Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at Community level of

Lenacil (ISO)

EC number: 218-499-0
CAS number: 2164-08-1

CLH-O-0000002461-82-02/A1

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

5 December 2013

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name:Lenacil

EC Number: **218-499-0 (EINECS)**

CAS Number: **2164-08-1**

Index Number: **Not listed on Annex VI**

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Lenacil [IUPAC: 3-cyclohexyl-6,7-dihydro-1-H-cyclopenta[d]pyrimidine-2,4(3H, 5H)-dione] is a uracil herbicide. The C.A. name is 3-cyclohexyl-6,7-dihydro-1H-cyclopentapyrimidine-2,4(3H,5H)-dione (CAS RN 2164-08-1). The formula is $C_{13}H_{18}N_2O_2$.

Table 1: Substance identity

• **Substance name:**	• ***Lenacil***
• **EC number:**	• ***218-499-0 (EINECS)***
• **CAS number:**	• ***2164-08-1***
• **Annex VI Index number:**	• ***Not listed in Annex VI of Regulation 1272/2008. Index No 182 in Annex I of Directive 91-414***
• **Degree of purity:**	• ***>975 g/kg***
• **Impurities:**	• ***No relevant impurities for classification***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	Not currently listed	Not currently listed
Current proposal for consideration by RAC	• Aquatic Acute category 1, H400, M-factor = 10; • Aquatic Chronic category 1, H410, M-factor = 10	• N, R50/53 • SCL: concentration Cn in % • N, R50/53 Cn≥2.5 • N, R51/53 0.25≤Cn<2.5 • R52/53 0.025≤Cn<0.25
Resulting harmonised classification (future entry in Annex VI,CLP Regulation)	• Aquatic Acute category 1, H400, M-factor = 10; • Aquatic Chronic category 1, H410, M-factor = 10	• N, R50/53 • SCL: concentration Cn in % • N, R50/53 Cn≥2.5 • N, R51/53 0.25≤Cn<2.5 • R52/53 0.025≤Cn<0.25

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification[1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	Not applicable	None	conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified			Data lacking
2.3.	Flammable aerosols	Not classified			Data lacking
2.4.	Oxidising gases	Not classified			Data lacking
2.5.	Gases under pressure	Not classified			Data lacking
2.6.	Flammable liquids	Not classified			Data lacking
2.7.	Flammable solids	Not classified	Not applicable	None	conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures				Data lacking
2.9.	Pyrophoric liquids	Not classified			Data lacking
2.10.	Pyrophoric solids	Not classified	Not applicable	None	conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	None	conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases				Data lacking
2.13.	Oxidising liquids	Not classified			Data lacking
2.14.	Oxidising solids	Not classified	Not applicable	None	conclusive but not sufficient for classification
2.15.	Organic peroxides	Not classified			Data lacking
2.16.	Substance and mixtures corrosive to metals	Not classified			Data lacking
3.1.	Acute toxicity - oral	Not classified	Not applicable	None	conclusive but not sufficient for classification
	Acute toxicity - dermal	Not classified	Not applicable	None	conclusive but not sufficient for classification
	Acute toxicity - inhalation	Not classified	Not applicable	None	conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Not classified	Not applicable	None	conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Not classified	Not applicable	None	conclusive but not sufficient for

					classification
3.4.	Respiratory sensitisation	Not classified	Not applicable	None	Data lacking
3.4.	Skin sensitisation	Not classified	Not applicable	None	conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	Not classified	Not applicable	None	conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	Not applicable	None	conclusive but not sufficient for classification
3.7.	Reproductive toxicity	Not classified	Not applicable	None	conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	Not classified	Not applicable	None	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	Not applicable	None	conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	Not applicable	None	conclusive but not sufficient for classification.
4.1.	Hazardous to the aquatic environment	Acute category 1 and aquatic chronic category 1	Acute M-factor = 10 Chronic M-factor = 10	None	
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	None	Data lacking

