations, a water control, and a DMF control were used. Nominal concentrations were 13, 22, 36, 60 and 100 % of saturation obtained in the 150 L stock tank, or 0.26, 0.44, 0.72, 1.2 and 2.0 mg/L, based on dilutions of the 2.0 mg/L measured concentration.

The juvenile fathead minnows were held at the laboratory for 188 - 194 days of age at test start. Glass aquaria (20 L) containing 15 liters of test solution (approximately 18-cm liquid depth) were employed. One replicate of each test concentration with ten fish was used. Fish were added to the test concentrations using random numbers. The fathead minnows ranged from 3.2 to 3.9 cm in standard length (mean 3.7 cm), 3.8 to 4.7 cm in total length (mean 4.4 cm), and 0.34 to 0.74 g in weight (mean 0.63 g). The loading was 0.42 g fish/L. Test solutions were held between 22.2 to 22.5 °C (mean 22.3 °C) and were unaerated. Fish were not fed 48 hours prior to nor during the test. A photoperiod of 16 hours light (387 lux) versus 8 hours darkness was employed. Observations were made every 24 hours. Dissolved oxygen and pH were measured in the water control and in all test concentrations before fish were added at the beginning of the test and approximately every 24 hours thereafter. Solutions for quantitative analysis were taken from controls and selected test solutions at the beginning and end of the study.

Findings:

Analytical results:
Measured concentrations of lenacil in test solutions are presented in Table 2.9.2.2.1-5.
Nominal lenacil concentrations were 13, 22, 36, 60 and 100 % of saturation, or 0.26, 0.44, 0.72, 1.2 and 2.0 mg a.s./L based on the 2.0 mg a.s./L measured concentration. Mean, measured concentrations were 0.38, 0.48, 0.80, 1.2 and 2.0 mg a.s./L.

Table 2.9.2.2.1-5: Measured concentrations of lenacil in test solutions

Nominal concentration (% of saturation, mg a.s./L)	Measured test concentration (mg a.s./L)		
	Day 0	Day 4	Average[1]
Control	0.00	0.00	0.00
Solvent control	0.00	0.00	0.00
13, 0.26	0.40	0.35	0.38
22, 0.44	0.51	0.45	0.48
36, 0.72	0.83	0.77	0.80
60, 1.2	1.20	1.19	1.20
100, 2.0	2.00	1.98	2.00

[1] *Average concentrations are reported to two significant figures*

Mortality:
The cumulative mortality during the test is presented in Table 2.9.9.2.1-6Table. No mortalities occurred in the controls or at any treatment level.

Table2.9.9.2.1-6: Mortality (%) of fathead minnow (*Pimephales promelas*) exposed to lenacil for 96 hours in a static, unaerated test

Mean measured concentration (mg/L)	Number of fish	Cumulative mortality			
		24 h	48 h	72 h	96 h
0 (control)	10	0	0	0	0
0 (solvent control)	10	0	0	0	0
0.38	10	0	0	0	0
0.48	10	0	0	0	0
0.80	10	0	0	0	0
1.2	10	0	0	0	0
2.0	10	0	0	0	0

Behavioural observations:
No unusual behaviour or signs of intoxication were observed at any treatment level.

Conclusions of the study:
Lenacil was not toxic at measured test concentrations up to 2.0 mg/L (apparent solubility limit in the diluation water) in a 96-hour, unaerated, static test using juvenile fathead minnows (*Pimephales promelas*).

RMS comments and conclusions:

The test design was in line with the OECD Test Guideline 203 and the validity criteria were met:

- The mortality in the control did not exceed 10 % (measured: 0 %)
- The dissolved oxygen concentration was at least 60 % of the air saturation value throughout the test (measured: 6.0 – 8.6 mg/L O_2, which corresponds to approximately 68 – 98 % of the air saturation value at 22 °C)
- The concentration of the substance being tested has been satisfactorily maintained (measured: > 100 % of nominal concentrations)

Therefore, the study is still considered acceptable and the endpoints reliable for use in the risk assessment.

The analytical method used could not be fully validated according to the EU Guidance SANCO/3029/99 rev. 4. No validation of the HPLC-UV method occurred in the study reports. The analytical phase only reports the results of the measured concentrations with some data on linearity and chromatograms. It is therefore difficult to state if the method was "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – studies no. 8.04, for further details). However, other HPLC-UV methods were validated with a LOQ that would cover the concentrations tested in this study. Further, the results for this study are not critical for the risk assessment. Therefore, the submission of additional validation data is not considered necessary.

LC_{50} (*Pimephales promelas*, 96 h) > 2.0 mg a.s./L (mean measured)
NOEC (*Pimephales promelas,* 96 h) = 2.0 mg a.s./L (mean measured)

2.9.2.2.2 Acute (short-term) toxicity to aquatic invertebrates

The information below was extracted from Volume 3 (CA), Section B.9.2 'Effect on aquatic organisms'. Two acute (short-term) toxicity studies with aquatic invertebrates are available for lenacil.

Report:	**CA8.2.4.1/01. Hutton D.G. (1989a)** **Static acute 48-hour EC50 of DPX-B634-84 to fed *Daphnia magna***

Report No.: HLR 86-89
Guidelines: OECD 202 Part I (1984), US EPA 72-2 (1985)
GLP: Yes

Previous evaluation: In DAR (November 2007); relevant for renewal application

Materials and methods:

Test substance: lenacil technical, blended batch numbers: 8802 and 8805, , chemical purity : 95.1 %
Test species: *Daphnia magna*
Number of organisms, age: 4 replicates with 5 daphnids for the control and per treatment group (20 daphnids per concentration), less than 24 hours old
Type of test: static acute toxicity test (48 hours)
Applied and measured concentrations:
Nominal concentrations: control; 50, 67, 89, 119, 158, 211, 281, 375, 500 mg a.s./L (all >> solubility limit).
measured concentrations after 48 h: 0.0; 4.3, 4.8, 4.7, 6.0, 5.5, 4.6, 5.2, 5.3, 8.4 mg a.s./L
Test conditions: temperature : 20.3 ºC
pH : 6.7 - 7.3
dissolved oxygen : 26 – 96 % O_2 saturation (2.4 - 8.9 mg/L O_2)
total hardness : 75 mg/L $CaCO_3$
photoperiod : 16/8 hours light/dark cycle
light intensity : 560 lux
Analytical methods: HPLC-UV.
Statistics: none performed

Test procedure:
Neonate daphnids, less than 24 hours old at the start of the test, were exposed to the test item for a period of 48 hours. They were kept in 250 mL glass beakers, filled with 200 mL test solution. The test solution consisted of filtered fish tank water (i.e. Haskell laboratory well water that had been used for fathead minnow culturing in a flow-through system, then filtered through a 0.8 micon filter), spiked with the required amount of test material to reach the test concentration. For each concentration, a 1-L volume of test solution was prepared, and ten split into 200 mL test volumes. The *Daphnia magna* were added to the test system at the start of the test, by randomly picking them from a single transfer tank.

Immobility counts and observations were made at approximately 24 and 48 hours after exposure was initiated. Dissolved oxygen and pH were measured in the water control and in all exposure concentrations at the beginning, at 24 h and at the end of the exposure period. The concentration of the test item in the test solutions were measured at the beginning and at the end of the test.

Findings:
Analytical results:
Nominal test concentrations for the study were 50 to 500 mg a.s./L. However, from Table 2.9.2.2.2-1, it is obvious that the solubility of DPX-B635-84 was very much less than that. Day 0 samples were taken right after sample preparation, when undissolved test material may still have been suspended in the samples. This would have accounted for the day 0 measured values being significantly higher than the expected solubility of lenacil. Based on the day 2 analytical results, it is felt that the solubility of lenacil in test water is about 4 to 8 mg/L. For this reason, the day 2 analysis are felt to be most representative of the measured test concentration, and thoe analysis are used in drawing the test conclusions.

Table 2.9.2.2.2-1: Measured concentrations of lenacil technical in the test solutions

Nominal concentration of test item (mg a.s./L)	Measured concentration of test item (µg a.s./L)					
	Day 0	% of nominal	Day 2	% of nominal	Arithmetic Mean	% of nominal
Water control	0.0	-	0.0	-	0.0	-
50	8.3	16.6	4.3	8.6	6.3	12.6
67	12.9	19.3	4.8	7.2	8.9	13.3
89	11.0	12.4	4.7	5.3	7.9	8.9
119	19.7	16.6	6.0	5.0	13	10.9
158	27.6	17.4	5.5	3.5	17	10.8
211	31.9	15.1	4.6	2.2	18	8.5
281	42.7	15.2	5.2	1.9	24	8.5
375	49.2	13.1	5.3	1.4	27	7.2
500	69.9	14.0	8.4	1.7	39	7.8

Biological results:
The biological results are presented in Table 2.9.2.2.2-2. No immobilisation occurred in the control or at any treatment level. The 48h was therefore > 8.4 mg a.s./L, the highest concentration tested.

Table 2.9.2.2.2-2: Cummulative observed immobility of *Daphnia magna* in a static acute toxicity test with lenacil

Nominal test concentrations (mg a.s./L)	Cumulative observed mortality (%)							
	24 h				48 h			
	A*	B*	C*	D*	A*	B*	C*	D*
Water control	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0
67	0	0	0	0	0	0	0	0
89	0	0	0	0	0	0	0	0
119	0	0	0	0	0	0	0	0
158	0	0	0	0	0	0	0	0
211	0	0	0	0	0	0	0	0
281	0	0	0	0	0	0	0	0

375	0	0	0	0	0	0	0	0
500	0	0	0	0	0	0	0	0

Conclusions:
Under the conditions of this test, lenacil was found to exhibit no toxicity to *Daphnia magna* at test concentrations up to 8.4 mg a.s./L (measured concentration after 48h), which is above the water solubility of lenacil.

RMS comments:
The validity criterion of the most recent version of OECD Test Guideline 202 are met:

- The immobilisation in the control did not exceed 10 % (measured: 0 %)

The dissolved oxygen concentration at the end of the test was > 3 mg/L in the control and in all test vessel (measured range 3.2-4.7), except for the treatment level of 67 mg a.s./L (nominal). However, there was no evidence of adverse effects on the test organisms at this test concentration. As in the control and in higher tested concentrations all validity criteria were met, this deviation is not considered to invalidate the results of this study.
The notifier argued that this study does not meet the requirements of the current OECD TG 202, as the concentrations tested (50 to 500 mg a.s./L) were higher than the limit test of 100 mg/L of test substance established in this guideline. However, the actual exposure, based on the measured concentrations, was below this limit of 100 mg/L.
Overall, this study is still considered acceptable for use in the risk assessment.

The analytical method used could not be fully validated according to the EU Guidance SANCO/3029/99 rev. 4. No validation of the HPLC-UV method occurred in the study reports. The analytical phase only reports the results of the measured concentrations with some data on linearity and chromatograms. It is therefore difficult to state if the method was "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – studies no. 8.06, for further details). However, other HPLC-UV methods were validated with a LOQ that would cover the concentrations tested in this study. Further, the results for this study are not critical for the risk assessment. Therefore, the submission of additional validation data is not considered necessary.

The following endpoints, based on concentrations measured after 48h, will be considered in the risk assessment:
EC_{50} (*Daphnia magna*, 48 h) > 8400 µg a.s./L
NOEC (*Daphnia magna*, 48 h) = 8400 µg a.s./L

Report:	**CA8.2.4.1/02. Renner P. (2016a)** **Acute toxicity of Lenacil technical to *Daphnia magna* in a 48-hour static test.**

Report No.: 15 10 48 031 W
Guidelines: OECD Guideline 202: *Daphnia* sp., Acute Immobilisation Test
GLP: Yes

Previous evaluation: None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application

Materials and methods:
Test substance: lenacil technical, batch no.: JUL14HE010, chemical purity: 98.82 %
Test species: *Daphnia magna*
Number of organisms, age: 4 replicates with 5 daphnids for the control and per treatment group (20 daphnids per concentration), less than 24 hours old
Type of test: static acute toxicity test (48 hours)
Applied and measured concentrations:
Nominal concentrations: untreated control; 100 mg a.s./L
Mean measured concentrations: 0; 3.11 mg a.s./L
Test conditions : temperature: 19.7 – 20.4 °C
pH: 7.58
oxygen content: 8.84 mg/L O_2
total hardness: 227 mg/L as $CaCO_3$

	photoperiod: 16 hours light : 8 hours dark cycle light intensity: 1480 lux
Analytical methods:	standard analytical methods (HPLC-MS/MS) were used to verify concentrations of the test item in the test solutions at the start and at the end of the test in the fresh and aged solutions.
Statistics:	Percentage immobility at 24 and 48 hours after application was calculated. Since a limit test was performed, calculations and statistical evaluations were not applicable.

Test procedure:

Neonate daphnids, less than 24 hours old at the start of the test, were exposed to the test item for a period of 48 hours. They were kept in a defined reconstituted water to which the test item has been added. Immobilisation of the daphnids was recorded at 3, 24 and 48 hours after the test start and was compared with the control values. The results (EC_{10}, EC_{20} and EC_{50}) were estimated from the data at 24 and 48 hours after application. As the test was performed as a limit-test, a single concentration of 100 mg/L, nominal, was used. Due to the low water solubility, this was equivalent to an initially measured test item concentration of 2.88 mg a.s./L and a mean measured concentration of 3.11 mg a.s./L.

At the start (0 hours) and at the end of the test (48 hours) pH and content of dissolved oxygen in test solutions were measured in freshly prepared and aged test solutions. The temperature was measured continuously.

Findings:

Analytical results:

Measured concentrations of lenacil in test solutions are presented in Table 2.9.2.2.2-3.

The geometric mean measured endpoint of 3.11 mg a.s./L was calculated based on the initially measured concentration of 2.88 mg a.s./L and the mean measured concentration of 6.36 mg a.s./L at the end of the exposure (aged test medium).

Table 2.9.2.2.2-3: Measured concentrations of lenacil technical in the test solutions

Nominal concentration (mg a.s./L)	Measured concentration (mg a.s./L) (% of nominal a.s.)		Geometric mean measured a.s. concentration over 48 hours (% of nominal a.s.)
	0 hours (fresh)	48 hours (aged)	
Control	n.d.* (-)	n.d.* (-)	3.11 (3.11)
100.0	2.88 (2.88)	3.36 (3.36)	

** not detected or detected concentration ≤ LOQ (LOQ: 50.4 µg a.s./L)*

Biological results:

The biological results are presented in Table 2.9.2.2.2-4.

No immobilization was observed in the control or in the treatment group of 3.11 mg a.s./L during the 48 hours of exposure to lenacil. No abnormal behavior or appearance of animals were observed, e.g. trapping at water surface or discoloration.

Table 2.9.2.2.2-4: Number of immobilised *Daphnia magna* and percentage immobility

Treatment group (mg a.s./L, nominal) (mg a.s./L, mean measured)	Immobilised Daphnia (number)		Immobility of Daphnia (%)	
	24 h	48 h	24 h	48 h
Control (test medium only)	0	0	0	0
100 (3.11)	0	0	0	0

Conclusions of the study:

The NOEC (no observed effect concentration) at 48 hours after application was determined to be ≥ 100 mg a.s./L, nominal (equivalent to ≥ 3.11 mg a.s./L, mean measured). The LOEC (lowest observed effect concentration) at 48 hours after application was determined to be > 100 mg a.s./L, nominal (equivalent to > 3.11 mg a.s./L, mean measured).

The EC_{10}, EC_{20} and EC_{50} values for immobilisation based on nominal concentrations were estimated to be > 100 mg a.s./L (equivalent to > 3.11 mg a.s./L, mean measured) at 48 hours.

RMS comments and conclusions:

The validity criteria of the most recent version of OECD Test Guideline 202 are met:

- The immobilisation in the control did not exceed 10 % (measured: 0 %)
- The dissolved oxygen concentration at the end of the test was > 3 mg/L (measured: 8.67 mg/L)

Therefore, this study is considered acceptable for use in the risk assessment.

The analytical method used could be fully validated according to the EU Guidance SANCO/3029/99 rev. 4., and is therefore considered "fit for purpose". The LOQ was set at 50.43 µg/L (before dilution: 50.43 mg/L) (please refer to Vol. 3 (CA), Section B.5.1.2.6 – study no. 10, for further details). The working range covers the test concentrations (taking into account the sample dilution of 1000).

The following endpoint, based on mean measured concentrations, will be considered in the risk assessment:

EC_{50} (*Daphnia magna*, 48h) > 3110 µg a.s./L

NOEC (*Daphnia magna*, 48 h) = 3110 µg a.s./L

2.9.2.2.3 Acute (short-term) toxicity to algae or aquatic plants

Please refer to Section 2.9.2.3.3 'Chronic toxicity to algae or aquatic plants' where both acute (short-term) and chronic toxicity to algae and aquatic plants are discussed.

2.9.2.2.4 Acute (short-term) toxicity to other aquatic organisms

No additional studies on the acute (short-term) toxicity of lenacil to other aquatic organisms are available.

2.9.2.3 Long-term aquatic hazard [equivalent to section 11.6 of the CLH report template]

The relevant studies on the acute aquatic toxicity of lenacil are shown in the table below. This table contains the the information included in the previous CLH report for lenacil (Dossier Submitted by Belgium in 2011), and was updated with the studies that were newly submitted for the current Annexs I renewal application for lenacil.

Table 2.9.2.3-1 Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Relevant study	Remarks	Reference
chronic fish juvenile growth study based on OECD 204 GLP	*Oncorhynchus mykiss*	lenacil, purity: 98.2%, batch n°: 9038	NOEC = 2.3 mg a.s/L (mean measured) based on mortality and growth	Acceptable. Supportive study	21 d flow-through fingerlings 5 fish/replicate 2 replicates/treatment	CA8.2.2.1/01 [redacted] 1991c
chronic fish early life stage study based on OECD 210 GLP	*Oncorhynchus mykiss*	lenacil, purity: 98.5%, batchn°: 9038	**NOEC = 0.160 mg a.s./L** (mean measured) based on mean standard length	Acceptable. Key study	90 d flow-through 20 embryos/cup 2 embryo cups/replicate 2 replicates/treatment	CA8.2.2.1/02 [redacted] 1996

chronic daphnia study based on OECD 211 GLP	*Daphnia magna*	lenacil, purity: 98.82%, batch n°: JUL14HE 010	**NOEC = 0.019 mg a.s./L** (mean measured) based on adult survival and total numbers of offspring	Acceptable. Key study	21 d semi-static 1 daphnid/replicate 10 replicates /treatment	CA8.2.5.1/ 02 Renner P., 2016b
algal growth inhibition study based on OECD 201, 92/69/EEC method C.3 and draft US EPA OPPTS 850.5400 GLP	*Pseudokircherniella subcapitata*	lenacil, purity: 98.6%, batch n°: 141712003	E_bC_{50} (72 h) = 0.0077 mg a.s./L E_bC_{50} (96 h) = 0.0065 mg a.s./L E_rC_{50} (72 h) = 0.016 mg a.s./L E_rC_{50} (96 h) = 0.015 mg a.s./L NOEC (96 h) = 0.0034 mg a.s./L (mean measured)	Acceptable. Supportive study	96 h static initial cell count: 1 x 10^4/mL 6 replicates for control and solvent control 3 replicates /treatment	CA8.2.6.1/ 01 Flatman D., 2003c
algal growth inhibition study based on OECD 201 and 92/69/EEC method C.3 GLP	*Navicula pelliculosa*	lenacil, purity: 98.6%, batch n°: 141712003	E_bC_{50} = 0.036 mg a.s./L E_rC_{50} = 0.096 mg a.s./L NOEC = 0.011 mg a.s./L (mean measured)	Acceptable. Supportive study	72 h static initial cell count: 1 x 10^4/mL 6 replicates for control 3 replicates/treatment	CA8.2.6.2/ 01 Flatman D., 2003b
algal growth inhibition study based on OECD 201 GLP	*Ankistrodesmus falcatus*	lenacil, purity: 99.3%, batch n°: 0190813	E_vC_{50} = 0.00856 mg a.s./L **E_rC_{50} = 0.0133 mg a.s./L NOEC = 0.00259 mg a.s./L** (initial measured)	Acceptable. Key study	72 h static initial cell count: 2500/mL 8 replicates for control and solvent control 4 replicates /treatment	CA8.2.6.2/ 02 Wenzel A., 2014a
algal growth inhibition study based on OECD 201	*Synechococcus leopoliensis*	lenacil, purity: 99.3%, batch n°: 0190813	E_vC_{50} = 0.0665 mg a.s./L E_rC_{50} = 0.209 mg a.s./L	Acceptable. Supportive study	72 h static initial cell count: 1 x 10^4/mL 6 replicates for control and solvent control	CA8.2.6.2/ 03 Wenzel A., 2014b

GLP			NOEC = 0.0296 mg a.s./L (initial measured)		4 replicates /treatment	
algal growth inhibition study based on OECD 201 GLP	*Anabaena flos-aquae*	lenacil, purity: 99.3%, batch n°: 0190813	E_yC_{50} = 0.345 mg a.s./L E_rC_{50} > 0.643 mg a.s./L NOEC = 0.113 mg a.s./L (initial measured)	Acceptable. Supportive study	72 h static initial cell count: 1 x 10^4/mL 6 replicates for control and solvent control 4 replicates /treatment	CA8.2.6.2/ 04 Wenzel A., 2014c
algal growth inhibition study based on OECD 201 GLP	*Closterium cornu*	lenacil, purity: 99.3%, batch n°: 0190813	E_yC_{50} = 0.0353 mg a.s./L E_rC_{50} = 0.0706 mg a.s./L NOEC = 0.0216 mg a.s./L (initial measured)	Acceptable. Supportive study	72 h static initial cell count: 2500/mL 8 replicates for control and solvent control 4 replicates /treatment	CA8.2.6.2/ 05 Wenzel A., 2014d
algal growth inhibition study based on OECD 201 GLP	*Xanthonema debile*	lenacil, purity: 99.3%, batch n°: 0190813	E_yC_{50} = 0.0298 mg a.s./L E_rC_{50} = 0.132 mg a.s./L NOEC = 0.0109 mg a.s./L (initial measured)	Acceptable. Supportive study	72 h static initial cell count: 1 x 10^4/mL 8 replicates for control and solvent control 4 replicates /treatment	CA8.2.6.2/ 06 Wenzel A., 2014e
Lemna growth inhibition study based on OECD draft and US EPA draft OPPTS 850.4400 GLP	*Lemna gibba*	lenacil, purity: 98.6%, batch n°: 141712003	E_bC_{50} = 0.019 mg a.s./L E_rC_{50} = 0.029 mg a.s./L NOEC = 0.0088 mg a.s./L (mean measured)	Acceptable. Supportive study	7 d semi-static inoculation with 4 plants bearing 3 fronds 3 replicates for control, solvent control and per treatment	CA8.2.7/ 01 Flatman D., 2003d
Maltby *et al.* (2010) (draft version of OECD 239)	*Chara globularis*	lenacil, purity: 99.1%, batch n°: 200010003	E_yC_{50} = 0.01354 mg a.s./L E_rC_{50} = 0.01357 mg a.s./L NOEC = 0.00753 mg a.s./L	Acceptable. Supportive study	7 d static A single pot containing 5 plants in each test vessel 6 replicates for control 3 replicates /treatment	CA8.2.7/ 02 Wenzel A., 2012a

			(mean measured)			
Maltby *et al.* (2010) (draft version of OECD 239)	*Elodea canadensis*	lenacil, purity: 99.1%, batch n°: 200010003	E_yC_{50} = 0.00378 mg a.s./L **E_rC_{50} = 0.00378 mg a.s./L NOEC = 0.00318 mg a.s./L** (initial measured)	Acceptable. Key study	7 d static A single pot containing 5 plants in each test vessel 6 replicates for control 3 replicates /treatment	CA8.2.7/ 03 Wenzel A., 2012b

2.9.2.3.1 Chronic toxicity to fish

The information below was extracted from Volume 3 (CA), Section B.9.2 'Effect on aquatic organisms'. Two long-term toxicity studies with fish are available for lenacil.

Report:	**CA8.2.2.1/01. [redacted] (1991c)** **Flow-through, 21-day toxicity of DPX-B634-91 (lenacil) to rainbow trout (*Oncorhynchus mykiss*).**

Report No.: HLR 200-91
Guidelines: OECD Guideline 204
GLP: Yes

Previous evaluation: In DAR (November 2007); relevant for renewal application

Material and Methods:

Test substance: lenacil, batch no.: 9038, chemical purity: 98.2 %
Test species: rainbow trout (*Oncorhynchus mykiss*)
Number of organisms, age, weight, length, loading: 2 replicates with 5 fish for the control and per treatment group, fingerlings, mean weight: 1.07 g, mean standard length: 3.8 cm, loading of 0.77 g fish/L passing through the replicate in 24 hours
Type of test: 21-day prolonged toxicity test, flow-through
Applied and measured concentrations:
Nominal concentrations: control; solvent control (dimethylformamide); 0.29, 0.58, 1.2 and 2.3 mg a.s./L (12.5, 25, 50 and 100 % saturation)
Mean measured concentrations: 0; 0; 0.33, 0.65, 1.1 and 2.3 mg a.s./L (92 – 11 % of nominal concentration)
Test conditions: temperature: 12.5 – 13.6 °C
pH: 6.9 – 7.4
oxygen content: 9.0 – 10.4 mg/L O_2 (85 – 98 % O_2 saturation)
total hardness: 74 mg/L $CaCO_3$
photoperiod: 16 hours light : 8 hours dark cycle
light intensity: 54 – 86 lux
Analytical methods: high performance liquid chromatography (HPLC) with UV detection (absorbance at 254 nm)
Statistics: For continuous or ratio data, such as length or standard length or weight of trout at study's end, a determination was made of whether parametric or non-parametric procedures should be used. Shapiro-Wilk's test was used to determine whether the data was normally distributed. For each variable, data were available on each individual surviving trout. Normality and homogeneous variance tests were done on the within-replicate data. Since the number of trout per replicate was not large, both tests were applied to residuals from separate statistical models for each concentration. In this case, between-replicate

variances were estimated by maximum likelihood methods. Bartlett's test of homogeneity of within-replicate variances was applied. Since there were only two replicate groups per concentration or control group, Bartlett's test is not reliable for testing homogeneity of between-replicate variances. However, maximum likelihood estimates of between-replicate variances were indistinguishable from 0, so no test was required.
For each property measured, the MATC (Maximum Allowable Toxic Concentration) was determined as the geometric mean of the highest concentration at which no effect was observed (NOEC) and the lowest concentration at which an effect was observed (LOEC).

Test procedure:
Initial attempts to dissolve the test substance were made by adding various amounts up to 20 mg/L of lenacil to 15 litres of dilution water in a 20 L aquarium, along with approximately 0.1 mL/L of DMF. The mixture was stirred for 6 hours and samples were taken periodically and analysed for lenacil. Lenacil maximum concentrations were approximately 2 mg/L at the end of 6 hours. Another solution was prepared by adding 1.5 g of lenacil to 150 litres of well water (10 mg/L) and mixing for approximately 6 hours. Again, samples were taken with time, and again the maximum concentration of lenacil found was approximately 2 mg/L. Precipitate was present in the stock solution.
A dilution water control, a DMF control, and four test concentrations were prepared and maintained using dilution water and a proportional diluter. Nominal test concentrations were 12.5, 25, 50 and 100 % of saturation obtained in the 300-liter stock tank, or 0.29, 0.58, 1.2 and 2.3 mg a.s./L based on dilutions of the 2.3 mg a.s./L measured concentration. Test solutions were delivered intermittently (about every 21 minutes) to replicate 7-liter glass exposure chambers (20.5 (length) x 21 (width) x 26 (height) cm; 18-cm liquid depth); the volume of each replicate was exchanged five times daily. Exposure chambers were held in a water bath at approximately 12 °C and were assigned to test concentrations using random numbers.
The fingerling rainbow trout were held in a 272 liter, circular, fiberglass holding tank at the laboratory for 49 days in continuously flowing well water. The water temperature was held at approximately 10 °C. The rainbow trout were fed frozen *Artemia* sp. Sickness, injury and abnormalities were not present.
Rainbow trout fingerlings of mean standard length (DMF control) 3.8 cm and mean weight 1.07 g were used. Five fish were added to each replicate (two replicates per concentration; total of 10 fish) using random numbers and were fed frozen *Artemia* sp. once daily during the test. Loading was 0.77 g/L passing through the replicate in 24 hours. Test solutions were held between 12.5 and 13.6 °C and were unaerated. A photoperiod of 16 hours light (54 – 86 lux) versus 8 hours darkness was employed with 25 minutes of transitional light (2 – 5 lux) preceding and following the beginning of the 16 hour light interval. Observations were made twice daily. Dissolved oxygen and pH were measured in the water and the DMF controls and all test concentrations before fish were added at the beginning of the test, twice weekly thereafter and at the end of the test. Temperature was measured in the water and DMF controls and all test concentrations before fish were added at the beginning of the test, daily thereafter and at the end of the test. Solutions for quantitative analysis were taken from controls and all replicates of all test concentrations at the beginning, at day 7, at day 14 and end of the study.

Findings:
Analytical results:
Measured concentrations of lenacil in test solutions are presented in Table 2.9.2.3.1-1.
Nominal lenacil concentrations were 12.5, 25, 50 and 100 % of saturation obtained in the 300-liter stock tank, or 0.29, 0.58, 1.2 and 2.3 mg a.s./L based on the dilutions of the 2.3 mg a.s./L measured concentration. Mean, measured concentrations of lenacil were 0.33, 0.65, 1.1 and 2.3 mg a.s./L.

Table 2.9.2.3.1-1: Measured concentrations of lenacil in test solutions

Nominal concentrations (% of saturation, mg a.s./L)	Measured test concentration (mg a.s./L)				
	Day 0	Day 7	Day 14	Day 21	Average[1]
Control A	0.00	0.00	0.00	0.00	0.00
Control B	0.00	0.00	0.00	0.00	0.00
Solvent control A	0.00	0.00	0.00	0.00	0.00
Solvent control B	0.00	0.00	0.00	0.00	0.00
12, 0.29 A	0.32	0.34	0.32	0.34	0.33
12, 0.29 B	0.32	0.34	0.35	0.34	0.34

25, 0.58 A	0.64	0.67	0.63	0.67	0.65
25, 0.58 B	0.64	0.67	0.64	0.65	0.65
50, 1.2 A	1.07	1.21	1.11	1.14	1.10
50, 1.2 B	1.07	1.21	1.06	1.17	1.10
100, 2.3 A	2.13	2.51	2.19	2.31	2.30
100, 2.3 B	2.19	2.51	2.29	2.28	2.30

[1] *Average concentrations are reported to two significant figures*

Mortality:

The cumulative mortality during the test is presented in Table 2.9.2.3.1-2Table . No mortalities occurred in the control, solvent control and the treatment levels of 0.33, 1.1 and 2.3 mg a.s./L. At the treatment level of 0.65 mg a.s./L, 40 % mortality was observed. Four of five fish in one replicate group at 0.65 mg a.s./L died, apparently from cannibalism by the remaining fish in that replicate.

Standard length:

The standard length data generated during the test is presented in Table 2.9.2.3.1-2Table . No statistical significant differences were observed.

Wet weight:

The wet weight data generated during the test is presented in Table 2.9.2.3.1-2. No statistical significant differences were observed.

Table 2.9.2.3.1-2: Summary of mortality and fish size data for 21-day prolonged toxicity test with rainbow trout (*Oncorhynchus mykiss*)

Measured concentration (mg a.s./L)	Mortality (%)	Standard length (cm)	Wet weight (g)
Control	0	3.8	1.070
Solvent control	0	3.9	0.989
0.33	0	3.6	0.963
0.65	40	4.0	1.318
1.1	0	3.7	1.047
2.3	0	3.7	1.022

Neither the control or any of the test concentrations were significantly different from the solvent control in terms of fish length or weight

Conclusions:

The LC_{50}, MATC and NOEC for rainbow trout (*Oncorhynchus mykiss*) exposed to lenacil for 21 days in a flow-through test were all > 2.3 mg a.s./L (the apparent solubility limit in the dilution water).

RMS comments:

The test design was in line with the OECD Test Guideline 204 are met:

- The mortality in the controls did not exceed 10 % (measured: 0 %)
- The dissolved oxygen concentration was at least 60 % of the air saturation value throughout the test (measured: 9.0 – 10.4 mg/L O_2, which corresponds to approximately 85 – 98 % of the air saturation value at 13 °C)

Therefore, the study is still considered acceptable and the endpoints reliable for use in the risk assessment.

It is noted that the mean measured concentrations were calculated as arithmetic mean values, which is acceptable for flow-through studies according to OECD 23.

The analytical method used could not be fully validated according to the EU Guidance SANCO/3029/99 rev. 4. No validation of the HPLC-UV method occurred in the study reports. The analytical phase only reports the results of the measured concentrations with some data on linearity and chromatograms. It is therefore difficult to state if the method was "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – studies no. 8.05, for further details). However, other HPLC-UV methods were validated with a LOQ that would cover the concentrations tested in this

study. Further, the results for this study are not critical for the risk assessment. Therefore, the submission of additional validation data is not considered necessary.

In accordance with the new data requirements (Commission Regulation EU No 283/2013), the EC_{10} and EC_{20} values should be calculated. Where these values cannot be estimated, an explanation should be provided. As the present study was already submitted for the initial Annex I inclusion of lenacil, this was not addressed in the study report. However, as there were no effects > 10% at the highest dose tested, the EC_{10} and EC_{20} cannot reliably be determined based on the results from this study.

The following endpoints will be used in the riks assessment:
LC_{50} (*Oncorhynchus mykiss*, 21 d) > 2.3 mg a.s./L (mean measured)
NOEC (*Oncorhynchus mykiss*, 21 d) = 2.3 mg a.s./L (mean measured)

Report:	**CA8.2.2.1/02. [redacted] 1996)** **Early life-stage toxicity of DPX-B634-91 (lenacil) to rainbow trout, *Oncorhynchus mykiss*.**

Report No.: HLR 235-96
Guidelines: OECD Guideline 210: Fish, Early-life Stage Toxicity Test
US EPA Pesticide Assessment Guidelines, Subdivision E, 72-4
GLP: Yes

Previous evaluation: In DAR (November 2007); relevant for renewal application

Materials and methods:

Test substance: lenacil, batch no: 9038, chemical purity: 98.5 %
Test species: rainbow trout (*Oncorhynchus mykiss*)
Number of organisms, age: 20 embryos were placed into each embryo cup, 2 embryo cups per replicate, 2 replicates per treatment (total of 40 embryos per replicate and 80 embryos per treatment); the surviving alevins and fingerlings were thinned (15 per replicate) and released into the appropriate test chamber replicate on day 45 when most of the fish had swum-up
Biological loading: 0.181 g fish/L/day at test end, based on the daily turnover rate of approximately 6 test solution volumes
Type of test: 90-day fish early-life stage toxicity test, flow-through
Applied and measured concentrations:
Nominal concentrations: control; solvent control (dimethylformamide);
20, 50, 130, 320, 800 and 2000 µg a.s./L
Mean measured concentrations: 0; 0; 31, 53, 160, 280, 640 and 1600 µg a.s./L (80 – 155 % of nominal concentration)
Test conditions: temperature: 10.6 – 11.7 °C
pH: 7.2 – 7.6
oxygen content: 8.5 – 11.4 mg/L O_2 (77 – 103 % O_2 saturation)
total hardness: 78 – 85 mg/L $CaCO_3$
photoperiod: relative darkness until hatch was completed; 16 hours light : 8 hours dark cycle from day 40 onwards
light intensity: 43 – 65 lux
Analytical methods: high performance liquid chromatography (HPLC) with UV detection (absorbance at 270 nm)
Statistics: For the first and last day of hatching, the Kruskal-Wallis test, the Mann-Whitney test and the Jonckheere-Terpstra test were used. The Cochran-Armitage trend test was used for cumulative dead eggs and number of larvae at end of hatching. For the first day of swim-up, the Kruskal-Wallis test, the Mann-Whitney test and the Jonckheere's test were used. The number of dead larvae from hatching to thinning, the number of abnormal larvae from hatching to thinning, the Cochran-

Armitage test was used. For the number of dead larvae from thinning to test end, the Fisher's exact test was used. For length data, the Dunnett's test and the Williams' test were used. For weight data, the Jonckheere's test was used. It was not possible to compute EC_{50} for any endpoint, since the highest percent affected in any concentration for any relevant endpoint did not exceed 21 %.

Test procedure:
A dilution water control, a dimethylformamide (DMF) control, and six test concentrations were used in this study. Nominal test concentrations were 20, 50, 130, 320, 800 and 2000 µg a.s./L. Maximum concentration of DMF in the DMF control and test solutions, with the exception of the water control, was 0.1 mL/L. Test chambers were assigned to test concentrations using random numbers. Test solutions were delivered intermittently (about every 17 minutes, 84 1-L volume additions every 24 hours) to test chambers from a proportional diluter. The DMF, for the DMF control, was delivered separately in a mixing chamber of the diluter. Test solutions in the mixing chamber were continuously stirred by a stir bar driven by a magnetic drive.
Test chambers were 20-L glass aquaria (40 [length] x 20 [width] x 25 [height] cm) split into two replicates, each holding approximately seven litres of solution (10-L replicate test chamber volume; 18-cm liquid depth) and fitted with a screen mesh-covered overflow pipe. A recirculating water bath was used to maintain temperature in the test chambers at 10 ± 2 °C, and test chambers were positioned in the water bath using random numbers. The volume of solution in each replicate chamber was exchanged approximately six times daily.
Two 212-mL screen mesh-bottom, glass embryo cups (5.5-cm diameter) were suspended into each replicate. Twenty embryos were placed into each embryo cup (total of 40 embryos per replicate and 80 embryos per test concentration). Embryo cups were assigned to each replicate and embryos were assigned to embryo cups using random numbers. Embryo cups oscillated at one cycle per 68 seconds (2- to 7-cm water depth in embryo cup). Embryo cups were inspected daily and unfertilized eggs and damaged or fungus-infected embryos were removed. The criterion for embryo death is opaque appearance.
The surviving alevins and fingerlings were thinned (15 per replicate) using random numbers and released into the appropriate test chamber replicate on day 45 when most of the fish had swum-up. Fish that swim-up are those that occupy and are able to maintain their position in the top half of the water column and in which the yolk sac has mostly been resorbed. The number of live fingerlings was determined daily by actual count. On day 45, feeding was initiated; the fish were fed newly-hatched brine shrimp (*Artemia* sp.) three times daily on weekdays, and twice daily on weekends and holidays. The fingerlings were last fed on day 89, approximately 20 hours prior to test conclusion on the following day. Uneaten food and/or debris were removed as needed. The criteria for alevin and fingerling death was the absence of opercular movement and lack of reaction to gentle prodding.
Diluter operation was checked daily. Test solution concentrations were verified by chemical analysis immediately prior to beginning the test, at test start, approximately weekly thereafter and at test end. Before thinning, water samples (approximately 15 mL) were taken from inside the embryo cup (one embryo cup per replicate was sampled, two samples per concentration); after thinning, water samples were taken at mid-depth from each test chamber replicate (two samples per concentration). Unscheduled samples were also taken in the event that test solution analysis was needed to verify diluter operation. Loading in the "A" replicate of the water control at the end of the test was 0.181 g fish per liter passing through the test chamber in 24 hours, based on the daily turnover rate of approximately six test solution volumes. Test solutions were held between 10.6 °C and 11.7 °C (mean = 11.2 °C), and were unaerated. The diluter was wrapped in black plastic to keep the embryos and alevins in relative darkness until hatching was completed. On day 40, the black plastic was removed. A 16-hour light (43 – 65 lux) and an 8-hour dark photoperiod with 25 minutes of transitional lighting (less than 3 lux) was used throughout the remainder of the study.
Dissolved oxygen, pH and temperature were measured in both replicates of the water and dimethylformamide (DMF) controls and all test concentrations at the beginning of the test before embryos were added, weekly, at total mortality in a replicate, and at the end of the test. A continuously-recording thermometer was used to check temperature variation in one of the control replicates. Total alkalinity, EDTA hardness and conductivity were measured in one replicate of the water control at the beginning of the test, weekly, and at the end of the test. Test solutions were not aerated during the study.

Findings:

Analytical results:
Measured concentrations of lenacil in test solutions are presented in Table 2.9.2.3.1-3.

Nominal test concentrations selected for the early life stage study were 20, 50, 130, 320, 800 and 2000 µg a.s./L, plus a dilution water and a DMF control. Mean, measured concentrations of lenacil, were 31, 53, 160, 280, 640 and 1600 µg a.s./L, respectively. The control samples contained no detectable concentrations of the active ingredient. The limit of quantification (LOQ) in this analysis was 7.6 µg/L and the limit of determination (LOD) was 2.3 µg/L. All in-life data are presented in terms of mean, measured concentrations.

Table 2.9.2.3.1-3: Measured concentrations of the active ingredient lenacil in aquatic test solutions

Nominal concentration (µg a.s./L)[b]	Measured test concentration (µg a.s./L)[a] (all results reported to two significant figures)					
	Day -1[c]	Day 0	Day 7	Day 14	Day 21	Day 28
Control	<LOD[d]	<LOD	<LOD	<LOD	<LOD	<LOD
Control		<LOD	<LOD	<LOD	<LOD	<LOD
DMF control	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
DMF control		<LOD	<LOD	<LOD	<LOD	<LOD
20	35	40	32	23	26	26
20		38	33	23	27	27
50	55	62	54	41	52	46
50		64	54	40	40	44
130	150	180	160	120	150	150
130		180	160	120	160	150
320	260	310	280	220	270	240
320		290	270	230	270	230
800	620	670	610	520	580	530
800		660	610	530	630	540
2000	1400	1500	1400	1300	1500	1400
2000		1500	1400	1200	1400	1300
2000 (stock)	1400	[e]	1200	1100	1300	1100

[a] *all values are averages of the results of two injections of the sample*

[b] *lenacil contains 98.5 % of the active ingredient*

[c] *day-1 values are not included in the mean, but were analysed to confirm test solution levels prior to startup*

[d] *LOD is the Limit of Detection, 2.3 µg/L for the analyte of interest*

[e] *no sample provided for analysis*

Table 2.9.2.3.1-3 continued: Measured concentrations of the active ingredient lenacil in aquatic test solutions

Nominal concentration (µg a.s./L)[b]	Measured test concentration (µg a.s./L)[a] (all results reported to two significant figures)					
	Day 35	Day 42	Day 49	Day 56	Day 63	Day 70
Control	<LOD[c]	<LOD	<LOD	<LOD	<LOD	<LOD
Control	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
DMF control	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
DMF contro	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20	33	31	38	36	34	30
20	31	30	34	40	31	31
50	48	45	67	67	57	48
50	52	50	65	66	59	51
130	160	200	180	170	170	150
130	160	200	190	160	170	160
320	270	310	360	310	320	290
320	280	300	340	300	300	0[d]
800	620	720	810	680	700	660
800	610	710	780	690	710	680
2000	1500	1700	2000	1700	1800	1600
2000	1600	1700	1900	1600	1700	1600

2000 (stock)	1500	1700	1900	1600	1800	1600

[a] *all values are averages of the results of two injections of the sample*

[b] *lenacil contains 98.5 % of the active ingredient*

[c] *LOD is the Limit of Detection, 2.3 µg/L for the analyte of interest*

[d] *The zero value was possibly the result of a sampling error. Resampling 3 days later gave values of 276 and 267 for the two replicates of this level. The original value was confirmed with the back-up, which also gave a zero value.*

Table 2.9.2.3.1-3 continued: Measured concentrations of the active ingredient lenacil in aquatic test solutions

Nominal concentration (µg a.s./L)[b]	Measured test concentration (µg a.s./L)[a] (all results reported to two significant figures)					
	Day 76	Day 84	Day 90	Mean measured	80-120 % of mean measured	Standard deviation
Control	<LOD[c]	<LOD	<LOD			
Control	<LOD	<LOD	<LOD			
DMF control	<LOD	<LOD	<LOD			
DMF contro	<LOD	<LOD	<LOD			
20	31	26	30	31	25-37	± 4.63
20	28	27	29			
50	49	47	57	53	42-64	± 8.15
50	55	48	56			
130	140	140	160	160	130-190	± 19.7
130	150	140	160			
320	270	280	310	280	220-340	± 31.8
320	270	280	300			
800	580	640	680	640	510-770	± 71.6
800	600	630	670			
2000	1500	1500	1700	1600	1300-1900	± 183
2000	1400	1500	1700			
2000 (stock)	1300	1500	1600	1500	1200-1800	± 259

[a] *all values are averages of the results of two injections of the sample*

[b] *lenacil contains 98.5 % of the active ingredient*

[c] *LOD is the Limit of Detection, 2.3 µg/L for the analyte of interest*

In-life data:

A summary of the in-life data is presented in Table 2.9.2.3.1-4.