[1)]Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Warning

Hazard statements: 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

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness	Not classified	Not applicable	None	conclusive but not sufficient for classification
Oxidising properties	Not classified	Not applicable	None	Data lacking
Flammability	Not classified	Not applicable	None	conclusive but not sufficient for classification
Other physico-chemical properties	Not classified	Not applicable	None	conclusive but not sufficient for classification
Thermal stability	Not classified	Not applicable	None	conclusive but not sufficient for classification
Acute toxicity	Not classified	Not applicable	None	conclusive but not sufficient for classification
Acute toxicity – irreversible damage after single exposure	Not classified	Not applicable	None	conclusive but not sufficient for classification
Repeated dose toxicity	Not classified	Not applicable	None	conclusive but not sufficient for classification
Irritation / Corrosion	Not classified	Not applicable	None	conclusive but not sufficient for classification
Sensitisation	Not classified	Not applicable	None	conclusive but not sufficient for classification
Carcinogenicity	Not classified	Not applicable	None	conclusive but not sufficient for classification[1],[2]
Mutagenicity – Genetic toxicity	Not classified	Not applicable	None	conclusive but not sufficient for classification
Toxicity to reproduction – fertility	Not classified	Not applicable	None	conclusive but not sufficient for classification
Toxicity to reproduction – development	Not classified	Not applicable	None	conclusive but not sufficient for classification
Toxicity to reproduction – breastfed babies. Effects on or via lactation	Not classified	Not applicable	None	conclusive but not sufficient for classification
Environment	N; R50/53	R50/53: ***Cn ≥2.5%*** N R51/53 ***0.25%≤Cn<2.5%*** R52/53: ***0.025%≤Cn<0.25%***		

[1)] Including SCLs [2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

[1] Although not currently classified, EFSA proposed the following labelling requirements: Xn; Carc. Cat 3; R40 Limited evidence of a carcinogenic effect. However these elements are not required on basis of experimental results – see endpoint discussions in Toxicology section below.

[2] EFSA conclusions (EFSA Journal 2009; 7(9):1326:

Using the historical control data provided by the company at the time when DAR was prepared it was concluded that:
"Increased incidence of malignant mammary adenocarcinoma were observed in rats and considered to be relevant to humans. In mice, increased incidences of lung single alveolar tumours (adenoma and carcinoma) and multiple liver adenomas were observed and were considered of equivocal relevance for humans. Based on mammary gland and lung tumours, the classification Carc. cat.3, R40 'Limited evidence of a carcinogenic effect' was proposed".
The company provided in April 2011 an updated database of historical control data performed at the test laboratory from 2001-2006. The range of these historical control data covers the experimental results of mammary adenocarcinoma which are within these updated historical control data. Therefore, RMS would propose that the classification is not more required. This conclusion is also applicable for the lung tumors reported in mice for which the company provided updated historical control data.

Labelling: Indication of danger: N
R-phrases: (R50/53) Dangerous for the environment; Very toxic to aquatic organisms, may cause long term adverse effects in the aquatic environment

S-phrases: (S35) This material and its container must be disposed of in a safe way
(S57) Use appropriate containment to avoid environmental contamination

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Lenacil is an herbicide. In January 2009, it was approved for Annex I listing as a third stage Part B Review compound under Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market, with Belgium as Rapporteur Member State. In accordance with Article 36(2) of the CLP Regulation, Lenacil should now be considered for harmonised classification and labelling. Therefore, this proposal considers all physical and chemical properties, human health and environmental endpoints. This Annex VI dossier presents a classification and labelling proposal based mainly on the information presented in the assessment of lenacil under Directive 91/414/EEC. This assessment (DAR) was based on one full data package submitted by the company Schirm GmbH on behalf of the Task Force DuPont/Schirm GmbH.

In the following, some references to expert meetings such as PRAPeR meetings are given. PRAPeR meetings are part of the peer review process of pesticide's active substances under Directive 91/414/EEC.

2.2 Short summary of the scientific justification for the CLH proposal

None of the physico-chemical properties displayed by Lenacil require classification according to the criteria applied under the Dangerous Substances Directive (DSD) or the Classification, Labelling and Packaging Regulation (CLP).

In mammals, Lenacil is not acutely toxic via oral, dermal or inhalation routes; is not irritating to skin or eyes nor shows sensitising potential. In short-term toxicity studies rats and dogs were the most sensitive species, showing alterations in the liver and thyroid function: the relevant oral NOAELs are 40.6 mg/kg bw/d and 44 mg/kg bw/d (rats and dogs, respectively; 13-week studies), which do not result in classification. Based on results from a battery of mutagenicity investigations Lenacil is unlikely to be genotoxic. None of these results necessitated classification.

Increased incidences of malignant mammary adenocarcinomas were observed in rats and were initially considered to be of relevance for humans. In mice, increased incidences of single alveolar tumours (adenoma and carcinoma) were observed in the lungs and were considered of equivocal relevance for humans. Based on mammary gland and lung tumour incidence in rats and mice, the EFSA proposed classification under the DSD for Lenacil as Carc. cat.3 (R40) '*Limited evidence of a carcinogenic effect*'.

However, supplementary evidence submitted to the RMS after the EU review, in the form of a review of potential tumorigenicity, indicated that there are no substantive data to indicate any carcinogenic effects of Lenacil administration which are relevant for the human hazard assessment. The 'Carc. Cat. 3' (Xn, R40) classification (according to DSD criteria) was proposed by the EFSA in the conclusions to the DAR. The proposed classification is not supported in the proposed CLP classification on the basis of insufficient evidence of human carcinogenic hazard. The current proposal of no classification is supported by a position paper prepared by D Andrew, TSGE (Lenacil: Review of Carcinogenicity and Proposed R40 Classification. Report No. TSGE 19-10-05. Andrew, D. 2011) which reviews extensive historical background data relating to both tumour types, and which concludes an absence of hazard for human health assessments.
The confidential document is added in chapter 13 of the IUCLID.

The relevant NOAEL from the long-term toxicity and carcinogenicity studies is 12 mg/kg bw/d (rat study). No specific effect on reproductive parameters was found in multi-generation studies with rats: the relevant parental NOAEL is 81.9 mg/kg bw/d, the offspring NOAEL is 1727 mg/kg bw/d and the reproductive toxicity NOAEL is 4300 mg/kg bw/d. When tested in developmental toxicity studies, Lenacil did not cause malformations in the rat and rabbits: the relevant maternal NOAEL in both species is 1000 mg/kg bw/d; the relevant developmental NOAELs are 1000 and 4000 mg/kg bw/d in rat and rabbits respectively (highest dose level tested). None of the reproductive or developmental toxicity investigations resulted in any classification requirements for Lenacil.

Hazard for aquatic organisms

Several studies (both acute and long-term) were available on aquatic organisms (fish, daphnia, algae and higher plants) for technical Lenacil, formulation product and the metabolites IN-KE 121 and IN-KF 313. Algae and aquatic plants were the most sensitive organisms. Regarding the degradability, Lenacil can not be considered rapidly degradable.

The endpoint driving the environmental classification was observed in a laboratory study with Lenacil and the unicellular green alga *Pseudokirchneriella subcapitata* (72h E_rC_{50} = 0.016 mg/L).

New data have been requested following the outcome of the EU review. These will not change the proposed classification and are therefore not discussed here.

An updated DAR was produced, which included clarifications on studies on the active substance, which were already mentioned in the original DAR. There were no new studies.

2.3 Current harmonised classification and labelling

No current harmonised classification in Annex VI of CLP.

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Lenacil is not currently listed in Annex VI, Table 3.1 of the CLP Regulation.

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

Lenacil is not currently listed in Annex VI, Table 3.2 of the CLP Regulation.

2.4 Current self-classificationand labelling

2.4.1 Current self-classification and labelling based on the CLP Regulationcriteria

The CLP self classification for Lenacil is derived via the DSD classification proposed by the EFSA, but taking account of the evaluation of carcinogenic observations in rats and mice. On this basis there is no justification for classifying Lenacil for physico-chemical properties; no classification was required on the basis of acute mammalian toxicity results and no STOT-SE indications were evident in the database. No adverse findings were evident neither in the battery of genotoxicity studies nor in the repeated administration toxicity studies, including investigations for reproductive or developmental toxicity. Consequently no STOT-RE classification is warranted. Initial concerns

regarding thyroid function changes in rats, induction of mammary gland tumours in rats and some indications of increased lung tumour incidence in mice were subject to re-evaluation by additional expert assessment against a more robust historical database.

The thyroid changes were not considered to be treatment–related and the incidence of rat and mouse tumours was shown to be within the range of new historical data for test animals. In particular the mammary adenocarcinoma incidence, on re-evaluation, was shown not to be associated with treatment.

Consequently the self-classification according to the criteria of the CLP Regulation involves only the environmental hazard and based on the results of the algal investigations, Lenacil is classified as Aquatic Acute Cat 1, Aquatic Chronic Cat 1, H410: Very Toxic to aquatic life with long lasting effects with the Signal word "Warning".