Hatch rate and survival averaged 86 % and 96 – 100 %, respectively in the water and DMF controls. There was no significant difference between the two controls for all endpoints with exception of length and weight, which were greater for the DMF control than for the water control. So, with the two exceptions noted, the controls were combined for further analysis.

Survival:

There were no statistical significant differences for the percentage hatch and survival between any of the test concentrations and the controls. The NOEC for cumulative dead eggs and number of larvae at end of hatching exceeded 1600 µg a.s./L. The NOEC for number of abnormal larvae from hatching to thinning exceeded 1600 µg a.s./L. The NOEC for number of dead larvae from thinning to test end exceeded 1600 µg a.s./L. A total of five fish was missing from two replicate chambers (one from 31 µg a.s./L, rep. B, and four from 640 µg a.s./L, rep. B) during the phase of the study from thinning to test end. The fish were presumed to be dead. The worst-case assumption that the loss of the fish was due to toxicant-related causes did not affect the statistical findings on fingerling survival at test end since this endpoint did not show a dose-response.

Sublethal effects and abnormalities:

No abnormalities were seen at test end while the occurrence of abnormalities (pale color, small size, curled fish) among the alevins at time of thinning (day 45) was low and averaged 3 % (in the DMF control) or less. The NOEC exceeded 1600 µg a.s./L

First day of hatching:
There were no statistical significant differences for the first day of hatching between any of the test concentrations and the controls. The NOEC exceeded 1600 µg a.s./L.

Last day of hatching:
Last day of hatching showed a small but statistically significant decrease with increasing test concentration. The difference between the first and last day of hatching was also smaller at 640 and 1600 µg a.s./L (one day) than at the remaining test concentrations and the controls (2 – 3 days). The NOEC was reported at 280 µg a.s./L (based on Jonckheere-Terpstra test).

First day of swim-up:
The first day of swim-pup showed a small but statistically significant decrease with increasing test concentration. The NOEC was reported at 280 µg a.s./L (based on Jonckheere-Terpstra test).

Weight:
The DMF control for mean weight was significantly higher than the water control mean weight, so only the DMF control was used for further analysis. The weight of surviving fingerlings at test end did not follow a monotonic dose-response. The NOEC was reported at 280 µg a.s./L (based on Jonckheere-Terpstra test).

Length:
The DMF control for mean length was significantly higher than the water control mean length, so only the DMF control was used for further analysis. The fingerling length decreased with increasing test concentration. An average length of 2.9, 2.9 and 2.9 cm was calculated for each of the 280, 640 and 1600 µg a.s./L test concentrations, compared to 3.3 cm for the DMF control. Length data was normally distributed and of homogeneous variances across concentrations. Dunnett's test found the 280 µg a.s./L concentration means to be significantly smaller than the DMF control mean. Since there was significant rep-to-rep variability in these data, Williams' test was applied to model the expected monotone dose-response, while capturing the effect of both variance components. By that test, the NOEC was reported at 31 µg a.s./L. There was a clear downward trend in the length data.

Table 2.9.2.3.1-4: Summary of hatching, survival, abnormalities, swim-up and growth for the 90-day early life-stage test with rainbow trout (*Oncorhynchus mykiss*)

Mean measured concentrations (µg a.s./L)	Mean hatching day[a]		Percent hatch[a]	Hatch to thinning[b]		Mean First Day of Swim-up[b]
	Start	End		Survival	Abnormalities	
				Number alive/Total (%)	Number affected/Number alive (%)	
Control	28	30	86	69/69 (100)	0/69 (0)	42
DMF control	28	30	86	66/69 (96)	2/66 (3.0)	42
31	27	30	85	68/68 (100)	1/68 (1.5)	42
53	27	29	84	67/67 (100)	1/67 (1.5)	42
160	27	30	83	64/66 (97)	1/64 (1.6)	41
280	28	30	89	71/71 (100)	1/71 (1.4)	42
640	28	29*	79	61/63 (97)	0/61 (0)	41*
1600	28	29*	88	70/70 (100)	0/70 (0)	41*

[a] *Based on four replicates per concentration; last observation was made at end of hatching. Last day of hatching was defined as day when live eggs remaining hatch to yield live larvae.*
[b] *Based on four replicates per concentration; last observation was made on day 45*
** Significantly different from combined controls ($p < 0.05$)*

Table 2.9.2.3.1-4 continued: Summary of hatching, survival, abnormalities, swim-up and growth for the 90-day early life-stage test with rainbow trout (*Oncorhynchus mykiss*)

Mean measured concentrations (µg a.s./L)	Thinning to test end[c]		Standard length (cm)	Wet weight (grams)
	Survival	Abnormalities		
	Number alive/Total (%)	Number affected/Number alive (%)	Mean (Standard Deviation)	
control	30/30 (100)	0/30 (0)	3.2 (0.2)	0.4974 (0.0702)
DMF control	30/30 (100)	0/30 (0)	3.3 (0.1)	0.5644 (0.0639)
31	28/30 (93)[d]	0/28 (0)	3.2 (0.2)	0.5596 (0.0831)
53	29/30 (97)	0/29 (0)	3.0 (0.1)	0.5049 (0.0499)
160	30/30 (100)	0/30 (0)	3.1 (0.2)	0.5285 (0.0566)
280	29/30 (97)	0/29 (0)	2.9 (0.1)*	0.5556 (0.0661)
640	26/30 (87)[e]	0/26 (0)	2.9 (0.2)*	0.5218 (0.1178)*
1600	30/30 (100)	0/30 (0)	2.9 (0.2)*	0.5199 (0.1010)*

[c] Based on day 90 data and two replicates per concentrtation.
[d] One fish was dead, and one fish was missing and presumed dead.
e Four fish were missing and presumed dead.
** Significantly different from DMF control ($p < 0.05$); trend testing found length at 160 and 53 µg/L also to be significantly different from the DMF control*

Lenacil had no effect on the hatch rate, first day of hatching, survival and abnormalities at the concentrations tested. Statistical analysis found the differences in the last day of hatching, first day of swim-up, and weight of surviving fingerlings at test end at 640 and 1600 µg a.s./L to be significant (i.e., NOEC for these endpoints was 280 µg a.s./L). The differences in length of suriving fingerlings at test end at 280 µg a.s./L and above were found to be significant (i.e., NOEC for length was 160 µg a.s./L) by Dunnett's test, and at 53 µg a.s./L and above were found to be significant (i.e., NOEC for length was 31 µg a.s./L) by Williams's test.

The biologically significant NOEC is considered to be 160 µg a.s./L. This conclusion is based on:

(1) The length data showed very little variation, with the standard error of the mean length only approximately 2 % of the DMF control mean length. This small variation made the Williams' test extremely sensitive, so that the 6 % and 5 % reductions in mean length at 53 and 160 µg a.s./L were found to be statistically significant although of doubtful biological relevance;
(2) The length effect was not supported by an effect on weight and, moreover, length was not highly correlated with weight (correlation coefficient of 0.62);
(3) The difference between the 31 µg a.s./L mean length and the DMF control mean length was 2.3 % of the control mean value (compared to 9.4, 9.8 and 11.8 % at 280, 640 and 1600 µg a.s./L, and that length of surviving fingerlings at test end is the most sensitive endpoint.

Conclusions:
The NOEC (No Observable Effect Concentration) of lenacil in a 90-day early life-stage toxicity study using rainbow trout (*Oncorhynchus mykiss*) was 160 µg a.s./L, based on mean, measured concentrations and standard length of surviving fingerlings at the end of the study. The MATC (Maximum Acceptable Toxicant Concentration) was 212 µg a.s./L, and the LOEC (Lowest Observed Effect Concentration) was 280 µg a.s./L. The EC_{50} could not be computed since the highest percent affected in any concentration for the endpoints measured did not exceed 21 %.

RMS comments and conclusions :
The validity criteria of the most recent version of OECD Test Guideline 210 are met:

- The dissolved oxygen concentration was > 60 % of the air saturation value throughout the test (measured: 8.5 - 11.4 mg/L O_2, which corresponds to approximately 77 – 103 % of the air saturation value at 10°C)
- The temperature did not differ by more than ± 1.5 °C between test chambers or between successive days at any time during the test.
- The overall survival of fertilised eggs and post-hatch success in the control was ≥ 75 % (measured: 86 % survival of fertilised eggs, 100 % post-hatch success)

Therefore, the study is still considered acceptable and the endpoints reliable for use in the risk assessment.

The analytical method used could not be fully validated according to the EU Guidance SANCO/3029/99 rev. 4. No validation of the HPLC-UV method occurred in the study reports. The analytical phase only reports the results of the measured concentrations with some data on linearity and chromatograms. It is therefore difficult to state if the method was "fit for purpose" (please refer to Vol. 3 (CA), Section B.5.1.2.6 – studies no. 8.08, for further details). However, other HPLC-UV methods were validated with a LOQ that would cover the concentrations tested in this study. Further, the results for this study are not critical for the risk assessment. Therefore, the submission of additional validation data is not considered necessary.

In accordance with the new data requirements (Commission Regulation EU No 283/2013), the EC_{10} and EC_{20} values should be calculated. Where these values cannot be estimated, an explanation should be provided. As the present study was already submitted for the initial Annex I inclusion of lenacil, this was not addressed in the study report. However, the RMS performed the additional calculations to determine the EC_{10} and EC_{20}. For the most sensitive endpoint (standard length of surviving fingerlings at the end of the study), the percent reduction compared to the control was the same for the three highest test concentrations, and amounted only 11%. Therefore, EC_{10} and EC_{20} values cannot reliably be estimated for this parameter.

The most sensitive endpoint (standard length of surviving fingerlings at the end of the study), which are based on mean measured concentrations, will be considered in the risk assessment:

NOEC (*Oncorhynchus mykiss*, 90 d) = 0.160 mg a.s./L

2.9.2.3.2 Chronic toxicity to aquatic invertebrates

The information below was extracted from Volume 3 (CA), Section B.9.2 'Effect on aquatic organisms'. Two long-term toxicity studies with fish are available for lenacil. However, only one of these studies was acceptable.

Report:	**CA8.2.5.1/01. Hutton D.G. (1989b)** **Chronic toxicity of DPX-B634-84 (Lenacil) to *Daphnia magna***

Report No.:	HLR 130-89
Guidelines:	OECD 202 Part II
GLP:	Yes
Previous evaluation:	In DAR (November 2007); not relevant for renewal application
Materials and methods:	
Test substance:	lenacil technical, blended batch numbers: 8802 and 8805, chemical purity: 95.1 %
Test species:	*Daphnia magna*
Number of organisms, age:	10 replicates with 4 daphnids per concentration (40 daphnids per treatment), less than 24 hours old at test start. The parent daphnids were 19 days old when the young were collected to start this test.
Type of test:	semi-static chronic toxicity test (21 days) (medium renewal 3 times per week)
Applied and measured concentrations:	
Nominal concentrations:	control; 0.15, 0.30, 0.6, 1.2, 2.5, 5.0 mg a.s./L
Measured concentrations:	0.00; 0.08, 0.13, 0.28, 0.48, 0.97, 1.7 mg a.s./L
Test conditions:	temperature: 19.6 – 20.5 °C pH: 7.2 - 7.6 (new medium), 7.1 - 7.4 (old medium) dissolved oxygen: fresh medium: 93 – 96 % O_2 saturation (8.6 - 8.8 mg/L O_2); old medium: 46 – 90 % O_2 saturation (4.2 - 8.3 mg/L O_2) total hardness : 77 ± 2 mg/L $CaCO_3$ photoperiod : 16/8 hours light/dark cycle light intensity : 560 lux

Test medium:	filtered fish tank water (i.e. Haskell Laboratory well water that had been used for fathead minnow culturing in a flow-through system, then filtered through a 0.8 micron filter)
Feeding:	three times per week (medium renewals) with a trout chow and yeast diet
Analytical methods:	HPLC-UV.
Statistics:	Survival data were analysed by the Fisher's Exact test to determine the NOEC/LOEC and by Probit analysis to derive the EC50. The EC50 for reproduction was calculated by Probit analysis using failure to produce embryos as the observation criterion. Reproduction data were analysed by one-way analysis of variance and Dunnett's method for multiple comparison (for total number of young produced and first day of reproduction) or by the Kruskal-Wallis test and its associated multiple comparison (for number of young produced per surviving parent).

Test procedure:

Neonate daphnids, less than 24 hours old at the start of the test, were exposed to the test item for a period of 21 days. They were kept in 250 mL glass beakers filled with approximately 200 mL test solution. Each test concentration was replicated ten times with four *Daphnia magna* per beaker for survival and reproduction measurements. The daphnids were added to the test beakers by randomly picking them from a single transfer tank. The parent *Daphnia magna* were transferred to fresh test solutions containing food three times per week. Fresh stock solutions were prepared on each renewal day. The source of the dilution water was filtered fish tank water (i.e. Haskell Laboratory well water that had been used for fathead minnow culturing in a flow-through system, then filtered through a 0.8 micron filter). Similar replicates of a dilution water control were set up as a measure of the quality of the test organisms, suitability of the dilution water, etc. The test beakers were not aerated.

Survival and reproduction data were recorded at the time of transferring the daphnids to fresh test solution (three times per week). Temperature was monitored continuously. Dissolved oxygen and pH were measured on the old and new test solutions on feeding and renewal days, as appropriate. Alkalinity, hardness and condicutivity of the water control were measured weekly. Analysis for the test substance in each test concentration was done at day 0, 2, 5, 7, 14 and 21 of the study. On days 7 and 14, both 'fresh' and 'old' samples were analysed.

Findings:

Analytical results:

The analytical results are shown in Table 2.9.2.3.2-1. Mean measured concentrations ranged from 34 to 53% of nominal concentrations. Lenacil proved extremely difficult to dissolve in the test water, and as a result, the measured test concentrations were significantly lower than nominal. The results were therefore expressed based on mean measured concentrations.

Table 2.9.2.3.2-1: Measured concentrations of lenacil technical in the test solutions

Nominal concentration (mg a.s./L)	Measured test concentration (mg a.s./L)								
	Day 0 fresh	Day 2 old	Day 5 old	Day 7 old	Day 7 fresh[a]	Day 14 old	Day 14 fresh[a]	Day 21 old	Arithmetic mean
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.15	0.05	0.05	0.04	0.09	0.16	0.14	0.05	0.11	0.08
0.30	0.07	0.05	0.09	0.18	0.22	0.16	0.09	0.20	0.13
0.60	0.17	0.12	0.18	0.35	0.48	0.34	0.20	0.43	0.28
1.2	0.33	0.24	0.33	0.62	0.62	0.63	0.38	0.70	0.48
2.5	0.85	0.69	0.65	1.0	1.5	1.0	0.72	1.4	0.97
5.0	1.6	1.5	1.5	1.8	2.3	1.5	1.7	1.8	1.7

[a]*On days 7 and 14, reported 'fresh' values are from samples taken before food was added*

Biological results:

The EC_{50} for immobilisation was calculated to be 1.2 mg a.s./L. The 21-day survival rates at concentrations of 0.97 mg a.s./L and higher were significantly lower than the control group. The NOEC for survival was 0.48 mg a.s./L.

The EC_{50} for reproduction was calculated to be 1.1 mg/L. The time to first brood was significantly different from the control at 1.7 mg a.s./L

Statistically significant ($p < 0.05$) reductions in total numbers of juveniles occurred at the treatment levels of 0.08, 0.97 and 1.7 mg a.s./L. The number of juveniles per adult was significantly significantly reduced compared to the control at treatment levels of 0.08 and 1.7 mg a.s./L. The significant differences at 0.08 mg a.s./L were considered not to be treatment-related since no effects were observed at the higher treatment levels of 0.13, 0.28 and 0.48 mg a.s./L. The NOEC for reproduction was therefore set at 0.48 mg a.s./L.

No males, winter eggs, or immobilized young were observed at any treatment level or in the control group during the test and no eggs were observed on the bottom at any treatment level during the test.

Table 2.9.2.3.2-2: Summary of effects of lenacil during the reproduction study with *Daphnia magna*

Measured test concentration (mg lenacil/L)	Adult survival (%)	Reproductive parameters		
		Time to first brood (days)	Total number of juveniles	Juveniles per adult
Control	85	9.0	535	139
0.08	70	8.6	324*	91*
0.13	80	8.8	397	101
0.28	80	9.0	511	134
0.48	75	9.0	551	144
0.97	55*	9.0	337*	110
1.7	35*	9.9*	126*	49*

* *Significantly different ($p < 0.05$) from the control group*

Conclusions:
Discounting the reproduction data seen for the 0.08 mg/L test concentration, the most sensitive parameters for the effect of lenacil on *Daphnia magna* were survival and total offspring. In both cases, the NOEC was 0.48 mg a.s./L (measured concentration). The EC_{50} (immobilisation) was 1.2 mg a.s./L (measured concentration) and the EC_{50} (reproduction) was 1.1 mg a.s./L (measured concentration).

RMS comments:
OECD Test Guideline 202 Part II was replaces by Test Guideline 211. The following validity criteria of the most recent version of this guideline are met:

- The mean number of live offspring produced per parent animal surviving at the end of the test in the control should be ≥ 60 (measured: 139).

However, the coefficient of variation of cumulative offspring per survivor was higher than the maximum level of 25 % as recommended by the test guideline (measured value of 29%). Further, the mortality of the parent animals in de control exceeded the maximum allowed level of 20 % in 4 out of 10 replicates. Therefore, this study is no longer considered acceptable for use in the risk assessment.

Report:	**CA8.2.5.1/02. Renner P. (2016b)** **Toxicity of lenacil technical to *Daphnia magna* in a 21-day semi-static reproduction test**
Report No.:	15 10 48 032 W
Guidelines:	OECD 211 (2012)
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Materials and methods:	
Test substance:	lenacil technical, batch no.: JUL14HE010, chemical purity: 98.82 %
Test species:	*Daphnia magna*

Number of organisms, age:	10 replicates with 1 daphnid per concentration, less than 24 hours old at test start and not first brood progeny
Type of test:	semi-static chronic toxicity test (21 days) (medium renewal 3 times per week)
Applied and measured concentrations:	
Nominal concentrations:	control; 0.01, 0.04, 0.13, 0.40 and 1.20 mg a.s./L
Measured concentrations:	0; 0.019, 0.054, 0.129, 0.389 and 1.250 mg a.s./L
Test conditions:	temperature: 19.7 – 20.6 °C pH: 7.18 – 7.43 oxygen content: 8.56 – 9.50 mg/L O_2 total hardness: 231 – 233 m/L as $CaCO_3$ photoperiod: 16 hours light: 8 hours dark cycle light intensity: 1406 lux
Test medium:	Elendt M4 medium
Feeding:	three times per week (medium renewals) with living algal cells of *Desmodesmus subspicatus*, the supplied diet (concentrated algal suspension) was based on the amount of organic carbon (C) provided to each parent animal: between 0.1 and 0.2 mg C/*Daphnia*/day
Analytical methods:	standard analytical methods were used to verify concentrations of the test item in the test solutions at the start and at the end of the test and once a week during the test in the aged and fresh prepared test solutions.
Statistics:	Data was analysed by appropriate statistical methods, using ToxRat Professional 3.2.1 (2015). The NOEC, LOEC and effect concentration EC_x at 21 days for reproductive output of animals, for mortality and intrinsic rate of parent animals were calculated. Additionally, LOEC/NOEC for the time of first reproduction of parent animals was determined.

Test procedure:

The aim of the test was to assess the effects of the test item on reproductive output of *Daphnia magna*. Neonate female daphnids (parent animals), aged less than 24 hours old at test start, were exposed to the test item added to test medium at a range of concentrations. The test duration was 21 days.

The offspring produced by each parent animal were removed and counted daily from the appearance of the first brood to prevent them consuming food intended for the adult. Mortality among the parent animals was recorded daily, at least as the same times as offspring are counted.

The test medium was renewed three times per week. Therefore, a second series of test vessels were prepared and the parent animals were transferred to them by a glass pipette of suitable diameter. The volume of the medium transferred with the *Daphnia* was minimised.

Dissolved oxygen concentration, pH values and water hardness (control and treatment group 0.40 mg a.s./L nominal) of test solutions were measured at test start, at each renewal and at test end in fresh and aged test solutions. Temperature was measured continuously using the equipment of the climatic chamber. Test solution concentrations were determined at test start, at week 1, week 2 and test end (week 3) in fresh and aged test solutions. Therefore, specimens for analysis were taken at the same time of a day. Test was terminated 21 days after parent animals have been transferred into test vessels.

At test end, total number of living offspring per parent animal alive was assessed. Reproductive output of exposed animals was compared to control level in order to determine NOEC and LOEC. Data wer modelled to estimate effect concentrations EC_{10}, EC_{20} and EC_{50}. Survival of parent animals and the time point of first brood appearance was documented.

The coefficient of variation on the mean number of living offspring per parent animal in control should be ≤ 25 %. This was documented by using individually held animals.

Algal suspension of *Desmodesmus* was added as food to result 0.1 – 0.2 mg/Carbon/*Daphnia*/day.

Findings:

Analytical results:

The measured test concentrations of lenacil technical in test solutions range from 95.2 to 133.3 % in fresh samples and 94.7 to 147.3 % in aged samples, respectively. Therefore, results are given as nominal and mean measured concentrations.

Table 2.9.2.3.2-3: Measured concentrations (mg a.s./L) of lenacil technical in the test solutions

Expected nominal concentration [a] (µg a.s./L)	0h fresh		1st week old		1st week fresh		2nd week old	
	Meas. conc. (µg a.s./L)	% of nominal	Meas. conc. (µg a.s./L)	% of nominal	Meas. conc. (µg a.s./L)	% of nominal	Meas. conc. (µg a.s./L)	% of nominal
Control	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-
14.63	17.20	117.6	19.92	136.2	18.44	126.1	18.63	127.4
43.92	52.35	119.1	56.51	128.7	52.70	120.0	55.10	125.5
131.8	125.4	95.2	132.29	100.4	126.5	96.0	124.76	94.7
395.3	401.9	101.7	376.80	95.3	389.4	98.5	441.30	111.6
1185.2	1193.5	100.6	1308.18	110.3	1216.6	102.6	1232.04	103.9

Expected nominal concentration [a] (µg a.s./L)	2nd week fresh		3rd week old		3rd week fresh		Test end	
	Meas. conc. (µg a.s./L)	% of nominal	Meas. conc. (µg a.s./L)	% of nominal	Meas. conc. (µg a.s./L)	% of nominal	Meas. conc. (µg a.s./L)	% of nominal
Control	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-
14.63	19.47	133.1	21.55	147.3	18.30	125.1	18.28	124.9
43.92	57.85	131.7	60.29	137.3	53.08	120.9	54.96	125.1
131.8	153.21	116.3	148.39	112.6	153.16	116.2	146.29	111.0
395.3	466.65	118.1	528.11	133.6	453.10	114.6	491.59	124.4
1185.2	-	-	-	-	-	-	-	-

[a]The expected nominal concentration was calculated based on the nominal concentration and a water solubility for lenacil of 3.6 mg a.s./L

Biological results:

The results are summarized in Table 2.9.2.3.2-4.

Parental mortality of 30 % and 100 % occurred in the two highest concentrations applied (0.40 and 1.20 mg a.s./L, nominal or 0.389 and 1.250 mg a.s./L, mean measured) evidently following a concentration-response pattern. Therefore, parent mortality was assigned as an effect of the test item. The replicates with parental mortality were not excluded from statistical analysis and were reported as cumulative offspring per introduced parent (additionally the cumulative offspring per survived parent was reported in Table 2.9.2.3.2-4).

Table 2.9.2.3.2-4: Effects of lenacil on *Daphnia magna* survival, reproduction and growth after 21-day exposure

Treatment group	Nominal concentration lenacil technical (mg a.s./L)					
	Control	0.01	0.04	0.13	0.40	1.20
	Mean measured concentrations lenacil technical (mg a.s./L)					
	control	0.019	0.054	0.129	0.389	1.250
Total number of living offspring 21 days after application (average introduced parents)	84.7	83.8	33.4	15.5	13.4	- [1]
SD	8.12	7.63	8.90	10.73	11.28	- [1]
Reduction (%) of cumulative offspring per introduced parent	-	1.10	60.6+	81.7+	84.2+	100.0+
Mortality of parent *Daphnia** (%)	0.0	0.0	0.0	0.0	30.0+	100.0+
Mean of Intrinsic rate r	0.305	0.300	0.203	0.151	0.140	- [1]
Inhibition of Intrinsic rate r (%)	-	1.60	33.4+	50.6+	54.1+	- [1]

SD standard deviation

+ significantly different from control (Multiple Sequentially-rejective Welch's t-test, α = 0.05, one sided smaller;

** Step-down Cochran-Armitage Test Procedure, α = 0.05, one sided greater)*

[1] 100 % mortality

Mean cumulative offspring per introduced parent

The NOEC (no observed effect concentration) 21 days after application was determined to be 0.01 mg a.s./L, nominal (0.019 mg a.s./L, mean measured). Hence, the LOEC (lowest observed effect concentration) was

determined to be 0.04 mg a.s./L, nominal (0.054 mg a.s./L, mean measured). An EC_{50} of 0.038 mg a.s./L, nominal (0.051 mg a.s./L, mean measured) was calculated.

Mortality of parents:
The NOEC for mortality of parent animals was determined to be 0.13 mg a.s./L, nominal (0.129 mg a.s./L, mean measured). Hence, the LOEC of 0.40 mg a.s./L, nominal (0.389 mg a.s./L, mean measured) was determined. An EC_{50} of 0.503 mg a.s./L, nominal (0.432 mg a.s./L, mean measured) was calculated.

Age at first reproduction:
The NOEC for the age at first reproduction was determined to be 0.01 mg a.s./L, nominal (0.019 mg a.s./L, mean measured). Hence, the LOEC of 0.04 mg a.s./L, nominal (0.054 mg a.s./L, mean measured) was determined. An EC_{50} of 0.178 mg a.s./L, nominal (0.196 mg a.s./L, mean measured) was calculated.
Abnormalities:
At any assessment, no visible signs of abnormalities of surviving animals were observed. Incidents in the course of the test which might have influenced results were not observed. No aborted brood and ephippia were observed in all treatment groups.

Validity criteria:
All validity criterioa for this type of study were met as followed:
Mortality of the parent animals (female *Daphnia*) at the test end: ≤ 20 % (observed: 0.0 % in the control). Mean number of live offspring produced per parent animal surviving at the end of the test: ≥ 60 (observed: 84.7 in controls). The coefficient of variation of cumulative offspring per survivor was ≤ 25 % with an actual value of 9.6 %.

Table 2.9.2.3.2-5: Effects of the test item lenacil technical: summary of statistical analysis

Effect concentration	**Lenacil technical (mg a.s./L)**			
	Mean cumulative offspring per introduced parent 21 days after application		**Mortality of parent *Daphnia***	**Intrinsic rate***
NOEC				
Test item, nominal	**0.01**		**0.13**	**0.01**
Test item, mean measured	0.019		0.129	0.019
LOEC				
Test item, nominal	**0.04**		**0.40**	**0.04**
Test item, mean measured	0.054		0.389	0.054
EC	**EC_{10}**	**EC_{20}**	**EC_{50}**	**EC_{50}**
And 95 % confidence intervals (lower – upeer)				
Test item, nominal	**0.008**	**0.038**	**0.503**	**0.178**
	(0.006 – 0.010)	(0.034 – 0.042)	(0.341 – 1.261)	(0.128 – 0.278)
Test item, mean measured	0.017	0.051	0.432	0.186
	(0.014 – 0.020)	(0.047 – 0.054)	(n.d.)	(0.149 – 0.245)

** the intrinsic rate is considered as a measure of population growth based on reproductive output and age-specific mortality. In steady state populations the intrinsic rate of population increase is zero, for growing populations it is positive and for shrinking populations it is negative*
n.d. not determined due to mathematical reasons or inappropriate data

Conclusions of the study:
After 21 days of exposure, the effects on mean cumulative offspring per introduced parent resulted in a NOEC of 0.01 mg a.s./L (nominal), LOEC of 0.04 mg a.s./L (nominal), EC_{10} of 0.008 mg a.s./L (nominal) and EC_{50} of 0.038 mg a.s./L (nominal). After 21 days of exposure, the effects on mortality of parent *Daphnia* resulted in a NOEC of 0.13 mg a.s./L (nominal), LOEC of 0.40 mg a.s./L (nominal) and EC_{50} of 0.503 mg a.s./L (nominal).

RMS comments:
The validity criteria of the most recent version of OECD Test Guideline 211 are met:

- The mortality of the parent animals (female Daphnia) did not exceed 20 % (measured: 0.0 %)
- The mean number of live offspring produced per parent animal surviving at the end of the test should be ≥ 60 (measured: 84.7 in control). The coefficient of variation of cumulative offspring per survivor was ≤ 25 % with an actual value of 9.6 %.

Consequently, this study is acceptable for use in the risk assessment.

The analytical method used could be fully validated according to the EU Guidance SANCO/3029/99 rev. 4., and is therefore considered "fit for purpose". The LOQ was set at 7.02 µg/L (please refer to Vol. 3 (CA), Section B.5.1.2.6 – study no. 11, for further details). The working range covers the test concentrations (taking into account sample dilution in some cases).

The most sensitive endpoints (for cumulative offpring per introduced parent after 21 days), based on mean measured concentrations, will be considered in the risk assessment:

EC_{10} (*Daphnia magna*, 21d) = 17 µg a.s./L
NOEC (*Daphnia magna*, 21d) = 19 µg a.s./L

2.9.2.3.3 Chronic toxicity to algae or aquatic plants

The information below was extracted from Volume 3 (CA), Section B.9.2 'Effect on aquatic organisms'. In total, nine studies on the toxicity of lenacil to algae are available. Two of these studies are however not acceptable. For aquatic plants, the toxicity of lenacil was tested in three studies, each performed with a different species.

Report:	**CA8.2.6.1/01. Flatman D. (2003c)** **Lenacil technical, algal growth inhibition assay *Selenastrum capricornutum***

Report No.:	ACD 034/022511
Guidelines:	92/69/EEC, method C.3 (1992), OECD 201 (1984), draft US EPA OPPTS 850.5400 (1996)
GLP:	Yes
Previous evaluation:	In DAR (November 2007); relevant for renewal application
Material and Methods:	
Test substance:	lenacil (Batch no.: 141712003, chemical purity: 98.6%)
Test species:	*Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*), unicellular freshwater green alga
Number of organisms:	3 replicates per test concentration, 6 replicates in the control and solvent control; 1 x 10^4 cells/mL at initiation
Type of test:	96 h static toxicity test
Applied and measured concentrations:	
Nominal test concentrations:	control; solvent control (dimethylformamide); serial dilutions (0.010, 0.022, 0.046, 0.10, 0.22, 0.46, 1.0 %) of a nominal concentration of 10 mg a.s./L, equivalent to 1.0, 2.2, 4.6, 10, 22, 46 and 100 µg a.s./L
Mean measured concentrations: 0.00; 0.00; 0.41, 0.79, 1.5, 3.4, 8.1, 17 and 36 µg a.s./L	
Test conditions:	Sterile synthetic nutrient test medium Conical flasks of 250 mL, containing 100 mL of test or control culture temperature: 22 – 24 ºC pH: 7.1 - 7.4 (initial), 7.6 - 7.8 (final) light regime: continuous illumination light intensity : 4210 - 4740 lux Incubation in an orbital shaker (140 cycles/min)
Analytical methods:	HPLC/UV
Statistics:	logistic regression for the calculation of the EC_{50} values. Williams test for the determination of the NOEC

Test procedure:

Samples were taken at 0, 24, 48, 72 and 96 hours and the cell densities determined by direct counting using a Coulter Multisizer II particle counter. Severely inhibited cultures were recultured to determine if the inhibitory effect of the test substance was algicidal or algistatic. Aliquots (0.5 mL) were taken from each replicate culture of the solvent control and the test concentrations resulting in severe growth inhibition (8.1, 17 and 36 µg a.s./L). The replicates were pooled, 200 mL of fresh sterile nutrient medium was added and the cultures were incubated at 22-24 °C for a further 9 days.

Findings:

Analytical results:

The measured concentrations at the beginning and the end of the exposure period are shown in Table 2.9.2.3.3-1. The nominal concentrations were not reached at the start of the test. However, the concentrations measured at the beginning of the test remained stable until the end of the exposure period. The results are expressed based on mean measured concentrations.

Table 2.9.2.3.3-1: Measured concentration of lenacil in the test media collected during the toxicity test with *Pseudokirchneriella subcapitata.*

Nominal concentration of test item (µg a.s./L)	Measured concentration of test item (µg a.s./L)					
	0 hour	% of nominal	96 hours [1]	% of nominal	Geometric Mean	% of nominal
Solvent control	<LOD [2]	-	< LOD [2]	-	< LOD [2]	-
1.0	0.4127	41.3	0.3989	39.9	0.41	40.6
2.2	0.8678	39.4	0.7084	32.2	0.79	35.6
4.6	1.453	31.6	1.501	32.6	1.5	32.1
10	3.962	39.6	2.803	28.0	3.4	33.3
22	8.234	37.4	8.056	36.6	8.1	37.0
46	16.52	35.9	17.07	37.1	17	36.5
100	34.88	34.9	38.00	38.0	36	34.4

[1]Samples were not filtered to remove algal cells before analysis; [2]LOD = Limit of Detection = 0.16 µg a.s./L

Biological results:

The effects on algal growth are summarized in Table 2.9.2.3.3-2 and Table 2.9.2.3.3-3. There was no statistically significant difference between the solvent control and the control for either biomass (area under the growth curve) or the growth rate at 72 and 96 hours. No statistically significant effect on growth rate or biomass were found for a test concentration of up to 3.4 µg a.s./L after 72h and 96h.

For biomass (area under the growth curve), the E_bC_{50} was calculated to be 7.7 µg a.s./L (95% confidence limits 6.7-8.7 µg a.s./L) and 6.5 µg a.s./L (95% confidence limits 5.8-7.3 µg a.s./L) for 72h and 96 h, respectively.

For growth rate, the E_rC_{50} was calculated to be 16 µg a.s./L (95% confidence limits 15-17 µg a.s./L) and 15 µg a.s./L (95% confidence limits 14-16 µg a.s./L) for 72h and 96 h, respectively.

Table 2.9.2.3.3-2: Reduction in biomass (area under the growth curve) for *Pseudokirchneriella subcapitata* exposed to a range of concentrations of lenacil.

Mean measured concentration (µg a.s./L)	Mean cell density (x 10^4 cells/mL)				Calculated mean area under the growth curve (A)		Percent inhibition [1]	
	24h	48h	72h	96h	0-72h	0-96h	0-72h	0-96h
Control	4.656	20.228	87.028	315.500	-	-	-	-
Solvent control	4.772	20.778	85.369	309.690	16	63	-	-
0.41	5.064	21.662	91.736	313.927	17	65	-6.8	-3.7
0.79	5.298	22.654	92.428	330.493	17	68	-8.9	-7.5
1.5	5.053	21.956	90.405	323.280	17	66	-6.2	-5.1

3.4	4.725	19.539	78.028	281.800	14	57	7.7	8.7
8.1	3.793	12.699	30.641	74.208	6.9	19	56*	69*
17	2.738	4.021	8.250	15.907	1.9	4.5	88*	93*
36	2.046	2.259	2.627	2.544	0.67	1.0	96*	98*

** Statistically significant difference compared to the control (Williams-test; α = 0.05); [1]negative values indicate an increase relative to the control*

Table 2.9.2.3.3-3: Reduction in average specific growth rate for *Pseudokirchneriella subcapitata* exposed to a range of concentrations of lenacil.

Mean measured concentration (µg a.s./L)	Mean cell density (x 10^4 cells/mL)				Calculated average specific growth rate µ ($hour^{-1}$)		Percent inhibition [1]	
	24h	48h	72h	96h	0-72h	0-96h	0-72h	0-96h
Control	4.656	20.228	87.028	315.500	-	-	-	-
Solvent control	4.772	20.778	85.369	309.690	0.059	0.058	-	-
0.41	5.064	21.662	91.736	313.927	0.061	0.058	-2.3	-0.68
0.79	5.298	22.654	92.428	330.493	0.060	0.058	-1.1	-0.48
1.5	5.053	21.956	90.405	323.280	0.061	0.059	-2.3	-1.4
3.4	4.725	19.539	78.028	281.800	0.058	0.057	2.5	2.1
8.1	3.793	12.699	30.641	74.208	0.045	0.043	25*	26*
17	2.738	4.021	8.250	15.907	0.027	0.027	55*	54*
36	2.046	2.259	2.627	2.544	0.012	0.0084	80*	86*

** Statistically significant difference compared to the control (Bonferroni t-test; α = 0.05); [1]negative values indicate an increase relative to the control; percentage inhibition values were calculated using non-rounded data*

All test and control cultures were inspected microscopically at 96 hours. No abnormalities were detected in any of the cultures examined. No cultures showed any signs of contamination by foreign algal cells or bacteria.

Following transfer to unamended control medium, regrowth was observed after 9 days for cultures previously inhibited by exposure to 8.1, 17 and 36 µg a.s./L during the definitive test. Consequently, the effect of lenacil was algistatic at these concentrations.

Conclusions:
The 96-hour toxicity of lenacil technical to the unicellular green alga *Pseudokircherniella subcapitata* was determined in a static system.
For biomass (area under the growth curve), the E_bC_{50} was calculated to be 7.7 µg a.s./L and 6.5 µg a.s./L for 72h and 96 h, respectively. For growth rate, the E_rC_{50} was calculated to be 16 µg a.s./L and 15 µg a.s./L for 72h and 96 h, respectively. The NOEC was determined to be 3.4 µg a.s./L.

RMS comments:
The validity criteria of the most recent version of OECD Test Guideline 201 (2011) are met:

- The biomass in the control and solvent control increased exponentially by a factor of at least 16 within the test period. This corresponds to a specific growth rate of at least 0.92 day^{-1} (measured: increase by a factor of 281 and 263 for the period 0-96h for the control and solvent control, respectively)
- The coefficient of variation of average specific growth rate during the whole test period in replicate control cultures did not exceed 7% (measured: 1.1 % in the control and 1.6% in the solvent control)
- The mean coefficient of variation for section-by-section growth rates in the control cultures did not exceed 35% (measured: 6.8% in the control and 5.7% in the solvent control).

Consequently, this study is considered acceptable for use in the risk assessment.

The analytical method used could not be fully validated according to the EU Guidance SANCO/3029/99 rev. 4. However, the available validation data from all studies where this method was used (CA8.2.1/02, CA8.2.6.1/01, CA8.2.6.2/01 and CA8.2.7/01) seem to indicate that the method works well and can be considered "fit for purpose". The LOQ for OECD medium was set at 2.5 µg/L (please refer to Vol. 3 (CA), Section B.5.1.2.6 – studies no. 3 to 6, for further details). The working range covers the test concentrations.

In accordance with the new data requirements (Commission Regulation EU No 283/2013), the EC_{10} and EC_{20} values should be calculated. Where these values cannot be estimated, an explanation should be provided. As the present study was already submitted for the initial Annex I inclusion of lenacil, this was not addressed in the study report. Therefore, RMS calculated the EC_{10} and EC_{20} for growth rate and biomass (area under the growth curve). EC_{10} and EC_{20} calculations were performed in the R statistical environment, Version 3.5.1 (R Development Core Team, 2018) using the R package 'drc'. Dose-response curve models were selected through goodness-of-fit metrics via the 'mselect' command, which compares the log likelihood value, Akaike's information criterion (AIC), the estimated residual standard error and the p-value from a lack-of-fit test for different models. The model with the best fit for the data was a five-parameter log-logistic function for biomass and Weibull Type 1 (3parameters) for growth rate. The calculated EC_x values are shown in Table 2.9.2.3.3-4.

Table 2.9.2.3.3-4: Calculated EC_{10} and EC_{20} values for the test with *Pseudokirchneriella subcapitata*.

Endpoint	EC_{10} (µg/L)*	EC_{20} (µg/L)*
Biomass (area under the growth curve)	3.45 (95% CL 3.01-3.89)	4.32 (95% CL 3.91-4.73)
Growth rate	5.87 (95% CL 5.03-6.71)	9.02 (95% CL 6.78-11.26)

**The model with best fit for the data was a five-parameter log-logistic function for biomass and a Weibull Type 1 (3 parameters) for growth rate.*

The following endpoints, based on mean measured concentrations, will be considered in the risk assessment:
E_bC_{50} (*Pseudokirchneriella subcapitata*, 96 h) = 6.5 µg a.s./L
E_bC_{20} (*Pseudokirchneriella subcapitata*, 96 h) = 4.32 µg a.s./L
E_bC_{10} (*Pseudokirchneriella subcapitata*, 96 h) = 3.45 µg a.s./L
E_rC_{50} (*Pseudokirchneriella subcapitata*, 96 h) = 15 µg a.s./L
E_rC_{20} (*Pseudokirchneriella subcapitata*, 96 h) = 9.02 µg a.s./L
E_rC_{10} (*Pseudokirchneriella subcapitata*, 96 h) = 5.87 µg a.s./L
NOEC (*Pseudokirchneriella subcapitata*, 96 h) = 3.4 µg a.s./L

Report:	**CA8.2.6.1/02. Douglas M.T. and Handley J.W. (1988)** **The algistatic activity of lenacil technical**

Report No.: DPT 171(K)/88189
Guidelines: OECD 201 (1984), US EPA 122-2
GLP: Yes

Previous evaluation: In DAR (November 2007); not relevant for renewal application

Material and Methods:

Test substance: lenacil (Batch no.: D231 206193 , chemical purity : 95.4 %)
Test species: *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*), unicellular freshwater green alga
Number of replicates: 3 replicates for the control and per treatment, initial mean measured cell count : 1.39×10^5/mL
Type of test: 120-hour static toxicity test
Applied and measured concentrations:
Nominal test concentrations: control; 0.01, 0.02, 0.04, 0.08, 0.16 mg a.s./L
Mean measured concentrations: exposure was not verified analytically
Test conditions: Sterile nutrient test medium
Conical flasks of 250 mL, containing 100 mL of test or control culture
temperature: 24 ± 1°C
pH: 7.6 - 7.7 (initial), 7.6 - 7.8 (final)
light regime: continuous illumination
light intensity: 7000 lux
Analytical methods: not performed

Statistics: Percentage inhibition values were plotted against the test concentration, a line fitted by eye and the E_bC_{50} or E_rC_{50} was read from the graph.

Test procedure: Samples were taken at 0, 24, 48, 72, 96 and 120 hours, and the absorbance was measured at 665 nm. The cell densities of the control cultures at initiation and at termination were determined by direct counting with the aid of a haemacytometer.

Severely inhibited cultures were recultured to determine if the inhibitory effect of the test substance was algicidal or algistatic. Aliquots (0.5 mL) were taken from the control and each replicate culture of the lowest test concentrations resulting in severe growth inhibition (0.04 and 0.08 mg a.s./L). The replicates were pooled, 100 mL of fresh sterile nutrient medium was added (to ensure that the test concentration was reduced below the inhibitory level) and the cultures were incubated at 24 °C for a further 9 days.

Findings:

The effects on algal growth are summarized in Table 2.9.2.3.3-5. The NOEC was estimated as 0.010 mg a.s./L. For biomass (area under the growth curve), the E_bC_{50} was estimated to be 0.012 mg a.s./L at 72h and 0.014 mg a.s./L at 120h. For growth rate, the E_rC_{50} was estimated to be 0.015 mg a.s./L for the period 24-48h.

Table 2.9.2.3.3-5: Reduction in biomass (area under the growth curve) and growth rate for *Pseudokirchneriella subcapitata* exposed to a range of concentrations of lenacil

Mean measured concentration (µg a.s./L)	Calculated mean area under the growth curve (A)		Percent inhibition [1]		Average specific growth rate µ ($hour^{-1}$)	Percent inhibition [1]
	0-72h	0-120h	0-72h	0-96h	24-48h	24-48h
Control	7.148	29.424	-	-	0.038	-
0.01	6.996	28.932	2	2	0.039	-1
0.02	0.840	5.796	88	80	0.009	76
0.04	-0.228	-0.496	103	102	-0.003	108
0.08	-0.536	-1.004	108	103	-0.007	118
0.16	-0.616	-1.120	109	104	-0.008	121

[1] *negative values indicate an increase relative to the control*

All test and control cultures were inspected at 120h. No abnormalities were observed in the control or at the treatment levels of 0.01, 0.02 and 0.04 mg a.s./L. However, colourless and deformed cells were observed at the treatment levels of 0.08 and 0.16 mg a.s./L.

Following transfer to unamended control medium, regrowth was observed after 9 days for control algae, but not for cultures previously inhibited by exposure to 0.04 and 0.08 mg a.s./L during the definitive test. Consequently, the effect of lenacil was algicidal at these concentrations.

Conclusions:

For biomass (area under the growth curve), the E_bC_{50} was estimated to be 0.012 mg a.s./L and 0.014 mg a.s./L for 72h and 120 h, respectively. For growth rate, the E_rC_{50} was estimated to be 0.015 mg a.s./L for 24-48h. The NOEC was determined to be 0.010 mg a.s./L.

RMS comments:

The following validity criteria of the most recent version of OECD Test Guideline 201 are met:

- The biomass in the control increased exponentially by a factor of at least 16 within the test period. This corresponds to a specific growth rate of at least 0.92 day^{-1} (measured: increase by a factor of 76 for the period 0-120h)

However, no analysis was performed to confirm initial exposure levels or to confirm stability during the test. Therefore, this study is not considered acceptable for use in the risk assessment.

Lenacil technical – Algal growth inhibition assay *Navicula pelliculosa*

Report No.:	ACD 036/024694
Guidelines:	92/69/EEC, method C.3 (1992), OECD 201 (1984)
GLP:	Yes
Previous evaluation:	In DAR (November 2007); relevant for renewal application

Material and Methods:

Test substance:	lenacil (Batch no.: 141712003, chemical purity: 98.6 %)
Test species:	*Navicula pelliculosa* (freshwater diatom)
Number of organisms:	6 replicates for the control and the solvent control; 3 replicates per test concentration; 1 x 10^4 cells/mL at initiation
Type of test:	72-hour static toxicity test
Applied and measured concentrations:	
Nominal test concentrations:	control; solvent control (dimethylformamide); serial dilutions (1.94, 4.27, 9.39, 20.7, 45.5, 100 %) of a nominal concentration of 10 mg a.s./L
Mean measured concentrations:	0.00; 0.00; 11, 22, 47, 105, 219, 468 µg a.s./L
Test conditions:	Sterile synthetic nutrient test medium Conical flasks of 250 mL, containing 100 mL of test or control culture temperature : 21.5-22.8°C. pH : 7.7 - 7.8 (initial), 7.5 - 7.8 (final) light regime : continuous illumination light intensity : 7870-8180 lux Incubation in an orbital shaker (150 cycles/min)
Analytical methods:	HPLC/UV
Statistics:	logistic regression for the calculation of the EC_{50} values. Williams test for the determination of the NOEC
Test procedure:	Samples were taken at 0, 24, 48, 72 and 96 hours and the cell densities determined by direct counting using a haemacytometer.

Findings:

Analytical results:

The measured concentrations at the beginning and the end of the exposure period are shown in Table 2.9.2.3.3-6. The results are expressed based on mean measured concentrations.

Table 2.9.2.3.3-6: Measured concentration of lenacil in the test media collected during the toxicity test with *Navicula pelliculosa.*

Nominal concentration of test item (% of prepeared solution)	Measured concentration of test item (µg a.s./L)		
	0 hour	72 hours [1]	Geometric Mean
Solvent control	<LOD [2]	< LOD [2]	< LOD [2]
1.94	10.57	10.74	11
4.27	21.24	22.71	22
9.39	46.95	46.26	47
20.7	107.5	102.1	105
45.5	221.6	216.2	219
100	476.1	460.0	468

[1]Samples were not filtered to remove algal cells before anal460.0ysis; [2]LOD = Limit of Detection = 0.7 µg a.s./L

Biological results:

Effects on algal growth are summarized in Table 2.9.2.3.3-7. There was a statistically significant between the control and solvent control for biomass (area under the growth curve) and growth rate at 72 hours (Student's t-test, respectively $p < 0.01$ and $p < 0.001$). The results from the test item treatment were therefore compared to the solvent control. Statisically significant differences compared to the control were found for both biomass and growth rate for test item concentration from 22.0 µg/L and higher. The NOEC was therefore set at 11 µg a.s./L.

For biomass (area under the growth curve), the E_bC_{50} was calculated to be 36 µg a.s./L (95% confidence limits 29-44 µg a.s./L) for 72h of exposure. For growth rate, the E_rC_{50} was calculated to be 96 µg a.s./L (95% confidence limits 85-110 µg a.s./L) for 72h of exposure.

Table 2.9.2.3.3-7: Reduction in biomass (area under the growth curve) and average specific growth rate for *Navicula pelliculosa* exposed to a range of concentrations of lenacil.

Mean measured concentration (µg/L)	Mean cell density (x 10^4 cells/mL)			Mean area under the growth curve (A)		Calculated average specific growth rate µ ($hour^{-1}$)	
	24h	48h	72h	0-72h	Percent inhibition [1]	0-72h	Percent inhibition [1]
Control	8.8125	25.771	88.625	-	-	-	-
Solvent control	8.6458	28.542	107.083	21	-	0.065	-
11	9.3333	37.875	130.750	26	-25	0.068	-4.2
22	8.3750	20.083	70.042	15	31*	0.059*	9.2
47	6.9167	11.708	24.792	6.9	68*	0.045*	31
105	4.2500	4.8750	8.1250	2.6	88*	0.029*	55
219	5.000	3.7500	3.5833	1.9	91*	0.017*	73
468	2.1667	2.6250	2.0417	0.8	96*	0.0099*	85

** Statistically significant difference compared to the control (Williams' test; $p<0.01$); [1]Percent inhibition compared to solvent control, negative values indicate an increase relative to the control*

All test and control cultures were inspected microscopically at 96 hours. No abnormalities were detected in any of the cultures examined.

Conclusions:

The 72-hour toxicity of lenacil to *Navicula pelliculosa* was determined in a static system. For biomass (area under the growth curve), the E_bC_{50} after 72h was calculated to be 36 µg a.s./L and the NOEC was 11 µg a.s./L. For growth rate, the E_rC_{50} after 72h was calculated to be 96 µg a.s./L and the NOEC was 11 µg a.s./L.

RMS comments:

The validity criteria of the most recent version of OECD Test Guideline 201 (2011) are met:

- The biomass in the control and solvent control increased exponentially by a factor of at least 16 within the test period. This corresponds to a specific growth rate of at least 0.92 day^{-1} (measured: increase by a factor of 89 and 107 for the period 0-72h for the control and solvent control, respectively)
- The coefficient of variation of average specific growth rate during the whole test period in replicate control cultures did not exceed 10% (measured: 1.1 % in the control and 1.0% in the solvent control)
- The mean coefficient of variation for section-by-section growth rates in the control cultures did not exceed 35% (measured: 32.9% in the control and 27.8% in the solvent control).

Consequently, this study is considered acceptable for use in the risk assessment.

The analytical method used could not be fully validated according to the EU Guidance SANCO/3029/99 rev. 4. However, the available validation data from all studies where this method was used (CA8.2.1/02, CA8.2.6.1/01, CA8.2.6.2/01 and CA8.2.7/01) seem to indicate that the method works well and can be considered "fit for purpose". The LOQ for OECD medium was set at 2.5 µg/L (please refer to Vol. 3 (CA), Section B.5.1.2.6 – studies no. 3 to 6, for further details). The working range covers the test concentrations.

In accordance with the new data requirements (Commission Regulation EU No 283/2013), the EC_{10} and EC_{20} values should be calculated. Where these values cannot be estimated, an explanation should be provided. As the present study was already submitted for the initial Annex I inclusion of lenacil, this was not addressed in the study report. Therefore, RMS calculated the EC_{10} and EC_{20} for growth rate and biomass (area under the growth curve).
EC_{10} and EC_{20} calculations were performed in the R statistical environment, Version 3.5.1 (R Development Core Team, 2018) using the R package 'drc'. Dose-response curve models were selected through goodness-of-fit metrics via the 'mselect' command, which compares the log likelihood value, Akaike's information criterion (AIC), the estimated residual standard error and the p-value from a lack-of-fit test for different models. The model with the

best fit for the data was Weibulll Type 1 (4 parameters) for both biomass and growth rate. The calculated EC_x values are shown in Table 2.9.2.3.3-8.

Table 2.9.2.3.3-8: Calculated EC_{10} and EC_{20} values for the test with *Navicula pelliculosa*.

Endpoint	EC_{10} (µg/L)*	EC_{20} (µg/L)*
Biomass (area under the growth curve)	13.07 (95% CL 7.38-18.76)	16.48 (95% CL 10.65-22.31)
Growth rate	20.44 (95% CL 17.23-23.65)	31.28 (95% CL 27.78-34.77)

**The model with best fit for the data was Weibull Tyoe 1 (4 parameters) for both biomass and growth rate.*

The following endpoints, based on mean measured concentrations, will be considered in the risk assessment:
E_bC_{50} (*Navicula pelliculosa*, 72 h) = 36 µg a.s./L
E_bC_{20} (*Navicula pelliculosa*, 72 h) = 16.5 µg a.s./L
E_bC_{10} (*Navicula pelliculosa*, 72 h) = 13.1 µg a.s./L
E_rC_{50} (*Navicula pelliculosa*, 72 h) = 96 µg a.s./L
E_rC_{20} (*Navicula pelliculosa*, 72 h) = 31.3 µg a.s./L
E_rC_{10} (*Navicula pelliculosa*, 72 h) = 20.4 µg a.s./L
NOEC (*Navicula pelliculosa*, 72 h) = 11 µg a.s./L

Report:	**CA8.2.6.2/02. Wenzel A. (2014a)** **Effect of Lenacil technical on the growth of *Ankistrodesmus falcatus***

Report No.: DPT-001/4-10/F
Guidelines: OECD 201 (2011)
GLP: Yes

Previous evaluation: None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application

Material and Methods:
Test substance: lenacil (Batch no.: 0190813, chemical purity: 99.3 %)
Test species: *Ankistrodesmus falcatus* (uni-cellular freshwater green algae); Chlorophyceae; Chlorophyta; SAG-No 202-2. *A. falcatus* is not one of the standard algal test species listed in OECD Guideline 201
Number of organisms: 8 replicates for the control and the solvent control; 4 replicates per test concentration; 2500 cells/mL at initiation
Type of test: 72-hour static toxicity test
Applied and measured concentrations:
Nominal test concentrations: control; solvent control (dimethylformamide); 0.801, 2.53, 8.01, 25.3, 80.0 µg a.s./L
Mean measured concentrations: 0.00, 0.00, 0.85, 2.56, 7.76, 21.7, 34.3 µg a.s./L
Initial measured concentrations: 0.00, 0.00, 0.87, 2.59, 7.72, 22.4, 38.3 µg a.s./L
Test conditions: Sterile synthetic nutrient test medium according to OECD 201
Conical flasks of 250 mL, containing 100 mL of test or control culture
temperature : 22.0°C.
pH : 8.13 – 8.25 (initial), 8.13 – 8.80 (final)
light regime : continuous illumination
light intensity : 6989-7052 lux
Incubation in an orbital shaker (150 rpm)
Analytical methods: LC-MS/MS
Statistics: Statistical evaluations were performed with ToxRat® Professional 2.09 software. E_rC_{50} and E_yC_{50} values and their corresponding 95% confidence limits were determined by probit analysis using linear maximum likelihood regression. After making Bonferroni-Holm adjustments, Welch's t-test and a Multiple

	Sequentially-rejective U-test were applied to the growth rate and yield data, respectively, to identify significant differences from the untreated control and determine the corresponding LOEC and NOEC values.
Test procedure:	Samples were taken at 0, 24, 48 and 72 hours and the cell densities determined by measurements of chlorophyll fluorescence. At the end of the test, the appearance of the algae was also checked by microscope to detect any visual effect of the test item.

Findings:

Analytical results:

At test start, the measured concentrations were 108, 103, 96.4, 88.6 and 47.8% of nominal. Analysis of fresh exposure media indicated that the intended test concentrations were reached, except for the highest concentration. Similarly, measured concentrations after 72 hours ranged from 83.5% to 105% of nominal, except at 80.0 µg a.s./L, where recovery was only 38.6%. The residual concentrations measured after 72 hours represented between 80.6% and 101% of the corresponding initial measured concentrations, indicating that the achieved concentrations of the test compound remained stable under the conditions of the test. Thus, the evaluation of the test was based on the initial measured concentrations.

Table 2.9.2.3.3-9: Measured concentration of lenacil in the test media collected during the toxicity test with *Ankistrodesmus falcatus*.

Nominal concentration of test item (µg a.s./L)	Measured 0 h		Measured 72 h			Geometric mean measured concentration	
	µg a.s./L	% of nominal	µg a.s./L	% of nominal	% of initial	µg a.s./L	% of nominal
Control	<LOQ	-	<LOQ	-	-	-	-
0.80	0.87	108	0.84	105	96.5	0.85	107
2.53	2.59	103	2.53	100	97.6	2.56	101
8.01	7.72	96.4	7.81	97.4	101	7.76	96
25.3	22.4	88.6	21.1	83.5	94.2	21.7	86
80.0	38.3	47.8	30.8	38.6	80.6	34.3	43

Measured concentrations were calculated from reported results of duplicate analysis at each timepoint; LOQ: Limit of Quantification (0.5 µg a.s./L)

Biological results:

Details of effects on algal growth are provided in Table 2.9.2.3.3-10. No statistically significant differences were found between the untreated and solvent control groups and the effects of the lenacil treatments were therefore evaluated by comparison with the solvent control alone. Statistically significant ($\alpha = 0.05$) inhibition of growth, based on specific growth rates and on cell yield, occurred at measured initial concentrations ≥ 7.72 µg a.s./L. The concentrations of lenacil causing 10%, 20% and 50% inhibition based on the various algal growth indices are compiled in Table 2.9.2.3.3-11.

Microscopic examination of algae taken from the various media after 72 hours showed the presence cellular debris in quantities that increased in accordance with the degree of growth inhibition, but otherwise the appearance of the algal cells exposed to lenacil was normal.

Table 2.9.2.3.3-10: Effects on growth of *Ankistrodesmus falcatus* following exposure to lenacil technical for 72 hours

Initial measured lenacil concentration (µg a.s./L)	Mean cell density (×10⁴ cells/mL)			Mean growth rate µ (day^{-1}) during 0 - 72 hours *[% inhibition]*[a]		Mean yield (×10⁴ cells/mL) after 72 hours *[% inhibition]*[a]	
	24 hours	48 hours	72 hours				
Control	0.7	3.0	7.7	1.141	*[-]*	7.443	*[-]*
Solvent control	0.8	3.2	7.8	1.144	*[-]*	7.537	*[-]*
0.87	1.1	4.2	9.2	1.202	*[-5.1]*	8.950	*[-18.7]*
2.59	1.1	4.3	8.2	1.161	*[-1.5]*	7.910	*[-4.9]*
7.72	1.0	2.5	4.7	0.974*	*[14.9]*	4.400*	*[41.6]*

22.4	0.7	0.6	0.4	0.190*	*[83.4]*	0.197*	*[97.4]*
38.3	0.3	0.2	0.2	-0.117*	*[110]*	-0.074*	*[101.0]*

*[a] Relative to the mean solvent control; *Significantly different (α = 0.05, one-sided smaller) from the solvent control, negative values indicate an increase relative to the control.*

Table 2.9.2.3.3-11: Growth inhibition endpoints for *Ankistrodesmus falcatus* following 72-hour exposure to lenacil technical

Parameter	Endpoint	Initial measured lenacil concentration (µg a.s./L)
Growth rate	E_rC_{10}	6.85 (95% CL: 6.4 to 7.29)
	E_rC_{20}	8.62 (95% CL: 8.15 to 9.06)
	E_rC_{50}	13.3 (95% CL:12.8 to 13.9)
	$NOEC_r$	2.59
Cumulative yield	E_yC_{10}	4.586 (95% CL: 4.211 to 4.899)
	E_yC_{20}	5.682 (95% CL: 5.381 to 5.932)
	E_yC_{50}	8.564 (95% CL: 8.370 to 8.789)
	$NOEC_y$	2.59

In the test performed most recently prior to this study with lenacil technical, the 72-hour E_rC_{50} endpoint for *A. falcatus* exposed to the reference toxicant 3,5-dichlorophenol was 3.68 mg/L (nominal).

Conclusions:
The 72-hour toxicity of lenacil technical to the unicellular green alga *Ankistrodesmus falcatus* was determined in a static system. Dose-related growth inhibition occurred and the 72-hour E_rC_{50} (based on specific growth rate) and E_yC_{50} (based on algal yield) were 13.3 and 8.56 µg lenacil/L, respectively, based on initial measured exposure concentrations. The NOEC and LOEC were 2.59 and 7.72 µg a.s./L, respectively, for both growth parameters.

RMS comments:
The validity criteria of the most recent version of OECD Test Guideline 201 (2011) are met:

- The biomass in the control and solvent control increased exponentially by a factor of at least 16 within the test period. This corresponds to a specific growth rate of at least 0.92 day^{-1} (measured: increase by a factor of 30.8 and 31.2 for the period 0-72h for the control and solvent control, respectively)
- The coefficient of variation of average specific growth rate during the whole test period in replicate control cultures did not exceed 10% (measured: 2.9 % in the control and 3.8% in the solvent control)
- The mean coefficient of variation for section-by-section growth rates in the control cultures did not exceed 35% (measured: 24.1% in the control and 20.5% in the solvent control).

Consequently, this study is considered acceptable for use in the risk assessment.

The analytical method used could be fully validated according to the EU Guidance SANCO/3029/99 rev. 4., and is therefore considered "fit for purpose". The LOQ was set at 0.5 µg/L (please refer to Vol. 3 (CA), Section B.5.1.2.6 – studies no. 12 to 17, for further details).

The following endpoints, based on initial measured concentrations, will be considered in the risk assessment:
E_yC_{50} (*Ankistrodesmus falcatus*, 72 h) = 8.56 µg a.s./L
E_yC_{20} (*Ankistrodesmus falcatus*, 72 h) = 5.68 µg a.s./L
E_yC_{10} (*Ankistrodesmus falcatus*, 72 h) = 4.59 µg a.s./L
E_rC_{50} (*Ankistrodesmus falcatus*, 72 h) = 13.3 µg a.s./L
E_rC_{20} (*Ankistrodesmus falcatus*, 72 h) = 8.62 µg a.s./L
E_rC_{10} (*Ankistrodesmus falcatus*, 72 h) = 6.85 µg a.s./L
NOEC (*Ankistrodesmus falcatus*, 72 h) = 2.59 µg a.s./L

Report:	**CA8.2.6.2/03. Wenzel A. (2014b)** **Effect of Lenacil technical on the growth of *Synechococcus leopoliensis***
Report No.:	DPT-001/4-10/C

Guidelines:	OECD 201 (2011)
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application

Material and Methods:

Test substance:	lenacil (Batch no.: 0190813, chemical purity: 99.3 %)
Test species:	*Synechococcus leopoliensis* (unicellular cyanobacteria); Cyanophyceae; Cyanobacteria. SAG-No 1402-1.
Number of organisms:	6 replicates for the control and the solvent control; 4 replicates per test concentration; 1 x 10^4 cells/mL at initiation
Type of test:	72-hour static toxicity test
Applied and measured concentrations:	
Nominal test concentrations:	control; solvent control (dimethylformamide); 30.7, 76.8, 192, 480, 1200 µg a.s./L
Mean measured concentrations:	0.00, 0.00, 29.9, 67.3, 106.4, 406.4, 592.1 µg a.s./L
Initial measured concentrations:	0.00, 0.00, 29.6, 69.0, 119.4, 399.8, 560.3 µg a.s./L
Test conditions:	Sterile synthetic nutrient test medium according to Bringmann and Kühn (1980) Conical flasks of 250 mL, containing 100 mL of test or control culture temperature : 22.0-23.0 °C. pH : 9.63-10.02 (initial), 8.45-8.55 (final) light regime : continuous illumination light intensity : 3010-3427 lux Incubation in an orbital shaker (150 rpm)
Analytical methods:	LC-MS/MS
Statistics:	Statistical evaluations were performed with ToxRat® Professional 2.10 software. E_rC_{50} and E_yC_{50} values and their corresponding 95% confidence limits were determined by probit analysis using linear maximum likelihood regression. After applying ANOVA, Williams' Multiple Sequential t-test was used to identify significant differences from the solvent control and determine the corresponding LOEC and NOEC values for the growth rate and yield data sets.
Test procedure:	Samples were taken at 0, 24, 48 and 72 hours and the cell densities determined by measurements of chlorophyll fluorescence. At the end of the test, the appearance of the cyanobacteria was also checked by microscope to detect any visual effect of the test item.

Findings:

Analytical results:

At test start, the measured concentrations ranged from 46.7 to 96.4% of nominal. Thus, the measured initial concentrations were outside the frame of ± 20% of nominal. Similarly, measured concentrations at the end of the test period ranged from 49.6 to 98.7% of nominal. The residual concentrations measured after 72 hours represented between 79.8% and 103% of the corresponding initial measured concentrations, indicating that the achieved concentrations of the test compound remained stable under the conditions of the test. Thus, the evaluation of the test was based on the initial measured concentrations.

Table 2.9.2.3.3-12: Measured concentration of lenacil in the test media collected during the toxicity test with *Synechococcus leopoliensis*.

Nominal concentration of test item (µg a.s./L)	Measured 0 h		Measured 72 h			Geometric mean measured concentration	
	µg a.s./L	% of nominal	µg a.s./L	% of nominal	% of initial	µg a.s./L	% of nominal
Control	<LOQ	-	<LOQ	-	-	-	-
Solvent control	<LOQ	-	<LOQ	-	-	-	-
30.7	29.6	96.4	30.3	98.7	102	29.9	97.6
76.8	69.0	89.8	65.7	85.6	95.2	67.3	87.7

192	119.4	62.2	95.2	49.6	79.8	106.4	55.4
480	399.8	83.3	412.9	86.0	103	406.4	84.7
1200	560.3	46.7	625.9	52.2	112	592.1	49.3

Measured concentrations were calculated from reported results of duplicate analysis at each timepoint; LOQ: Limit of Quantification (0.5 µg a.s./L)

Biological results:

Details of effects on algal growth are provided in Table 2.9.2.3.3-13. The effects of the lenacil treatments were evaluated by comparison with the solvent control alone. Statistically significant ($\alpha = 0.05$) inhibition of growth, based on specific growth rates and on cell yield, occurred at measured initial concentrations ≥ 69.0 µg a.s./L. The concentrations of lenacil causing 10%, 20% and 50% inhibition based on the various algal growth indices are compiled in Table 2.9.2.3.3-14.

Microscopic examination of algae taken from the various media after 72 hours showed the presence cellular debris in quantities that increased in accordance with the degree of growth inhibition, but otherwise the appearance of the algal cells exposed to lenacil was normal.

Table 2.9.2.3.3-13: Effects on growth of *Synechococcus leopoliensis* following exposure to lenacil technical for 72 hours

Initial measured lenacil concentration (µg a.s./L)	Mean cell density (×10⁴ cells/mL)			Mean growth rate µ (day^{-1}) during 0 - 72 hours *[% inhibition]*[a]		Mean yield (×10⁴ cells/mL) after 72 hours *[% inhibition]*[a]	
	24 hours	48 hours	72 hours				
Control	4.87	16.63	58.01	1.35	*[-]*	57.01	*[-]*
Solvent control	4.99	15.96	60.72	1.367	*[-]*	59.72	*[-]*
29.6	7.04	24.09	61.43	1.372	*[-0.39]*	60.43	*[-1.19]*
69.0	7.34	16.84	26.49	1.092*	*[20.1]*	25.49*	*[57.3]*
119.4	4.70	7.96	12.39	0.832*	*[39.1]*	11.39*	*[80.9]*
399.8	3.18	4.71	5.11	0.542*	*[60.4]*	4.11*	*[93.1]*
560.3	2.30	2.58	1.99	0.229*	*[83.3]*	0.99*	*[98.3]*

*[a] Relative to the mean solvent control; *Significantly different ($\alpha = 0.05$, one-sided smaller) from the solvent control, negative values indicate an increase relative to the control.*

Table 2.9.2.3.3-14: Growth inhibition endpoints for *Synechococcus leopoliensis* following 72-hour exposure to lenacil technical

Parameter	Endpoint	Initial measured lenacil concentration (µg a.s./L)
Growth rate	E_rC_{10}	39.3 (95% CL: 27.5 to 51.3)
	E_rC_{20}	69.7 (95% CL: 53.7 to 85.1)
	E_rC_{50}	209 (95% CL:180 to 242)
	$NOEC_r$	29.6
Cumulative yield	E_yC_{10}	33.1 (95% CL: 26.7 to 38.4)
	E_yC_{20}	42.0 (95% CL: 35.8 to 47.1)
	E_yC_{50}	66.5 (95% CL: 61.5 to 71.3)
	$NOEC_y$	29.6

In the test performed most recently prior to this study with lenacil technical, the 72-hour E_rC_{50} endpoint for *S. leopoliensis* exposed to the reference toxicant 3,5-dichlorophenol was 3.37 mg/L (nominal).

Conclusions:

The 72-hour toxicity of lenacil technical to the unicellular cyanobacteria *Synechococcus leopoliensis* was determined in a static system. Dose-related growth inhibition occurred and the 72-hour E_rC_{50} (based on specific growth rate) and E_yC_{50} (based on algal yield) were 209 and 66.5 µg lenacil/L, respectively, based on initial measured exposure concentrations. The NOEC and LOEC were 29.6 and 69.0 µg a.s./L, respectively, for both growth parameters.

RMS comments:
The validity criteria of the most recent version of OECD Test Guideline 201 (2011) are met:

- The biomass in the control and solvent control increased exponentially by a factor of at least 16 within the test period. This corresponds to a specific growth rate of at least 0.92 day^{-1} (measured: increase by a factor of 58.0 and 60.7 for the period 0-72h for the control and solvent control, respectively)
- The coefficient of variation of average specific growth rate during the whole test period in replicate control cultures did not exceed 10% (measured: 2.6 % in the control and 2.4% in the solvent control)
- The mean coefficient of variation for section-by-section growth rates in the control cultures did not exceed 35% (measured: 18.3% in the control and 13.9% in the solvent control).

It is noted that the initial cell concentration was only 1 x 10^4 cells/mL, while OECD 201 recommends in initial cell concentration from 5 x 10^4 – 10^5 cells/mL. However, as an exponential increase in cell density was obtained, this is not considered to invalidate the results of this study. Consequently, this study is considered acceptable for use in the risk assessment.

The analytical method used could be fully validated according to the EU Guidance SANCO/3029/99 rev. 4., and is therefore considered "fit for purpose". The LOQ was set at 0.5 µg/L (please refer to Vol. 3 (CA), Section B.5.1.2.6 – studies no. 12 to 17, for further details).

It is noted that for the 119.4 µg a.s./L (nominal) treatment, the measured concentration at the end of the test was only 79.8% of the initial measured concentration. However, as this is very close to the limit of 80%, RMS agrees that the results can be expressed based on initial measured concentrations.

The following endpoints, based on initial measured concentrations, will be considered in the risk assessment:
E_yC_{50} (*Synechococcus leopoliensis*, 72 h) = 66.5 µg a.s./L
E_yC_{20} (*Synechococcus leopoliensis*, 72 h) = 42.0 µg a.s./L
E_yC_{10} (*Synechococcus leopoliensis*, 72 h) = 33.1 µg a.s./L
E_rC_{50} (*Synechococcus leopoliensis*, 72 h) = 209 µg a.s./L
E_rC_{20} (*Synechococcus leopoliensis*, 72 h) = 69.7 µg a.s./L
E_rC_{10} (*Synechococcus leopoliensis*, 72 h) = 39.3 µg a.s./L
NOEC (*Synechococcus leopoliensis*, 72 h) = 29.6 µg a.s./L

Report:	**CA8.2.6.2/04. Wenzel A. (2014c)** **Effect of Lenacil technical on the growth of *Anabaena flos-aquae***
Report No.:	DPT-001/4-10/E
Guidelines:	OECD 201 (2011)
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Material and Methods:	
Test substance:	lenacil (Batch no.: 0190813, chemical purity: 99.3 %)
Test species:	*Anabaena flos-aquae* (filamentous cyanobacteria); Cyanophyceae; Cyanobacteria. UTEX Strain No. UTEX 1444.
Number of organisms:	6 replicates for the control and the solvent control; 4 replicates per test concentration; 1 x 10^4 cells/mL at initiation
Type of test:	72-hour static toxicity test
Applied and measured concentrations:	
Nominal test concentrations:	control; solvent control (dimethylformamide); 188, 375, 750, 1500, 3000 µg a.s./L
Mean measured concentrations:	0.00, 0.00, 101.5, 115.5, 127.5, 652.3, 673.2 µg a.s./L
Initial measured concentrations:	0.00, 0.00, 97.0, 113.0, 120.6, 599.2, 634.3 µg a.s./L
Test conditions:	Sterile synthetic nutrient test medium according to OECD 201 Conical flasks of 250 mL, containing 100 mL of test or control culture

temperature : 22.0 °C.
pH : 7.62-7.99 (initial), 8.08-8.16 (final)
light regime : continuous illumination
light intensity : 3113-3528 lux
Incubation in an orbital shaker (150 rpm)

Analytical methods: LC-MS/MS

Statistics: Statistical evaluations were performed with ToxRat® Professional 2.10 software. E_rC_{50} and E_yC_{50} values and their corresponding 95% confidence limits were determined by probit analysis using linear maximum likelihood regression. After applying ANOVA, Williams' Multiple Sequential t-test was used to identify significant differences from the solvent control and determine the corresponding LOEC and NOEC values for the growth rate and yield data sets.

Test procedure: Samples were taken at 0, 24, 48 and 72 hours and the cell densities determined by measurements of chlorophyll fluorescence. At the end of the test, the appearance of the cyanobacteria was also checked by microscope to detect any visual effect of the test item.

Findings:

Analytical results:

At test start, the measured concentrations ranged from 16.1 to 51.6% of nominal. Thus, the measured initial concentrations were outside the frame of ± 20% of nominal. Similarly, measured concentrations at the end of the test period ranged from 18.0 to 56.5% of nominal. The residual concentrations measured after 72 hours represented between 104% and 118% of the corresponding initial measured concentrations, indicating that the achieved concentrations of the test compound remained stable under the conditions of the test. Thus, the evaluation of the test was based on the initial measured concentrations.

Table 2.9.2.3.3-15: Measured concentration of lenacil in the test media collected during the toxicity test with *Anabaena flos-aquae*.

Nominal concentration of test item (µg a.s./L)	Measured 0 h		Measured 72 h			Geometric mean measured concentration	
	µg a.s./L	% of nominal	µg a.s./L	% of nominal	% of initial	µg a.s./L	% of nominal
Control	<LOQ	-	<LOQ	-	-	-	-
Solvent control	<LOQ	-	<LOQ	-	-	-	-
30.7	97.0	51.6	106	56.5	110	101.5	54.0
76.8	113.0	30.1	118	31.5	104	115.5	30.8
192	120.6	16.1	135	18.0	112	127.5	17.0
480	599.2	39.9	710	47.3	118	652.3	43.5
1200	634.3	21.1	714	23.8	113	673.2	22.4

Measured concentrations were calculated from reported results of duplicate analysis at each timepoint; LOQ: Limit of Quantification (0.5 µg a.s./L)

Biological results:

Details of effects on algal growth are provided in Table 2.9.2.3.3-16. Although it is recommended in the guideline to use the solvent control rather than the control for test evaluation, the present test was evaluated using the control (growth medium only) due to slight growth inhibition observed in the solvent control. Statistically significant ($\alpha = 0.05$) inhibition of growth, based on specific growth rates and on cell yield, occurred at measured initial concentrations ≥ 120.6 µg a.s./L. The concentrations of lenacil causing 10%, 20% and 50% inhibition based on the various algal growth indices are compiled in Table 2.9.2.3.3-17.

Microscopic examination of algae taken from the various media after 72 hours showed the presence cellular debris in quantities that increased in accordance with the degree of growth inhibition, but otherwise the appearance of the algal cells exposed to lenacil was normal.

Table 2.9.2.3.3-16: Effects on growth of *Anabaena flos-aquae* following exposure to lenacil technical for 72 hours

Initial measured lenacil concentration (µg a.s./L)	Mean cell density (×10⁴ cells/mL)			Mean growth rate µ (day^{-1}) during 0 - 72 hours *[% inhibition]*[a]		Mean yield (×10⁴ cells/mL) after 72 hours *[% inhibition]*[a]	
	24 hours	48 hours	72 hours				
Control	3.76	9.58	24.15	1.06	*[-]*	23.15	*[-]*
Solvent control	3.56	8.75	19.90	0.99	*[-]*	18.90	*[-]*
97.0	5.69	13.95	35.81	1.19	*[-12.5]*	34.81	*[-50.4]*
113.0	5.11	11.38	25.61	1.08	*[-1.88]*	24.61	*[-6.31]*
120.6	4.82	9.71	16.22	0.93*	*[12.5]*	15.22*	*[34.3]*
599.2	4.46	7.20	8.38	0.71*	*[33.3]*	7.38*	*[68.1]*
634.3	4.19	7.62	6.79	0.64*	*[40.0]*	5.79*	*[75.0]*

[a] *Relative to the mean solvent control; *Significantly different (α = 0.05, one-sided smaller) from the solvent control, negative values indicate an increase relative to the control.*

Table 2.9.2.3.3-17: Growth inhibition endpoints for *Anabaena flos-aquae* following 72-hour exposure to lenacil technical

Parameter	Endpoint	Initial measured lenacil concentration (µg a.s./L)
Growth rate	E_rC_{10}	186 (95% CL: 126 to 237)
	E_rC_{20}	325 (95% CL: 260 to 379)
	E_rC_{50}	945[a] (95% CL: 802 to 1209)
	$NOEC_r$	113
Cumulative yield	E_yC_{10}	97.0 (95% CL: 58.5 to 134)
	E_yC_{20}	150 (95% CL: 103 to 194)
	E_yC_{50}	345 (95% CL: 277 to 433)
	$NOEC_y$	113

[a] *Extrapolated beyond the highest measured concentration tested.*

In the test performed most recently prior to this study with lenacil technical, the 72-hour E_rC_{50} endpoint for *A. flos-aquae* exposed to the reference toxicant 3,5-dichlorophenol was 5.85 mg/L (nominal).

Conclusions:
The 72-hour toxicity of lenacil technical to the filamentous cyanobacteria *Anabaena flos-aquae* was determined in a static system. Dose-related growth inhibition occurred and the 72-hour E_rC_{50} (based on specific growth rate) and E_yC_{50} (based on algal yield) were 945 and 345 µg lenacil/L, respectively, based on initial measured exposure concentrations. The NOEC and LOEC were 113.0 and 120.6 µg a.s./L, respectively, for both growth parameters.

RMS comments:
The validity criteria of the most recent version of OECD Test Guideline 201 (2011) are met:

- The biomass in the control and solvent control increased exponentially by a factor of at least 16 within the test period. This corresponds to a specific growth rate of at least 0.92 day^{-1} (measured: increase by a factor of 24.2 and 19.9 for the period 0-72h for the control and solvent control, respectively)
- The coefficient of variation of average specific growth rate during the whole test period in replicate control cultures did not exceed 10% (measured: 2.9 % in the control and 5.5% in the solvent control)
- The mean coefficient of variation for section-by-section growth rates in the control cultures did not exceed 35% (measured: 23.1% in the control and 21.2% in the solvent control).

Consequently, this study is considered acceptable for use in the risk assessment.

The analytical method used could be fully validated according to the EU Guidance SANCO/3029/99 rev. 4., and is therefore considered "fit for purpose". The LOQ was set at 0.5 µg/L (please refer to Vol. 3 (CA), Section B.5.1.2.6 – studies no. 12 to 17, for further details).

It is noted that the E_rC_{50} reported in the study report was extrapolated beyond the highest measured concentration tested. Therefore, an E_rC_{50} of > 643 µg a.s./L will be used instead of the calculated value. The following endpoints, based on initial measured concentrations, will be considered in the risk assessment:
E_yC_{50} (*Anabaena flos-aquae*, 72 h) = 345 µg a.s./L

E_yC_{20} (*Anabaena flos-aquae*, 72 h) = 150 µg a.s./L
E_yC_{10} (*Anabaena flos-aquae*, 72 h) = 97.0 µg a.s./L
E_rC_{50} (*Anabaena flos-aquae*, 72 h) > 643 µg a.s./L
E_rC_{20} (*Anabaena flos-aquae*, 72 h) = 325 µg a.s./L
E_rC_{10} (*Anabaena flos-aquae*, 72 h) = 186 µg a.s./L
NOEC (*Anabaena flos-aquae*, 72 h) = 113 µg a.s./L

Report:	**CA8.2.6.2/05. Wenzel A. (2014d)** **Effect of Lenacil technical on the growth of *Closterium cornu***

Report No.:	DPT-001/4-10/G
Guidelines:	OECD 201 (2011)
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application

Material and Methods:

Test substance:	lenacil (Batch no.: 0190813, chemical purity: 99.3 %)
Test species:	*Closterium cornu* (unicellular alga); Zygnematophyceae; Streptophyta (Charophyta). SAG-No 132.80.
Number of organisms:	8 replicates for the control and the solvent control; 4 replicates per test concentration; 2500 cells/mL at initiation
Type of test:	96-hour static toxicity test
Applied and measured concentrations:	
Nominal test concentrations:	control; solvent control (dimethylformamide); 4.00, 12.7, 40, 127, 400 µg a.s./L
Mean measured concentrations:	0.00, 0.00, 2.21, 7.34, 21.6, 61.6, 188 µg a.s./L
Test conditions:	Sterile synthetic nutrient test medium (Bacillariophycean medium; No. 11, SAG medium) Conical flasks of 250 mL, containing 97 mL of test or control culture temperature : 21.5-22.0 °C. pH : 6.47-6.52 (initial), 7.58-8.64 (final) light regime : continuous illumination light intensity: 61.54-62.78 µmol/m².s Incubation in an orbital shaker (150 rpm)
Analytical methods:	LC-MS/MS
Statistics:	Statistical evaluations were performed with ToxRat® Professional 2.09 software. E_rC_{50} and E_yC_{50} values and their corresponding 95% confidence limits were determined by probit analysis using linear maximum likelihood regression. After making Bonferroni-Holm adjustments, Welch's t-test was applied to the growth rate and yield data, to identify significant differences from the untreated control and determine the corresponding LOEC and NOEC values.
Test procedure:	Samples were taken at 0, 24, 48, 72 and 96 hours and the cell densities determined by counting a sample under the microscope taken directly from the test vessels. Fuchs-Rosenthal chamber with chamber factor of 5000 x cells/square (1 mm² x 0.2 mm depth) was used for microscopic counting. At the end of the test, the appearance of the algae was also checked by microscope to detect any visual effect of the test item.

Findings:

Analytical results:

At test start, the measured concentrations ranged from 45.5 to 71.4% of nominal. Thus, the measured initial concentrations were outside the frame of ± 20% of nominal. Measured concentrations at the end of the test period (after 96h) ranged from 38.3 to 48.7% of nominal, and generally represented less than 80% of the corresponding

initial measured concentration. The results of the test were therefore based on the geometric mean measured concentrations of lenacil in the test medium.

Table 2.9.2.3.3-18: Measured concentration of lenacil in the test media collected during the toxicity test with *Closterium cornu*.

Nominal concentration of test item (µg a.s./L)	Measured 0 h		Measured 96 h			Geometric mean measured concentration	
	µg a.s./L	% of nominal	µg a.s./L	% of nominal	% of initial	µg a.s./L	% of nominal
Control	<LOQ	-	<LOQ	-	-	-	-
Solvent control	<LOQ	-	<LOQ	-	-	-	-
4.00	2.8	70.0	1.7	42.5	60.7	2.21	55.3
12.7	9.1	71.6	6.0	47.2	65.9	7.34	57.8
40.0	27.1	67.8	17.2	43.0	63.5	21.6	54
127	78.1	61.5	48.6	38.3	62.2	61.6	48.5
400	182	45.5	195	48.7	107.1	188	47

Measured concentrations were calculated from reported results of duplicate analysis at each timepoint; LOQ: Limit of Quantification (0.5 µg a.s./L)

Biological results:

Details of effects on algal growth are provided in Table 2.9.2.3.3-19. A statistically significant difference was found between the untreated and solvent control groups in terms of both growth rate and algal yield. The effects of the lenacil treatments were therefore evaluated by comparison with the solvent control alone. Statistically significant ($\alpha = 0.05$) inhibition of growth, based on specific growth rates and on cell yield, occurred at geometric mean measured concentrations ≥ 21.6 µg a.s./L. However, for growth rate, the inhibition in the 21.6 µg a.s./L treatment was only 7.27%. As inhibition below 10% is not considered ecotoxicologically relevant, the NOEC for growth rate was was set at 21.6 µg a.s./L. The concentrations of lenacil causing 10%, 20% and 50% inhibition based on the various algal growth indices are compiled in Table 2.9.2.3.3-20.

Microscopic examination of algae taken from the various media after 96 hours showed the presence cellular debris in quantities that increased in accordance with the degree of growth inhibition, but otherwise the appearance of the algal cells exposed to lenacil was normal.

Table 2.9.2.3.3-19: Effects on growth of *Closterium cornu* following exposure to lenacil technical for 96 hours

Geometric mean measured lenacil concentration (µg a.s./L)	Mean cell density ($\times 10^3$ cells/mL)				Mean growth rate µ (day^{-1}) during 0 - 96 hours *[% inhibition]*[a]		Mean yield ($\times 10^4$ cells/mL) after 96 hours *[% inhibition]*[a]	
	24 hours	48 hours	72 hours	96 hours				
Control	6.0	14.2	43.3	93.6	0.905	*[-]*	91.1	*[-]*
Solvent control	6.3	13.5	35.6	76.7	0.854	*[-]*	74.2	*[-]*
2.21	7.5	12.8	32.3	72.3	0.837	*[2.08]*	69.8	*[5.98]*
7.34	6.9	12.3	29.1	78.9	0.862	*[-0.92]*	76.4	*[-2.95]*
21.6	5.2	9.73	22.2	59.6	0.792*	*[7.27]*	57.1*	*[23.0]*
61.6	4.3	4.26	7.50	17.5	0.482*	*[43.6]*	15.0*	*[79.8]*
188	3.1	2.17	3.20	3.88	0.101*	*[88.1]*	1.38*	*[98.1]*

*[a] Relative to the mean solvent control; *Significantly different ($\alpha = 0.05$, one-sided smaller) from the solvent control, negative values indicate an increase relative to the control.*

Table 2.9.2.3.3-20: Growth inhibition endpoints for *Closterium cornu* following 96-hour exposure to lenacil technical

Parameter	Endpoint	Geometric mean measured lenacil concentration (µg a.s./L)
Growth rate	E_rC_{10}	24.6 (95% CL: 19.4 to 29.3)
	E_rC_{20}	35.3 (95% CL: 29.7 to 40.3)
	E_rC_{50}	70.6 (95% CL:64.0 to 78.0)

	$NOEC_r$	21.6
Cumulative yield	E_yC_{10}	14.9 (95% CL: 10.1 to 18.8)
	E_yC_{20}	20.0 (95% CL: 15.1 to 24.1)
	E_yC_{50}	35.3 (95% CL: 30.2 to 41.4)
	$NOEC_y$	7.34

In the separate reference test, the 96-hour E_rC_{50} endpoint for *C. cornu* exposed to 3,5-dichlorophenol was 3.62 mg/L (nominal).

Conclusions:
The 96-hour toxicity of lenacil technical to the unicellular alga *Closterium cornu* was determined in a static system. Dose-related growth inhibition occurred and the 96-hour E_rC_{50} (based on specific growth rate) and E_yC_{50} (based on algal yield) were 70.6 and 35.3 µg lenacil/L, respectively, based on initial measured exposure concentrations. The NOEC was 21.6 µg a.s./L for growth rate and 7.34 µg a.s./L for yield.

RMS comments:
The validity criteria of the most recent version of OECD Test Guideline 201 (2011) are met:

- The biomass in the control and solvent control increased exponentially by a factor of at least 16 within the test period. This corresponds to a specific growth rate of at least 0.92 day^{-1} (measured: increase by a factor of 17.3 and 14.6 over the 0-72h period in the control and solvent control, respectively. As over this period the validity criterion was not met, the test duration was prolonged for further 24h. After 96h, the cell number increased by a factor 37.4 and 30.7 in the control and solvent control, respectively)
- The coefficient of variation of average specific growth rate during the whole test period in replicate control cultures did not exceed 10% (measured: 2.5 % in the control and 2.6% in the solvent control)
- The mean coefficient of variation for section-by-section growth rates in the control cultures did not exceed 35% (measured: 13.8% in the control and 20.0% in the solvent control).

Consequently, this study is considered acceptable for use in the risk assessment.

The analytical method used could be fully validated according to the EU Guidance SANCO/3029/99 rev. 4., and is therefore considered "fit for purpose". The LOQ was set at 0.5 µg/L (please refer to Vol. 3 (CA), Section B.5.1.2.6 – studies no. 12 to 17, for further details).