No further classification is considered necessary based on the available data for Lenacil.

2.4.2 Current self-classification and labelling based on DSD criteria

Lenacil is currently self-classified by DuPont with N, R50/53. Lenacil is currently labelled by DuPont with S35 "This material and its container must be disposed of in a safe way." and S57 "Use appropriate container to avoid environmental contamination."

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Lenacil is a pesticide active ingredient and there is no need for a justification at this point according to Article 36(3) of the CLP Regulation.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

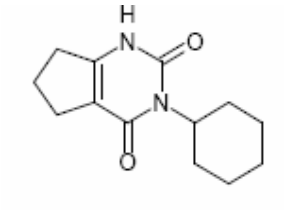
1.1 Name and other identifiers of the substance

Lenacil [IUPAC name: 3-cyclohexyl-6,7-dihydro-1-H-cyclopenta[d]pyrimidine-2,4(3H, 5H))-dione] is a uracil herbicide. The Chemicals Abstract name is 3-cyclohexyl-6,7-dihydro-1H-cyclopentapyrimidine-2,4(3H,5H)-dione (CAS Registry Number 2164-08-1). The chemical formula is $C_{13}H_{18}N_2O_2$.

Table 5: Substance identity

EC number:	218-499-0 (EINECS)
EC name:	Lenacil
CAS number (EC inventory):	2164-08-1
CAS number:	2164-08-1
CAS name:	3-cyclohexyl-6,7-dihydro-1Hcyclopentapyrimidine-2,4(3H,5H)-dione
IUPAC name:	3-cyclohexyl-6,7-dihydro-1H-cyclopenta[d]pyrimidine-2,4(3H, 5H)-dione
CLP Annex VI Index number:	Lenacil is not listed in Annex VI
Molecular formula:	$C_{13}H_{18}N_2O_2$
Molecular weight range:	234.3 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

CONSTITUENT	TYPICAL CONCENTRATION	CONCENTRATION RANGE	REMARKS
LENACIL	**MIN. 97.5% (W/W) (975 G/KG)**		

Current Annex VI entry: Not applicable

Table 7: Impurities (non-confidential information)

Impurity identity and levels are confidential. See confidential annex.
Current Annex VI entry: Not applicable.

Table 8: Additives (non-confidential information)

Additives are confidential. See confidential annex.
Current Annex VI entry: Not applicable.

1.2.1 Composition of test material

Information on the test material used in the different physico-chemical and (eco-)toxicological studies is given in each chapter respectively.

However, a summary for the (eco-)toxicological studies can be found in Table 9 which gives a global overview :

Test batch identities - Table 9

Study	Reference	Batch n°	Purity %
Acute toxicity study			
Oral	Sarver, 1989	4581-752	99.4
Oral	Blanchard 2001a	141712003	98.6
Percutaneous	Blanchard, 2001b	141712003	98.6
Inhalation	Coombs, 2001	141712003	98.6
Skin irritation	Blanchard 2001c	141712003	98.6
Eye irritation	Blanchard 2001d	141712003	98.6
Skin sensitization	Armondi, 1992	9038	98.2