The following endpoints, based on geometric mean measured concentrations, will be considered in the risk assessment:
E_yC_{50} (*Closterium cornu*, 96 h) = 35.3 µg a.s./L
E_yC_{20} (*Closterium cornu*, 96 h) = 20.0 µg a.s./L
E_yC_{10} (*Closterium cornu*, 96 h) = 14.9 µg a.s./L
$NOEC_y$ (*Closterium cornu*, 96 h) = 7.34 µg a.s./L
E_rC_{50} (*Closterium cornu*, 96 h) = 70.6 µg a.s./L
E_rC_{20} (*Closterium cornu*, 96 h) = 35.3 µg a.s./L
E_rC_{10} (*Closterium cornu*, 96 h) = 24.6 µg a.s./L
$NOEC_r$ (*Closterium cornu*, 96 h) = 21.6 µg a.s./L

Report:	**CA8.2.6.2/06. Wenzel A. (2014e)** **Effect of Lenacil technical on the growth of *Xanthonema debile***

Report No.: DPT-001/4-10/I
Guidelines: OECD 201 (2011)
GLP: Yes

Previous evaluation: None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application

Material and Methods:
Test substance: lenacil (Batch no.: 0190813, chemical purity: 99.3 %)

Test species:	*Xanthonema debile* (unicellular yellow-green alga); Xanthophyceae; Heterokontophyta. SAG-No 836-1.
Number of organisms:	8 replicates for the control and the solvent control; 4 replicates per test concentration; 1 x 10^4 cells/mL at initiation
Type of test:	72-hour static toxicity test
Applied and measured concentrations:	
Nominal test concentrations:	control; solvent control (dimethylformamide); 10.0, 31.6, 100, 316, 1000 µg a.s./L
Mean measured concentrations:	0.00, 0.00, 10.8, 33.8, 63.2, 303, 576 µg a.s./L
Initial measured concentrations:	0.00, 0.00, 10.9, 34.3, 62.9, 310, 600 µg a.s./L
Test conditions:	Sterile synthetic nutrient test medium according to OECD 201 Conical flasks of 250 mL, containing 99 mL of test or control culture temperature : 22.0 °C. pH : 8.09-8.47 (initial), 8.16-8.27 (final) light regime : continuous illumination light intensity : 62.75-64.69 µmol/m².s Incubation in an orbital shaker (150 rpm)
Analytical methods:	LC-MS/MS
Statistics:	Statistical evaluations were performed with ToxRat® Professional 2.10 software. E_rC_{50} and E_yC_{50} values and their corresponding 95% confidence limits were determined by probit analysis using linear maximum likelihood regression and assuming log-normal distribution. Williams' Multiple Sequential t-test was applied to the growth rate and yield data, to identify significant differences from the untreated control and determine the corresponding LOEC and NOEC values.
Test procedure:	Samples were taken at 0, 24, 48 and 72 hours and the cell densities determined by measurements of chlorophyll fluorescence. At the end of the test, the appearance of the algae was also checked by microscope to detect any visual effect of the test item.

Findings:

Analytical results:

At test start, the measured concentrations ranged from 60.0 to 109% of nominal. Thus, the measured initial concentrations were outside the frame of ± 20% of nominal. Similarly, measured concentrations at the end of the test period ranged from 55.3 to 108% of nominal. The residual concentrations measured after 72 hours represented between 92.2% and 101% of the corresponding initial measured concentrations, indicating that the achieved concentrations of the test compound remained stable under the conditions of the test. Thus, the evaluation of the test was based on the initial measured concentrations.

Table 2.9.2.3.3-21: Measured concentration of lenacil in the test media collected during the toxicity test with *Xanthonema debile*.

Nominal concentration of test item (µg a.s./L)	Measured 0 h		Measured 72 h			Geometric mean measured concentration	
	µg a.s./L	% of nominal	µg a.s./L	% of nominal	% of initial	µg a.s./L	% of nominal
Control	<LOQ	-	<LOQ	-	-	-	-
Solvent control	<LOQ	-	<LOQ	-	-	-	-
10.0	10.9	109	10.8	108	98.4	10.8	108
31.6	34.3	109	33.3	105	97.1	33.8	107
100	62.9	62.9	63.6	63.6	101	63.2	63.2
316	310	98.2	295	93.4	95.2	303	95.8
1000	600	60.0	553	55.3	92.2	576	57.6

Measured concentrations were calculated from reported results of duplicate analysis at each timepoint; LOQ: Limit of Quantification (0.5 µg a.s./L)

Biological results:

Details of effects on algal growth are provided in Table 2.9.2.3.3-22. There was no significant difference between the control and solvent control. As recommended by OECD 201, the solvent control was used for test evaluation in preference of the untreated control or the mean of the two control groups. Statistically significant ($\alpha = 0.05$) inhibition of growth, based on specific growth rates and on cell yield, occurred at measured initial concentrations $\geq$ 34.3 µg a.s./L. The concentrations of lenacil causing 10%, 20% and 50% inhibition based on the various algal growth indices are compiled in Table 2.9.2.3.3-23.

Microscopic examination of algae taken from the various media after 72 hours showed the presence cellular debris in quantities that increased in accordance with the degree of growth inhibition, but otherwise the appearance of the algal cells exposed to lenacil was normal.

Table 2.9.2.3.3-22: Effects on growth of *Xanthonema debile* following exposure to lenacil technical for 72 hours

Initial measured lenacil concentration (µg a.s./L)	Mean cell density ($\times 10^4$ cells/mL)			Mean growth rate µ (day^{-1}) during 0 - 72 hours *[% inhibition]*[a]		Mean yield ($\times 10^4$ cells/mL) after 72 hours *[% inhibition]*[a]	
	24 hours	48 hours	72 hours				
Control	2.8	6.8	17.7	0.957	*[-]*	16.71	*[-]*
Solvent control	3.1	7.2	18.2	0.964	*[-]*	17.16	*[-]*
10.9	4.0	9.6	17.1	0.946	*[1.86]*	16.13	*[6.05]*
34.3	3.4	5.7	7.7	0.678*	*[29.7]*	6.66*	*[61.2]*
62.9	3.1	3.9	4.5	0.500*	*[48.2]*	3.49*	*[79.7]*
310	2.8	3.1	3.0	0.364*	*[62.3]*	1.98*	*[88.5]*
600	2.6	2.9	2.3	0.283*	*[70.7]*	1.34*	*[92.2]*

*[a] Relative to the mean solvent control; *Significantly different ($\alpha = 0.05$, one-sided smaller) from the solvent control, negative values indicate an increase relative to the control.*

Table 2.9.2.3.3-23: Growth inhibition endpoints for *Xanthonema debile* following 72-hour exposure to lenacil technical

Parameter	Endpoint	Initial measured lenacil concentration (µg a.s./L)
Growth rate	E_rC_{10}	7.03 (95% CL: 3.27 to 11.88)
	E_rC_{20}	19.2 (95% CL: 11.28 to 28.19)
	E_rC_{50}	132 (95% CL: 101.39 to 174.75)
	$NOEC_r$	10.9
Cumulative yield	E_yC_{10}	11.2 (95% CL: 7.36 to 14.43)
	E_yC_{20}	15.6 (95% CL: 11.46 to 19.07)
	E_yC_{50}	29.8 (95% CL: 25.72 to 33.85)
	$NOEC_y$	10.9

In the test performed most recently prior to this study with lenacil technical, the 72-hour E_rC_{50} endpoint for *X. debile* exposed to the reference toxicant 3,5-dichlorophenol was 0.97 mg/L (nominal).

Conclusions:
The 72-hour toxicity of lenacil technical to the unicellular yellow-green alga *Xanthonema debile* was determined in a static system. Dose-related growth inhibition occurred and the 72-hour E_rC_{50} (based on specific growth rate) and E_yC_{50} (based on algal yield) were 132 and 29.8 µg lenacil/L, respectively, based on initial measured exposure concentrations. The NOEC and LOEC were 10.9 and 34.3 µg a.s./L, respectively, for both growth parameters.

RMS comments:
The validity criteria of the most recent version of OECD Test Guideline 201 (2011) are met:

- The biomass in the control and solvent control increased exponentially by a factor of at least 16 within the test period. This corresponds to a specific growth rate of at least 0.92 day^{-1} (measured: increase by a factor of 17.7 and 18.2 for the period 0-72h for the control and solvent control, respectively)
- The coefficient of variation of average specific growth rate during the whole test period in replicate control cultures did not exceed 10% (measured: 3.7 % in the control and 4.2% in the solvent control)

- The mean coefficient of variation for section-by-section growth rates in the control cultures did not exceed 35% (measured: 11.0% in the control and 13.4% in the solvent control).

Consequently, this study is considered acceptable for use in the risk assessment.

The analytical method used could be fully validated according to the EU Guidance SANCO/3029/99 rev. 4., and is therefore considered "fit for purpose". The LOQ was set at 0.5 µg/L (please refer to Vol. 3 (CA), Section B.5.1.2.6 – studies no. 12 to 17, for further details).

The following endpoints, based on initial measured concentrations, will be considered in the risk assessment:
E_yC_{50} (*Xanthonema debile*, 72 h) = 29.8 µg a.s./L
E_yC_{20} (*Xanthonema debile*, 72 h) = 15.6 µg a.s./L
E_yC_{10} (*Xanthonema debile*, 72 h) = 11.2 µg a.s./L
E_rC_{50} (*Xanthonema debile,* 72 h) = 132 µg a.s./L
E_rC_{20} (*Xanthonema debile*, 72 h) = 19.2 µg a.s./L
E_rC_{10} (*Xanthonema debile*, 72 h) = 7.03 µg a.s./L
NOEC (*Xanthonema debile*, 72 h) = 10.9 µg a.s./L

Report:	**CA8.2.6.2/07. Wenzel A. (2014f)** **Effect of Lenacil technical on the growth of *Nannochloropsis limnetica***
Report No.:	DPT-001/4-10/H
Guidelines:	OECD 201 (2011)
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); not relevant for renewal application
Material and Methods:	
Test substance:	lenacil (Batch no.: 0190813, chemical purity: 99.3 %)
Test species:	*Nannochloropsis limnetica* (unicellular alga); Eustigmatophyceae; Heterokontophyta. SAG-No 18.99.
Number of organisms:	8 replicates for the control and the solvent control; 4 replicates per test concentration; 1 x 10^5 cells/mL at initiation
Type of test:	113-hour static toxicity test
Applied and measured concentrations:	
Nominal test concentrations:	control; solvent control (dimethylformamide); 18.75, 37.5, 75.0, 150, 300 µg a.s./L
Arithmetic mean measured concentrations:	0.00, 0.00, 23.1, 42.3 70.8, 153, 301 µg a.s./L
Test conditions:	Sterile synthetic nutrient test medium according to OECD 201 Conical flasks of 250 mL, containing 100 mL of test or control culture temperature : 22.0 °C. pH : 9.93-10.28 (initial), 8.43-8.59 (final) light regime : continuous illumination light intensity : 91.84-95.51 µmol/m².s Incubation in an orbital shaker (150 rpm)
Analytical methods:	LC-MS/MS
Statistics:	Statistical evaluations were performed with ToxRat® Professional 2.10 software. E_rC_{50} and E_yC_{50} values and their corresponding 95% confidence limits were determined by probit analysis using linear maximum likelihood regression and assuming log-normal distribution. After applying ANOVA, Williams' Multiple Sequential t-test was used to identify significant differences from the solvent control and determine the corresponding LOEC and NOEC values for the growth rate and yield data sets.
Test procedure:	Samples were taken at 0, 24, 48, 70, 89 and 113 hours and the cell densities determined by measurements of chlorophyll fluorescence. At the end of the test,

the appearance of the algae was also checked by microscope to detect any visual effect of the test item.

Findings:

Analytical results:

At test start, the measured concentrations ranged from 84.1 to 123.9% of nominal. Thus, the measured initial concentrations were outside the frame of ± 20% of nominal. Similarly, measured concentrations at the end of the test period (after 113 hours) ranged from 91.5 to 123% of nominal. The residual concentrations measured after 113 hours represented between 81.9% and 138.3% of the corresponding initial measured concentrations. As the deviation from the nominal and measured initial concentrations was greater than ± 20% at test end, the evaluation of the test was based on arithmetic mean concentrations.

Table 2.9.2.3.3-24: Measured concentration of lenacil in the test media collected during the toxicity test with *Nannochloropsis limnetica*.

Nominal concentration of test item (µg a.s./L)	Measured 0 h		Measured 113 h			Arithmetic mean measured concentration	
	µg a.s./L	% of nominal	µg a.s./L	% of nominal	% of initial	µg a.s./L	% of nominal
Control	<LOQ	-	<LOQ	-	-	-	-
Solvent control	<LOQ	-	<LOQ	-	-	-	-
18.75	23.2	124	23.0	123	99.1	23.1	123.2
37.5	46.5	124	38.1	102	91.9	42.3	112.8
75	73.0	97.3	68.6	91.5	94.0	70.8	94.4
150	171	114	147	98.1	86.1	159	106.1
300	252	84.1	349	116	138.3	301	100.2

Measured concentrations were calculated from reported results of duplicate analysis at each timepoint; LOQ: Limit of Quantification (0.5 µg a.s./L)

Biological results:

Details of effects on algal growth are provided in Table 2.9.2.3.3-25. There was no significant difference between the control and solvent control. As recommended by OECD 201, the solvent control was used for test evaluation in preference of the untreated control or the mean of the two control groups. Statistically significant ($\alpha = 0.05$) inhibition of growth, based on specific growth rates and on cell yield, occurred at measured initial concentrations ≥ 70.8 µg a.s./L. The concentrations of lenacil causing 10%, 20% and 50% inhibition based on the various algal growth indices are compiled in Table 2.9.2.3.3-26.

Microscopic examination of algae taken from the various media after 72 hours showed the presence cellular debris in quantities that increased in accordance with the degree of growth inhibition, but otherwise the appearance of the algal cells exposed to lenacil was normal.

Table 2.9.2.3.3-25: Effects on growth of *Nannochloropsis limnetica* following exposure to lenacil technical for 113 hours

Arithmetic mean measured lenacil concentration (µg a.s./L)	Mean cell density (×10^5 cells/mL)					Mean growth rate µ (day^{-1}) during 0 - 113 hours *[% inhibition]*[c]		Mean yield (×10^4 cells/mL) after 113 hours *[% inhibition]*[c]	
	24 hours	48 hours	70 hours	89 hours	113 hours				
Control	3.3[a]	5.2[a]	9.5[a]	17.4[a]	32.1[a]	0.73	*[-]*	31.10	*[-]*
Solvent control	2.7[a]	4.6[a]	9.4[a]	17.4[a]	33.0[a]	0.73	*[-]*	31.97	*[-]*
23.1	4.9[b]	6.8[b]	8.6[b]	19.2[b]	35.6[b]	0.76	*[-4.8]*	34.60	*[-8.2]*
42.3	4.5[b]	5.6[b]	8.0[b]	14.1[b]	25.9[b]	0.67	*[7.9]*	24.90	*[22.1]*
70.8	4.1[b]	4.5[b]	6.0[b]	9.2[b]	14.1[b]	0.56*	*[22.6]*	13.07*	*[59.1]*
159	4.9[b]	4.0[b]	5.5[b]	8.7[b]	15.9[b]	0.59*	*[18.6]*	14.93*	*[53.3]*
301	2.9	0.5	0.0	0.1	0.0	-1.97*	*[371.4]*	-1.00*	*[103.1]*

[a] Excluding anomalously low counts from two replicates that were considered to be outliers; [b] excluding anomalously low counts from one replicate that was considered to be an outlier; [c] Relative to the mean solvent

*control; *Significantly different (α = 0.05, one-sided smaller) from the solvent control, negative values indicate an increase relative to the control.*

Table 2.9.2.3.3-26: Growth inhibition endpoints for *Nannochloropsis limnetica* following 113-hour exposure to lenacil technical

Parameter	Endpoint	Arithmetic mean measured lenacil concentration (µg a.s./L)
Growth rate	E_rC_{10}	105 (95% CL: 57.6 to 135)
	E_rC_{20}	127 (95% CL: 81.7 to 156)
	E_rC_{50}	184 (95% CL:148 to 226)
	$NOEC_r$	42.3
Cumulative yield	E_yC_{10}	17.5 (95% CL: 3.56 to 31.2)
	E_yC_{20}	28.8 (95% CL: 9.45 to 45.4)
	E_yC_{50}	75.1 (95% CL: 48.6 to 116)
	$NOEC_y$	42.3

Conclusions:
The 113-hour toxicity of lenacil technical to the unicellular fresh-water alga *Nannochloropsis limnetica* was determined in a static system. Dose-related growth inhibition occurred and the 113-hour E_rC_{50} (based on specific growth rate) and E_yC_{50} (based on algal yield) were 184 and 75.1 µg lenacil/L, respectively, based on arithmetic mean measured exposure concentrations. The NOEC and LOEC were 42.3 and 70.8 µg a.s./L, respectively, for both growth parameters.

RMS comments:
The following validity criteria of the most recent version of OECD Test Guideline 201 (2011) are met:

- The biomass in the control and solvent control increased exponentially by a factor of at least 16 within the test period. This corresponds to a specific growth rate of at least 0.92 day^{-1} (measured: increase by a factor of 9.46 and 9.39 over the 0-70h period in the control and solvent control, respectively. As over this period the validity criterion was not met, the test duration was prolonged for until 113h. After 113h, the cell number increased by a factor 32.1 and 33.0 in the control and solvent control, respectively)

However, the mean coefficient of variation for section-by-section growth rates in the control cultures was 43.1%, and thus exceeded the maximum allowed value of 35%. For the solvent control, the mean coefficient of variation for section-by-section growth rates was 34.1%, which was just below the trigger of 35%. Further, the mean coefficient of variation of average specific growth rate during the whole test period (113h) in replicate control cultures exceeded the trigger of 10%, with a value of 12.08% in the control and 15.39% in the solvent control.
In the study report, the author argues that the validity criterion for section-by-section growth rate was met for the solvent control. Further, the author states that, taking into account the prolonged test period over 113 hours and that *N. limnetica* is a non-standard algal species, the slightly higher coefficient of variation over the whole test period in the controls is without influence on the acceptability of the test and its relevant results. However, the RMS does not agree with the study authors, and considers that the fact that two out of three validity criteria were not met is sufficient to invalidate the results from this study.

It is further noted that 2 replicates in the control and solvent control, and one replicate in the 23.1, 42.3, 70.8 and 159 µg a.s./L (arithmetic mean measured concentrations) treatments were excluded from evaluation, as they were considered an outlier based on expert judgement due to irregular growth (the number of cells counted were anomalously low). This further indicates that the performance of the control was not sufficiently stable, and that therefore the results of this study might be questioned.

Overall, this study is not considered acceptable for use in risk assessment.

Report:	**CA8.2.7/01. Flatman D. (2003d)** **Lenacil technical - Higher plant (*Lemna*) growth inhibition test**

Report No.: ACD 039/023827
Guidelines: OECD draft (2000), US EPA draft OPPTS 850.4400 (1996)

GLP: Yes

Previous evaluation: In DAR (November 2007); relevant for renewal application

Material and Methods:

Test substance: lenacil (Batch no.: 141712003, chemical purity: 98.6%)

Test species: *Lemna gibba* (common duckweed)

Number of organisms: 4 colonies consisting of 3 fronds were placed in each test vessel, resulting in 12 fronds per replicate at test initiation; 3 replicates for the control, the solvent control and per treatment,

Type of test: 7-day semi-static toxicity test (media renewal at 48 to 72-hour intervals)

Applied and measured concentrations:

Nominal concentrations: control; solvent control (100 µL/L dimethylformamide); serial dilutions (0.10, 0.20, 0.40, 0.60, 1.8 %) of a nominal concentration of 10 mg a.s./L, equivalent to 10, 20, 40, 60 and 180 µg a.s./L

Mean measured concentrations: 0.0; 0.0; 3.7, 8.8, 15, 24, 71 µg a.s./L

Test conditions:
Conical flasks of 500 mL, test volume 200 mL; 20 x-AAP medium
temperature: 23.5 - 26.2 °C
pH: 7.6 - 7.7 (fresh media), 7.7 - 8.6 (old media)
light regime: continuous illumination
light intensity: 4870 - 5610 lux

Analytical methods: HPLC/UV

Statistics: An analysis was carried out to determine any significance between the control and solvent control groups using ordinary t-tests. The F1 test for monotonicity was first applied. If this was not significant at the 1% level, then each of the test compound treated groups was compared with the solvent control using a two-sided Williams test for monotonic trends. If the monotonicity test was significant at the 1% level, indicating a non-monotone dose-response, then a two-sided Dunnet's test was applied instead. To estimate ECx values, a two-parameter logistig regression curve versus log10(concentration) was fitted to the individual percentage inhibition values, with 0% inhibition assumed at zero dose (solvent control) and 100% inhibition at infinite dose.

Test procedure:

At media renewal (day 2, 5 and 7), all plants were observed and any difference in growth habit or general health were noted in comparison to the control. Frond numbers were counted as an assessment of growth in each test vessel on days 2, 5 and 7 of the exposure period.

At the end of the exposure phase, all plant material in each replicate was carefully blotted dry on paper towel. The fronds from each test vessel were then placed into a pre-weighed, pre-filter paper. The fronds were then oven-dried at approximately 60°C for 48 hours. After cooling under dry conditions, the filter paper together with the dry fronds was re-weighed. The dry weights of each individual frond were then calculated from the number of fronds in each vessel.

Findings:

Analytical results:

The measured concentrations in the fresh and expired test media are shown in Table 2.9.2.3.3-27. The nominal concentrations were never obtained within ± 20%, but the measured concentrations were relatively constant during the exposure period. The results are expressed based on mean measured concentrations.

Table 2.9.2.3.3-27: Measured concentration of lenacil in the test media collected during the toxicity test with *Lemna gibba*

Nominal concentration of test item (% of	Measured concentration of test item (µg a.s./L)						
	Day 0 fresh	Day 2 expired	Day 2 fresh	Day 5 expired	Day 5 fresh	Day 7 expired	Geometric mean

prepared solution)							
Solvent control	<LOD [1]	<LOD [1]	<LOD [1]	<LOD [1]	<LOD [1]	<LOD [1]	-
0.1	3.51	3.80	3.39	3.51	3.93	4.34	3.73
0.2	8.43	7.91	8.92	8.67	9.83	9.05	8.78
0.4	15.22	15.42	15.64	13.87	15.93	15.21	15.20
0.6	23.73	24.89	23.11	22.26	23.96	23.82	23.6
1.8	67.44	73.10	69.85	66.59	73.29	76.64	71.06

[1] *LOD = Limit of Detection = 0.7 µg a.s./L*

Biological results:

There was no significant difference between the control and solvent control for any of the parameters tested. After 7 days of exposure, the number of fronds was significantly reduced at the treatment levels of 8.8, 15, 24 and 71 µg a.s./L, compared to the solvent control. Frond dry weight showed a more variable response after exposure to lenacil: dry weight was significantly reduced at the treatment level of 15 µg a.s./L. Above and below this concentration, no significant differences in frond dry weights were observed between the solvent control group and test plants.

The effects of growth inhibition were evident from visual inspections of the plants at the end of the exposure period. There were no visible effects on growth for fronds exposed to 15 µg a.s./L and below. However, cultures exposed to the treatment level of 24 µg a.s./L showed a higher incidence of small and dead fronds. At the treatment level of 71 µg a.s./L, the fronds had become detached from their colonies and existed as separate entities, some plants had no visible root growth, and roots that were present were brittle.

Table 2.9.2.3.3-28: Inhibition of biomass (area under the growth curve) and growth rate in the toxicity test with *Lemna gibba*

Mean measured concentration (µg a.s./L)	Mean number of fronds	Mean % inhibition (0-7 days) [1]		Mean dry weight of fronds (mg)	Mean % inhibition (0-7 days) [1]
		Area under the growth curve	Growth rate		
Control	136	-	-	0.11	-
Solvent control	151	-	-	0.12	-
3.7	153	3.20	-0.61	0.12	5.54
8.8	146	5.66	1.31	0.098	20.11
15	86	43.74**	22.67**	0.082	32.95*
24	59	59.56**	37.51**	0.10	18.84
71	15	93.45**	90.33**	0.15	-18.97

** Statistically significant different compared to the control (Dunnett's test, $p < 0.05$); ** Statistically significant different compared to the control (Williams' test, $p < 0.01$); [1]negative values indicate an increase relative to the control*

Based on the percent inhibition observed for frond number, an EC_bC_{50} (for area under the growth curve) and E_rC_{50} (for growth rate) of 19.20 µg a.s./L (95% CI: 16.90-21.82 µg a.s./L) and 28.62 µg a.s./L (95% CI: 25.93-31.58 µg a.s./L), respectively, were calculated. The NOEC for frond number was 8.8 µg a.s./L. For dry weight, an EC_{50} could not be calculated (no effects > 50% were observed).

Conclusions:

Lenacil technical was inhibitory to the growth of *Lemna gibba*, at concentrations in excess of 8.8 µg a.s./L after seven days of exposure. The EC_bC_{50} (for area under the growth curve) for seven days of exposure was 19.20 µg a.s./L and the E_rC_{50} (for growth rate) was 28.62 µg a.s./L.

RMS comments:

Although the study was performed according to a draft version of this test guideline, the validity criteria of OECD Test Guideline 221 are met:

- The frond number doubling time in the control was less than 2.5 days (60h), corresponding to approximately an average specific growth rate of 0.275 day^{-1} (measured: doubling time of frond number in

the control and solvent control was 2.0 and 1.9 days, respectively; the average specific growth rate in the control and solvent control between day 0 and 7 was 0.346 and 0.362 day^{-1}, respectively)

Consequently, this study is considered acceptable for use in the risk assessment.

The analytical method used could not be fully validated according to the EU Guidance SANCO/3029/99 rev. 4. However, the available validation data from all studies where this method was used (CA8.2.1/02, CA8.2.6.1/01, CA8.2.6.2/01 and CA8.2.7/01) seem to indicate that the method works well and can be considered "fit for purpose". The LOQ for lemna medium was set at 1.011 µg/L (please refer to Vol. 3 (CA), Section B.5.1.2.6 – studies no. 3 to 6, for further details). The working range covers the test concentrations.

In accordance with the new data requirements (Commission Regulation EU No 283/2013), the EC_{10} and EC_{20} values should be calculated. Where these values cannot be estimated, an explanation should be provided. As the present study was already submitted for the initial Annex I inclusion of lenacil, this was not addressed in the study report. Therefore, RMS calculated the EC_{10} and EC_{20} for growth rate and biomass (area under the growth curve) for the parameter 'frond number'. EC_{10} and EC_{20} calculations were performed in the R statistical environment, Version 3.5.1 (R Development Core Team, 2018) using the R package 'drc'. The same model and assumptions were used as in the study report: a two-parameter logistig regression curve versus log10(concentration) was fitted to the individual percentage inhibition values, with 0% inhibition assumed at zero dose (solvent control) and 100% inhibition at infinite dose. The calculated EC_x values are shown in Table 2.9.2.3.3-29.

Table 2.9.2.3.3-29: Calculated EC_{10} and EC_{20} values for the test with *Lemna gibba*.

Endpoint	EC_{10} (µg/L)	EC_{20} (µg/L)
Biomass (area under the growth curve)	7.51 (95% CL 2.87-12.16)	10.62 (95% CL 6.19-15.04)
Growth rate	11.32 (95% CL 7.54-15.11)	15.94 (95% CL 12.20-19.69)

The following endpoints for frond number, based on mean measured concentrations, will be considered in the risk assessment:

E_bC_{50} (*Lemna gibba*, 7 d) = 19.2 µg a.s./L
E_bC_{20} (*Lemna gibba*, 7 d) = 10.6 µg a.s./L
E_bC_{10} (*Lemna gibba*, 7 d) = 7.5 µg a.s./L
E_rC_{50} (*Lemna gibba*, 7 d) = 28.6 µg a.s./L
E_rC_{20} (*Lemna gibba*, 7 d) = 15.9 µg a.s./L
E_rC_{10} (*Lemna gibba*, 7 d) = 11.3 µg a.s./L
NOEC (*Lemna gibba*, 7 d) = 8.8 µg a.s./L

Report:	**CA8.2.7/02. Wenzel A. (2012a)** **Macrophytes, growth inhibition test - effect of Lenacil technical on the growth of *Chara globularis* in the presence of sediment**
Report No.:	DPT-001/4-80/D
Guidelines:	Maltby *et al.* (2010)[17]
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Material and Methods:	
Test substance:	lenacil (Batch no.: 200010003, chemical purity: 99.1%)

[17] Maltby, L., Arnold, D., Arts, G., Davies, J., Heimbach, F., Pickl, C. and Poulsen, V. (eds) (2010): Aquatic Macrophyte Risk Assessment for Pesticides. Chapter 5.2: Development of a proposed method for the rooted aquatic macrophyte, *Myriophyllum* sp., pp. 47-63. SETAC Europe Workshop AMRAP Wageningen (The Netherlands), January 2008. SETAC CRC Press, Taylor & Francis Group, Boca Raton London New York.

Test species:	*Chara globularis* (also known as *Chara fragilis*)
	Algae, Charophyta, Charales, Characeae. Due to its stem-like and leaf-like structures it is generally accepted as a "macrophyte". The algae are anchored in the substrate.
Number of organisms:	a single pot containing 5 plants was placed in each test vessel; 6 replicates for the control, 3 replicates per treatment.
Type of test:	7-day static toxicity test
Applied and measured concentrations:	
Nominal concentrations:	control (nutrient medium only); 3.13, 12.5, 50, 200, 800 µg a.s./L
Arithmetic mean measured concentrations: 0.0; 2.18, 7.73, 24.1, 94.1, 475.6 µg a.s./L	
Sediment:	Artificial, prepared at least two days prior to use according to OECD guideline 219.
	'**Standard' sediment** comprised 5.0% powdered sphagnum peat (dry weight, according to 2 ± 0.5% organic carbon), 19.8% (dry weight) kaolin clay, 75.1% (dry weight) fine quartz sand, pH was adjusted to 7.0 ± 0.5 with 50 g $CaCO_3$ and moisture content was adjusted with deionised water to *ca* 30%. **'Nutrient-supplemented' sediment** was prepared as above, but was fortified with nutrients and moisture-adjusted by using an aqueous solution containing ammonium chloride and sodium phosphate instead of deionised water
Test vessels:	2-L glass beakers (*ca.* 24 cm tall and 11 cm diameter). Each contained a single small plastic plant pot (*ca.* 8 cm tall, with 9 cm diameter and 350 mL capacity) with plants potted in sediment. The depth of medium overlying the sediment surface at the top of the pots was at least 12 cm.
	Plant pots were filled by first covering the holes at the base with a filter paper before adding a 1 cm layer of standard sediment. A 4 cm layer of nutrient-supplemented sediment was placed on top and this was covered with a further 1 cm standard sediment. A *ca*. 2 mm layer of coarse quartz sand was placed on top of the sediment to minimise the flotation of sediment into the overlying medium. After planting, test vessels were carefully filled with 2 L of the respective medium preparation and the initial level marked on the outside of each unit. Evaporation losses > 10% were restored by adding distilled water. The growth medium used was prepared according to Smart & Barko (1985), which has been shown to be suitable for macrophytes.
Test conditions:	temperature: 18.5 – 22.0 °C
	pH: Initial: 7.98 – 8.07; Final: 8.82 - 9.05 in the control, 8.03 - 9.03 L in the lenacil treatments ≤ 24.1 µg/L, 7.72 – 7.83 in the lenacil treatments ≥ 94.1 µg/L dissolved oxygen: Initial: 8.10 - 8.46 mg O_2/L; Final: 10.63 - 11.30 mg O_2/L in the control, 10.02 - 10.92 mg O_2/L in the lenacil treatments ≤ 24.1 µg/L, 7.54 to 8.06 mg O_2/L in the lenacil treatments ≥ 94.1 µg/L.
	light regime: 16:8 h light:dark light intensity: 7791 - 7973 lux
Analytical methods:	LC-MS/MS
Statistics:	Calculations were applied to the data for replicate vessels, not individual plants. Data evaluation was based on relative gain and growth rate for each of the measured length and biomass parameters. Endpoints were determined with ToxRat® software. Concentrations causing 10%, 20% and 50% inhibition relative to the control, based on the various growth indices, were determined by probit analysis modified for continuous data. ANOVA followed by Williams' test was used to identify statistically significant differences from growth in the control and to locate the LOEC and NOEC concentrations for each of the growth indices.
Test procedure:	

Just before the start of the test, 25 plants of the pre-culture were harvested and plant fresh weight and subsequently dry weight were determined to obtain the respective data for day 0. During the 7-day exposure, shoot length was recorded at the start of the test and on days 4 and 7 (test termination). Shoot length (apical and side shoots) was determined using a ruler positioned within the vessel close to the plant to be measured. At the end of the exposure period, all plants were harvested. Total plant fresh weight was determined after absorbing remaining test medium attached to the plants by means of tissue paper. Dry weight was determined subsequently after weighing the fresh plants. The five plants per replicate were combined and the plants were dried in aluminium weighing boats at 60°C for 48h. Any symptoms (such as chlorosis and necrosis) or other observations were recorded.

Findings:

Analytical results:

The measured lenacil concentrations ranged from 28.1% to 48.0% of the nominal values at test start and increased to between 66.0% and 109.6% of nominal in samples taken on Day 7. Since the highest test concentration was prepared directly in growth medium without using a solubiliser/solvent, the low recovery at test start suggests that the test item was not completely dissolved and was probably inhomogeneously distributed in the visually clear solution. The other treatment levels were prepared by dilution of the highest test concentration. Further dissolution of lenacil during the exposure period is thought to have caused the concentrations at termination to have exceeded those measured at Day 0. The results of the test were related to overall arithmetic mean measured concentrations of lenacil in the test medium; these were considered to represent exposure to dissolved, bioavailable lenacil and therefore to provide the basis for worst-case endpoints.

Table 2.9.2.3.3-30: Measured concentrations in a growth inhibition test with *Chara globularis* exposed to lenacil technical under static conditions in the presence of sediment for 7 days

Nominal concentration of test item (µg a.s./L)	Start exposure period (day 0)		End exposure period (day 7)		Arithmetic mean measured concentration	
	µg a.s./L	% of nominal	µg a.s./L	% of nominal	µg a.s./L	% of nominal
Control	<LOQ	-	<LOQ	-	-	-
3.13	0.92	29.5	3.43	109.6	2.18	69.5
12.5	6.00	48.0	9.46	75.7	7.73	61.8
20	14.66	29.3	33.45	66.9	24.1	48.1
200	56.18	28.1	131.99	66.0	94.1	47.0
800	308.70	38.6	642.48	80.3	475.6	59.4

LOQ: Limit of Quantification (0.1 µg a.s./L)

Biological results:

Concentration-dependent effects of lenacil were recorded for all the measured growth parameters of *C. globularis* and are shown in Table 2.9.2.3.3-31 to Table 2.9.2.3.3-33. Statistically significant ($p \leq 0.05$) effects were encountered at mean measured concentrations ≥ 24.1 µg a.s./L for all growth parameters with the exception of growth rate based on fresh weight measurements, where significant effects were limited to the highest treatment.

In the 94.1 µg a.s./L treatment, all lateral whorls of all plants appeared brownish on Day 7, but the apical whorls were unaffected. This effect was absent in all other lenacil treatments, including that containing the highest concentration (475.6 µg a.s./L). At the termination of the test, one out of five plants was necrotic in each of the three replicate vessels of the 475.6 µg a.s./L treatment.

Table 2.9.2.3.3-31: Effects on *Chara globularis* shoot length following exposure to lenacil technical under static conditions in the presence of sediment for 7 days

Mean measured lenacil concn (µg a.s./L)	Mean[a] shoot length (cm)			Mean relative increase (%) in shoot length after 7 days *[% inhibition]*		Mean growth rate based on shoot length during 0 - 7 days *[% inhibition]*	
	Day 0	Day 4	Day 7				
Control	3.06	4.79	9.15	194.127	*[-]*	0.153	*[-]*
2.18	2.92	4.22	9.27	218.360	*[-12.48]*	0.165	*[-7.64]*
7.73	3.10	4.45	8.69	178.693	*[7.95]*	0.146	*[4.33]*
24.1	2.95	3.95	5.69	95.587*	*[50.76]*	0.096*	*[37.46]*

94.1	2.85	3.68	7.07	148.547*	*[23.48]*	0.130*	*[15.21]*
475.6	2.70	3.43	6.10	95.140*	*[50.99]*	0.093*	*[39.02]*

*[a] Based on 6 replicates in the control and 3 replicates per lenacil treatment.; * Significantly different (p < 0.05, Williams' t-test) from the control, negative values indicate an increase relative to the control.*

Table 2.9.2.3.3-32: Effects on *Chara globularis* fresh weight following exposure to lenacil technical under static conditions in the presence of sediment for 7 days

Mean measured lenacil concn (µg a.s./L)	Meana fresh weight (mg)		Mean relative increase in fresh weight after 7 days [% inhibition]		Mean growth rate based on fresh weight during 0 - 7 days [% inhibition]	
	Day 0 [b]	Day 7				
Control	36.2	76.85	112.113	*[-]*	0.105	*[-]*
2.18	36.2	96.34	165.927	*[-48.00]*	0.140	*[-32.65]*
7.73	36.2	87.85	142.487	*[-27.09]*	0.126	*[-19.86]*
24.1	36.2	63.17	74.347*	*[33.69]*	0.079	*[24.59]*
94.1	36.2	65.31	80.287*	*[28.39]*	0.084	*[20.05]*
475.6	36.2	59.60	64.517*	*[42.45]*	0.070*	*[33.42]*

*[a] Based on 6 replicates in the control and 3 replicates per lenacil treatment; [b] Representative plants subjected to identical preculture, but used for initial wet and dry weight measurements only; * Significantly different (p < 0.05, Williams' t-test) from the control, negative values indicate an increase relative to the control.*

Table 2.9.2.3.3-33: Effects on *Chara globularis* dry weight following exposure to lenacil technical under static conditions in the presence of sediment for 7 days

Mean measured lenacil concn (µg a.s./L)	Mean[a] dry weight (mg)		Mean relative increase in dry weight after 7 days *[% inhibition]*		Mean growth rate based on dry weight during 0 - 7 days *[% inhibition]*	
	Day 0 [b]	Day 7				
Control	9.00	16.64	87.083	*[-]*	0.089	*[-]*
2.18	9.00	21.49	89.267	*[-2.5]*	0.091	*[-2.3]*
7.73	9.00	17.53	98.900	*[-13.6]*	0.097	*[-9.2]*
24.1	9.00	11.82	28.233*	*[67.6]*	0.033*	*[62.5]*
94.1	9.00	14.68	66.433*	*[23.7]*	0.073*	*[18.1]*
475.6	9.00	9.96	23.867*	*[72.6]*	0.029*	*[67.2]*

*[a] Based on 6 replicates in the control and 3 replicates per lenacil treatment; [b] Representative plants subjected to identical preculture, but used for initial wet and dry weight measurements only; * Significantly different (p < 0.05, Williams' t-test) from the control, negative values indicate an increase relative to the control.*

The concentrations of lenacil causing 10%, 20% and 50% inhibition based on the various growth indices, and the corresponding LOEC and NOEC values, are compiled in Table 2.9.2.3.3-34.

Table 2.9.2.3.3-35: Growth inhibition endpoints for *Chara globularis* following exposure to lenacil technical under static conditions in the presence of sediment for 7 days

Parameter		Mean measured lenacil concentration (µg a.s./L) *[95% confidence limits]*				
		EC_{50}	EC_{20}	EC_{10}	LOEC	NOEC
Shoot length	relative gain	450 [103 - >476]	11.5 [0 – 48.8]	1.68[a] [0 – 12.4]	24.1	7.73
	growth rate	> 476	37.53 [0.01 - 194.8]	4.60 [0 – 27.6]	24.1	7.73
Fresh weight	relative gain	> 476	32.3 [2.59 – 87.2]	6.36 [0.04 – 24.2]	24.1	7.73
	growth rate	> 476	77.0 [10.4 – 242]	13.1 [0.06 – 45.7]	476	94.1
Dry weight	relative gain	113 [18.0 - >476]	7.22 [n.d. – 35.2]	1.71[a] [n.d. – 13.0]	24.1	7.73
	growth rate	n.d.[b]	n.d.[b]	n.d.[b]	24.1	7.73

[a] Extrapolated below the lowest test concentration (2.18 µg a.s./L); [b] No meaningful concentration/response relationship ((F) > 0.05, i.e. slope of the relationship is not significantly different from zero); n.d.: Not determined

Conclusions

The 7-day toxicity of lenacil technical to the charophyte *Chara globularis* (also known as *C. fragilis*) was determined in the presence of sediment in a static system. Dry weight gain was the most sensitive growth parameter, with an E_yC_{50} value of 113 µg a.s./L (mean measured), but the corresponding endpoint based on growth rate could not be determined. E_yC_{50} values of 450 and >476 µg a.s./L were calculated for shoot length gain and fresh weight gain, respectively, and the corresponding endpoints based on growth rate (E_rC_{50}) for both parameters were both >476 µg a.s./L. The NOEC was 7.73 µg a.s./L for all parameters except that for growth rate based on fresh weight, which was 94.1 µg a.s./L.

RMS comments

This study was performed according to a draft test guideline for the rooted aquatic macrophyte *Myriophyllum sp.*, as published in Maltby *et al.* (2010). This draft test guideline formed the basis of the more recently published OECD Test Guideline 239 (Water-sediment *Myriophyllum spicatum* toxicity test). As stated in this test guideline, the method is in principle applicable to any other aquatic macrophyte species, provided that adequate growth occurs in the untreated controls under the test conditions. For the present study with *Chara globularis*, the validity criteria of OECD TG 239 are met:

- The mean total shoot length and mean total shoot fresh weight in control plants at least doubled during the exposure phase of the test (measured: shoot length and total shoot fresh weight in the control increased by a factor of 3 and 2.1, respectively)
- The control plants did not show any visual symptoms of chlorosis and were visibly free from contamination by other organisms such as algae and/or bacterial films on the plants, at the surface of the sediment and in the test medium.
- The mean coefficient of variation for yield based on measurements of shoot fresh weight (i.e. from test initiation to test termination) in the control cultures did not exceed 35% between replicates (measured: 15.0)

The analytical method used could be fully validated according to the EU Guidance SANCO/3029/99 rev. 4., and is therefore considered "fit for purpose". The LOQ was set at 0.1 µg/L (please refer to Vol. 3 (CA), Section B.5.1.2.6 – studies no. 18 and 19, for further details).

It is however noted that the measured concentration at the end of the test was increased compared to the measured concentration at test start, which is unexpected. According to the explanation provided by the study author, this is the result of the test item not being completely dissolved in the (visually clear) test solution (no solvent was used), and a subsequent further dissolution during the test period. As the analytical method is fully validated, it is expected that the measured concentration values are reliable. Nevertheless, the strange behaviour of the test item results in some uncertainty regarding the exposure in the test.

It further is noted that results for dry weight (the most sensitive parameter) do not follow a clear dose-response pattern (i.e. statistically significant effect at the three highest test concentrations, but 62.5 and 67.2% effect at 24.1 and 475.6 µg a.s./L, respectively, and only 18.1% effect at 94.1 µg a.s./L). As a result, the concentration response function fitted to the data showed a poor fit. The study report mentions that for dry weight no significant concentration response function could be calculated for growth rate (p-value above the significance level of 0.05).

The issues discussed above limit the reliability of this study. Following discussion and consultation with the notifier, and given the fact that this study is crucial in the (higher tier) risk assessment for aquatic macrophytes (see Volume 3 (PPP), Section B.9.4.3), it was agreed that the notifier will repeat this study in order to confirm its results.

For the time being, the results from the current study will nevertheless be considered in the risk assessment. RMS recalculated the endpoints for dry weight, the most sensitive parameter. To be able to calculate an acceptable dose-response curve, the anomalous results found at the treatment level of 94.1 µg a.s./L were removed from the dataset used for the calculations.

In the study report, the results were expressed based on arithmetic mean measured concentrations. However, OECD TG 239 recommends that the geometric mean measured concentration is used. As the geometric mean measured concentrations are significantly lower compared to the arithmetic mean measured concentrations (see Table 2.9.2.3.3-36), RMS recalculated the endpoints based on geometric mean measured concentrations. EC_x calculations were performed in R statistical environment, Version 3.5.1 (R Development Core Team, 2018) using the R package 'drc'. Dose-response curve models were selected through goodness-of-fit metrics via the 'mselect' command, which compares the log likelihood value, Akaike's information criterion (AIC), the estimated residual standard error and the p-value from a lack-of-fit test for different models. The model with the best fit for the data was a Weibull Type 1 (3parameters) for both yield and growth rate.

The model used was a 2-parameter log-logistic function (with the lower limit fixed at 0 and the upper limit at 100; inhibition values lower than 0% or greater than 100% were replaced by 0 and 100, respectively). The calculated EC_x values are shown in Table 2.9.2.3.3-37. These EC_x values are considered acceptable for use in the risk assessment.

Table 2.9.2.3.3-36: Comparison between nominal, arithmetic mean measured and geometric mean measured concentrations in the toxicity study with *Chara globularis*.

Nominal concentration of test item (µg a.s./L)	Start exposure period (day 0)		End exposure period (day 7		Arithmetic mean measured concentration		Geometric mean measured concentrations	
	µg a.s./L	% of nominal	µg a.s./L	% of nominal	µg a.s./L	% of nominal	µg a.s./L	% of nominal
Control	<LOQ	-	<LOQ	-	-	-	-	-
3.13	0.92	29.5	3.43	109.6	2.18	69.5	1.78	56.8
12.5	6.00	48.0	9.46	75.7	7.73	61.8	7.53	60.3
20	14.66	29.3	33.45	66.9	24.1	48.1	22.1	110.7
200	56.18	28.1	131.99	66.0	94.1	47.0	86.1	43.1
800	308.70	38.6	642.48	80.3	475.6	59.4	445.3	55.7

Table 2.9.2.3.3-37: Calculated EC_{10}, EC_{20} and EC_{50} values for the test with *Chara globularis*, based on geometric mean measured concentrations.

Endpoint	EC_{10} (µg/L)	EC_{20} (µg/L)	EC_{50} (µg/L)
Yield	10.46 (95% CL 9.11-11.80)	11.29 (95% CL 9.99-12.60)	13.54 (95% CL 12.40-14.69)
Growth rate	10.46 (95% CL 9.11-11.82)	11.31 (95% CL 10.00-12.62)	13.57 (95% CL 12.42-14.71)

The following endpoints for the most sensitive parameter (dry weight), based on geometric mean measured concentrations, will be considered in the risk assessment:

E_yC_{50} (*Chara globularis*, 7 day) = 13.54 µg a.s./L
E_yC_{20} (*Chara globularis*, 7 day) = 11.29 µg a.s./L
E_yC_{10} (*Chara globularis*, 7 day) = 10.46 µg a.s./L
E_rC_{50} (*Chara globularis*, 7 day) = 13.57 µg a.s./L
E_rC_{20} (*Chara globularis*, 7 day) = 11.31 µg a.s./L
E_rC_{10} (*Chara globularis*, 7 day) = 10.46 µg a.s./L
NOEC (*Chara globularis*, 7 day) = 7.53 µg a.s./L

Report:	**CA8.2.7/03. Wenzel A. (2012b)** **Macrophytes, growth inhibition test - effect of Lenacil technical on the growth of *Elodea canadensis* in the presence of sediment**
Report No.:	DPT-001/4-80/C
Guidelines:	Maltby *et al.* (2010)[18]

[18] Maltby, L., Arnold, D., Arts, G., Davies, J., Heimbach, F., Pickl, C. and Poulsen, V. (eds) (2010): Aquatic Macrophyte Risk Assessment for Pesticides. Chapter 5.2: Development of a proposed method for the rooted aquatic

GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Material and Methods:	
Test substance:	lenacil (Batch no.: 200010003, chemical purity: 99.1%)
Test species:	*Elodea canadensis* (Canadian waterweed)
	Hydrocharitaceae, Alismatales, Monocotyledonae. The plant is rooted in the sediment.
Number of organisms:	a single pot containing 5 plants was placed in each test vessel; 6 replicates for the control, 3 replicates per treatment.
Type of test:	7-day static toxicity test
Applied and measured concentrations:	
Nominal concentrations:	control (nutrient medium only); 3.13, 12.5, 50, 200, 800 µg a.s./L
Arithmetic mean measured concentrations:	0.0; 6.15, 8.24, 20.1, 74.7, 418 µg a.s./L
Sediment:	Artificial, prepared at least two days prior to use according to OECD guideline 219.
	'**Standard' sediment** comprised 5.0% powdered sphagnum peat (dry weight, according to 2 ± 0.5% organic carbon), 19.8% (dry weight) kaolin clay, 75.1% (dry weight) fine quartz sand, pH was adjusted to 7.0 ± 0.5 with 50 g $CaCO_3$ and moisture content was adjusted with deionised water to *ca* 30%.
	'Nutrient-supplemented' sediment was prepared as above, but was fortified with nutrients and moisture-adjusted by using an aqueous solution containing ammonium chloride and sodium phosphate instead of deionised water
Test vessels:	2-L glass beakers (*ca.* 24 cm tall and 11 cm diameter). Each contained a single small plastic plant pot (*ca.* 8 cm tall, with 9 cm diameter and 350 mL capacity) with plants potted in sediment.
	Plant pots were filled by first covering the holes at the base with a filter paper before adding a 1 cm layer of standard sediment. A 4 cm layer of nutrient-supplemented sediment was placed on top and this was covered with a further 1 cm standard sediment. A *ca*. 2 mm layer of coarse quartz sand was placed on top of the sediment to minimise the flotation of sediment into the overlying medium.
	After planting, test vessels were carefully filled with 2 L of the respective medium preparation and the initial level marked on the outside of each unit. Evaporation losses > 10% were restored by adding distilled water.
	The growth medium used was prepared according to Smart & Barko (1985), which has been shown to be suitable for macrophytes.
Test conditions:	temperature: 18.0 – 21.0 °C
	pH: Initial: 7.68 – 7.94; Final: 8.74 - 9.16 in the control, 8.00 – 8.73 L in the lenacil treatments ≤ 20.1 µg/L, 7.71 – 7.80 in the lenacil treatments ≥ 74.7 µg/L dissolved oxygen: Initial: 8.47 - 8.77 mg O_2/L; Final: 8.79 - 11.33 mg O_2/L in the control, 9.02 - 10.45 mg O_2/L in the lenacil treatments ≤ 20.1 µg/L, 7.80 to 8.13 mg O_2/L in the lenacil treatments ≥ 74.7 µg/L.
	light regime: 16:8 h light:dark
	light intensity: 7794 - 7962 lux
Analytical methods:	LC-MS/MS
Statistics:	Calculations were applied to the data for replicate vessels, not individual plants. Data evaluation was based on relative gain and growth rate for each of the measured length and biomass parameters. Endpoints were determined with ToxRat® software. Concentrations causing 10%, 20% and 50% inhibition

relative to the control, based on the various growth indices, were determined by probit analysis modified for continuous data. ANOVA followed by Williams' test was used to identify statistically significant differences from growth in the control and to locate the LOEC and NOEC concentrations for each of the growth indices.

Test procedure:

Just before the start of the test, 25 plants of the pre-culture were harvested and plant fresh weight and subsequently dry weight were determined to obtain the respective data for day 0. During the 7-day exposure, shoot length was recorded at the start of the test and on days 0, 3, 5 and 7. Shoot length (apical and side shoots) was determined using a ruler positioned within the vessel close to the plant to be measured. At the end of the exposure period, all plants were harvested. Total plant fresh weight was determined after absorbing remaining test medium attached to the plants by means of tissue paper. Dry weight was determined subsequently after weighing the fresh plants. The five plants per replicate were combined and the plants were dried in aluminium weighing boats at 60°C for 48h. Any symptoms (such as chlorosis and necrosis) or other observations were recorded. The length of the main root was also determined at day 7. The relative increase in root length was calculated by assuming an initial root length of 0.1 cm.

Findings:

Analytical results:

The measured lenacil concentrations ranged from 21.0% to 101.6% of the nominal values at test start and increased to between 53.8% and 291.3% of nominal in samples taken on Day 7. Since the highest test concentration was prepared directly in growth medium without using a solubiliser/solvent, the low recovery at test start suggests that the test item was not completely dissolved and was probably inhomogeneously distributed in the visually clear solution. The other treatment levels were prepared by dilution of the highest test concentration. Further dissolution of lenacil during the exposure period is thought to have caused the concentrations at termination to have exceeded those measured at Day 0. The results of the test were related to overall arithmetic mean measured concentrations of lenacil in the test medium; these were considered to represent exposure to dissolved, bioavailable lenacil and therefore to provide the basis for worst-case endpoints.

Table 2.9.2.3.3-38: Measured concentrations in a growth inhibition test with *Elodea canadensis* exposed to lenacil technical under static conditions in the presence of sediment for 7 days

Nominal concentration of test item (µg a.s./L)	Start exposure period (day 0)		End exposure period (day 7		Arithmetic mean measured concentration	
	µg a.s./L	% of nominal	µg a.s./L	% of nominal	µg a.s./L	% of nominal
Control	<LOQ	-	<LOQ	-	-	-
3.13	3.18	101.6	9.12	291.3	6.15	196.5
12.5	4.49	35.9	12.00	96.0	8.24	66.0
20	12.64	25.3	27.56	55.1	20.1	40.2
200	41.96	21.0	107.50	53.8	74.7	37.4
800	241.7	30.2	595.03	74.4	418	52.3

LOQ: Limit of Quantification (0.1 µg a.s./L)

Biological results:

Concentration-dependent effects of lenacil were recorded for all the measured growth parameters of *E. canadensis* and are shown in Table 2.9.2.3.3-39 to Table 2.9.2.3.3-42. Statistically significant ($p \leq 0.05$) effects were encountered at mean measured concentrations ≥ 74.7 µg a.s./L for shoot length (both relative increase and growth rate) and for growth rate based on root length. Statistically significant ($p \leq 0.05$) effects were encountered at mean measured concentrations ≥ 20.1 µg a.s./L for fresh weight (both relative increase and growth rate) and for relative increase based on root length. Statistically significant ($p \leq 0.05$) effects were encountered at mean measured concentrations ≥ 8.24 µg a.s./L for dry weight (both relative increase and growth rate).

Table 2.9.2.3.3-39: Effects on *Elodea canadensis* shoot length following exposure to lenacil technical under static conditions in the presence of sediment for 7 days

Mean measured lenacil concn (µg a.s./L)	Mean[a] shoot length (cm)				Mean relative increase (%) in shoot length after 7 days *[% inhibition]*		Mean growth rate based on shoot length during 0 - 7 days *[% inhibition]*	
	Day 0	Day 3	Day 5	Day 7				
Control	3.18	4.19	4.65	6.06	91.15	*[-]*	0.09	*[-]*
6.15	3.09	3.47	3.69	5.19	66.18	*[27.4]*	0.072	*[19.8]*
8.24	3.39	4.23	4.71	6.97	105.13	*[-15.3]*	0.102	*[-13.7]*
20.1	3.28	3.58	3.75	5.48	64.13	*[29.6]*	0.071	*[21.5]*
74.7	3.28	3.64	3.93	5.00	51.75*	*[43.2]*	0.06*	*[34.3]*
418	3.15	3.32	3.59	3.88	22.35*	*[75.5]*	0.028*	*[68.6]*

*[a] Based on 6 replicates in the control and 3 replicates per lenacil treatment; * Significantly different (p < 0.05, Williams' t-test) from the control, negative values indicate an increase relative to the control.*

Table 2.9.2.3.3-40: Effects on *Elodea canadensis* fresh weight following exposure to lenacil technical under static conditions in the presence of sediment for 7 days

Mean measured lenacil concn (µg a.s./L)	Mean[a] fresh weight (mg)		Mean relative increase in fresh weight after 7 days [% inhibition]		Mean growth rate based on fresh weight during 0 - 7 days [% inhibition]	
	Day 0[b]	Day 7				
Control	142.3	212.2	49.08	*[-]*	0.056	*[-]*
6.15	142.3	207.5	45.79	*[6.7]*	0.054	*[4.1]*
8.24	142.3	206.8	45.30	*[7.7]*	0.053	*[4.9]*
20.1	142.3	172.5	18.47*	*[62.4]*	0.024*	*[57.4]*
74.7	142.3	190.6	33.97*	*[30.8]*	0.042*	*[25.8]*
418	142.3	143.5	0.86*	*[98.2]*	0.000*	*[99.8]*

*[a] Based on 6 replicates in the control and 3 replicates per lenacil treatment; [b] Representative plants subjected to identical preculture, but used for initial wet and dry weight measurements only; * Significantly different (p < 0.05, Williams' t-test) from the control, negative values indicate an increase relative to the control.*

Table 2.9.2.3.3-41: Effects on *Elodea canadensis* dry weight following exposure to lenacil technical under static conditions in the presence of sediment for 7 days

Mean measured lenacil concn (µg a.s./L)	Mean[a] dry weight (mg)		Mean relative increase in dry weight after 7 days [% inhibition]		Mean growth rate based on dry weight during 0 - 7 days [% inhibition]	
	Day 0[b]	Day 7				
Control	28.56	33.53	17.38	*[-]*	0.021	*[-]*
6.15	28.56	40.04	40.17	*[-131.1]*	0.048	*[-127.4]*
8.24	28.56	26.62	-6.8*	*[139.1]*	-0.01*	*[148.4]*
20.1	28.56	21.78	-23.77*	*[236.7]*	-0.039*	*[286.3]*
74.7	28.56	20.01	-29.93*	*[272.2]*	-0.051*	*[341.9]*
418	28.56	16.03	-43.9*	*[352.2]*	-0.086*	*[503.7]*

*[a] Based on 6 replicates in the control and 3 replicates per lenacil treatment; [b] Representative plants subjected to identical preculture, but used for initial wet and dry weight measurements only.; * Significantly different (p < 0.05, Williams' t-test) from the control, negative values indicate an increase relative to the control. Inhibition <100 indicates decrease of the observed parameter relative to initial dry weight and compared to controls during the test.*

Table 2.9.2.3.3-42: Effects on *Elodea canadensis* root length (of the longest root) following exposure to lenacil technical under static conditions in the presence of sediment for 7 days

Mean measured lenacil concn (µg a.s./L)	Mean[a] root length (cm)		Mean relative increase in root length after 7 days [% inhibition]	Mean growth rate based on root length during 0 - 7 days [% inhibition]
	Day 0[b]	Day 7		

Control	0.1	3.18	3.08	*[-]*	0.490	*[-]*
6.15	0.1	2.98	2.88	*[6.49]*	0.482	*[1.70]*
8.24	0.1	2.81	2.71	*[12.1]*	0.473	*[3.50]*
20.1	0.1	1.71	1.61*	*[47.8]*	0.402	*[18.0]*
74.7	0.1	1.11	1.01*	*[67.3]*	0.341*	*[30.5]*
418	0.1	0.37	0.27*	*[91.1]*	0.152 [c]	*[69.0]*

*[a] Based on 6 replicates in the control and 3 replicates per lenacil treatment; [b] assumption, not actually measured; * Significantly different ($p < 0.05$, Williams' t-test) from the control, negative values indicate an increase relative to the control; [c] non-significance of the highest treatment is caused by very high variability of the replicates and therefore not considered*

The concentrations of lenacil causing 10%, 20% and 50% inhibition based on the various growth indices, and the corresponding LOEC and NOEC values, are compiled in Table 2.9.2.3.3-43

Table 2.9.2.3.3-43: Growth inhibition endpoints for *Elodea canadensis* following exposure to lenacil technical under static conditions in the presence of sediment for 7 days

Parameter		Mean measured lenacil concentration (µg a.s./L) *[95% confidence limits]*				
		EC_{50}	EC_{20}	EC_{10}	LOEC	NOEC
Shoot length	relative gain	93.3 49.8 – 234	12.5 3.23 – 25.1	4.37 0.55 – 10.9	74.7	20.1
	growth rate	154 86.9 – 361	21.9 7.32 – 40.7	7.88 1.47 – 17.8	74.7	20.1
Fresh weight	relative gain	64.2 20.5 – 703	9.18 0.08 – 26.8	3.32 0 – 12.6	20.1	8.24
	growth rate	81.6 26.2 – 1022	11.9 0.13 – 34.9	4.36 0 – 16.2	20.1	8.24
Dry weight	relative gain	7.18 6.83 – 7.53	6.35 5.91 – 6.69	5.96 5.45 – 6.32	8.24	6.15
	growth rate	7.18 6.83 – 7.53	6.35 5.91 – 6.69	5.96 5.45 – 6.32	8.24	6.15
Root length	relative gain	31.0 20.1 – 50.6	7.29 3.24 – 11.6	3.42 1.04 – 6.3	20.1	8.24
	growth rate	170 106 - 319	32.4 13.6 – 54.6	13.6 3.77 – 26.8	74.7	20.1

Conclusions:

The 7-day toxicity of lenacil technical to the macrophyte *Elodea canadensis* was determined in the presence of sediment in a static system. Dry weight gain was the most sensitive growth parameter, with an EC_{50} value of 7.18 µg a.s./L for both increase in dry weight and dry weight growth rate. For fresh weight, the EC_{50} values for increase and growth rate were 64.2 and 81.6 µg a.s./L, respectively. Shoot length was less sensitive than fresh weight with EC_{50} values for increase and growth rate of 93.3 and 154 µg a.s./L, respectively. For root length (i.e. length of the longest root), the EC_{50} values for increase and growth rate were 31.0 and 170 µg a.s./L, respectively.

RMS comments:

This study was performed according to a draft test guideline for the rooted aquatic macrophyte *Myriophyllum sp.*, as published in Maltby *et al.* (2010). This draft test guideline formed the basis of the more recently published OECD Test Guideline 239 (Water-sediment *Myriophyllum spicatum* toxicity test). As stated in this test guideline, the method is in principle applicable to any other aquatic macrophyte species, provided that adequate growth occurs in the untreated controls under the test conditions. For the present study with *Elodea canadensis*, the following validity criteria of OECD TG 239 are met:

- The control plants did not show any visual symptoms of chlorosis and were visibly free from contamination by other organisms such as algae and/or bacterial films on the plants, at the surface of the sediment and in the test medium.
- The mean coefficient of variation for yield based on measurements of shoot fresh weight (i.e. from test initiation to test termination) in the control cultures did not exceed 35% between replicates (measured: 13.3%)

The mean total shoot length and mean total shoot fresh weight in control plants, however, increased by only a factor 1.91 and 1.49, respectively, while OECD TG 239 requires that these parameters should at least double during the exposure phase of the test. The increase in shoot length is however close to the required factor of 2. Further, the results show a clear dose-response relationship for all of the tested parameters. Therefore, the observed deviations from the validity criteria pontentially have no impact on the validity of the test results

The analytical method used could be fully validated according to the EU Guidance SANCO/3029/99 rev. 4., and is therefore considered "fit for purpose". The LOQ was set at 0.1 µg/L (please refer to Vol. 3 (CA), Section B.5.1.2.6 – studies no. 18 and 19, for further details).

It is however noted that the measured concentration at the end of the test was increased compared to the measured concentration at test start, which is unexpected. According to the explanation provided by the study author, this is the result of the test item not being completely dissolved in the (visually clear) test solution (no solvent was used), and a subsequent further dissolution during the test period. As the analytical method is fully validated, it is expected that the measured concentration values are reliable. Nevertheless, the strange behaviour of the test item results in some uncertainty regarding the exposure in the test. Furthermore, at the lowest test concentration, the measured concentration at the end of the test was about 300% of the nominal concentration, which is even more surprising, and adds up to the uncertainty regarding the exposure.

The issues discussed above limit the reliability of this study. Following discussion and consultation with the notifier, and given the fact that this study is crucial in the (higher tier) risk assessment for aquatic macrophytes (see Volume 3 (PPP), Section B.9.4.3), it was agreed that the notifier will repeat this study in order to confirm its results.

For the time being, the results from the current study will nevertheless be considered in the risk assessment. To take into account the doubts regarding the measured concentrations at the end of the test, the RMS and co-RMS consider that the most appropriate way to express the endpoints from this study is based on initial measured concentrations, as this represents a worst case. Therefore, RMS recalculated the endpoints for dry weight, the most sensitive parameter, based on initial measured concentrations. EC_x calculations were performed in R statistical environment, Version 3.5.1 (R Development Core Team, 2018) using the R package 'drc'. The model used was a 2-parameter log-logistic function (with the lower limit fixed at 0 and the upper limit at 100; inhibition values lower than 0% or greater than 100% were replaced by 0 and 100, respectively). The calculated EC_x values are shown in Table 2.9.2.9.9-44Table .

Table 2.9.2.3.3-44: Calculated EC_{10}, EC_{20} and EC_{50} values for the test with *Elodea canadensis*, based on initial measured concentrations.

Endpoint	EC_{10} (µg/L)	EC_{20} (µg/L)	EC_{50} (µg/L)
Yield	3.49 (95% CL 3.31-3.67)	3.59 (95% CL 3.42-3.78)	3.78 (95% CL 3.60-3.96)
Growth rate	3.49 (95% CL 3.31-3.67)	3.59 (95% CL 3.42-3.78)	3.78 (95% CL 3.60-3.96)

The following endpoints for the most sensitive parameter (dry weight), based on initieal measured concentrations, will be considered in the risk assessment:
E_yC_{50} (*Elodea canadensis*, 7 day) = 3.78 µg a.s./L
E_yC_{20} (*Elodea canadensis*, 7 day) = 3.59 µg a.s./L
E_yC_{10} (*Elodea canadensis*, 7 day) = 3.49 µg a.s./L
E_rC_{50} (*Elodea canadensis*, 7 day) = 3.78 µg a.s./L
E_rC_{20} (*Elodea canadensis*, 7 day) = 3.59 µg a.s./L
E_rC_{10} (*Elodea canadensis*, 7 day) = 3.49 µg a.s./L
NOEC (*Elodea canadensis*, 7 day) = 3.18 µg a.s./L

2.9.2.3.4 Chronic toxicity to other aquatic organisms

No additional studies on the chronic toxicity of lenacil to other aquatic organisms are available.

2.9.2.4 Comparison with the CLP criteria

2.9.2.4.1 Acute aquatic hazard

Table 2.9.4.2.1 Summary of information on acute aquatic toxicity relevant for classification

Method	Species	Test material	Results	Remarks	Reference
acute fish study based on OECD 203 and US EPA 72-1 GLP	*Oncorrhynchus mykiss*	lenacil, purity: 98.2%, batch n°: 9038	**LC_{50}> 2.0 mg a.s./L** (mean measured)	Acceptable. Key study	96 h static fingerlings 10 fish/replicate 1 replicate/treatment
acute daphnia study based on OECD 202 GLP	*Daphnia magna*	lenacil, purity: 98.82%, batch n°: JUL14HE 010	**EC_{50}> 3.11 mg a.s./L** (mean measured)	Acceptable. Key study	48 h static 5 daphnids /replicate 4 replicates /treatment
algal growth inhibition study based on OECD 201 GLP	*Ankistrodesmus falcatus*	lenacil, purity: 99.3%, batch n°: 0190813	E_yC_{50} = 0.00856 mg a.s./L **E_rC_{50} = 0.0133 mg a.s./L** **NOEC = 0.00259 mg a.s./L** (initial measured)	Acceptable. Key study	72 h static initial cell count: 2500/mL 8 replicates for control and solvent control 4 replicates /treatment
Maltby *et al.* (2010) (draft version of OECD 239)	*Elodea canadensis*	lenacil, purity: 99.1%, batch n°: 200010003	E_yC_{50} = 0.00378 mg a.s./L **E_rC_{50} = 0.00378 mg a.s./L** **NOEC = 0.00318 mg a.s./L** (mean measured)	Acceptable. Key study	7 d static A single pot containing 5 plants in each test vessel 6 replicates for control 3 replicates /treatment

Based on the available acute (short-term) aquatic toxicity studies, aquatic plants were identified as the most sensitive species with an E_rC_{50} of 0.00378 mg a.s./L.

Based on this endpoint, the following classification for lenacil according to CLP is proposed:

- **Aquatic acute category 1 (based on E_rC_{50} aquatic plants ≤ 1 mg/L)**
- **M-factor = 100 (based on 0.001 mg/L < E_rC_{50} ≤ 0.01 mg/L**
- **H400**

2.9.2.4.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Table 2.9.2.4.2 Summary of information on long-term aquatic toxicity relevant for classification

Method	Species	Test material	Results	Remarks	Reference
chronic fish early life stage study based on OECD 210 GLP	*Oncorhynchus mykiss*	lenacil, purity: 98.5%, batchn°: 9038	**NOEC = 0.160 mg a.s./L** (mean measured) based on mean standard length	Acceptable. Key study	90 d flow-through 20 embryos/cup 2 embryo cups/replicate 2 replicates/treatment

chronic daphnia study based on OECD 211 GLP	*Daphnia magna*	lenacil, purity: 98.82%, batch n°: JUL14HE 010	**NOEC = 0.019 mg a.s./L** (mean measured) based on adult survival and total numbers of offspring	Acceptable. Key study	21 d semi-static 1 daphnid/replicate 10 replicates /treatment
algal growth inhibition study based on OECD 201 GLP	*Ankistrodesmus falcatus*	lenacil, purity: 99.3%, batch n°: 0190813	E_yC_{50} = 0.00856 mg a.s./L **E_rC_{50} = 0.0133 mg a.s./L** **NOEC = 0.00259 mg a.s./L** (initial measured)	Acceptable. Key study	72 h static initial cell count: 2500/mL 8 replicates for control and solvent control 4 replicates /treatment
Maltby *et al.* (2010) (draft version of OECD 239)	*Elodea canadensis*	lenacil, purity: 99.1%, batch n°: 200010003	E_yC_{50} = 0.00378 mg a.s./L **E_rC_{50} = 0.00378 mg a.s./L** **NOEC = 0.00318 mg a.s./L** (mean measured)	Acceptable. Key study	7 d static A single pot containing 5 plants in each test vessel 6 replicates for control 3 replicates /treatment

Based on the available long-term aquatic toxicity studies, algae and aquatic plants were identified as the most sensitive species with a lowest NOEC of 0.00259 mg a.s./L for algae and 0.00539 mg a.s./L for aquatic plants.

Lenacil is not rapidly degradable and the potential for aquatic bioaccumulation is low

Based on this information, the following classification for lenacil according to CLP is proposed:

- **Aquatic chronic category 1 (based on NOEC algae and aquatic plants ≤ 0.1 mg/L)**
- **M-factor = 10 (based on 0.001 mg/L < NOEC ≤ 0.01 mg/L**
- **H410**

2.9.2.5 Conclusion on classification and labelling for environmental hazards

Classification:
Aquatic Acute category 1 (based on E_rC_{50} algae and aquatic plants ≤ 1 mg/L)
H400
M-factor = 100 (based on 0.001 mg/L < $L(E)C_{50}$ ≤ 0.01 mg/L)

Aquatic Chronic category 1 (based on NOEC algae and aquatic plants ≤ 0.1 mg/L)
H410
M-factor = 10 (based on 'not readily degradable' and 0.001 mg/L < NOEC ≤ 0.01 mg/L)

Labelling:

GHS pictogram:	yes	
Signal word:	warning	
Hazard assessment:	H410 Very toxic to aquatic life with long lasting effects	
Precautionary statements:	Prevention – P273	Avoid release to the environment
	Response – P391	Collect spillage
	Disposal – P501	Dispose of contents / container to … in accordance with local regulations

2.9.3 Summary of effects on arthropods

2.9.3.1 Bees

Laboratory toxicity studies with honeybees or bumblebees were conducted for the representative formulation Lenacil 500 g/L SC, containing the active substance lenacil. The endpoints of all available studies are summarized in Table 2.9.3.1-1.

Table 2.9.3.1-1: Summary of bee toxicity data on Lenacil

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Honeybees					
Honeybee (*Apis mellifera*)	Lenacil 500 g/L SC	48h acute contact and oral toxicity test	LD_{50} (contact) LD_{50} (oral)	**> 498.4 µg product/bee (> 227.2 µg a.s./bee)** **> 452.3 µg product/bee (> 206.2 µg a.s./bee)**	CP10.3.1.1/01 Haupt S., 2016a
Honeybee (*Apis mellifera*)	Lenacil 500 g/L SC	48h acute contact and oral toxicity test	LD_{50} (contact) LD_{50} (oral)	> 240 µg product/bee (> 100 µg a.s./bee) > 264 µg product/bee (> 110 µg a.s./bee)	CP10.3.1.1/02 Schmitzer S., 2006a
Honeybee (*Apis mellifera*)	Lenacil 500 g/L SC	10 day chronic adult oral toxicity test	LC_{50} NOEC LDD_{50} NOED	> 15078 mg product/kg food (> 6874 mg a.s./kg food) 15708 mg product/kg food (= 6874 mg a.s./kg food) **> 312.1 µg product/bee/day (> 142.3 µg a.s./bee/day)** 312.1 µg product/bee/day (= 142.3 µg a.s./bee/day	CP10.3.1.2/01 Haupt S. and Knebel N., 2016a
Honeybee (*Apis mellifera*)	Lenacil 500 g/L SC	8 day chronic larval toxicity test, repeated exposure	8 day LC_{50} 8 day NOEC 8 day LD_{50} 8 day NOED	> 2937.1 mg product/kg food (> 1339 mg a.s./kg food) 2937.1 mg product/kg food (= 1339 mg a.s./kg food) > 435.2 µg product/larva (> 198.4 µg a.s./larva) **435.2 µg product/larva (= 198.4 µg a.s./larva)**	CA8.3.1.3/01 Haupt S. and Knebel N., 2016c
Bumblebees					

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Bumblebee (*Bombus terrestris*)	Lenacil 500 g/L SC	48h acute contact and oral toxicity test	LD_{50} (contact) LD_{50} (oral)	**> 438.7 µg product/bee (> 200.0 µg a.s./bee)** **> 428.6 µg product/bee (> 195.4 µg a.s./bee)**	CP10.3.1.1/03 Haupt S., 2016b
Bumblebee (*Bombus terrestris*)	Lenacil 500 g/L SC	10 day chronic adult oral toxicity test	LC_{50} NOEC LDD_{50} NOED	> 1228 mg product/kg food (> 560 mg a.s./kg food) 1228 mg product/kg food (= 560 mg a.s./kg food) **> 208.6 µg product/bee/day (> 95.1 µg a.s./bee/day)** 208.6 µg product/bee/day (= 95.1 µg a.s./bee/day	CP10.3.1.2/02 Haupt S. and Knebel N., 2016b

***bold** - values used in the risk assessment*

2.9.3.2 Other non-target arthropods

Laboratory studies were performed with the representative formulation Lenacil 500 g/L SC, containing the active substance lenacil. The endpoints of these laboratory studies are shown in Table 2.9.3.2-1 below.

Table 2.9.3.2-1: Summary of arthropod toxicity data on lenacil in laboratory studies

Species	Test substance	Study type	Endpoint	Reference
Standard laboratory studies				
Aphidius rhopalosiphi	Lenacil 500 g/L SC	7 days, laboratory test, artificial substrate, 2D exposure to nymphs	LR_{50} > 4000 mL product/ha (> 1900 g a.s./ha) Unacceptable effects (> 50%) on reproduction of 54.4%, 64.1% and 63.3% at 119 g a.s./ha, 475 g a.s./ha and 950 g a.s./ha, respectively. Only 38.2% effects on reproduction at 1900 g a.s./ha. ($NOER_{repr.}$ < 119 g a.s./ha)	CP10.3.2.1/01 Moll M., 2005
Typhlodromus pyri	Lenacil 500 g/L SC	48h, laboratory test, artificial substrate, 2D exposure to adults	LR_{50} > 4000 mL product/ha (> 1900 g a.s./ha) No unacceptable effects on reproduction up to 4000 mL product/ha (= 1900 g a.s./ha) (ER_{50} > 1900 g a.s./ha)	CP10.3.2.1/02 Rosenkranz B., 2006
Poecilus cupreus	Lenacil 500 g/L SC	14 days, laboratory test, artificial substrate, 2D exposure to adults	LR_{50} > 3580 mL product/ha (> 1700 g a.s./ha) No effects on food consumption were observed up to 3580 mL product/ha (=	CP10.3.2.1/03 Schmitzer S., 2006b

Species	Test substance	Study type	Endpoint	Reference
			1700 g a.s./ha). Effects on reproduction were not assessed.	

2.9.4 Summary of effects on non-target soil meso- and macrofauna

2.9.4.1 Earthworms

Acute and long-term toxicity studies with earthworms were performed for lenacil, the representative formulation Lenacil 500 g/L SC, and the metabolites IN-KE121 and IN-KF313. The endpoints are summarized in Table 2.9.4.1-1 and 2.9.4.1-2.

Table 2.9.4.1-1: Summary of non-target soil meso- and macrofauna toxicity data on lenacil and the representative formulation Lenacil 500 g/L SC

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Earthworms					
earthworm (*Eisenia fetida*)	lenacil	14 day, acute toxicity test, 10% organic matter	LC_{50} NOEC	**> 1000 mg a.s./kg soil dw** 1000 mg a.s./kg soil dw	CA8.4.1/01 Rodgers M.H., 2002
earthworm (*Eisenia fetida*)	lenacil	56 day, earthworm reproduction test, 10% organic matter	EC_{50} EC_{20} EC_{10} NOEC	> 1000 mg a.s./kg soil dw ND ND **1000 mg a.s./kg soil dw**	CA8.4.1/02 Pavic B., 2016
earthworm (*Eisenia fetida*)	Lenacil 500 g/L SC	14 day, acute toxicity test, 10% organic matter	LC_{50} NOEC	**> 1000 mg form./kg soil dw** (> 417 mg a.s./kg soil dw) 1000 mg form./kg soil dw (= 417 mg a.s./kg soil dw)	CP10.4.1.1/01 Lührs U., 2005
earthworm (*Eisenia fetida*)	Lenacil 500 g/L SC	56 day, earthworm reproduction test, 10% organic matter	EC_{50} EC_{20} EC_{10} NOEC	> 96 mg form. kg soil dw (> 40 mg a.s./kg soil dw) ND ND **96 mg form./kg soil dw** **(= 40 mg a.s./kg soil dw)**	CP10.4.1.1/02 Lührs U., 2006

Notes: ***bold*** *– values used for risk assessment*
ND – could not be determined

Table 2.9.4.1-2: Summary of non-target soil meso- and macrofauna toxicity data on IN-KE121 and IN-KF313, the relevant metabolites of lenacil in soil.

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Earthworms					
earthworm (*Eisenia fetida*)	IN-KE121	14 day, acute toxicity test, 10% organic matter	LC_{50} NOEC	**> 1000 mg a.s./kg soil dw** 556 mg a.s./kg soil dw	CA8.4.1/04 Rodgers M.H. 2004b
earthworm (*Eisenia fetida*)	IN-KE121	56 day, earthworm reproduction test,	EC_{50} EC_{20} EC_{10} NOEC	> 40 mg a.s./kg soil dw ND ND **40 mg a.s./kg soil dw**	CA8.4.1/05 Pavic B., 2015a

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
		10% organic matter			
earthworm (*Eisenia fetida*)	IN-KF313	14 day, acute toxicity test, 10% organic matter	LC_{50} NOEC	**> 1000 mg a.s./kg soil dw** 309 mg a.s./kg soil dw	CA8.4.1/03 Rodgers M.H., 2004a
earthworm (*Eisenia fetida*)	IN-KF313	56 day, earthworm reproduction test, 10% organic matter	EC_{50} EC_{20} EC_{10} NOEC	> 40 mg a.s./kg soil dw ND ND **40 mg a.s./kg soil dw**	CA8.4.1/06 Pavic B., 2015b

Notes: ***bold*** *– values used for risk assessment*

2.9.4.2 Other non-target soil macro-organisms

Given that lenacil and its two major metabolites do not persist in soil beyond 100 days, and that the acute risk to earthworms, sensitive indicator species for non-target arthropods (*Aphidius rhopalosiphi* and *Typhlodromus pyri*) and soil microflora were shown to be acceptable, further studies on other soil non-target meso- and macrofauna are not considered to be required.

2.9.5 Summary of effects on soil nitrogen transformation

Studies on the effects on soil nitrogen and carbon transformation of lenacil and the representative formulation Lenacil 500 g/L SC are available. The endpoints derived from these studies are shown in Table 2.9.5-1.

Table 2.9.5-1: Summary of data on the effect of lenacil on soil nitrogen transformation and soil respiration

Test system	Test substance	Test soil	Duration of exposure	NOEC	References
N transformation	lenacil	Sandy loam	28 days	**6.7 mg a.s./kg soil dw**	CA8.5/01 Carter J.N., 2002
C transformation	lenacil	Sandy loam	28 days	**6.7 mg a.s./kg soil dw**	CA8.5/01 Carter J.N., 2002
N transformation	Lenacil 500 g/L SC	Loamy sand	28 days	**8.0 mg form./kg soil dw (= 3.3 mg a.s./kg soil dw)**	CP10.5/01 Reis K.H.., 2006
C transformation	Lenacil 500 g/L SC	Loamy sand	28 days	**8.0 mg form./kg soil dw (= 3.3 mg a.s./kg soil dw)**	CP10.5/01 Reis K.H.., 2006
N transformation	Lenacil 500 g/L SC	Sandy loam	28 days	**79.24 mg form./kg soil dw (= 35 mg a.s./kg soil dw)**	CP10.5/02 Schulz L., 2016

bold: *values used for risk assessment*

2.9.6 Summary of effects on terrestrial non-target higher plants

Studies on the effects on seedling emergence and vegetative vigour of lenacil in the representative formulation Lenacil 500 g/L SC are available. The endpoints from these studies are shown in Table 2.9.6-1.

Table 2.9.6-1: Summary of plant toxicity data on lenacil

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value [a]	Reference
6 non-target plant species	Lenacil 500 g/L SC	Seedling emergence test	ER_{50} NOER	**372.99 mL prod./ha (177.2 g a.s./ha)** < 250 mL prod./ha (< 118.8 g a.s./ha)	CP10.6.2/01 Goßmann A. and Meinerling M., 2006
10 non-target plant species	Lenacil 500 g/L SC	Vegetative vigour test	ER_{50} NOER	**1497 mL prod./ha (= 777 g a.s./ha)** 120 mL prod./ha (= 62.5 g a.s./ha)	CP10.6.2/02 Stürtz S. and Knebel N., 2016

*Notes: **bold** – values used in the risk assessment; [a]only the lowest value for any of the tested species is shown*

2.9.7 Summary of effects on other terrestrial organisms (flora and fauna)

No specific information was submitted.

2.9.8 Summary of effects on biological methods for sewage treatment

The inhibitory effect of lenacil on the oxygen consumption of activated sludge suspension (4 g sludge (dry matter)/L water) was determined. The 3-hour EC_{50} for total O_2 uptake was > 100 mg a.s./L. It should however be noted that the lowest oxygen uptake in the control after 3 hours was equivalent to 12.3 mg O_2/g/hour. As this value is below the threshold value of 20 mg O_2/g/hour, as set by the most recent version of OECD Test Guideline 209, the available test is not acceptable for use in the risk assessment.

2.9.9 Summary of product exposure and risk assessment

2.9.9.1 Birds

The risk assessment included in Volume 3 (PPP) section B.9.2.1 indicated an acceptable acute and long-term risk to birds for the proposed use in sugar and fodder beet. The risk assessment for effects on birds has been performed according to the latest EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009).

Table 2.9.9.1-1: Summary of the endpoints used in the risk assessment for birds

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Mallard duck (*Anas platyrhynchos*)	Lenacil technical	Single dose (Acute oral toxicity)	LD_{50}	**> 2000 mg a.s./kg bw**	CA8.1.1.1/01 [redacted] 2002a
Bobwhite quail (*Colinus virginianus*)	Lenacil technical	Single dose (Acute oral toxicity)	LD_{50}	**> 2000 mg a.s./kg bw**	CA8.1.1.1/02 [redacted] 2002b
Bobwhite quail (*Colinus virginianus*)	Lenacil technical	21 weeks (Sub chronic and reproduction)	NOEL EC_{10} EC_{20}	1024 mg a.s./kg diet **(= 100.4 mg a.s./kg bw/d)** NE NE	CA8.1.1.3/01 [redacted] *et al.*, 1996

*Note: NE – not estimated; **bold** – endpoint used for the current risk assessment*

Two acute toxicity studies for birds are available ([redacted] 2002a, CA8.1.1.1/01; [redacted] 2002b, CA8.1.1.1/02), with mallard duck and bobwhite quail, resulting in LD_{50} > 2000 mg a.s./kg bw for both species. The endpoint **LD_{50} > 2000 mg a.s./kg bw** is used in the acute risk assessment.

For birds, no avian toxicity study is available with the formulation Lenacil 500 g/L SC. In the section B.6 on Mammalian toxicology, acute oral toxicity studies in rat were performed for the active substance lenacil (LD_{50} > 5000 mg a.s./kg bw) and the representative formulation Lenacil 500 g/L SC (LD_{50} > 2000 mg form./kg bw). Since studies showed no effects at the highest dose tested, it is appropriate to base the risk assessment on the active substance, which is also covering the risk assessment for the representative formulation Lenacil 500 g/L SC.

In order to assess the possible relevance of metabolites in food items of wild birds and mammals, the occurrence of soil, water and plant metabolites was taken into consideration. IN-KE 121 and IN-KF 313 are the relevant metabolites in soil and water. These metabolites were taken into account in the assessment for secondary poisoning. In the available plant metabolism studies, the metabolite IN-KC943 and its glucosides were identified. However, based on structural similarty with the parent lenacil, they were considered unlikely to be of higher toxicity than lenacil itself. Therefore, they are considered covered by the risk assessment performed for lenacil.

For the acute risk to birds following exposure to lenacil, the TER_A values for the proposed use in sugar and fodder beet (at either 1 x 500 g a.s./ha or 4 x 125 g a.s./ha) exceed the Annex VI trigger of 10 at the screening step (see Table 2.9.9.1-2), indicating an **acceptable acute risk to birds**.

Table 2.9.9.1-2: Screening step – estimates of acute exposure to lenacil and the risk to birds from such exposure following application of Lenacil 500 g/L SC in sugar and fodder beet

Crop group	Indicator species	Shortcut value (mg a.s./kg bw)	App. rate (kg a.s./ha)	MAF	DDD (mg a.s./kg bw)	LD_{50} (mg a.s./kg bw)	TER_A
Sugar beet	small omnivorous bird	158.8	0.500	1.0	79.4	> 2000	> 25
			0.125	1.8	35.7	> 2000	> 56

Note: TER shown in bold falls below the relevant trigger

The TER_{LT} values for the proposed use in sugar and fodder beet (at either 1 x 500 g a.s./ha or 4 x 125 g a.s./ha) exceed the Annex VI trigger of 5 at the screening step (see Table 2.9.9.1-3), indicating an **acceptable reproductive risk to birds** from exposure to lenacil following the proposed use of Lenacil 500 g/L SC.

Table 2.9.9.1-3: Screening step – estimates of long-term exposure to lenacil and the risk to birds from such exposure following application of Lenacil 500 g/L SC in sugar and fodder beet

Crop group	Indicator species	Shortcut value (mg a.s./kg bw/day)	App. rate (kg a.s./ha)	MAF	f_{twa}	Long-term DDD (mg a.s./kg bw/day)	NOEL (mg a.s./kg bw/day)	TER_{LT}
Sugar beet	small omnivorous bird	64.8	0.500	1.0	0.53	17.2	100.4	5.8
			0.125	2.2	0.53	9.44	100.4	10.6

Note: TER shown in bold falls below the relevant trigger

The ratio of effective application rate to the endpoints for lenacil is clearly below the trigger value of 3000 (see Table 2.9.9.1-4) indicating that the **acute and long-term risk to birds via the consumption of drinking water (puddle scenario) can be considered acceptable** without further calculations.

Table 2.9.9.1-4: Ratios of effective application rate to endpoints for lenacil following the use of Lenacil 500 g/L SC in sugar and fodder beet

Intended use	App. rate (g a.s./ha)	MAF	AR_{eff} (g a.s/ha)	LD_{50} (mg a.s./kg bw)	Ratio of AR_{eff} to LD_{50}	NOEL (mg a.s./kg bw/day)	Ratio of AR_{eff} to NOEL	Ratio trigger
Sugar and fodder beet	500	1.0	500	> 2000	< 0.25	100.4	5	3000

According to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009), substances with a log P_{OW} greater than 3 have potential for bioaccumulation. The log P_{OW} of lenacil was determined to be 1.70 at

pH 7, 25 °C (see Volume 3, Section B.2). The calculated log P_{OW} values for the metabolites IN-KE 121 and IN-KF 313 are 1.04 and 3.111. The log P_{OW} for metabolite IN-KF 313 is above the trigger of 3 and therefore, an assessment of the risk from bioaccumulation has to be performed.

For IN-KF 313, the TER values for bioconcentration in earthworms and fish exceed the trigger of 5, indicating that the **risk from bioaccumulation of IN-KF 313 in fish-eating and earthworm-eating birds is acceptable.**

Table 2.9.9.1-5: Estimates of exposure to the metabolite IN-KF313 through bioconcentration in earthworms, and the risk from such exposure following the application of Lenacil 500 g/L SC according to the proposed uses

Substance	BCF_{worm}	PEC_{soil} [1] (mg/kg)	PEC_{worm}	DDD	NOEL [2] (mg a.s./kg bw/day)	TER	Trigger
IN-KF313	12.9	0.123	1.59	1.67	10.04	6.01	5

[1] Worst-case initial PEC_{soil} values, calculated for and application of 1 x 500 g a.s./ha in sugar and fodder beet;
[2] As no specific toxicity endpoint for IN-KF313 is available, the endpoint of the parent, divided by a factor 10, is used as a conservative approach in the risk assessment.

Table 2.9.9.1-6: Estimates of exposure and risk to metabolite IN-KF313 through bioconcentration in fish following the application of Lenacil 500 g/L SC in sugar and fodder beet

Crop	PEC_{water} [1] (mg/L)	BCF_{fish} [2]	TWA	PEC_{fish} (mg/L)	DDD	NOEL [3] (mg a.s./kg bw/day)	TER	Trigger
Sugar beet	0.06522	52.4	1	3.42	0.54	10.04	18.5	5

[1] Worst case $PEC_{SW, max}$ for metabolite IN-KF 313, calculated for application rates of 4 x 125 g a.s./ha or 1 x 500 g a.s./ha in sugar and fodder beet; [2] BCF_{fish} was estimated as 52.4; [3] As no specific toxicity endpoint for IN-KF313 is available, the endpoint of the parent, divided by a factor 10, is used as a conservative approach in the risk assessment

2.9.9.2 Mammals

The risk assessment included in Volume 3 (PPP) section B.9.2.2 indicated an acceptable acute and long-term risk to mammals for the proposed use in sugar and fodder beet. The risk assessment for effects on mammals has been performed according to the latest EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009).

Table 2.9.9.2-1: Summary of the endpoints used in the risk assessment for mammals

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Rat	Lenacil technical	Single dose (Acute oral)	LD_{50}	**> 5000 mg a.s./kg bw**	[redacted] 2001
Rat	Lenacil technical	2-genereation reproduction study	NOAEL	**82 mg a.s./kg bw/day**	[redacted] 2003a

In the section B.6 on Mammalian toxicology, acute oral toxicity studies in rat were performed for the active substance lenacil (LD_{50} > 5000 mg a.s./kg bw) and the representative formulation Lenacil 500 g/L SC (LD_{50} > 2000 mg form./kg bw). Since studies showed no effects at the highest dose tested, it is appropriate to base the risk assessment on the active substance, which is also covering the risk assessment for the representative formulation Lenacil 500 g/L SC.

Following the EFSA Guidance Document (2009), all available short-term dietary and long-term reproduction studies are considered to determine the ecotoxicologically relevant endpoint for **reproductive/developmental toxicity** to mammals. The following three studies were identified as the most relevant for the risk assessment (all studies with rats):

- 90-day short-term repeated-dose oral toxicity test with NOAEL = 41 mg a.s./kg bw/day and LOAEL = 412 mg a.s./kg bw/day based on a decreased body weight gain in males
- 2-generation reproduction study with NOAEL = 82 mg a.s./kg bw/day and LOAEL = 817 mg a.s./kg bw/day based on reduced body weight gain in females during gestation and reduction in body weight gain of offspring during lactation (F_1 and F_2), as well as reduction in terminal body weight of offspring (F_1)
- Developmental toxicity study with NOAEL = 100 mg a.s./kg bw/day and LOAEL = 300 mg a.s./kg bw/day based on developmental toxicity in offspring such as increased skeletal variations

Although the 90-day short-term repeated-dose oral toxicity test and the 2-generation reproduction study are not quite similar in test design, it seems that effects on parental toxicity on reduced body weight gain are demonstrated, at 412 mg a.s./kg bw/day and at 817 mg a.s./kg bw/day, respectively. Moreover, adverse effects on offspring on body weight gain were observed in the 2-generation reproduction study at 817 mg a.s./kg bw/day. Also, adverse effects on offspring on skeletal variations were observed in the developmental toxicity study at 300 mg a.s./kg bw/day. Overall, it seems appropriate to set the ecotoxicologically relevant **NOAEL at 82 mg a.s./kg bw/day**, covering up for the parental toxicity from 412 mg a.s./kg bw/day onwards and covering up for the de offspring toxicity from 300 mg a.s./kg bw/day onwards. This proposal of endpoint was discussed with co-RMS AT and agreed upon.

In order to assess the possible relevance of metabolites in food items of wild birds and mammals, the occurrence of soil, water and plant metabolites was taken into consideration. IN-KE 121 and IN-KF 313 are the relevant metabolites in soil and water. These metabolites were taken into account in the assessment for secondary poisoning. In the available plant metabolism studies, the metabolite IN-KC943 and its glucosides were identified. However, based on structural similarty with the parent lenacil, they were considered unlikely to be of higher toxicity than lenacil itself. Therefore, they are considered covered by the risk assessment performed for lenacil.

The TER_A values for lenacil exceed the Annex VI trigger of 10 for all proposed uses at the screening step (see Table 2.9.9.2-2). This indicates **an acceptable acute risk to mammals** from exposure to lenacil following the proposed use of Lenacil 500 g/L SC in sugar and fodder beet.

Table 2.9.9.2-2: Screening step – estimates of acute exposure to lenacil and the risk to mammals from such exposure following application of Lenacil 500 g/L SC in sugar and fodder beet

Crop group	Indicator species	Shortcut value (mg a.s./kg bw)	App. rate (kg a.s./ha)	MAF	DDD (mg a.s./kg bw)	LD_{50} (mg a.s./kg bw)	TER_A
Sugar beet	Small herbivorous mammal	118.4	0.500	1.0	59.2	> 5000	84.5
			0.125	1.8	26.6	> 5000	188

Note: TER shown in bold falls below the relevant trigger

The TER_{LT} values for the proposed use in sugar and fodder beet (at either 1 x 500 g a.s./ha or 4 x 125 g a.s./ha) exceed the Annex VI trigger of 5 at the screening step (see Table 2.9.9.2-3), indicating an **acceptable reproductive risk to mammals** from exposure to lenacil following the proposed use of Lenacil 500 g/L SC.

Table 2.9.9.2-3: Screening step – estimates of long-term exposure to lenacil and the risk to mammals from such exposure following application of Lenacil 500 g/L SC in sugar and fodder beet

Crop group	Indicator species	Shortcut value (mg a.s./kg bw/day)	App. rate (kg a.s./ha)	MAF	f_{twa}	Long-term DDD (mg a.s./kg bw/day)	NOEL (mg a.s./kg bw/day)	TER_{LT}
Sugar beet	Small herbivorous mammal	48.3	0.500	1.0	0.53	12.8	82	6.4
			0.125	2.2	0.53	7.04	82	11.6

Note: TER shown in bold falls below the relevant trigger

The ratio of effective application rate to the endpoints for lenacil is clearly below the trigger value of 3000 (see Table 2.9.9.2-4) indicating that the **acute and long-term risk to mammals via the consumption of drinking water (puddle scenario) can be considered acceptable** without further calculations.

Table 2.9.9.4-4: Ratios of effective application rate to endpoints for lenacil following the use of Lenacil 500 g/L SC in sugar and fodder beet

Intended use	App. rate (g a.s./ha)	MAF [1]	AR_{eff} (g a.s/ha)	LD_{50} (mg a.s./kg bw)	Ratio of AR_{eff} to LD_{50}	NOAEL (mg a.s./kg bw/day)	Ratio of AR_{eff} to NOEL	Ratio trigger
Sugar and fodder beet	500	1.0	500	> 5000	< 0.1	82	6.1	3000

According to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009), substances with a log Pow greater than 3 have potential for bioaccumulation. The log Pow of lenacil was determined to be 1.70 at pH 7, 25 °C (see Volume 3, Section B.2). The calculated log Pow values for the metabolites IN-KE 121 and IN-KF 313 are 1.04 and 3.111. The log Pow for metabolite IN-KF 313 is above the trigger of 3 and therefore, an assessment of the risk from bioaccumulation has to be performed.

For IN-KF 313, the TER values for bioconcentration in fish exceed the trigger of 5, indicating that the **risk from bioaccumulation of IN-KF 313 in fish-eating mammals is acceptable.** The TER value for bioconcentration in earthworms is slightly below the trigger of 5, indicating a potential risk. However, the available data on the toxicity of IN-KF313 for other groups of non-target organisms indicates that this metabolite is less toxic than the parent lenacil. Therefore, the TER as calculated in Table is likely to be too conservative. As the TER based on very conservative assumptions is close to the trigger, it is expected that when more realistic parameter values are taken into account, the trigger will be exceeded. Therefore, **the risk to earthworm-eating mammals for IK-KF313 is considered acceptable**.

Table 2.9.9.2-5: Estimates of exposure to the metabolite IN-KF 313 through bioconcentration in earthworms, and the risk from such exposure following the application of Lenacil 500 g/L SC according to the proposed uses

Substance	BCF_{worm}	PEC_{soil} [1] (mg/kg)	PEC_{worm}	DDD	NOAEL [2] (mg a.s./kg bw/day)	TER	Trigger
IN-KF313	12.9	0.123	1.59	2.03	8.2	4.03	5

[1] Worst-case initial PEC_{soil} values, calculated for and application of 1 x 500 g a.s./ha in sugar and fodder beet;
[2] As no specific toxicity endpoint for IN-KF313 is available, the endpoint of the parent, divided by a factor 10, is used as a conservative approach in the risk assessment.

Table 2.9.9.2-6: Estimates of exposure and risk to metabolite IN-KF313 through bioconcentration in fish following the application of Lenacil 500 g/L SC in sugar and fodder beet

Crop	PEC_{water} [1] (mg/L)	BCF_{fish} [2]	TWA	PEC_{fish} (mg/L)	DDD	NOEL [3] (mg a.s./kg bw/day)	TER	Trigger
Sugar beet	0.06522	52.4	1	3.42	0.49	8.2	16.9	5

[1] Worst case $PEC_{SW, max}$ for metabolite IN-KF 313, calculated for application rates of 4 x 125 g a.s./ha or 1 x 500 g a.s./ha in sugar and fodder beet; [2] BCF_{fish} was estimated as 52.4; [3] As no specific toxicity endpoint for IN-KF313 is available, the endpoint of the parent, divided by a factor 10, is used as a conservative approach in the risk assessment

2.9.9.3 Aquatic organisms

The risk assessment for aquatic organisms included in Volume 3 (PPP) section B.9.4 indicated that the acute and chronic risk to fish and the acute risk to aquatic invertebrates can be considered acceptable based on FOCUS Step 3 PEC values for the proposed use in sugar and fodder beet (both for 1 x 500 g a.s./ha and 4 x 125 g a.s./ha). The chronic risk to aquatic invertebrates was acceptable at FOCUS Step 4, if appropriate risk mitigation measures are taken into account. Based on a Higher Tier SSD-RAC, the risk to algae and aquatic invertebrates was acceptable for all relevant scenarios at FOCUS Step 3 or Step 4, with the exception of the R1 stream and R3 stream scenarios. For the R1 stream and R3 stream scenarios, a 10 m vegetated filter strip or 5 m drift buffer in combination with 10 m vegetated filter strip was not sufficient to obtain an acceptable risk after application of 1 x 500 g a.s./ha. Similarly, a 20 m vegetated filter strip was not sufficient for an acceptable risk after application of 4 x 125 g a.s./ha. Further risk mitigation measures should be considered at Member State level for these scenarios.

The risk assessments for aquatic organisms (fish, aquatic invertebrates, algae and aquatic plants) were conducted in accordance to the new EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (2013).

Table 2.9.9.3-1 shows the Tier 1 Regulatory Acceptable Concentrations (RAC) for lenacil and the metabolites IN-KE 121 and IN-KF 313 in surface water. From the Tier 1 data it is clear that the risk assessment will be driven by the chronic risk to algae and aquatic plants.

Table 2.9.9.3-1: Tier 1 RAC values for lenacil and its metabolites IN-KE121 and IN-KF313 for surface water for the different groups of aquatic organisms

	Species group	Endpoint	Assessment factor	RAC
Lenacil				
Acute effect assessment	Fish	LC_{50} > 2000 µg a.s./L	100	> 20 µg a.s./L
	Aquatic invertebrates	EC_{50} > 3110 µg a.s./L	100	> 31.1 µg a.s./L
			Overall acute RAC	**> 20 µg a.s./L**
Chronic effect assessment	Fish	NOEC = 160 µg a.s./L	10	16 µg a.s./L
	Daphnia magna	EC_{10} = 17 µg a.s./L	10	1.7 µg a.s./L
	Algae	E_rC_{50} = 13.3 µg a.s./L	10	1.33 µg a.s./L
	Aquatic plants	E_rC_{50} = 3.78 µg a.s./L	10	0.378 µg a.s./L
			Overall chronic RAC (surface water)	**0.378 µg a.s./L**
IN-KE121				
Chronic effect assessment	Algae	E_rC_{50} = 27800 µg/L	10	2780 µg /L
			Overall chronic RAC (surface water)	**2780 µg/L**
IN-KF313				
Chronic effect assessment	Algae	E_rC_{50} = 4270 µg/L	10	427 µg /L
			Overall chronic RAC (surface water)	**427 µg/L**

Notes: RAC = Regulatory Acceptable Concentration

A microcosm study is available in which the effects of lenacil on populations of macrophytes and phytoplankton have been investigated. In this microcosm study, lenacil was applied as a single application of the formulation Venzar 80 WP. As the toxicity of both Venzar 80 WP and the representative formulation Lenacil 500 g/L SC is driven by the active ingredient, the results from this study would be representative for Lenacil 500 g/L SC. However, a number of deficiencies and uncertainties were identified in this study. As a consequence, this study was not considered sufficiently reliable for use in the risk assessment. Even if this microcosm study would be sufficiently reliable, it would only be representative for a GAP with one application of lenacil. Multiple applications (up to 4 applications with an interval of 7 days are possible according to the proposed GAP) are not covered in the microcosm.

Based on the available laboratory toxicity data for seven algae and three aquatic macrophyte species, a Species Sensitivity Distribution (SSD) was constructed, resulting in an HC_5 value of 3.19 µg a.s./L. Taking into account the assessment factor (AF) of 3 as recommended by the EFSA Guidance Document on aquatic organisms (2013) for primary producers, an **SSD-RAC$_{sw,ch}$ of 1.06 µg a.s./L** was obtained. This RAC value is used in the higher tier risk assessment for algae and aquatic plants.

For each group of aquatic organisms, the Tier 1 RAC values were compared to the PEC_{SW}, to determine whether the risk is acceptable or not. When a Higher Tier RAC was available, an additional comparison was made based on this Higher Tier RAC (see Table 2.9.9.3-2 to Table 2.9.9.3-5). For the active substance lenacil, the acute and chronic risk to fish and the acute risk to aquatic invertebrates can be considered acceptable based on FOCUS Step 3 PEC values for the proposed use in sugar and fodder beet (both for 1 x 500 g a.s./ha and 4 x 125 g a.s./ha). The chronic risk to aquatic invertebrates was acceptable at FOCUS Step 4, if appropriate risk mitigation measures are taken into account. Based on a Higher Tier SSD-RAC, the risk to algae and aquatic invertebrates was acceptable for all relevant scenarios at FOCUS Step 3 or Step 4, with the exception of the R1 stream and R3 stream scenarios. For the R1 stream and R3 stream scenarios, a 10 m vegetated filter strip or 5 m drift buffer in combination with 10 m vegetated

filter strip was not sufficient to obtain an acceptable risk after application of 1 x 500 g a.s./ha. Similarly, a 20 m vegetated filter strip was not sufficient for an acceptable risk after application of 4 x 125 g a.s./ha. Further risk mitigation measures should be considered at Member State level for these scenarios.

Table 2.9.9.3-2: Comparison of the relevant RAC values and the maximum PEC_{SW} (ug a.s./L) for lenacil at FOCUS Steps 1 to 3 following application of Lenacil 500 g/L SC in sugar and fodder beet at 1 x 500 g a.s./ha.

Scenario	fish acute	fish chronic	Aquatic invertebrates	Aquatic invertebrates prolonged	Algae	Higher plant	Algae and aquatic plants
	Oncorhynchus mykiss	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Ankistrodesmus falcatus*	*Elodea canadensis*	*SSD approach*
Level of assessment	Tier 1	Tier 1	Tier 1	Tier 1	Tier 1	Tier 1	Higher Tier
RAC	> 20 µg/L	16 µg/L	> 31.1 µg/L	1.7 µg/L	1.33 µg/L	0.378 µg/L	1.06 µg/L
FOCUS Step 1 PEC values	**149.140**	**149.140**	**149.140**	**149.140**	**149.140**	**149.140**	**149.140**
FOCUS Step 2 PEC values							
North Europe	**21.828**	**21.828**	21.828	**21.828**	**21.828**	**21.828**	**21.828**
South Europe	**39.587**	**39.587**	**39.587**	**39.587**	**39.587**	**39.587**	**39.587**
FOCUS Step 3 PEC values							
D3 / ditch	2.624	2.624	2.624	**2.624**	**2.624**	**2.624**	**2.624**
D4 / pond	0.111	0.111	0.111	0.111	0.111	0.111	0.111
D4 / stream	2.143	2.143	2.143	**2.143**	**2.143**	**2.143**	**2.143**
R1 / pond	0.178	0.178	0.178	0.178	0.178	0.178	0.178
R1 / stream	2.067	2.067	2.067	**2.067**	**2.067**	**2.067**	**2.067**
R3 / stream	3.672	3.672	3.672	**3.672**	**3.672**	**3.672**	**3.672**

Notes: PEC values in bold indicate that the $PEC_{SW/SED}$ exceeds the RAC, and thus that further consideration is necessary

Table 2.9.9.3-3: Comparison of the relevant RAC values and the maximum PEC_{SW} (µg a.s./L) for lenacil at FOCUS Steps 1 to 3 following application of Lenacil 500 g/L SC in sugar and fodder beet at 4 x 125 g a.s./ha.

Scenario	fish acute	fish chronic	Aquatic invertebrates	Aquatic invertebrates prolonged	Algae	Higher plant	Algae and aquatic plants
	Oncorhynchus mykiss	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Ankistrodesmus falcatus*	*Elodea canadensis*	*SSD approach*
Level of assessment	Tier 1	Tier 1	Tier 1	Tier 1	Tier 1	Tier 1	Higher Tier
RAC	> 20 µg/L	16 µg/L	> 31.1 µg/L	1.7 µg/L	1.33 µg/L	0.378 µg/L	1.06 µg/L
FOCUS Step 1 PEC values	**149.140**	**149.140**	**149.140**	**149.140**	**149.140**	**149.140**	**149.140**
FOCUS Step 2 PEC values							
North Europe	12.680	12.680	12.680	**12.680**	**12.680**	**12.680**	**12.680**
South Europe	**22.789**	**22.789**	22.789	**22.789**	**22.789**	**22.789**	**22.789**
FOCUS Step 3 PEC values							
D3 / ditch	0.656	0.656	0.656	0.656	0.656	**0.656**	0.656
D4 / pond	0.065	0.065	0.065	0.065	0.065	0.065	0.065
D4 / stream	0.536	0.536	0.536	0.536	0.536	**0.536**	0.536
R1 / pond	0.337	0.337	0.337	0.337	0.337	0.337	0.337
R1 / stream	5.544	5.544	5.544	**5.544**	**5.544**	**5.544**	**5.544**
R3 / stream	5.597	5.597	5.597	**5.597**	**5.597**	**5.597**	**5.597**

Notes: PEC values in bold indicate that the $PEC_{SW/SED}$ exceeds the RAC, and thus that further consideration is necessary

Table 2.9.9.3-4: Comparison of the relevant RAC values for the chronic risk to aquatic invertebrates, algae and aquatic plants, and the maximum PECsw (ug a.s./L) for lenacil at FOCUS Step 4 following application of Lenacil 500 g/L SC in sugar and fodder beet at 1 x 500 g a.s./ha.

Scenario	Aquatic invertebrates-prolonged			Algae			Higher plant			Algae and aquatic plants		
	Daphnia magna			*Ankistrodesmus falcatus*			*Elodea canadensis*			*SSD approach*		
Level of assessment	Tier 1			Tier 1			Tier 1			Higher Tier		
RAC	1.7 µg/L			1.33 µg/L			0.378 µg/L			1.06 µg/L		
Mitigation options	**5 m DB**	**10 m VFS**	**5 m DB + 10 m VFS**	**5 m DB**	**10 m VFS**	**5 m DB + 10 m VFS**	**5 m DB**	**10 m VFS**	**5 m DB + 10 m VFS**	**5 m DB**	**10 m VFS**	**5 m DB + 10 m VFS**
FOCUS Step 4 PEC values												
D3 / ditch	0.860	-	-	0.860	-	-	**0.860**	-	-	0.860	-	-
D4 / stream	0.905	-	-	0.905	-	-	**0.905**	-	-	0.905	-	-
R1 / stream	-	1.812		-	**1.812**		-	**1.812**		-	**1.812**	
R3 / stream	-		1.675	-		**1.675**	-		**1.675**	-		**1.675**

Notes: PEC values in bold indicate that the PEC$_{SW/SED}$ exceeds the RAC, and thus that further consideration is necessary; DB: drift buffer; VFS: vegetated filter strip

Table 2.9.9.3-5: Comparison of the relevant RAC values for the chronic risk to aquatic invertebrates, algae and aquatic plants, and the maximum PECsw (ug a.s./L) for lenacil at FOCUS Step 4 following application of Lenacil 500 g/L SC in sugar and fodder beet at 4 x 125 g a.s./ha.

Scenario	Aquatic invertebrates prolonged	Algae	Higher plant	Algae and aquatic plants
	Daphnia magna	*Ankistrodesmus falcatus*	*Elodea canadensis*	*SSD approach*
Level of assessment	Tier 1	Tier 1	Tier 1	Higher Tier
RAC	1.7 µg/L	1.33 µg/L	0.378 µg/L	1.06 µg/L
Mitigation options	**20 m VFS**	**20 m VFS**	**20 m VFS**	**20 m VFS**
FOCUS Step 4 PEC values				
R1 / stream	1.315	1.315	**1.315**	**1.315**
R3 / stream	1.339	**1.339**	**1.339**	**1.339**

Notes: PEC values in bold indicate that the PEC$_{SW/SED}$ exceeds the RAC, and thus that further consideration is necessary; VFS: vegetated filter strip

The risk to aquatic organisms from exposure to the metabolites IN-KE 121 and IN-KF 313 was acceptable at FOCUS Step 1 or 2 (Table 2.9.9.3-6 and Table 2.9.9.3-7).

Table 2.9.9.3-6: Comparison of the relevant RAC values and the maximum PECsw for IN-KE121 at FOCUS Steps 1 to 2 following application of Lenacil 500 g.L SC in sugar and fodder beet at 1 x 500 g a.s./ha.

Metabolite	**IN-KE121**	**IN-KF313**
Scenario	**Algae**	**Algae**
	Pseudokircherniella subcapitata	*Pseudokircherniella subcapitata*
Level of assessment	Tier 1	Tier 1
RAC	2780 µg/L	427 µg/L
FOCUS Step 1 PEC values (µg/L)	32.671	65.220
FOCUS Step 2 PEC values (µg/L)		
North Europe	3.951	8.748
South Europe	7.657	16.676

Notes: PEC values in bold indicate that the $PEC_{SW/SED}$ exceeds the RAC, and thus that further consideration is necessary

Table 2.9.9.3-7: Comparison of the relevant RAC values and the maximum PECsw for IN-KE121 at FOCUS Steps 1 to 2 following application of Lenacil 500 g.L SC in sugar and fodder beet at 4 x 125 g a.s./ha.

Metabolite	**IN-KE121**	**IN-KF313**
Scenario	**Algae**	**Algae**
	Pseudokircherniella subcapitata	*Pseudokircherniella subcapitata*
Level of assessment	Tier 1	Tier 1
RAC	2780 µg/L	427 µg/L
FOCUS Step 1 PEC values (µg/L)	32.671	65.220
FOCUS Step 2 PEC values (µg/L)		
North Europe	2.063	5.082
South Europe	3.960	9.616

Notes: PEC values in bold indicate that the $PEC_{SW/SED}$ exceeds the RAC, and thus that further consideration is necessary

2.9.9.4 Bees

The risk assessment included in Volume 3 (PPP) sections B.9.6.1 indicated an acceptable risk to honeybees for the proposed used of Lenacil 500 g/L SC in sugar and fodder beet. The risk assessment was conducted according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) and the revised EPPO scheme (2010). In addition, a risk assessment according to the EFSA Guidance Document on the risk assessment of plant protection on bees (2013) was performed for the chronic risk to adult honeybees and honeybee larvae and for the risk to bumblebees. This Guidance Document is not yet noted by the Standing Committee on Plants, Animals, Food and Feed. Nevertheless, as SANCO/10329/2002 does not take into account chronic honeybee and bumblebee data, this additional assessment allows to take all available data on bees into account in a risk assessment.

Table 2.9.9.4-1: Summary of bee toxicity data used in the risk assessment for lenacil

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Honeybees					
Honeybee (*Apis mellifera*)	Lenacil 500 g/L SC	48h acute contact and oral toxicity test	LD_{50} (contact) LD_{50} (oral)	**> 498.4 µg product/bee (> 227.2 µg a.s./bee)** **> 452.3 µg product/bee (> 206.2 µg a.s./bee)**	CP10.3.1.1/01 Haupt S., 2016a
Honeybee (*Apis mellifera*)	Lenacil 500 g/L SC	10 day chronic adult oral toxicity test	LDD_{50}	**> 312.1 µg product/bee/day (> 142.3 µg a.s./bee/day)**	CP10.3.1.2/01 Haupt S. and Knebel N., 2016a
Honeybee (*Apis mellifera*)	Lenacil 500 g/L SC	8 day chronic larval toxicity test, repeated exposure	8 day NOED	**435.2 µg product/larva (= 198.4 µg a.s./larva)**	CA8.3.1.3/01 Haupt S. and Knebel N., 2016c
Bumblebees					
Bumblebee (*Bombus terrestris*)	Lenacil 500 g/L SC	48h acute contact and oral toxicity test	LD_{50} (contact) LD_{50} (oral)	**> 438.7 µg product/bee (> 200.0 µg a.s./bee)** **> 428.6 µg product/bee (> 195.4 µg a.s./bee)**	CP10.3.1.1/03 Haupt S., 2016b
Bumblebee (*Bombus terrestris*)	Lenacil 500 g/L SC	10 day chronic adult oral toxicity test	LDD_{50}	**> 208.6 µg product/bee/day (> 95.1 µg a.s./bee/day)**	CP10.3.1.2/02 Haupt S. and Knebel N., 2016b

The Tier 1 acute risk assessment according to SANCO/10329/2002 is shown in Table 2.9.9.4-2. As the HQ values are all below the trigger of 50, the acute risk to honeybees can be considered acceptable for the proposed use in sugar and fodder beet.

Table 2.9.9.4-2: Acute risk to adult honeybees from oral and contact exposure to lenacil following the proposed uses of Lenacil 500 g/L SC.

Exposure route	Crop	Application rate (g a.s./ha)	LD_{50} (µg a.s./bee)	HQ	Trigger value
Oral	Sugar and fodder beet	500	> 206.2	< 2.42	50
Contact	Sugar and fodder beet	500	> 227.2	< 2.20	50

Note: HQ values shown in bold exceed the trigger.

The results of the Tier 1 chronic risk assessment according to the revised EPPO scheme (2010) are shown in Table 2.9.9.4-3. The chronic TER exceeds the trigger of 1 for adult bees and larvae, indicating the risk can be considered acceptable.

Table 2.9.9.4-3: Chronic risk to adult bees and larvae from oral and contact exposure to lenacil following the use of Lenacil 500 g/L SC in sugar and fodder beet.

Honeybee stage	Exposure route	NOED	Worst case residue intake	TER_{ch}	Trigger value
Adult	Oral	142.3 µg a.s./bee/day	4.82 µg a.s./bee/day	29.52	1
Larvae	Oral	198.4 µg a.s./larva	2.14 µg a.s./larva	92.71	1

Note: TER values shown in bold are below the trigger.

The Screening Step assessment for honeybees according to the EFSA Guidance Document on bees (2013) is shown in Table 2.9.9.4-4 (contact exposure risk assessment) and 2.9.9.4-5 (oral exposure risk assessment). All HQ and ETR values are below the relevant trigger, indicating the risk can be considered acceptable.

Table 2.9.9.4-4: Acute contact exposure of adult honeybees to lenacil following the proposed uses of Lenacil 500 g/L SC – screening step.

Test substance	Crop	Application rate (g/ha)	LD_{50} (µg/bee)	HQ	Trigger value
Lenacil 500 g/L SC	Sugar and fodder beet	500	> 227.2	< 2.20	42

HQ values shown in bold exceed the trigger.

Table 2.9.9.4-5: Acute and chronic oral exposure of adult honeybees and honeybee larvae to lenacil following the proposed uses of Lenacil 500 g/L SC – screening step.

Type of assessment	Test substance	Crop	Application rate (kg a.s./ha)	SV	Endpoint	ETR	Trigger value
Acute oral exposure adult bees	Lenacil 500 g/L SC	Sugar and fodder beet	0.500	7.6	> 206.2 µg a.s./bee	< 0.018	0.2
Chronic oral exposure adult bees	Lenacil 500 g/L SC	Sugar and fodder beet	0.500	7.6	> 142.3 µg a.s./bee/day	< 0.027	0.03
Chronic oral exposure larvae	Lenacil 500 g/L SC	Sugar and fodder beet	0.500	4.4	198.4 µg a.s./ larva	0.011	0.2

*SV: Shortcut value; **bold** values exceed the trigger, indicating a potential risk.*

In Table 2.9.9.4-6 and 2.9.9.4-7, the risk assessment for exposure to contaminated guttation water and surface water according to the EFSA Guidance Document on bees (2013) is shown. As the ETR values are below the trigger, the risk from exposure to contaminated water can also be considered acceptable.

Table 2.9.9.4-6: Risk to adult honeybees and honeybee larvae following the consumption of guttation water contaminated with lenacil following application of Lenacil 500 g/L SC in sugar and fodder beet.

Type of assessment	Water consumption (µL)	PEC (µg/µL) [1)]	Endpoint	ETR	Trigger
Acute oral exposure adult bees	11.4	0.004	> 206.2 µg a.s./bee	< 0.00022	0.2
Chronic oral exposure adult bees	11.4	0.00216	> 142.3 µg a.s./bee/day	< 0.00017	0.03
Chronic oral exposure larvae	111	0.00288	198.4 µg a.s./ larvae	0.0016	0.2

*[1)] based on a maximum water solubility of 4 mg/L for lenacil; **bold** values exceed the trigger, indicating a potential risk*

Table 2.9.9.4-7: Risk to adult honeybees and honeybee larvae following the consumption of surface water contaminated with lenacil following application of Lenacil 500 g/L SC in sugar and fodder beet.

Type of assessment	Crop	Water consumption (µL)	PEC (µg/µL)	Endpoint	ETR	Trigger
Acute oral exposure adult bees	All proposed uses	11.4	149.14×10^{-6}	> 206.2 µg a.s./bee	8.25×10^{-6}	0.2
Chronic oral exposure adult bees	All proposed uses	11.4	149.14×10^{-6}	> 142.3 µg a.s./bee/day	0.119×10^{-6}	0.03
Chronic oral exposure larvae	All proposed uses	111	149.14×10^{-6}	198.4 µg a.s./ larvae	0.834×10^{-6}	0.2

***bold** values exceed the trigger, indicating a potential risk*

2.9.9.5 Other non-target arthropods

The risk assessment included in Volume 3 (PPP) section B.9.6.2 indicated an acceptable risk to non-target arthropods. The risk assessment was conducted according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002).

The Tier 1 risk assessment for in-field and off-field exposure is shown in Table 2.9.9.5-1 and 2.9.9.5-2, respectively. The HQ values for both in-field and off-field exposure are below the trigger of 2, indicating an acceptable risk for non-target arthropods from exposure to lenacil following an application of Lenacil 500 g/L according to the proposed use in sugar and fodder beet.

Table 2.9.9.5-1: In-field risk to non-target terrestrial arthropods based on laboratory studies (Tier I) from exposure to lenacil following application of the formulation Lenacil 500 g/L SC in sugar and fodder beet.

Test substance	Test species	LR_{50} (g a.s./ha)	In-field PER (g a.s./ha)	HQ	Trigger value
Lenacil 500 g/L SC	*Aphidius rhopalosiphi*	> 1900	500	0.263	2
	Typhlodromus pyri	> 1900		0.263	
	Poecilus cupreus	> 1700		0.294	

Table 2.9.9.5-2: Off-field risk to non-target terrestrial arthropods based on laboratory studies (Tier I) from exposure to lenacil following application of the formulation Lenacil 500 g/L SC in sugar and fodder beet.

Test substance	Test species	LR_{50} (g a.s./ha)	Off-field foliar			Trigger value
			Off-field PER (g a.s./ha)	Correction factor	HQ	
Lenacil 500 g/L SC	*Aphidius rhopalosiphi*	> 1900	1.385	10	0.0073	2
	Typhlodromus pyri	> 1900			0.0073	
	Poecilus cupreus	> 1700			0.0081	

2.9.9.6 Earthworms

The risk assessment included in Volume 3 (PPP) section B.9.8.1 indicated an acceptable risk to earthworms from the formulation, the active substance and the relevant metabolites IN-KE 121 and IN-KF313. The risk assessment was conducted according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002).

The TER values for both the acute and chronic risk assessment are shown in Table 2.9.9..6-1 and 2.9.9.6-2, respectively. These TER values exceed the trigger values of 10 and 5, respectively, indicating an acceptable risk for earthworms from exposure to lenacil following an application of Lenacil 500 g/L according to the proposed use in sugar and fodder beet.

Table 2.9.9.6-1: Acute risk (TER_A) to earthworms for lenacil, its metabolite IN-KE121 and IN-KF313 and the formulation Lenacil 500 g/L SC, based on worst-case max PEC_{SOIL} values for the proposed uses.

Test species	Test substance	LC_{50} (mg a.s./kg)	$LC_{50,CORR}$ (mg a.s./kg)	Max PEC_{SOIL} (mg a.s./kg)	TER_A
Eisenia fetida	lenacil	> 1000	-	0.533	1876
	Lenacil 500 g/L SC	> 417	-	0.533	782
	IN-KE121	> 1000	-	0.079	12658
	IN-KF313	> 1000	> 500	0.123	4065

Table 2.9.9.6-2: Chronic risk (TER_{LT}) to earthworms for lenacil, its metabolite IN-KE121 and IN-KF313 and the formulation Lenacil 500 g/L SC, based on worst-case max PEC_{SOIL} values for the proposed uses.

Test species	Test substance	NOEC (mg a.s./kg)	$NOEC_{CORR}$ (mg a.s./kg)	Max PEC_{SOIL} (mg a.s./kg)	TER_{LT}
Eisenia fetida	lenacil	1000	-	0.533	1876

	Lenacil 500 g/L SC	40	-	0.533	75
	IN-KE121	40	-	0.079	506
	IN-KF313	40	20	0.123	163

2.9.9.7 Other soil macro-organisms

No studies with other soil non-target meso- and macrofauna are available. Therefore, no risk assessment for these species was performed. Given that lenacil and its two major metabolites do not persist in soil beyond 100 days, and that the risk to earthworms, sensitive indicator species for non-target arthropods (*Aphidius rhopalosiphi* and *Typhlodromus pyri*) and soil microflora were shown to be acceptable, further studies on other soil non-target meso- and macrofauna were not considered to be required.

2.9.9.8 Soil micro-organisms

The risk assessment included in Volume 3 (PPP) section B.9.10 indicated an acceptable risk to soil nitrogen transformation processes from exposure to lenacil and the formulation Lenacil 500 g/L SC. The risk assessment was conducted according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002).

Table 2.9.9.8-1: Summary of the soil nitrogen transformation endpoints for lenacil and the formulation Lenacil 500 g/L SC, and risk assessment based on worst-case PEC_{soil} values for the proposed uses of Lenacil 500 g/L SC.

Test substance	Test rate (mg a.s./kg soil d.w.)	% effect on soil nitrogen transformation at 28 days after treatment [1)]	PEC_{SOIL} (mg/kg soil d.w.)	Acceptable risk
lenacil	6.7	+23.4	0.533	yes
Lenacil 500 g/L SC	3.3	+2.3	0.533	yes
	35	+3.1	0.533	yes

[1)] % effect – negative values indicate a reduction in the treated sample compared to the control

Table 2.9.9.8-2: Summary of the soil carbon transformation endpoints for lenacil and the formulation Lenacil 500 g/L SC, and risk assessment based on worst-case PEC_{soil} values for the proposed uses of Lenacil 500 g/L SC

Test substance	Test rate (mg a.s./kg soil d.w.)	% effect on soil carbon transformation at 28 days after treatment [1)]	PEC_{SOIL} (mg/kg soil d.w.)	Acceptable risk
lenacil	6.7	-18.5	0.533	yes
Lenacil 500 g/L SC	3.3	+10.24	0.533	yes

[1)] % effect – negative values indicate a reduction in the treated sample compared to the control

2.9.9.9 Non-target plants

The risk assessment included in Volume 3 (PPP) section B.9.12 indicated an acceptable risk to non-target plants. The risk assessment was conducted according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002).

Using the standard distance of 1m for the proposed use in sugar and fodder beer, the calculated TER values exceed the trigger of 5, as shown in Table 2.9.9.9-1. This indicates an acceptable risk.

Table 2.9.9.9-1: TER values for non-target plants following the proposed use Lenacil 500 g/L SC in sugar and fodder beet.

Crop use	Drift distance (m)	PER (g a.s./ha)	ER_{50} (g a.s./ha)	TER
Sugar and fodder beet	1	13.85	177.2	12.79

2.9.9.10 Biological methods for sewage treatment

For biological methods for sewage treatment no risk assessment could be performed, due to the lack of an acceptable endpoint.

A study on the impact of metconazole on the respiration of sewage sludge is available from the original DAR for metconazole (November 2007), from which an EC_{50} > 100 mg a.s./L was derived. However, the available study does not comply to all validity criteria of the most recent version of the test guideline. Therefore, this endpoint is no longer considered acceptable for use in the risk assessment. As no other studies are available, the risk assessment could not be performed.

2.10 ENDOCRINE DISRUPTING PROPERTIES

An assessment of the endocrine disrupting (ED) properties of lenacil was performed in line with the ECHA/EFSA Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. The full assessment was included in Volume 3 (AS) Section B.6.8.3 for human health and in Volume 3 (AS) Section B.9.1.5 for the environment. Below, a summary of the outcome of this assessment is included.

All available data relevant for the ED assessment for lenacil were populated in the Excel template provided as Appendix E to the ECHA/EFSA guidance document. This Excel file was included as an Annex to the Assessment Report, under the agreed nomenclature of the documents used in the PPR review (EFSA), under: "**Lenacil_DRAR_23_Appendix E_2019_05_28.XLSM**"

2.10.1 ED assessment for human health

Notifier considered that lenacil fulfilled all conditions to propose a scenario "1a" (*i.e.* sufficiently investigated, no adversity indicated by "EATS-mediated" parameters. Taking into account all data of the dossier, **RMS** is of the opinion that lenacil fulfilled the condition of scenario **"1b"** (EATS-mediated adversity observed, postulate MoA).

Rationale:
The potential of lenacil to induce adverse effects on components of the endocrine system has initially been assessed in published *in vitro* assays. Based on the available receptor-based *in vitro* studies it was suggested that lenacil has no PXR, mPPARα, mPPARγ or AhR agonistic activities at concentrations of $\leq 10^{-5}$M, *in vitro.* Further OECD Level-2 studies were submitted at the occasion of the renewal in 2018-2019.
Potential endocrine-related adverse findings were also identified in in the guideline short-term and long-term feeding studies, and in the multi-generation reproduction study. The adverse effect pertained to (i) thyroid findings, but also some indications of (ii) mammary tumours and (iii) uterus adversity, with a possible underlying endocrine MoA.

No effects on fertility, reproductive performance and/or development and sexual maturation of the offspring was seen even when lenacil was orally administered at dose levels exceeding the limit dose level of 1000 mg/kg b.w./d.
Regarding the observed potential endocrine adverse findings:

(i) In both the sub-chronic and the chronic feeding studies with rats (**B.6.3.2.1.1** -ACD 002/013903 and **B.6.5.1.1**- ACD 045/024288-), adverse effects on the thyroid were observed, without any evidence of organ atrophy, but with evidence of histopathologically diagnosed blackening, possibly indicating lipofuscin deposition. Follicular tumours were weakly induced, however without being eligible for carcinogenicity classification, as the incidence was low, comprised into HCD and because the likely MoA was UDPGT induction, with relevance for rodents but not for the human.

The effect of the substance on thyroid pathways was not entirely elucidated. A perchlorate discharge study with lenacil showed no direct toxicity to the thyroid which could be explained via an inhibition of deiodinase or peroxidase. The key findings from this test was that lenacil at doses up to >4000 mg/kg b.w./d did not affect the ability of the thyroid to take-up and organify iodide. It was demonstrated that lenacil did not act as an inhibitor of the deiodinase and/or peroxidase which converts T_4 to T_3. A separate peroxidase inhibition assay on porcine thyrocytes demonstrated that lenacil did not act via this pathway. Inconsistent effects on thyroid hormone levels were reported, but overall, the thyroid hormone modifications were not indicating a clear effect of the substance.

No MoA could unequivocally be identified for the finding of the thyroids blackening, but the existing mechanistic study *suggests* that a direct thyrotropic effect is unlikely. In a WoE evaluation, **RMS** agrees that there was no consistent pattern suggestive of a clear impact on the T modality.

However, there is no DNT study, thus it remains unclear what the impact could be of subtle thyroid hormone changes (if relevant) on the behavioural development in young rats, and **RMS** suggests to discuss it more in detail in the peer review process.

(ii) Based on an increased incidence of malignant mammary adenocarcinomas in the rat carcinogenicity study, which was considered to be *possibly* relevant for humans in the EFSA

conclusion on the peer review of lenacil (EFSA, 2009) and more recently by the ECHA Risk Assessment Committee (RAC) lenacil was classified as a category 2 carcinogen (Carc. 2; H351). This harmonised classification proposal has meanwhile been adopted.

On the other hand, the mode of action (MoA) for the induction of mammary tumours observed in the rat carcinogenicity study with lenacil is still unknown. The absence of a genotoxic potential of lenacil does, however, not suggest that mammary gland tumours are caused be a genotoxic MoA and for this reason an epigenetic, endocrine-mediated MOA could be the cause for the induction of these tumours.

However, as the mechanistic *in-vitro* studies aiming to investigate effects on EAS-modalities demonstrated no interaction, the emergence of mammary tumours was considered most likely not endocrine-mediated. The mammary tumours may be either of equivocal relevance, or an epigenetic MoA could be at the basis of the tumourigenicity.

(iii) Uterus changes occur in several studies in the present DRAR which could be considered possibly treatment-related. The data with effect on the uterus pertain to findings observed in the Wistar rat, and increased uterus weight, along with fluid distention and/or luminal dilatation, or hyperplasia were observed. It is observed that the effects occur at relatively high dose (mostly ≥ 4000 mg/kg b.w./d), and that a dose-response was observed at times but not consistently. In the key study (2G), top-dose findings are poorly dose-responsive and/or increased histopathological findings are only borderline when compared to controls. No effects were observed in the dog, and in mice it was not investigated.

In addition, a the newly submitted uterotrophic assay was conducted up to a dose of 1000 mg/kg b.w./d (guideline limit dose) but also with another rat strain (Sprague-Dawley derived), and the assay turned out negative for uterus findings. As mentioned in (ii), lower-tier *in-vitro* studies do not indicate interference with EAS pathways. **RMS** concludes that the observed uterus findings are unlikely caused by an endocrine-dependent pathway. The overall WoE would suggest that the effect of lenacil on uterus was limited.

In conclusion, adverse endocrine findings possibly involving EAS-pathways were identified and further investigated. Both *in-vitro* and *in-vivo* mechanistic studies were performed in order to investigate an EATS-mediated MoA, and the latter could on the basis of these studies be excluded.

Thyroid adverse effects were also identified, and existing and new *in-vivo* mechanistic studies indicated that lenacil is not a primary thyrotoxicant. It was demonstrated that lenacil induced CYP4501B metabolic enzymes, as well as UDPGT, possibly explaining a number of adverse findings. Overall, the data showed that the adverse findings were mainly associated to systemic toxicity at doses nearby or >MTD, exceeding the limit dose of 1000 mg/kg b.w./d.

It is therefore plausible that no primary endocrine MoA for the EATS-modalities would be at the basis of all observations. For the thyroid findings, no other explanation than top-dose toxicity and UDPGT induction could be formulated. Therefore, **RMS** would be inclined to consider lenacil **not meeting the criteria for endocrine disruption**. While potentially adverse EATS findings have been detected, subsequent mechanistic studies and a WoE consideration leads to the conclusion that the ED criteria are not met.

One residual doubt subsist, for which a final discussion is seeked, namely the absence of a developmental neurotoxicity study. It is not excluded that any thyroidal effect could have more impact on young animals. Therefore, the question whether or not a DNT is necessary needs further discussion.

2.10.2 ED assessment for the environment

For wild mammals, the main source of information are the regulatory toxicological studies performed with mammals in the laboratory for human safety purposes, which are summarized in Volume 3 (AS) Section B.6. The relevant studies are listed in Table 2.10.2-1. A summary of these studies is included in Volume 3 (AS) Section B.9.1.1.3 and Section B.9.2.2.1.

Table 2.10.2-1: Studies relevant for the ED assessment of lenacil in non-target organisms other than mammals.

Study type	Species	Guideline	OECD conceptual framework level	Endpoint	Value	Reference
Avian reproduction test	*Colinus virginianus*	EPA 71-4; OECD 206	4	NOEL (22 weeks)	1024 mg a.s./kg diet (100.4 mg a.s./kg bw/day)	CA8.1.1.3/01 [redacted] *et al.*, 1996
Fish early lifestage (ELS)	*Oncorhynchus mykiss*	EPA 72-4; OECD 210	4	90d NOEC	0.160 mg a.s./L	CA8.2.2.1/02 [redacted] 1996

For mammals as non-target organisms, the same conclusion as for the human health assessment can be drawn (see above). Adverse endocrine findings possibly involving EAS-pathways were identified and further investigated. Both *in-vitro* and *in-vivo* mechanistic studies were performed in order to investigate an EATS-mediated MoA, and the latter could on the basis of these studies be excluded.

For other non-target vertebrates (i.e. birds and aquatic vertebrates), the available database is limited to an avian reproduction study and a fish early lifestage (ELS) toxicity study. In these studies, effects on parameters 'sensitive to, but not diagnostic of, EAS' were generally observed at dose levels where there was also systemic toxicity. However, as no specific EAS-mediated parameters are investigated in the available avian and fish reproductive studies, the available data can only be considered supportive for the lack of ED related adversity.Overall, according to Section 3.4.1 of the ECHA/EFSA GD, adversity based on EAS-mediated parameters is not considered to be sufficiently investigated.

To investigate endocrine activity of lenacil, *in vitro* mechanistic data are available for the EAS-modalities, which are summarised in detail in Volume 3 (AS) Section B.6.8.3. Based on these data, the RMS considered that there is no indication that there is interference of lenacil with the E or A modality. Overall, there is no evidence for EAS-mediated activity of lenacil.

In conclusion for the **EAS-modalities**: although adversity based on EAS-mediated parameters is not sufficiently investigated, the available *in vitro* mechanistic data indicate that there is no EAS-mediate endocrine activity. Consequently, scenario 2a (ii) from Table 5 from the ECHA/EFSA GD applies here, and it is possible to conclude that for the EAS-modalities, **the ED criteria for non-target organisms are not met for lenacil**. Further testing is therefore not considered required.

For assessing the ED properties through the **T-modality**, no *in vivo* studies with amphibians are available. Consequently, adversity based on T-mediated parameters is not sufficiently investigated.

A number of *in vivo* mechanistic studies with mammals is available, which were assessed in the toxicology section (see Volume 3 (AS) Section B.6.8.3 for details). Although the results of these studies were not fully inconsistent, they indicated that lenacil is not a primary thyrotoxicant. However, no developmental neurotoxicity study is available, and therefore it is not excluded that any thyroidal effect could have more impact on young mammals. Given that there is still some doubt on T-mediated endocrine activity, RMS considers that the conclusions for mammals cannot be extrapolated to other non-target vertebrates. Therefore, the available evidence is not sufficient to conclude on the T-mediated endocrine activity in non-target organisms.

Based on the above, scenario 2a (iii) from Table 5 from the ECHA/EFSA GD applies here. Therefore, **additional data should be generated**. In line with the ECHA/EFSA GD, RMS proposes the following testing strategy: In a first instance, a level 3 study according to OECD TG 231 (Amphibian Metamorphosis Assay - AMA) would be required. Two cases are possible:

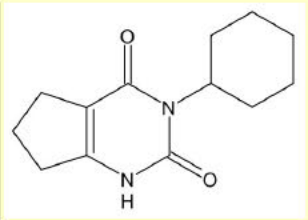
1. If this study is negative, it can be concluded that the ED criteria are not met for the T-modality.
2. If positive, however, a larval amphibian growth and development assay (LAGDA, OECD TD 241) will be needed to support the mode of action (MoA) analysis.

2.11 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA *[SECTIONS 1-6 OF THE CLH REPORT]*

2.11.1 Identity of the substance [section 1 of the CLH report]

2.11.1.1 Name and other identifiers of the substance

Table 2.10.1.1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	*[The Guidance for identification and naming of substances under REACH and CLP can be found at the following link: http://echa.europa.eu/guidance-documents/guidance-on-reach]* 3-cyclohexyl-1,5,6,7-tetrahydrocyclopentapyrimidine-2,4(*3H*)-dione
Other names (usual name, trade name, abbreviation)	DPX-B0634 (synonym : B10048563)
ISO common name (if available and appropriate)	Lenacil
EC number (if available and appropriate)	218-499-0 (EINECS)
EC name (if available and appropriate)	3-cyclohexyl-6,7-dihydro-1H-cyclopentapyrimidine-2,4(*3H,5H*)-dione
CAS number (if available)	2164-08-1
Other identity code (if available)	CIPAC : 163
Molecular formula	$C_{13}H_{18}N_2O_2$
Structural formula	
SMILES notation (if available)	/
Molecular weight or molecular weight range	234.3 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not relevant
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not relevant
Degree of purity (%) (if relevant for the entry in Annex VI)	*[The minimum and maximum values should be specified.]* Min. 975 g/kg

2.11.1.2 Composition of the substance

Table 2.10.1.2-1: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
3-cyclohexyl-1,5,6,7-tetrahydrocyclopentapyrimidine-2,4(*3H*)-dione (Lenacil)	Min. 97.5	Hazard Class and Category Code(s): Carc. 2 Aquatic Acute 1 Aquatic Chronic 1 Hazard statements : H351, H400 and H410 Pictograms: GHS08, GHS09 and Wng	

Table 2.10.1.2-2: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
/				

Table 2.10.1.2-3: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
/					

Table 2.10.1.2-4.1: Test substances (non-confidential information) used in toxicological tests

Study	Reference	Report n°	a.s.	Batch n°	Purity %
B.6.1 ADME studies					
ADME, main study	Ghantous. (1996)	HLR 62-94	[^{14}C] Lenacil, Lenacil	No 391, B634-88 No19615,20300	>98% 98%
Interspecies *in vitro* metabolism	Pineiro Costas (2016)	WIL Research Project 512721	[^{14}C]Lenacil	CFQ42525	97.6%
B.6.2 Acute toxicity studies					
Oral	[redacted] (2001a)	ACD 004/013224/AC	Lenacil	141712003	98.6
Percutaneous	[redacted] (2001b)	ACD 005/013220/AC	Lenacil	141712003	98.6
Inhalation	Coombs (2001)	ACD 021/013229	Lenacil	141712003	98.6
Skin irritation	[redacted] (2001c)	ACD 006/013201/SE	Lenacil	141712003	98.6

Study	Reference	Report n°	a.s.	Batch n°	Purity %
Eye irritation	[redacted] (2001d)	ACD 007/013273/SE	Lenacil	141712003	98.6
Skin sensitisation	[redacted] (1992)	HLO 34-92	Lenacil	B634-91	98.2
Phototoxicity	Westerink (2016)	WIL Research Project 511052	Lenacil	B0634-142	98.6
B.6.3 Short term studies – 6.8.2 supplementary studies(°)					
28-d rat, oral	[redacted] (2002a)	ACD 001/010098	Lenacil	141712003	98.6
28-d dog, oral	[redacted] (2001)	ACD 003/013230	Lenacil	141712003	98.6
90-d (4-wk rec.) rat, oral	[redacted] (2002b)	ACD 002/013903	Lenacil	141712003	98.6
Additional 90-d (4-wk rec.) rat, oral (°)	[redacted] (2004)	ACD 055/024499	Lenacil	141712003	98.6
90-d mouse, oral	[redacted] (1991)	HLR 293-91	Lenacil	B634-91	98.2
90-d dog, oral	[redacted] (2002)	ACD 022/014297	Lenacil	141712003	98.6
B.6.4 Genotoxicity studies					
Salmonella/microsome assay (*in vitro*)	Russell (1977)	HLR 601-77	Lenacil	B634-50	n.s.
Salmonella/microsome assay (*in vitro*)	D'Amico (1994)	HLR 413-94	Lenacil	B634-107	n.s.
Salmonella/microsome assay (*in vitro*)	May (2001)	ACD 016/013217	Lenacil	141712003	98.6
UDS study (*in vitro*)	Riach (1989)	IRI 6135	Lenacil	8906	n.s.
Chromosomal aberrations (*in vitro*)	Allais (2001)	ACD 017/013707	Lenacil	14171003	98.6
Chromosomal aberrations (*in vitro*)	Kellum (2017)	047303003	Lenacil	047303003	98.6
Mouse lymphoma (*in vitro*)	Clare (2003)	ACD 053/023530	Lenacil	141712003	98.6
Mouse micronucleus assay (*in vivo*)	[redacted] (2001)	ACD 018/013472	Lenacil	141712003	98.6
B.6.5 Long term studies					
Long-term toxicity and carcinogenicity, rat, oral	[redacted] 2003	ACD 045/024288	Lenacil	141712003	98.6
Long-term toxicity and carcinogenicity, rat, oral	[redacted] (2004)	ACD 045/042214	Lenacil	141712003	98.6
Oncogenicity, mice, oral	[redacted] (1994)	HLR 336-93	Lenacil	B634-91	98.2
B.6.6 Reproduction studies					
2-generation, rat, oral	[redacted] (2002)	ACD 019/010186	Lenacil	141712003	98.6
2-generation, rat, oral	[redacted] (2003)	ACD 020/023865	Lenacil	141712003	98.6

Study	Reference	Report n°	a.s.	Batch n°	Purity %
Developmental, rat, oral	[redacted] (1978)	HLR 405-78	Lenacil	B634-61	*Ca.*100
Developmental, rat, oral	[redacted] (1996)	HLR 996-96	Lenacil	DP B 634091 [redacted] 18759	98.5
Embryo-foetal development, rat, oral (preliminary)	[redacted] (2003)	ACD 057/030001	Lenacil	141712003	98.6
Embryo-foetal development, rat, oral	[redacted] (2003)	ACD 058/032316	Lenacil	141712003	98.6
Developmental toxicity, rabbit, oral	[redacted] (1991)	HLR 626-91	Lenacil	B634-91	98.5
B.6.8.2 Supplementary studies					
Thyroid study, 20 weeks, rat, oral	[redacted] (2004)	ACD 060/033946	Lenacil	141712003	98.6
B.6.8.3 Studies assessing endocrine effects					
ER binding assay, *in-vitro*	Nabb (2018a)	DuPont R. No.: 49349	Lenacil	047303003	99.33
AR binding assay, *in-vitro*	Nabb (2018b)	DuPont R. No.: 49367	Lenacil	047303003	99.33
ER transactivation assay, *in-vitro*	Rijk (2018a)	DuPont R. No.: 49351	Lenacil	047303003	99.33
AR transactivation assay, *in-vitro*	Rijk (2018b)	DuPont R. No.: 50113	Lenacil	047303003	99.33
6-d uterotrophic assay, rat, oral	[redacted] (2018)	DuPont R. No.: 49350	Lenacil	036402003	99.33

n.s.: not specified (despite GLP study)

Table 2.10.1.2-4.2: Test substance (non-confidential information) used in the ecotoxicological tests

Test species	Test substance	Chemical purity	Batch No	Reference	Report No
Birds – Acute oral toxicity					
Mallard duck (*Anas platyrhynchos*)	Lenacil	98.6 %	141712003	CA8.1.1.1/01 [redacted] (2002a)	ACD 048/022425
Bobwhite quail (*Colinus virginianus*)	Leancil	98.6 %	141712003	CA8.1.1.1/02 [redacted] (2002b)	ACD 049/022426
Birds – Short-term dietary toxicity					
Bobwhite quail (*Colinus virginianus*)	Lenacil	98.9 %	141712003	CA8.1.1.2/01 [redacted] (2004a)	DPT 637/033931
Birds – Sub-chronic and reproductive toxicity					
Bobwhite quail (*Colinus virginianus*)	Lenacil	98.4 %	DPX-B634-91 (9038)	CA8.1.1.3/01 [redacted] *et al.* (1996)	AMR 3419-95
Aquatic organisms – Toxicity of the active substance and metabolites					
Common carp (*Cyprinus carpio*)	Lenacil	98.6 %	141712003	CA8.2.1/01 [redacted] (2003a)	ACD 035/022512

Rainbow trout (*Oncorhynchus mykiss*)	Lenacil	98.2 %	DPX-B634-91 (9038)	CA8.2.1/02 [redacted] (1991a)	HLR 199-91
Fathead minnow (*Pimephales promelas*)	Lenacil	98.2 %	DPX-B634-91 (9038)	CA8.2.1/03 [redacted] (1991b)	HLR-198-91
Rainbow trout (*Oncorhynchus mykiss*)	Lenacil	98.2 %	DPX-B634-91 (9038)	CA8.2.2.1/01 [redacted] (1991c)	HLR 200-91
Rainbow trout (*Oncorhynchus mykiss*)	Lenacil	98.5 %	DPX-B634-91 (9038)	CA8.2.2.1/02 [redacted] (1996)	HLR 235-96
Daphnia magna	Lenacil	95.1 %	Blended batches 8802 and 8805	CA8.2.4.1/01 Hutton D.G. (1989a)	HLR 86-89
Daphnia magna	**Lenacil**	**98.82 %**	**JUL14HE010**	**CA8.2.4.1/02 Renner P. (2016a)**	**15 10 48 031 W**
Dapnia magna	**Lenacil**	**98.82 %**	**JUL14HE010**	**CA8.2.5.1/02 Renner P. (2016b)**	**15 10 48 032 W**
Green alga (*Pseudokirchneriella subcapitata*)	Lenacil	98.6 %	141712003	CA8.2.6.1/01 Flatman D. (2003c)	ACD 034/022511
Green alga (*Pseudokirchneriella subcapitata*)	IN-KE121	96.7 %	7X-0245	CA8.2.6.1/03 Jenkins C.A. (2004a)	ACD 064/0427300
Geen alga (*Pseudokirchneriella subcapitata*)	IN-KF313	99.6 %	1Y-0622	CA8.2.6.1/04 Jenkins C.A. (2004b)	ACD 066/042848
Diatom (*Navicula pelliculosa*)	Lenacil	98.6 %	141712003	CA8.2.6.2/01 Flatman D. (2003b)	ACD 036/024694
Green alga (*Ankistrodesmus falcatus*)	**Lenacil**	**99.3 %**	**B0634-159 (0190813)**	**CA8.2.6.2/02 Wenzel A. (2014a)**	**DPT-001/4-10/F**
Cyanobacteria (*Synechococcus leopoliensis)*	**Lenacil**	**99.3 %**	**B0634-159 (0190813)**	**CA8.2.6.2/03 Wenzel A. (2014b)**	**DPT-001/4-10/C**
Cyanobacteria (*Anabaena flos-aquae*)	**Lenacil**	**99.3 %**	**B0634-159 (0190813)**	**CA8.2.6.2/04 Wenzel A. (2014c)**	**DPT-001/4-10/E**
Alga (*Closterium cornu*)	**Lenacil**	**99.3 %**	**B0364-159 (0190813)**	**CA8.2.6.2/05 Wenzel A. (2014d)**	**DPT-001/4-10/G**
Yellow-green alga (*Xanthonema debile*)	**Lenacil**	**99.3 %**	**B0364-159 (0190813)**	**CA8.2.6.2/06 Wenzel A. (2014e)**	**DPT-001/4-10/I**
Common duckweed (*Lemna gibba*)	Lenacil	98.6 %	141712003	CA8.2.7/01 Flatman D. (2003d)	ACD 039/023827
Macrophyte (*Chara globularis*)	**Lenacil**	**99.1 %**	**B0634-158 (200010003)**	**CA8.2.7/02 Wenzel A. (2012a)**	**DPT-001/4-80/D**
Canadian waterweed (*Elodea canadensis*)	**Leancil**	**99.1 %**	**B0364-158 (200010003)**	**CA8.2.7/03 Wenzel A. (2012b)**	**DPT-001/4-80/C**
Aquatic organisms – Toxicity of the preparations					

Green alga (*Pseudokirchneriella subcapitata*)	**Lenacil 500 g/L SC**	**475 g a.s./L**	**0870805 V1-NF1**	**CP10.2.1/01 Pawlowski S. and Wydra V. (2006)**	**26801210**
Common duckweed (*Lemna gibba*)	**Lenacil 500 g/L SC**	**475 g a.s./L**	**0870805 V1-NF1**	**CP10.2.1/02 Pawlowski S. (2006)**	**26802240**
Outdoor microcosm study	Venzar 80 WP	80.5 % w/w a.s.	NOV00HE037	CP10.2.3/01 Jenkins C.A. (2005)	ACD 075/043691
Bees – Acute and chronic toxicity of the preparations					
Honey bee (*Apis mellifera*)	**Lenacil 500 g/L SC**	**519.2 g a.s./L**	**DEC14HE004**	**CP10.3.1.1./01 Haupt S. (2016a)**	**95231035**
Honey bee (*Apis mellifera*)	**Lenacil 500 g/L SC**	**475 g a.s./L**	**0870805 V1-NF1**	**CP10.3.1.1./02 Schmitzer S. (2006a)**	**268013035**
Bumble bee (*Bombus terrestris*)	**Lenacil 500 g/L SC**	**519.2 g a.s./L**	**DEC14HE004**	**CP10.3.1.1/03 HauptS. (2016b)**	**95231105**
Honey bee (*Apis mellifera*)	**Lenacil 500 g/L SC**	**519.2 g a.s./L**	**DEC14HE004**	**CP10.3.1.2/01 Haupt S. and Knebel N. (2016a)**	**95231136**
Bumble bee (*Bombus terrestris)*	**Lenacil 500 g/L SC**	**519.2 g a.s./L**	**DEC14HE004**	**CP10.3.1.2/02 Haupt S. and Knebel N. (2016b)**	**95231107**
Honey bee (*Apis mellifera*)	**Lenacil 500 g/L SC**	**519.2 g a.s./L**	**DEC14HE004**	**CP10.3.1.3/01 Haupt S. and Knebel N. (2016c)**	**95231032**
Non-target terrestrial arthropds – laboratory tests					
Parasitic wasp (*Aphidius rhopalosiphi*)	**Lenacil 500 g/L SC**	**475 g a.s./L**	**0870805 V1-NF1**	**CP10.3.2.1/01 Moll M. (2005)**	**26811001**
Predatory mite (*Typhlodromus pyri*)	**Lenacil 500 g/L SC**	**475 g a.s./L**	**0870805 V1-NF1**	**CP10.3.2.1/02 Rosenkranz B. (2006)**	**26812063**
Ground beetle (*Poecilus cupreus*)	**Lenacil 500 g/L SC**	**475 g a.s./L**	**0870805 V1-NF1**	**CP10.3.2.1/03 Schmitzer S. (2006b)**	**26814006**
Soil macro-organisms – toxicity of the active substance and metabolites					
Earthworm (*Eisenia fetida*)	Lenacil	98.6 %	141712003	CA8.4.1/01 Rodgers M.H. (2002)	ACD 027/014409
Earthworm (*Eisenia fetida*)	**Lenacil**	**98.82 %**	**JUL14HE010**	**CA8.4.1/02 Pavic B. (2016)**	**95231022**
Earthworm (*Eisenia fetida*)	IN-KF313	99.6 %	1Y-0622	CA8.4.1/03 Rodgers M.H. (2004a)	ACD 062/043039
Earthworm (*Eisenia fetida*)	IN-KE121	96.7 %	7X-0245	CA8.4.1/04 Rodgers M.H. (2004b)	ACD 061/043033
Earthworm (*Eisenia fetida*)	**IN-KE121**	**95 %**	**EXP-15-DA9895**	**CA8.4.1/05 Pavic B. (2015a)**	**100471022**

Earthworm (*Eisenia fetida*)	**IN-KF313**	**99 %**	**70409**	**CA8.4.1/06 Pavic B. (2015b)**	**100461022**
Soil macro-organisms – Toxicity of the preparations					
Earthworm (*Eisenia fetida*)	**Lenacil 500 g/L SC**	**475 g a.s./L**	**0870805 V1-NF1**	**CP10.4.1.1/01 Lührs U. (2005)**	**26816021**
Earthworm (*Eisenia fetida* / *Eisenia andrei*)	**Lenacil 500 g/L SC**	**475 g a.s./L**	**0870805 V1-NF1**	**CP10.4.1.1/02 Lührs U. (2006)**	**26817022**
Soil micro-organisms – Toxicity of the active substance and metabolites					
Soil micro-organisms	Lenacil	98.6 %	141712003	CA8.5/01 Carter J.N. (2002)	ACD/026
Soil micro-organisms – Toxicity of the preparations					
Soil micro-organisms	**Lenacil 500 g/L SC**	**475 g a.s./L**	**0870805 V1-NF1**	**CP10.5/01 Reis K.H. (2006)**	**26815080**
Soil micro-organisms	**Lenacil 500 g/L SC**	**500.6 g a.s./L**	**DEC14HE004**	**CP10.5/02 Schulz L. (2016)**	**15 10 48 058 N**
Non-target terrestrial plants – Toxicity of the preparations					
Several plant species	Lenacil 500 g/L SC	475 g a.s./L	0870805 V1-NF1	CP10.6.2/01 Goβmann A. and Meinerling M. (2006)	26803086
Several plant species	**Lenacil 500 g/L SC**	**519.2 g a.s./L**	**DEC14HE004**	**CP10.6.2/02 Stürtz S. and Knebel N. (2016)**	**95231087**
Activated sludge – Toxicity of the active substance					
Study not acceptable					

For physico-chemical tests, the material used was:

- Pure grade active ingredient PGAI: Lenacil 99% pure (Batch No. 066406003)
- Technical grade active ingredient TGAI: Lenacil 98.6% pure (Batch No. 141712003).
- Technical grade active ingredient: Leancil 98.9% pure (Batch No. B0634-88).

For Fate, please refer to Table 2.8.2.1-1.

2.11.2 Proposed harmonized classification and labelling

2.11.2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 2.10.2.1: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	613-320-00-6	3-cyclohexyl-1,5,6,7-tetrahydrocyclopentapyrimidine-2,4(*3H*)-dione	218-499-0	2164-08-1	Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H400 H410	GHS08 GHS09 Wng	H351 H410		M=10 M=10	
Dossier submitters proposal	613-320-006	lenacil (ISO); 3-cyclohexyl-6,7-dihydro1H-cyclopenta[d]pyrimidi ne-2,4(3H,5H)-dione	218-499-0	2164-08-1	Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H400 H410	GHS08 GHS09 Wng	H351 H410	Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	Modify the M-factor for aquatic acute to 100	
Resulting Annex VI entry if agreed by RAC and COM	613-320-006	lenacil (ISO); 3-cyclohexyl-6,7-dihydro1H-cyclopenta[d]pyrimidi ne-2,4(3H,5H)-dione	218-499-0	2164-08-1	Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H400 H410	GHS08 GHS09 Wng	H351 H410		M=100 M=10	

2.11.2.2 Additional hazard statements / labelling

None

Table 2.10.2.2: Reason for not proposing harmonised classification and status under CLH public consultation

Hazard class	Reason for no classification	Within the scope of CLH public consultation
Explosives	data conclusive but not sufficient for classification;	Yes/No
Flammable gases (including chemically unstable gases)	Hazard class not applicable	Yes/No
Oxidising gases	Hazard class not applicable	Yes/No
Gases under pressure	Hazard class not applicable	Yes/No
Flammable liquids	Hazard class not applicable	Yes/No
Flammable solids	data conclusive but not sufficient for classification;	Yes/No
Self-reactive substances	Data lacking	Yes/No
Pyrophoric liquids	Hazard class not applicable	Yes/No
Pyrophoric solids	Data lacking	Yes/No
Self-heating substances	data conclusive but not sufficient for classification	Yes/No
Substances which in contact with water emit flammable gases	Data lacking	Yes/No
Oxidising liquids	Hazard class not applicable	Yes/No
Oxidising solids	data conclusive but not sufficient for classification;	Yes/No
Organic peroxides	Data lacking	Yes/No
Corrosive to metals	Data lacking	Yes/No
Acute toxicity via oral route		Yes/No
Acute toxicity via dermal route		Yes/No
Acute toxicity via inhalation route		Yes/No
Skin corrosion/irritation		Yes/No
Serious eye damage/eye irritation		Yes/No
Respiratory sensitisation		Yes/No
Skin sensitisation		Yes/No
Germ cell mutagenicity		Yes/No
Carcinogenicity		Yes/No
Reproductive toxicity		Yes/No
Specific target organ toxicity-single exposure		Yes/No
Specific target organ toxicity-repeated exposure		Yes/No
Aspiration hazard		Yes/No
Hazardous to the aquatic environment	Harmonised classification proposed	**Yes**

Hazard class	Reason for no classification	Within the scope of CLH public consultation
Hazardous to the ozone layer		Yes/No

2.11.3 History of the previous classification and labelling

According to the harmonised classification and labelling (ATP07) approved by the European Union, this substance is very toxic to aquatic life, is very toxic to aquatic life with long lasting effects and is suspected of causing cancer. The current C&L may be maintained, except for an adaptation in the section ecotoxicology, where a revision of the M-factor is proposed.

2.11.4 Identified uses

Plant protection product (herbicide) under Reg (EC) no 1107/2009

2.11.5 Data sources

Plant protection product (herbicide) evaluated under AIR-4, under Reg (EC) no 1107/2009

2.12 RELEVANCE OF METABOLITES IN GROUNDWATER

Two major soil metabolites were detected in the aerobic laboratory soil degradation studies:

- IN-KF313 with a maximum occurrence of 21.7% AR and
- IN-KE121 with a maximum occurrence of 13.9% AR.

Further metabolite fractions were only detected in minor amounts (< 5% AR) and are, therefore, considered not relevant. Thus, the relevance for groundwater must only be assessed for IN-KF313 and IN-KE121.

According to SANCO/221/20001, degradation products must be characterised and identified and their relevance must be assessed, if one of the following conditions applies:
a) Metabolites, which account for more than 10% of the amount of active substance added in soil at any time during the studies; or
b) which account for more than 5% of the amount of active substance added in soil in at least two sequential measurements during the studies; or
c) for which at the end of soil degradation studies the maximum of formation is not yet reached.
All metabolites found in lysimeter studies at annual average concentrations exceeding 0.1 µg/L in the leachate should be identified and subject to further assessment.

2.12.1 STEP 1: Exclusion of degradation products of no concern

The metabolites (IN-KE121, IN-KF313, metabolite fractions M1, M2 and M3 isolated in the leachates of the lysimeter study (these fractions are postulated to consists of the six polar metabolites IPM1 and PM1-PM5)) mentioned above do not meet the criteria for metabolites of no concern outlined in Part 4, Step 1 of the SANCO/221/20001:
a) it is CO_2 or an inorganic compound, not containing a heavy metal; or,
b) it is an organic compound of aliphatic structure, with a chain length of 4 or less, which consists only of C, H, N or O atoms and which has no "alerting structures" such as epoxide, nitrosamine, nitrile or other functional groups of known toxicological concern;
c) it is a substance, which is known to be of no toxicological or ecotoxicological concern, and which is naturally occurring at much higher concentrations in the respective compartment

Therefore, it can be concluded that further assessment at Step 2 is required.

2.12.2 STEP 2: Quantification of potential groundwater contamination

The leaching behaviour of lenacil and its major soil metabolites (IN-KE121 and IN-KF313) was assessed by means of simulation runs with the FOCUS leaching models FOCUS PEARL 4.4.4, FOCUS PELMO 5.5.3, and FOCUS MACRO 5.5.4. The predicted environmental concentrations in groundwater were assessed in accordance with the FOCUS groundwater guidance (20002; 20113; 2014a4; European Commission, 20145) and FOCUS Kinetics (FOCUS, 20066; 2014b7). Interception values were taken from FOCUS (FOCUS, 2014a4) based on the recommended growth stage for the application.
Single and multiple applications as well as different application rates to sugar beets were simulated in consideration of the recommended use patterns and in order to cover the worst case application (Table 2.11.2-1).

Table 2.11.2-1: Harmonised assumptions relevant to groundwater exposure assessments for the use of lenacil on sugar beets

FOCUS Crop	Appl. timing	Appl. rate (g a.s./ha)	No. of appl. (-)	Appl. interval (days)	Inter-ception (%)	Resulting soil load (g a.s./ha)	Appl. method
Sugar beets	Emerg. + 7 d	500	1	-	20	400	Ground Spray
Sugar beets	Emerg. + 7 d	125	4	7	20	100	Ground Spray

The sorption behaviour of the metabolites IN-KF313 and IN-KE12 proved to be pH dependent. Therefore calculations under acidic and alkaline soil conditions were carried out.
The simulations conducted with the current versions of FOCUS PEARL, FOCUS PELMO, and FOCUS MACRO indicate that the use of lenacil is not likely to pose an unacceptable risk to groundwater if the active substance is used in compliance with label recommendations. The 80thh percentile predicted environmental concentrations in groundwater (PECgw) of the active substance lenacil and its metabolites IN-KF313 and IN-KE121 were calculated to be < 0.1 µg/L for all scenario / model combinations.

Furthermore the results of a lysimeter study confirmed that lenacil, IN-KF313 as well as IN-KE121 had leachate concentrations below the trigger of 0.1 µg/L.

However, polar metabolites M1, M2 and M3 were observed at levels above 0.1 µg/L in leachates during the first (M1, M2, M3) and second year (M1, M3) of the lysimeter study. It is highly unlikely based on chromatographic behaviour that M1, M2 and M3 represent individual components. Evidence from soil degradation studies has shown that polar material contains metabolites that result from a more extensive breakup of the lenacil molecule. This involves loss of the cyclohexyl ring and/or opening of the central lenacil pyrimidine ring structure. The resulting polar fragments are chemically unstable which leads to further degradation in soil resulting in numerous low molecular weight metabolites. The evidence for this further degradation is the very high level of CO_2 that is formed in soil degradation studies (47.6-77% after 120 days). A proportion of the polar fragments formed are low molecular weight carboxylic acids and these substances have the potential to be incorporated into naturally occurring amino acids. Similarly, low molecular weight amino acids may be formed directly as lenacil degradation products. Molecules of this type will be polar in nature and represent the most likely nature of the residue found in lysimeter leachate.
In a position paper ("Identification of polar metabolites formed from the degradation of lenacil in soil and their presence in soil lysimeter leachate", Goodyear, 2012), the key characteristics of the potential polar metabolites were brought forward and it was postulated that the fractions M1, M2 and M3 consists of an identified polar metabolite (IPM1) and further postulated metabolites PM1-PM5.
To support the conclusion that the polar residue fractions consist of several individual metabolites a new microlysimeter study was conducted (Hein, 2016). The results of the microlysimeter study indicated that the fractions M1, M2 and M3 consist of a high number of polar individual compounds. Up to 33 sub-fractions are separated, suggesting a non-relevance of the polar fractions in leachates for exposure assessment. Furthermore one fraction could possibly be related to IPM1. Therefore, the polar metabolites were not considered any further in PECgw assessment.

2.12.3 STEP 3: Hazard assessment – identification of relevant metabolites

2.12.3.1 STEP 3, Stage 1: screening for biological activity

Based on current data, there are no indications of comparable biological activity when metabolites are compared to the parent compound.

2.12.3.2 STEP 3, Stage 2: screening for genotoxicity

According to SANCO/221/20001, genotoxicity of the metabolites has to be investigated by in-vitro genotoxicity studies.

In a previously submitted position paper (Kurubaran, S., 2016), the genotoxicity potential of the identified polar metabolite IPM1 and the proposed polar metabolites PM1- PM5 was evaluated. The OECD Toolbox v3.1 was used to profile the 6 polar metabolites on the basis of a number of different profiling schemes to characterize DNA binding alerts (OECD and OASIS) and potential *in vitro* mutagenicity (ISS alerts) (Benigni and Bossa, 2008). The DNA Binding alerts for OASIS and OECD characterize potential for genotoxicity based on organic chemistry reaction principles. The endpoint schemes for these same alerts together with the ISS alerts are substantiated by experimental toxicity data, typically Ames data. Predictions were also made using the expert system TOPKAT and TIMES.
In short, no genotoxic concern was predicted in one or more (Q)SAR analyses for the compounds IPM1, PM1, PM2, PM3, PM4, while the outcome of the PM5 was uncertain. The relevance of the genotoxicity prediction of PM5 was disputable since it was based on aniline/phenylene diamine structures, which are not found in PM5. On balance, taking into account that some structures of the polar metabolites share alerts found in lenacil itself, which tested negative in the battery of genotoxicity studies, it is concluded that the polar metabolites are devoid of genotoxicity potential.

2.12.3.3 STEP 3, Stage 3: screening for toxicity

According to SANCO/221/20001, for the metabolites of active substances which are classified as category 1, 2 or 3 carcinogens according to Directive 67/548/EEC8, it has to be checked if their toxicological properties lead to a classification equal or even more severe than that of the parent compound.

The active substance lenacil is classified as a Category 2 carcinogen under CLP. The carcinogenicity classification of lenacil is based on an increased incidence of malignant mammary adenocarcinomas in a rat carcinogenicity study which were considered to be relevant for humans in the EFSA conclusion on the peer review of lenacil (EFSA, 2009) and more recently by the ECHA Risk Assessment Committee (RAC). As the mode of action (MOA) for the induction of mammary tumours in female rats is unknown, and as the absence of a genotoxic potential of lenacil does not suggest that mammary gland tumours are caused by a genotoxic mode of action, an epigenetic, endocrine-mediated MOA could be the cause for the induction of these tumours.
In order to prove or disprove an endocrine-mediated MOA of carcinogenicity, the potential endocrine disrupting (ED) properties of lenacil was investigated in a first step using the OECD toolbox, considering the profilers estrogen receptor (ER) binding and rtER Expert System ver. 1 – USEPA (Kurubaran, S., 2016). Similarly, for the assessment of the potential toxicity of the groundwater metabolites, a weight-of-evidence approach has been adopted, based on QSAR screening and structural alerts for endocrine disruption and carcinogenicity in the OECD toolbox. Carcinogenicity (genotoxic and non-genotoxic) alerts by ISS and oncologic primary classification profilers in the OECD Toolbox have been used to screen for carcinogenicity structural alerts (Kurubaran, S., 2016).
The QSAR system used did not reveal structural alerts regarding ED properties of both lenacil and its groundwater metabolites. With respect to the carcinogenicity potential of the groundwater metabolites, all polar metabolites (PM1-PM5) except IPM1 were found to have no structural alerts for either genotoxic or nongenotoxic carcinogenicity. Due to the presence of an alpha, beta-unsaturated carbonyl function, IPM1 was shown to reveal a structural alert for genotoxic carcinogenicity. However, the genotoxic potential of the identified polar metabolite IPM1 has already been addressed in depth in another previously submitted position paper and it was concluded to have low potential for genotoxicity (Kurubaran, S., 2016). Based on the QSAR results, considering the high hydrophilicity of IPM1 and the negative genotoxicity of parent lenacil, there is presently no conclusive evidence to suggest that IPM1 is a genotoxic carcinogen when applying a weight-of-evidence approach. However, since IPM1 is extremely unstable, and cannot be synthetized, it is considered that a further hazard assessment is neither possible not appropriate.

Overall, when applying a weight-of-evidence approach taking into account the QSAR results obtained for polar metabolites PM1-PM5 and based on polarity/lipophilicity considerations, it can be concluded that based on their structural similarity to lenacil, the groundwater metabolites are not expected to be of higher toxicity as compared to the parent active substance.
Therefore, all polar metabolites (PM1-PM5) as well as IPM1 are not considered as relevant metabolites regarding toxicity.

2.12.4 STEP 4: Exposure assessment – threshold of concern approach

According to SANCO/221/20001, following the concept of a "threshold approach ", metabolites which have passed Step 3 and do not exceed estimated or actual concentrations in groundwater of 0.75 µg/L (and which passed all stages of Step 3) are considered as non-relevant.

For substances of unknown structure with no alerts for carcinogenicity/mutagenicity the Scientific Committee on Plants (Munro, I., 1999) proposed a toxicological threshold of concern (TTC) of 1.5 µg/person/day or 0.02 µg/kg bw/day (75 kg/person) and 0.025 µg/kg bw/day (60 kg/person) which is in line with the threshold developed by the US-FDA. Assuming a consumption of 2 liters of water/day for an adult, all of which comes from the upper soil layer, such an acceptable exposure level relates to an acceptable estimated upper limit of 0.75 µg/L for a metabolite. The maximum estimated total metabolite concentration in groundwater of 0.519 µg/L which is based on the measured concentration in the lysimeter leachate for fraction M2 is well below 0.75 µg/L and, thus, below the toxicological threshold of concern of 1.5 µg/person/day.

Furthermore, the theoretical ingestion by a person drinking 2 liters of water per day would be equivalent to 0.512, 1.038 and 0.546 µg for metabolite fractions M1, M2 and M3 respectively, corresponding to 0.0085, 0.0173 and 0.0091 µg/kg bw/d for M1, M2 and M3 (assuming a default weight of 60 kg/person). The calculated exposure values are below the toxicological threshold of concern of 0.02 -0.025 µg/kg bw/day or 1.5 µg/person/day as set by the Scientific Committee on Plants and are in line with the threshold developed by the US-FDA for substances of unknown structures.

Taking into account the conservative threshold of toxicological concern approach, it can be concluded that the estimated groundwater concentration of polar fractions M1, M2 and M3, respectively, present no unacceptable health risk to consumers via drinking water consumption. It is further noted that this approach is additionally conservative in that in the absence of knowledge on the composition of the metabolite fractions, the groundwater concentrations of the whole fractions were taken into account for this assessment as a worst-case.

2.12.5 STEP 5: Refined risk assessment

Based on the results of the QSAR screens for genotoxicity and carcinogenicity and when applying a weight-of-evidence approach, it can be concluded that based on their structural similarity with lenacil, the polar metabolites or metabolite fractions are not expected to reveal a more severe toxicity profile and to be toxicologically more relevant than the parent active substance which is classified as a category 2 carcinogen under the CLP (Carc. 2; H351). Since all metabolites are closely related to lenacil from the structural point of view, did not reveal an alert for endocrine disrupting properties in the QSAR screen for estrogen receptor binding and considering the logPow values of these metabolites which are all comparable to or even lower than the logPow of lenacil, a comparable toxicity profile to lenacil can be assumed. As a result, it is justified to perform a strictly human health-based risk assessment and to consider for this purpose the reference values as derived for lenacil. For the purpose of a strictly human health-based exposure and risk assessment, the maximum annual average concentrations of polar fractions M1, M2 and M3 have been compared with the ADI of 0.12 mg/kg bw/day as derived for lenacil in the context of the plant protection dossier. The theoretical ingestion by a person drinking 2 liters of water per day would be 0.512, 1.038 and 0.546 µg for M1, M2 and M3 respectively, corresponding to 0.0085, 0.0173 and 0.0091 µg/kg bw/d for M1, M2 and M3 (assuming a default weight of 60 kg/person). Compared to the ADI of 0.12 mg/kg bw/d, the theoretical ingestion of M1, M2 and M3 via the groundwater would represent 0.007, 0.014 and 0.008 % of the ADI.
Based on these results it is not anticipated that either of the polar fractions M1, M2 and M3 or a combination of these will represent an unacceptable health risk for consumers via drinking water consumption.

2.12.6 Overall conclusion

Major soil metabolites IN-KE121 and IN-KF313 are not expected to exceed concentrations of 0.1 µg/L in groundwater at 1 m and are therefore considered non-relevant metabolites.

Based on the available data and considerations, it cannot be excluded that the polar metabolites (in M1, M2 and M3) can exceed concentrations of 0.1 µg/L in groundwater at 1 m. Based on the results of the QSAR screens for genotoxicity and carcinogenicity and when applying a weight-of-evidence approach, it can be concluded that based on their structural similarity with lenacil, the polar metabolites or metabolite fractions are not expected to reveal a more severe toxicity profile and to be toxicologically more relevant than the parent active substance which is classified as a category 2 carcinogen under the CLP (Carc. 2; H351)., Consequently they are not considered to present an unacceptable health risk for consumers via drinking water consumption.

2.13 CONSIDERATION OF ISOMERIC COMPOSITION IN THE RISK ASSESSMENT

2.13.1 Identity and physical chemical properties

No consideration of isomeric composition is necessary: Lenacil does not display diastereo- or enantioisomerism and thus exists as a unique stereoisomer. All physical-chemical studies were performed on purified or technical material containing > 97.5% w/w of lenacil.

2.13.2 Methods of analysis

Lenacil has no stereoisomers and thus no consideration of isomeric composition is necessary.

2.13.3 Mammalian toxicity

Lenacil has no stereoisomers and thus no consideration of isomeric composition is necessary.

2.13.4 Operator, Worker, Bystander and Resident exposure

Lenacil has no stereoisomers and thus no consideration of isomeric composition is necessary.

2.13.5 Residues and Consumer risk assessment

Lenacil has no stereoisomers and thus no consideration of isomeric composition is necessary.

2.13.6 Environmental fate

Lenacil has no stereoisomers and thus no consideration of isomeric composition is necessary.

2.13.7 Ecotoxicology

Lenacil has no stereoisomers and thus no consideration of isomeric composition is necessary.

2.14 RESIDUE DEFINITIONS

2.14.1 Definition of residues for exposure/risk assessment

Food of plant origin: lenacil [post-emergence use on root crops & pre-emergence use on leafy crops]

Food of animal origin: inconclusive

Soil: lenacil, IN-KF313 and IN-KE121

Groundwater: lenacil, IN-KF313 and IN-KE121

Surface water: lenacil, IN-KF313 and IN-KE121

Sediment: lenacil, IN-KF313 and IN-KE121

Air: lenacil

2.14.2 Definition of residues for monitoring

Food of plant origin: lenacil

Food of animal origin: inconclusive

Soil: lenacil

Groundwater: lenacil

Surface water: lenacil

Sediment: lenacil

Air: lenacil

Level 3

LENACIL

3 PROPOSED DECISION WITH RESPECT TO THE APPLICATION

3.1 BACKGROUND TO THE PROPOSED DECISION

3.1.1 Proposal on acceptability against the decision making criteria – Article 4 and annex II of regulation (EC) No 1107/2009

3.1.1.1 Article 4				
		Yes	No	
i)	It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with. Specifically the RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.	X		RMS considers that Lenacil can be renewed and that authorisations of PPP may be considered in at least one member State.
3.1.1.2 Submission of further information				
		Yes	No	
i)	It is considered that a complete dossier has been submitted	X		RMS considers that a complete dossier of lenacil was submitted. However, please refer to section 3.1.4 where additional information is needed
ii)	It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because: (a) the data requirements have been amended or refined after the submission of the dossier; or (b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.			RMS considers that a complete dossier of lenacil was submitted. However, please refer to section 3.1.4 where additional information is needed
3.1.1.3 Restrictions on approval				
		Yes	No	
	It is considered that in line with Article 6 of Regulation (EC) No 1107/2009 approval should be subject to conditions and restrictions.		X	*Not applicable*
3.1.1.4 Criteria for the approval of an active substance				
Dossier				
		Yes	No	
	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).	X		Please refer to Level 2.6

	It is considered that the dossier contains the information necessary to carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on feed or food crops or leads indirectly to residues in food or feed). In particular it is considered that the dossier: (a) permits any residue of concern to be defined; (b) reliably predicts the residues in food and feed, including succeeding crops (c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing; (d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals; (e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.	X	X	Please refer to Level 2.7 On the one hand, for fodder and sugar beet (representative uses), a residue definition can be derived, an MRL can be proposed (for sugar beet root) and a consumer dietary risk assessment could be performed. On the other hand, due to significant residue levels of lenacil in some rotational crops that may be used as (major) livestock feed items, the livestock dietary burden trigger is exceeded. However, the available data package does not cover a significant intake by livestock animals, *i.e.* no metabolism studies are available and, hence, a residue definition and MRLs for animal products cannot be proposed. As a consequence, mitigation measures (restriction with regard to rotational/succeeding crops) appear to be necessary. See 2.7.2, 2.7.3, 2.7.5.2 and 2.7.7.3.
	It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.	X		Please refer to Level 2.8 and Level 2.9
Efficacy				
		Yes	No	
	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.	X		The efficacy was not assessed for the renewal process of lenacil. Plant protection products based on lenacil are currently authorised for representative uses in some MS. Lenacil based products will be re-assessed in case of the renewal of lenacil.
Relevance of metabolites				
		Yes	No	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.			Toxicological data and rationales, including QSAR's, are in the opinion of both RMS and co-RMS sufficient to conclude on the toxicological profile of metabolites.
Composition				
		Yes	No	
	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.	X		A new 5 batch analysis based on large scale production has been submitted for the purpose of renewal. The proposed minimum purity for renewal is 975 g/kg. For details and discussion on the specification for the current production and the reference specification to be considered for renewal, please refer to Vol. 4.

				Impurities potentially relevant have been identified. Further data are necessary (please refer to Vol. 4).
	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.			Not relevant. FAO specifications do not exist for lenacil.
	It is considered for reasons of protection of human or animal health or the environment, stricter specifications than that provided for by the FAO specification should be adopted			Not relevant
Methods of analysis				
		Yes	No	
	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which are present in quantities greater than 1 g/kg in the active substance, safener or synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.	X		The lenacil content in technical material is determined by the validated method B0634.220.03.ES using reversed-phase HPLC-UV or UPLC-UV. Validated methods for determining impurities are also available (see Vol. 4). A validated method using reversed-phase HPLC-UV is also available to determine lenacil content in the representative formulation. **Potential data gap for validated analytical methods to determine the relevant impurities in the formulation (see level 2.5.1.1)**
	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.	X	X	For details, please refer to level 2.5.2. The following methods are available and considered acceptable for monitoring in the different matrices: - DFG S19 (LC-MS/MS) in plant matrices, LOQ = 0.01 mg/kg for lencail (high water, acidic, oily and dry [high starch] matrices), independently validated. - LC-MS/MS based on QuEChERS in animal matrices, LOQ = 0.01 mg/kg for lenacil (milk, meat, fat, liver and egg), independently validated. - **Open for a fully validated method of monitoring in soil.** - LC-MS/MS in drinking and surface water, LOQ = 0.1 µg/L for lenacil, independently validated. - LC-MS/MS in air, LOQ = 0.1 mg/m^3 for lenacil.

				- **Open for a monitoring method in body fluids.**
	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		
Impact on human health				
Impact on human health - ADI, AOEL, ARfD				
		Yes	No	
	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be established with an appropriate safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.	X		The **ADI** of 0.12 mg/kg bw/d is proposed based on the application of a standard safety factor of 100 to the NOAEL of 12 mg/kg bw/d, identified in the rat 2-yr chronic toxicity and carcinogenicity study. A developmental NOAEL was established (lowest most relevant developmental NOAEL = 300 mg/kg b.w./d in the main rat developmental study, based upon skeletal ossification delays at 1000 mg/kg b.w./d). The **ARfD** of lenacil could be derived at 300 mg/kg bw/d ÷100 = 3 mg/kg b.w./d. The **AAOEL** could likewise be established at the same level. The **AOEL** of 0.4 mg/kg bw/d is proposed based on the application of the standard safety factor of 100 to the NOAEL of 40 mg/kg bw/d identified in 90 days dog study. No correction factor for oral absorption is considered necessary.
Impact on human health – proposed genotoxicity classification				
		Yes	No	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as mutagen category 1A or 1B.**		X	Based on the results of in vitro and in vivo genotoxicity studies, lenacil is not considered to be genotoxic (see level 2.6.4).
Impact on human health – proposed carcinogenicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for**		X	Lenacil was classified Carc. Cat. 2 but not Carc. Cat. 1, on the basis of mammary adenocarcinoma in the rat.

	classification, in accordance with the provisions of Regulation (EC) No 1272/2008, **as carcinogen category 1A or 1B.**			
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			Not relevant
Impact on human health – proposed reproductive toxicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 1A or 1B.**		X	Lenacil caused no reprotoxicity findings qualifying for C&L under CLP.
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			Not relevant
Impact on human health – proposed endocrine disrupting properties classification				
		Yes	No	
i)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **carcinogenic category 2 and toxic for reproduction category 2 and on that basis shall be considered to have endocrine disrupting properties**		X	Harmonised classification Carc. Cat. 2 exists but no reprotoxicity C&L is necessary, thus interim criteria are not met. The ED potential was evaluated taking into account the EFSA guidance, and based upon this evaluation, both RMS and co-RMS are of the opinion that ED criteria are not met either. Potential endocrine-related adverse findings were observed in different studies (increased incidence of malignant mammary adenocarcinomas in the rat carcinogenicity study, uterus changes in different studies, thyroid effects in

				sub-chronic and chronic feeding studies with rats). The overall Weight of Evidence, based on all available studies, would suggest that the effect of lenacil on uterus was very limited. In addtion, lower-tier studies were performed upond request by the RMS, which do not indicate interference with neither oestrogenic nor androgenic pathways. Therefore, it was concluded that the observed uterus and mammary findings are unlikely caused by an endocrine-dependent pathway. Since unfortunately no lower-tier *in-vitro* studies were conducted on the thyroid-pathway, observed thyroid findings *in-vivo* remain unexplained.
ii)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **toxic for reproduction category 2 and** in addition the RMS considers the substance **has toxic effects on the endocrine organs and on that basis shall be considered to have endocrine disrupting properties**		X	No reprotoxicity C&L is necessary, and toxic effects on the endocrine organs (especially thyroid) are insufficient to conclude that thus interim criteria are met. The ED potential was evaluated taking into account the EFSA guidance, and based upon this evaluation, both RMS and co-RMS are of the opinion that ED criteria are not met either. Potential endocrine-related adverse findings were observed in different studies (increased incidence of malignant mammary adenocarcinomas in the rat carcinogenicity study, uterus changes in different studies, thyroid effects in sub-chronic and chronic feeding studies with rats). The overall Weight of Evidence, based on all available studies, would suggest that the effect of lenacil on uterus was very limited. In addtion, lower-tier studies were performed upond request by the RMS, which do not indicate interference with neither oestrogenic nor androgenic pathways. Therefore, it was concluded that the observed uterus and mammary findings are unlikely caused by an endocrine-dependent pathway. Since unfortunately no lower-tier *in-vitro* studies were conducted on the thyroid-pathway, observed thyroid findings *in-vivo* remain unexplained.
iii)	Linked to either i) or ii) immediately above. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			Not relevant
Fate and behaviour in the environment				

Persistent organic pollutant (POP)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1.		X	Water = no DT_{50} water = 32.3 days (worst case) < 2 months. Soil = no Field soil degradation studies carried out in EU show that DT_{50} values of lenacil are < 6 months (worst-case DT_{50} = 88 days) Sediment = no Water/sediment studies show that DT_{50} values of lenacil are < 6 months (DT_{50} whole system = 122.9 days (worst-case), DT_{50} water = no data). ➔ Based on available data, lenacil is not considered as Persistent regarding the POP criteria Log Pow for lenacil is 1.70 (below trigger value of 3), no study was requested to determine the BCF. The bioconcentration factor (BCF) in aquatic organisms for the metabolite IN-KF313 (log Pow 3.111) is 52.4 (considered as worst-case compared to lenacil), which is < 5000 ➔ lenacil is not considered as potentially bioaccumulative regarding the POP criteria Air DT_{50} < 2 days Lenacil is not considered as potential for long-range environmental transport
Persistent, bioaccumulative and toxic substance (PBT)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2.		X	Water = no DT_{50} water = 32.3 days (worst case) < 2 months. Sediment = no Water/sediment study shows that DT_{50} values of lenacil are < 6 months (DT_{50} whole system = 122.9 days (worst-case), DT_{50} water = no data). Soil = no ➔ Field soil degradation studies carried out in EU show that DT_{50} values of lenacil are < 120 days (worst-case DT_{50} = 88 days) ➔ Based on available data, lenacil is not considered as Persistent regarding the PBT criteria Log Pow for lenacil is 1.70 (below trigger value of 3), no study was requested to determine the BCF. The bioconcentration factor (BCF) in aquatic organisms for the metabolite IN-KF313 (log Pow 3.111) is 52.4 (considered as worst-case compared to lenacil), which is < 5000

				➔ lenacil is not considered as potentially bioaccumulative regarding the POP criteria The lowest NOEC for the most sensitive aquatic species is < 0.01 mg/L (NOEC = 0.00222 mg a.s./L, based on a microcosm study). Lenacil is not considered toxic regarding the PBT criteria
Very persistent and very bioaccumulative substance (vPvB).				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a a very persistent and very bioaccumulative substance (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3.		X	Water = no DT_{50} water = 32.3 days (worst case) < 2 months. Sediment = no Water/sediment studies show that DT_{50} values of lenacil are < 180 days (DT_{50} whole system = 122.9 days (worst-case), DT_{50} water = no data). Soil = no Field soil degradation studies carried out in EU show that DT_{50} values of lenacil are < 180 days (worst-case DT_{50} = 88 days). ➔ Based on available data, lenacil is not considered as Persistent regarding the vPvB criteria Log Pow for lenacil is 1.70 (below trigger value of 3), no study was requested to determine the BCF. The bioconcentration factor (BCF) in aquatic organisms for the metabolite IN-KF313 (log Pow 3.111) is 52.4 (considered as worst-case compared to lenacil), which is < 5000 ➔ lenacil is not considered as potentially bioaccumulative regarding the POP criteria
Ecotoxicology				
		Yes	No	
	It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The RMS is content that the assessment takes into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.	X		Due to the lack of an acceptable endpoint, no risk assessment for biological method for sewage treatment could be performed. The acute and long-term risk to birds and mammals was acceptable at the screening step for all proposed uses. For the active substance lenacil, the acute and chronic risk to fish and acute risk to aquatic invertebrates is acceptable based on the Tier 1 RAC value and FOCUS Step 3 PEC values for all proposed uses. The chronic risk to aquatic invertebrates was acceptable based on the Tier 1 RAC and FOCUS Step 4 PEC values, if appropriate risk mitigation measured are taken into account. Based on a higher tier ETO-RAC, the risk to algae and aquatic plants was acceptable for all relevant scenarios at FOCUS Step 3 or Step 4, with the exception of the R1 stream and R3 stream scenarios. For the R1 stream and R3 stream scenarios, a 10 m vegetated filter strip or 5 m drift buffer in combination with

				10 m vegetated filter strip was not sufficient to obtain an acceptable risk after application of 1 x 500 g a.s./ha in sugar and fodder beet. Similarly, a 20 m vegetated filter strip was not sufficient for an acceptable risk after application of 4 x 125 g a.s./ha in sugar and fodder beet. Further risk mitigation measures should be considered at Member State level for these scenarios. The risk to aquatic organisms from exposure to the metabolites IN-KE 121 and IN-KF 313 was considered acceptable at FOCUS Step 1 or Step 2, based on the Tier 1 RAC. For all proposed uses, the risk to bees and other non-target arthropods was acceptable at Tier 1. A low risk to soil organisms and non-target plants was demonstrated for all representative uses of the product Lenacil 500 g/L SC. See sections B.9.1 to B.9.14 of Volume 3 (PPP) for further details.
	It is considered that, on the basis of the assessment of Community or internationally agreed test guidelines, the substance **HAS** endocrine disrupting properties that may cause adverse effects on non-target organisms.		X	For mammals as non-target organisms, the same conclusion as for the human health assessment can be drawn. Adverse endocrine findings possibly involving EAS-pathways were identified and further investigated. Both *in-vitro* and *in-vivo* mechanistic studies were performed in order to investigate an EATS-mediated MoA, and the latter could on the basis of these studies be excluded. Thyroid adverse effects were also identified, and existing and new *in-vivo* mechanistic studies indicated that lenacil is not a primary thyrotoxicant. It was demonstrated that lenacil induced CYP4501B metabolic enzymes, as well as UDPGT, possibly explaining a number of adverse findings. Overall, the data showed that the adverse findings were mainly associated to systemic toxicity at doses nearby or >MTD, exceeding the limit dose of 1000 mg/kg b.w./d. It is therefore plausible that no primary endocrine MoA for the EATS-modalities would be at the basis of all observations. For the thyroid findings, no other explanation than top-dose toxicity and UDPGT induction could be formulated. Therefore, RMS would be inclined to consider lenacil not meeting the criteria for endocrine disruption. While potentially adverse EATS findings have been detected, subsequent mechanistic studies and a WoE consideration leads to the conclusion that the ED criteria are not met. For other non-target vertebrates (i.e. birds and aquatic vertebrates), adversity based on EAS-mediated parameters is not considered sufficiently investigated, the available studies do not specifically address parameters directly related to an endocrine MoA. However, based on *in vitro* mechanistic data, it is considered that there is no indication that there is interference of lenacil with the E or A modality. Overall, there is no evidence for EAS-mediated activity of lenacil. Consequently, for the EAS modalities, the ED criteria for non-target organisms are not considered to be met for lenacil. For the T-modality, no data

				investigating T-mediated adversity is available. Further, the available evidence is not considered sufficient to conclude on the T-mediated endocrine activity in non-target organisms. Therfore, for the T-modality, additional data should be generated.
	Linked to the consideration of the endocrine properties immediately above. It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.		X	Non-target organisms are potentially exposed to the active substance lenacil when used according to the proposed use of Lenacil 500 g/L SC in sugar and fodder beet.
	It is considered that it is established following an appropriate risk assessment on the basis of Community or internationally agreed test guidelines, that the use under the proposed conditions of use of plant protection products containing this active substance, safener or synergist: — will result in a negligible exposure of honeybees, or — has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.	X		The risk assessment for honeybees indicated an acceptable risk based on Tier 1 laboratory studies. The first tier risk assessment was conducted according to SANCO/10329/2002, the guidance available at the time of the assessment. Therefore, no formal consideration of effects on colony survival and development has been conducted, as this is not part of the SANCO/10329/2002 risk assessment procedure. For the same reason, no formal consideration of effects on non-*Apis* bees has been conducted. Studies have been submitted and evaluated, investigating the chronic toxicity of lenacil (in the formulation Lenacil 500 g/L SC) to adult honeybees and honeybee larvae, in line with the data requirements. Further, acute and chronic toxicity studies with adult bumblebees have been submitted and evaluated. In order to take all available data into account, a first tier assessment following the revised EPPO guideline for bees (2010) was performed for the chronic risk to adult honeybees and honeybee larvae. This assessment indicated an acceptable chronic risk to honeybees. Further, a first tier assessment for the chronic risk to adult honeybees and honeybee larvae, and for the acute and chronic risk to adult bumblebees was performed according to the EFSA guidance document for bees (2013). As this guidance document is not yet noted by the Standing Committee on Plants, Animals, Food and Feed, this risk assessment was included for information only. This assessment indicated an acceptable chronic risk to adult honeybees, honeybee larvae, and bumblebees (please note that no assessment was performed for the chronic risk to bumblebee larvae and the risk to solitary bees, as no data on the toxicity is available).
Residue definition				
		Yes	No	

	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.	X	X	See 2.7.3 and 2.7.10. A residue definition for risk assessment and monitoring can be derived for the plant matrices relevant to the representative uses (parent compound lenacil only). However, a residue definition cannot be proposed for animal products, as no livestock metabolism studies were provided, although the livestock dietary burden trigger was exceeded. However, exceedance of the livestock dietary burden trigger may be avoided by implementation of appropriate mitigation measures (restriction with regard to rotational/succeeding crops).
Fate and behaviour concerning groundwater				
		Yes	No	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites, degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		Please refer to 2.8.6. PEC_{GW} were below 0.1 µg/L for lenacil and its metabolites IN-KF313 and IN-KE121 in all scenario / model combinations.

3.1.2 Proposal – Candidate for substitution

Candidate for substitution				
		Yes	No	
	It is considered that the active substance shall be approved as a candidate for substitution		X	Lenacil ADI, ARfD or (A)AOEL is <u>not</u> significantly lower than those of the majority of the approved active substances within groups of substances/use categories, — it does <u>not</u> meet two of the criteria to be considered as a PBT substance — there are <u>no</u> reasons for concern linked to the nature of the critical effects (such as developmental neurotoxic or immunotoxic effects) which, in combination with the use/exposure patterns, amount to situations of use that could still cause concern, for example, high potential of risk to groundwater; even with very restrictive risk management measures (such as extensive personal protective equipment or very large buffer zones), — it contains <u>no</u> significant proportion of non-active isomers,

				— it is not classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as carcinogen category 1A or 1B. — it is not classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as toxic for reproduction category 1A or 1B —it is not considered to have endocrine disrupting properties that may cause adverse effects in humans

3.1.3 Proposal – Low risk active substance

Low-risk active substances				
		Yes	No	
	It is considered that the active substance **shall be considered of low risk.** If the active substance is not a micro-organism, in particular it is considered that: (a) the substance **should NOT be classified or proposed for classification** in accordance to Regulation (EC) No 1272/2008 as any of the following: — carcinogenic category 1A, 1B or 2, — mutagenic category 1A, 1B or 2, — toxic to reproduction category 1A, 1B or 2, — skin sensitiser category 1, — serious damage to eye category 1, — respiratory sensitiser category 1, — acute toxicity category 1, 2 or 3, — specific Target Organ Toxicant, category 1 or 2, — toxic to aquatic life of acute and chronic category 1 on the basis of appropriate standard tests, — explosive, — skin corrosive, category 1A, 1B or 1C; (b) it has **not been identified as priority substance under Directive 2000/60/EC**; (c) it is **not deemed to be an endocrine disruptor** in accordance to Annex II of Regulation (EC) No 1107/2009; (d) it **has no neurotoxic or immunotoxic effects**; (e) it **is not persistent** (half-life in soil is more than 60 days) or its **bio-concentration factor is lower than 100**. (f) it is a **semiochemical** and verifies points (a) to (d).		X	Based on the cited hazard criteria, and on the need to use PPE during application with the representative products, lenacil should not be considered of low risk.

	Paragraph (e) doesn't apply to naturally occurring active substances. If the active substance is a micro-organism, in particular it is considered that at strain level the micro-organism has not demonstrated multiple resistance to anti-microbials used in human or veterinary medicine. If the active substance is a baculovirus, in particular it has not demonstrated adverse effects on non-target insects.			

3.1.4 List of studies to be generated, still ongoing or available but not peer reviewed

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going.	Study on-going and anticipated date of completion	Study available but not peer-reviewed
3.1.4.1 Identity of the active substance or formulation				
Further data are requested regarding two impurities (including o.a. (eco)toxicological relevance) (please refer to Vol. 4).	Relevant to all representative uses	X		
3.1.4.2 Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation				
None				
3.1.4.3 Data on uses and efficacy				
None				
3.1.4.4 Data on handling, storage, transport, packaging and labelling				
None				
3.1.4.5 Methods of analysis				
RMS is of the opinion that further validation data should be provided at a higher level (specification	Relevant to all representative uses	X		

level) for the analytical method to determine one impurity in lenacil (see Vol. 4 for details).				
Several tox. studies were performed in DMSO as vehicle for which a validated analytical method has not been submitted.	Relevant to all representative uses	X		
Extraction efficiency of the monitoring method in plant matrices: in the course of the assessment, the notifier referred to Study AMR-4193-96 (Radiovalidation of Trace Analytical Method for Lenacil Residues in Sugar Beet Root and Top – 1998) which was not part of the renewal dossier. The study should be submitted.	Relevant to all representative uses			X (study needs however to be submitted to RMS)
Open for a fully validated monitoring method in soil (lenacil).	Relevant to all representative uses		X (date of submission has not been provided)	
Open for a fully validated monitoring method in body fluids. Study Report No. 17S07170-01-VMBF – Gaag S. (2017) – Validation of an Analytical Method for the Determination of Residues of Lenacil in Body Fluids should be submitted.	Relevant to all representative uses			X (study needs however to be submitted to RMS)
3.1.4.6 Toxicology and metabolism				
Further data are requested regarding two impurities (including o.a. (eco)toxicological relevance) (please refer to Vol. 4). See 3.1.4.1.	Relevant to all representative uses	X		
Since unfortunately no lower-tier *in-vitro* studies were conducted on the thyroid-pathway, observed thyroid findings in-vivo remain unexplained	Relevant to all representative uses		X (date of submission has not been provided)	
Whereas no overt signs of immunotoxic action could be identified in any repeated toxicity assay, there is still some uncertainty, since the lymphoid blood line and related organs such as spleen and/or	Relevant to all representative uses	X		

thymus are consistently altered, albeit not always in a non-dose-dependent way. However, it seems quite obvious that high-dose administrations were consistently freight with leukopenia and some uncertainty remains as regards the interpretation of the observed findings throughout the repeated toxicity studies. Therefore, RMS requests the conduct of a guideline GLP-immunotoxicity study, guideline according to e.g. OPPTS 870.7800 Immunotoxicity (EPA) or equivalent.				
3.1.4.7 Residue data				
Livestock metabolism studies (ruminants, poultry) are required to investigate the potential transfer and metabolism of lenacil (and metabolites) from livestock feed items into animal commodities. However, exceedance of the livestock dietary burden trigger may be avoided by implementation of appropriate mitigation measures (restriction with regard to rotational/succeeding crops).	Relevant to all representative uses	X		
3.1.4.8 Environmental fate and behaviour				
Structural elucidation of lenacil metabolites in the leachates of the microlysimeter study (Gärtner, 2016)	Identification of unknown metabolites found in the microlysimeter study (Hein, 2016)		X (only interim report was submitted, provisional deadline for submission of final report: March 2019)	
3.1.4.9 Ecotoxicology				
The study on the effect of lenacil on activated sewage sludge evaluated in the original DAR is not in line with the most recent version of the test guideline and therefore no longer acceptable. Consequently, no acceptable endpoint for use in	All representative uses	X		

the ecotoxicological risk assessment is available, and no risk assessment for biological methods for sewage treatment could be performed.				

3.1.5 Issues that could not be finalised

An issue is listed as an issue that could not be finalised where there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) No 546/2011, and where the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

Area of the risk assessment that could not be finalised on the basis of the available data	Relevance in relation to representative use(s)
1. Consumer dietary risk assessment could not be finalized, since potential transfer of residues from livestock feed items (derived from rotational crops) into animal commodities was not addressed.	Relevant to the representative use on sugar beet and fodder beet without any mitigation measures in place to avoid residues in rotational crops. Exceedance of the livestock dietary burden trigger may be avoided by implementation of appropriate mitigation measures (restriction with regard to rotational/succeeding crops).
2. Toxicology: thyroid effects and uncertainty on the need for a supplemental DNT study	These measures relate to all representative uses
3. Toxicology: there is uncertainty on the immunotoxicity of lenacil	These measures relate to all representative uses
4. Ecotoxicology: The risk assessment for biological methods for sewage treatments could not be performed, due to the lack of an acceptable endpoint (the available study does not comply with the most recent version of the test guideline)	These measures relate to all representative uses

3.1.6 Critical areas of concern

An issue is listed as a critical area of concern:
(a) where the substance does not satisfy the criteria set out in points 3.6.3, 3.6.4, 3.6.5 or 3.8.2 of Annex II of Regulation (EC) No 1107/2009 and the applicant has not provided detailed evidence that the active substance is necessary to control a serious danger to plant health which cannot be contained by other available means including non-chemical methods, taking into account risk mitigation measures to ensure that exposure of humans and the environment is minimised, or
(b) where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) 546/2011, and where this assessment does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

Critical area of concern identified	Relevance in relation to representative use(s)
None	

3.1.7 Overview table of the concerns identified for each representative use considered

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in 3.3.1, has been evaluated as being effective, then 'risk identified' is not indicated in this table.)
All columns are grey as the material tested in the toxicological studies has not been demonstrated to be representative of the technical specification.

Representative use		Sugar and fodder beet
Operator risk	Risk identified	
	Assessment not finalised	
Worker risk	Risk identified	
	Assessment not finalised	
Bystander risk	Risk identified	
	Assessment not finalised	
Consumer risk	Risk identified	
	Assessment not finalised	X[1]
Risk to wild non target terrestrial vertebrates	Risk identified	
	Assessment not finalised	
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified	
	Assessment not finalised	
Risk to aquatic organisms	Risk identified	
	Assessment not finalised	
Groundwater exposure active substance	Legal parametric value breached	
	Assessment not finalised	
Groundwater exposure metabolites	Legal parametric value breached	
	Parametric value of 10µg/L[(a)] breached	
	Assessment not finalised	
Comments/Remarks		

The superscript numbers in this table relate to the numbered points indicated within chapter 3.1.5 and 3.1.6. Where there is no superscript number, see level 2 for more explanation.
(a): Value for non relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003

3.1.8 Area(s) where expert consultation is considered necessary

It is recommended to organise a consultation of experts on the following parts of the assessment report:

Area(s) where expert consultation is considered necessary	Justification
Residues	Because the necessary livestock metabolism studies are not available, the RMS elaborated detailed mitigation measures with regard to succeeding/rotational crops, in order to avoid significant livestock exposure to residues: see **2.7.5.2** and **2.7.7.3**. The co-RMS expressed its reservations to this approach and noted that the elaboration of such detailed mitigation measures is rather uncommon at EU level; they are defined at the national level. The co-RMS indicated to be more in favor of asking for a ruminant metabolism study, due to the fact that without mitigation measures there is an extreme exceedance of the livestock dietary burden trigger. However, RMS is of the opinion that the acceptability of possible mitigation measures at EU level could be considered; it may be helpful for risk managers.
Toxicology – ED assessment	Pending peer review and submission of requested studies Issue on potential need of DNT study due to the equivocal thyroid findings
Ecotoxicology – higher tier risk assessment for aquatic organisms (algae and macrophytes)	The higher tier data package for algae and macrophytes consists of a microcosm study and a number of laboratory studies with additional species. After consultation, the RMS and co-RMS agreed not to use the microcosm study, as is had a number of deficiencies and was therefore not considered sufficiently reliable for use in the risk assessment. However, if a proper MDD-analysis is provided by the applicant, and this would demonstrate that the study has a sufficient statistical power, this conclusion might be reconsidered. The additional laboratory studies were used for SSD calculations. Two of the studies with aquatic macrophytes had some shortcomings, and will be repeated by the applicant. For the time being, the endpoints from the available studies were used. Given that additional information relevant for the higher tier risk assessment for algae and aquatic plants might become available following peer review, RMS is of the opinion that a discussion through expert consultation would be helpful.

3.1.9 Critical issues on which the Co RMS did not agree with the assessment by the RMS

Points on which the co-rapporteur Member State did not agree with the assessment by the rapporteur member state. Only the points relevant for the decision making process should be listed.

Issue on which Co-RMS disagrees with RMS	Opinion of Co-RMS	Opinion of RMS

None : most sections have been discussed between RMS and co-RMS	-	-

3.2 PROPOSED DECISION

[redacted]

3.3 RATIONAL FOR THE CONDITIONS AND RESTRICTIONS TO BE ASSOCIATED WITH THE APPROVAL OR AUTHORISATION(S), AS APPROPRIATE

3.3.1 Particular conditions proposed to be taken into account to manage the risks identified

[redacted]	[redacted]
[redacted]	[redacted]

3.4 APPENDICES

3.4.1 GUIDANCE DOCUMENTS USED IN THIS ASSESSEMENT

General

See *"Commission Communication in the framework of the implementation of Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market"* (2013/C 95/01)

See *"Commission communication in the framework of the implementation of Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market"* (2013/C 95/02)

Section identity, physical chemical and analytical methods

Section identity and physico chemical properties

Guidance document on the assessment of the equivalence of technical materials of substances regulated under Regulation (EC) No. 1107/2009, SANCO/10597/2003, rev.10.1

Technical material and preparations: guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of Directive 91/414, SANCO/3030/99 rev.4.

WHO/FAO. 2016. Manual on development and use of FAO and WHO specifications for pesticides. Third revision of the first edition. Rome, 2016

Section analytical methods

Technical material and preparations: guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of Directive 91/414, SANCO/3030/99 rev.4.

Guidance document on pesticides residue analytical methods, SANCO/825/00 rev. 8.1

Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414, SANCO/3029/99 rev.4.

Section Data on application and efficacy

EPPO Guideline PP 1/213 (4) Resistance risk analysis (Bulletin OEPP/EPPO Bulletin (2015) 45 (3), 371–38).

Guidelines for the Preparation of a Biological Assessment Dossier, 7600/VI/95 rev. 6 (as amended by PSD), v1.03 04/11/2010.

Cleaning Application Equipment – Efficacy Aspects, Efficacy Guideline 302, PSD December 2001

Section Toxicology

Guidance document on the assessment of the relevance of metabolites in groundwater of substances regulated under council directive 91/414/EEC; Sanco/221/2000 –rev.10- final 25 February 2003

Guidance on Dermal Absorption, EFSA Panel on Plant Protection Products and their Residues (PPR) - European Food Safety Authority (EFSA), EFSA Journal 2012;10(4):2665-2695.

Section Residue and consumer risk assessment

EC, 2013. Working document on the nature of pesticide residues in fish (European Commission, 31 January 2013 – SANCO/11187/2013 Rev. 3)

EC, 2017. European Commission – Guidance Document: Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs. SANCO 7525/VI/95-rev. 10.3. 13 June 2017

OECD, 2013. OECD guidance document No 73 on residue in livestock (Sept. 2013). *[Series on Pesticides No. 73; ENV/JM/MONO(2013)8]*

Section fate and behavior in environment

FOCUS (2000): FOCUS groundwater scenarios in the EU review of active substances. Forum for the co-ordination of pesticide fate models and their use. EU Commission Document SANCO/321/2000 version 2002.

FOCUS (2011): Generic guidance for Tier 1 FOCUS ground water assessments, version 2.0. FOCUS groundwater scenarios working group.

FOCUS (2014a): Generic Guidance for Tier 1 FOCUS Ground Water Assessments, version 2.2. May 2014. 66 pp.

European Commission (EC) (2014): Assessing Potential for Movement of Active Substances and their Metabolites to Ground Water in the EU. Report of the FOCUS Ground Water Work Group, EC Document Reference Sanco/13144/2010 version 3, 613 pp.

FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. The Final Report of the Work Group on Degradation Kinetics of FOCUS Sanco/10058/2005, version 2.0, June, 2006.

FOCUS (2014b): Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration. Version 1.1, 440 pp.

Section ecotoxicology

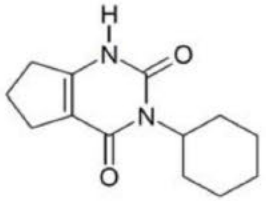
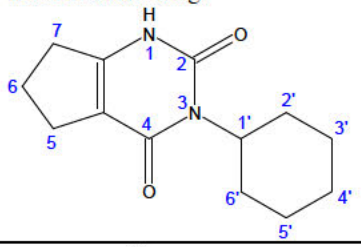
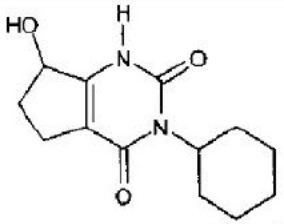
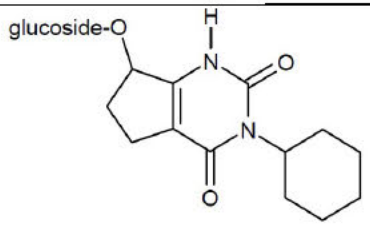
European Food Safety Authority, 2009; Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA, EFSA Journal 2009; 7(12):1438.

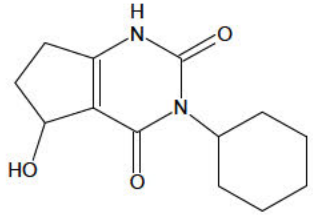
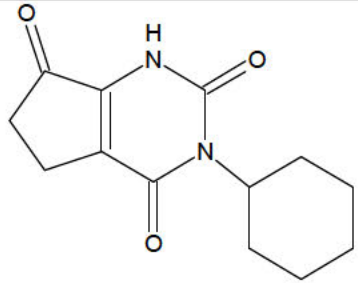
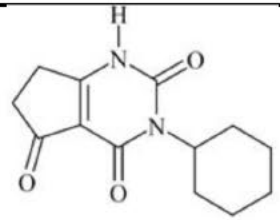
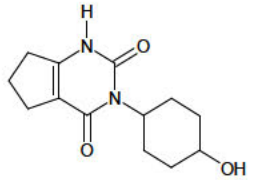
EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013; 11(7):3290.

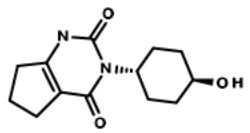
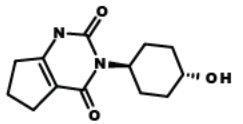
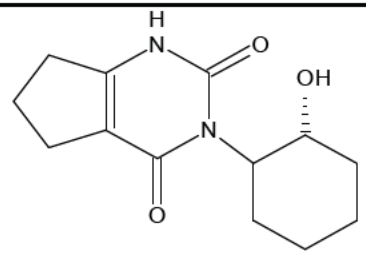
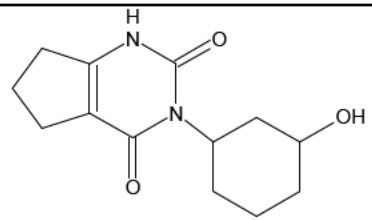
Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, SANCO/10329/2002, rev 2 (final) 17 October 2002.

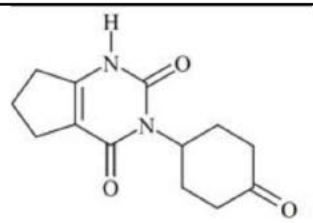
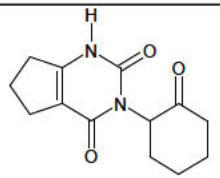
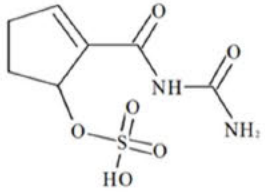
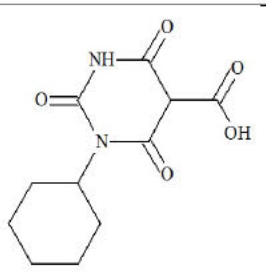
Candolfi *et al*. (2001). Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. ESCORT 2 workshop (European Standard Characteristics of Non-Target Arthropod Regulatory Testing), Wageningen, NL, 21-23 March 2000, SETAC Europe. SETAC publication, August 2001.

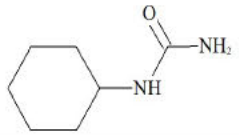
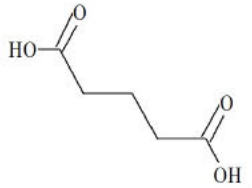
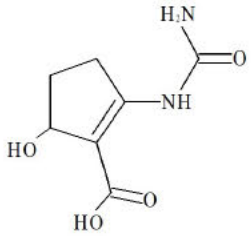
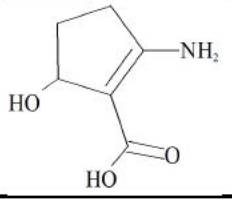
3.4.2 SUBSTANCES AND METABOLITES : STRUCTURES, CODES, SYNONYMS

Code Number (Synonyms)	Description	Compound found in:	Structure
Lenacil DPX-B0634	**Chemical name (IUPAC):** 3-cyclohexyl-1,5,6,7-tetrahydrocyclopentapyrimidine-2,4(3H)-dione **Common name:** Lenacil **CAS number**: 2164-08-1 **Molecular weight:** 234.3 g/mol	Soil, water and sediment; Plant (sugar beet); Rat (Ghantous, 1996: peak 6 (ND); Piñeiro, 2016 (*in-vitro*): Rat <Mouse <Dog = Human)	 IUPAC-numbering: 
IN-KC943	**Chemical name (IUPAC):** 3-cyclohexyl-6,7-dihydro-7-hydroxy-1-H-cyclopentapyrimidine-2,4(3H,5H)-dione **CAS number**: Not available **Molecular weight:** 251.3 g/mol	Plant (sugar beet foliage 3.1%TRR/<0.01ppm; *spinach 1.1%TRR***) Rat (Piñeiro, 2016: M33/M35: hydroxylated on C5, C6 or C7)	
IN-KC943-glucoside	**CAS number**: Not available **Molecular weight:** 414.46 g/mol	Plant (sugar beet foliage 11%TRR/<0.02ppm)	

Code Number (Synonyms)	Description	Compound found in:	Structure
IN-KQ961	**Chemical name (IUPAC):** 3-Cyclohexyl-6,7-dihydro-5-hydroxy-1H-cyclopentapyrimidine-2,4-(3H,5H)-dione **CAS number**: Not available **Molecular weight:** 251.3 g/mol	*Plant (spinach 1.1%TRR**)* Rotational crops (spinach, turnip, wheat); Rat (Ghantous, 1996: peak 5 (3-31%) hydroxylated on C5 or C6; Piñeiro, 2016: M33/M35: hydroxylated on C5, C6 or C7); Water (aquatic photolysis at alkaline conditions, but not relevant for risk assessment)	
IN-KD302	**Chemical name (IUPAC):** 3-Cyclohexyl-5,6-dihydro-1H-cyclopentapyrimidine-2,4,7-(3H)-trione	*Plant (spinach <1%TRR**)*	
IN-KF313 (KF 313-1, 5-oxo-lenacil, M20.5)	**Chemical name (IUPAC):** 3-Cyclohexyl-6,7-dihydro-1H-cyclopentapyrimidine-2,4,5-(3H)-trione **CAS number**: Not available **Molecular weight:** 248.28 g/mol	Soil, water and sediment Not recovered in rat	
IN-KD304 *Z*-isomer (KD304 - *Z*-isomer; IN-G2172) *E*-isomer	**Chemical name (IUPAC):** *Cis*-6,7-dihydro-3-(4-hydroxycyclohexyl)-1H-cyclopentapyrimidine-2,4-(1H,3H)-dione *Trans*-6,7-dihydro-3-(4-hydroxycyclohexyl)-1H-cyclopentapyrimidine-2,4-(3H,5H)-dione	*Plant (spinach 11%TRR**);* Rotational crops (spinach, turnip, wheat); Rat (Ghantous, 1996: peak 4 (0.4-20%); Piñeiro, 2016: M25/M26: hydroxylated on C1' to C6')	

Code Number (Synonyms)	Description	Compound found in:	Structure
(KD304 - *E*-isomer)			 *Z*-isomer  *E*-isomer
IN-KD305 (*E*-isomer)	**Chemical name (IUPAC):** *Trans*-1,5,6,7-Tetrahydro-3-(2-hydroxycyclohexyl)-2H-cyclopentapyrimidine-2,4-(3H)-dione	Rat (Piñeiro, 2016: M25/M26: hydroxylated on C1' to C6')	
IN-KC939 (Z/E 2:1)	**Chemical name (IUPAC):** 6,7-Dihydro-3-(3-hydroxycyclohexyl)-1H-cyclopentapyrimidine-2,4-(3H,5H)-dione	Rat (Piñeiro, 2016: M25/M26: hydroxylated on C1' to C6')	

Code Number (Synonyms)	Description	Compound found in:	Structure
IN-KE121 (LN5 oxo-lenacil M14.0/M15.0)	**Chemical name (IUPAC):** 6,7-Dihydro-3-(4-oxocyclohexyl)-1H-cyclopentapyrimidine-2,4-(3H,5H)-dione **CAS number**: Not available **Molecular weight:** 248.28 g/mol	Soil, water and sediment Rat (Ghantous, 1996: ND; Piñeiro, 2016: M24: oxo- on C1' to C6')	
IN-KQ957 (M14.0/M15.0)	**Chemical name (IUPAC):** 6,7-Dihydro-3-(2-oxocyclohexyl)-1H-cyclopentapyrimidine-2,4-(3H,5H)-dione	Soil (tentative identification in soil of confined rotational crop study)**; Rat (Ghantous, 1996: ND; Piñeiro, 2016: M24: oxo- on C1' to C6')	
IPM1	**Chemical name:** 2-(carbamoylcarbamoyl)cyclopent-2-en-1-yl hydrogen sulfate **CAS number**: Not available **Molecular weight:** 250.23 g/mol	Soil, groundwater# (not relevant for risk assessment)	
PM1*	**Chemical name:** 1-cyclohexyl-2,4,6-trioxo-1,3-diazinane-5-carboxylic acid **CAS number**: Not available **Molecular weight:** 254.24 g/mol	Soil, groundwater# (not relevant for risk assessment)	

Code Number (Synonyms)	Description	Compound found in:	Structure
PM2*	**Common name:** 1-cyclohexylurea **CAS number**: 698-90-8 **Molecular weight:** 142.20 g/mol	Soil, groundwater# (not relevant for risk assessment)	
PM3*	**Common name:** Glutaric acid **CAS number**: CAS 110-94-1 **Molecular weight:** 132.11 g/mol	Soil, groundwater# (not relevant for risk assessment)	
PM4*	**Chemical name:** 2-(carbamoylamino)-5-hydroxycyclopent-1-ene-1-carboxylic acid **CAS number**: Not available **Molecular weight:** 186.17 g/mol	Soil, groundwater# (not relevant for risk assessment)	
PM5*	**Chemical name:** 2-amino-5-hydroxycyclopent-1-ene-1-carboxylic acid **CAS number**: Not available **Molecular weight:** 143.14 g/mol	Soil, groundwater# (not relevant for risk assessment)	

* Postulated structure.

** tentative (identity and occurrence based on co-chromatography with standards; not confirmed by other means)

Polar metabolites found in groundwater were described in DAR (first approval) as M1, M2 and M3. Metabolite fractions M1, M2, M3 were detected in the leachates of lysimeters and were postulated to consist of the six polar metabolites IPM1 and PM1-PM5. The polar structure IPM1 has been identified in soil via confirmatory data and acknowledged in the EFSA Conclusion

(EFSA, 2013[19])). However, the other five polar metabolites (PM1, PM2, PM3, PM4 and PM5) have not been elucidated nor confirmed yet. The results of the new microlysimeter study indicate that the fractions M1, M2 and M3 consist of a high number of polar individual compounds, up to 33 sub-fractions are separated. Furthermore one fraction could possibly be related to IPM1.

[19] EFSA. (2013). Conclusion on the Peer Review of the Pesticide Risk Assessment of Confirmatory Data Submitted for the Active Substance Lenacil. EFSA Journal 11(9): 3354.

3.5 REFERENCE LIST

Belgium, 2007. Draft Assessment Report (DAR) on the active substance lenacil prepared by the rapporteur Member State, Belgium, in the framework of Directive 91/414/EEC, November 2007.

Belgium, 2009. Final Addendum to the DAR on lenacil, compiled by EFSA, July 2009.

Belgium, 2012. Addendum to the Draft Assessment Report section B8, confirmatory data, December 2012.

Belgium, 2013. Addendum to the Draft Assessment Report section B7, confirmatory data, March 2013.

Belgium, 2016. Addendum to the assessment report on lenacil, confirmatory data, relevance of groundwater metabolites. June 2016. Available online: www.efsa.europa.eu.

EFSA, 2009. Conclusion on the peer review of the pesticide risk assessment of the active substance lenacil on request from the European Commission. EFSA Journal 2009; 7(10):1326. [84 pp.]. doi:10.2903/j.efsa.2009.1326. Available online: www.efsa.europa.eu

EFSA, 2013. Conclusion on the peer review of the pesticide risk assessment of confirmatory data submitted for the active substance lenacil. EFSA Journal 2013,11(9):3354, 19 pp. doi:10.2903/j.efsa.2013.3354

EFSA, 2016. Technical report on the outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for lenacil in light of confirmatory data. EFSA supporting publication 2016:EN-1105. 21 pp.

Section identity, physical chemical and analytical methods

Guidance document on the assessment of the equivalence of technical materials of substances regulated under Regulation (EC) No. 1107/2009, SANCO/10597/2003, rev.10.1

Technical material and preparations: guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of Directive 91/414, SANCO/3030/99 rev.4.

WHO/FAO. 2016. Manual on development and use of FAO and WHO specifications for pesticides. Third revision of the first edition. Rome, 2016

Guidance document on pesticides residue analytical methods, SANCO/825/00 rev. 8.1

Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414, SANCO/3029/99 rev.4.

Section data on application and efficacy

EPPO Guideline PP 1/213 (4) Resistance risk analysis (Bulletin OEPP/EPPO Bulletin (2015) 45 (3), 371–38).

Guidelines for the Preparation of a Biological Assessment Dossier, 7600/VI/95 rev. 6 (as amended by PSD), v1.03 04/11/2010.

Cleaning Application Equipment – Efficacy Aspects, Efficacy Guideline 302, PSD December 2001.

Section toxicology

Guidance document on the assessment of the relevance of metabolites in groundwater of substances regulated under council directive 91/414/EEC; Sanco/221/2000 –rev.10- final 25 February 2003

Guidance on dermal absorption. EFSA Panel on Plant Protection Products and their Residues (PPR) - European Food Safety Authority (EFSA), EFSA Journal 2017;15(6): 4873, 60pp.

Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products, EFSA Journal 2014;12(10):3874, 55 pp.

Section residue and consumer risk assessment

EC, 2013. Working document on the nature of pesticide residues in fish (European Commission, 31 January 2013 – SANCO/11187/2013 Rev. 3)

EC, 2017. European Commission – Guidance Document: Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs. SANCO 7525/VI/95-rev. 10.3. 13 June 2017.

Section fate and behavior in environment

/

Section ecotoxicology

EPPO/OEPP (2010). Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees (PP 3/10(2)). Bulletin OEPP/EPPO Bulletin 40: 323-331.

EFSA Panel on Plant Protection Products and their Residues (2012). Scientific opinion on the science behind the development of a risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2012;10(5):2668, 275 pp., doi:10.2903/j.efsa.2012.2668

EFSA (European Food Safety Authority) (2015). Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924, 62 pp