Short term studies			
28-day oral rat	Thirlwill, 2002a	141712003	98.6
4 week dog, oral	Geary, 2001	141712003	98.6
90 day rat study	Thirlwill, 2002b	141712003	98.6
90 day mouse study	Malley, 1991	9038	98.2
90 day dog study	Geary, 2002	141712003	98.6
Genotoxicity:			
Bacterial assays	Russell, 1977	Code IBN-634-50	Not specified
	Reynolds, 1989	Haskell N° 17980	99.4
	D'Amici, 1994	DPX-B634-107 ? Lenacil?	Not specified
	May 2001	141712003	98.6
UDS	Riach, 1989	Batch n° 8906	Not specified
Chromosomal aberrations	Allias, 2001	14171003	98.6
Mouse lymphoma	Clare, 2003	141712003	98.6
MN	Mehmood, 2001	141712003	98.6
Long term studies			
rat	Thirlwill, 2002c	141712003	98.6
rat	Thirlwill, 2002c	141712003	98.6
mice	Malek, 1994	9038 (Lenacil?)	98.2
Reproduction studies			
2 generation rat study	Patten, 2002	141712003	98.6
Developmental rat	Smith, 1978	INB-634-61	100
	Munley, 1996	DP B 634091 Haskell 18759	98.5
	Patten, 2003c	141712003	98.6
Developmental rabbit	Hurtt, 1991	DP B 634091 Batch n° 9038	98.5
Aquatic toxicity studies			
96 h acute fish *Oncorhynchus mykiss*	Hutton D.G., 1991a	9038	98.2
96 h acute fish *Pimephales promelas*	Hutton D.G., 1991b	9038	98.2
96 h acute fish *Cyprinus carpio*	Flatman D., 2003a	141712003	98.6
21 d fish juvenile growth study *Oncorhynchus mykiss*	Hutton D.G., 1991c	9038	98.2
90 d fish early life stage study *Oncorhynchus mykiss*	Kreamer G.-L.C., 1996	9038	98.5
48 d *Daphnia magna* study	Hutton D.G., 1989a	blended: 8802 and 8805	95.1
21 d *Daphnia magna* study	Hutton D.G., 1989b	blended: 8802 and 8805	95.1
72 h algal growth inhibition study *Navicula pelliculosa*	Flatman D., 2003b	141712003	98.6
96 h algal growth inhibition study *Pseudokirchneriella subcapitata*	Flatman D., 2003c	141712003	98.6
7 d*Lemna gibba* growth inhibition study	Flatman D., 2003d	141712003	98.6

1.3 Physico-chemical properties

For physico-chemical tests the material used was:
- Pure grade active ingredient PGAI 1: Lenacil, 99% pure (Batch number 066406003)
- Technical grade active ingredient TGAI 1: Lenacil, 98.6% pure (Batch number 141712003)

Table 10: Summary of physico- chemical properties

Property	Method	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa		Fine powder, light beige solid	Hamroll, K. 2003	purity 99%
Melting/freezing point	EEC-Method A1	Not applicable	ACD 025/014039 Comb, A.L. 2002a	Decomposition starts at 270°C (purity 99%)
Boiling point	EEC-method A2	Not applicable	ACD 025/014039 Comb, A.L. 2002a	Decomposition starts at 270°C (purity 99%)
Temperature of decomposition	EEC-Method A1 (heated block) GLP	> 270°C	ACD 025/014039 Comb, A.L. 2002a	Decomposition starts at 270°C (purity 99%)
Relative density	EEC-method A3 (Pyknometer solvent displacement) GLP	1.31kg/L	ACD 025/014039 Comb, A.L. 2002a	
Vapour pressure	EEC-method A4 (vapour pressure balance method) GLP	1.7 x 10^{-9} Pa at 25 °C	ACD 025/014039 Comb, A.L. 2002a	purity 99%
Surface tension	EEC-method A5 GLP	62.5 mN/m	ACD 025/014039 Comb, A.L. 2002a	90% saturated solution, 24°C, purity 99%
Water solubility	EEC-method A6 GLP	pH 5: 2.9 mg/L pH 7: 2.9 mg/L pH 9: 3.6 mg/L	Bell, A. (2005)	99 % pure. All at 20°C (Batch 108906003)
Partition coefficient n-octanol/water	EEC-method A8 GLP	pH 4 : Log Pow = 1.70 pH 7 : Log Pow = 1.70 pH 9 : Log Pow = 1.25	ACD 025/014039 Comb, A.L. 2002a	99 % pure. All at 25°C
Flash point		Not applicable		Decomposition starts at 270°C (purity 99%).
Flammability	EEC-method A10 (burning rate test)	not highly flammable	ACD 024/013898 Comb, A.L. 2002b	Decomposition starts at 270°C (purity 98.6%)
Auto-flammability	EEC-method A16 (relative self ignition) GLP	Not self-igniting	ACD 024/013898 Comb, A.L. 2002b	
Explosive properties	EEC-method A14 (thermal,	not explosive	ACD 024/013898 Comb, A.L.	(purity 98.6%)

Property	Method	Value	Reference	Comment (e.g. measured or estimated)
	shock and friction) GLP		2002b	
Self-ignition temperature		No data		
Oxidising properties	EEC-method A17 + statement GLP	not oxidising	ACD 024/013898 Comb, A.L. 2002b	(purity 98.6%)
Granulometry		No data		not required for active substances according to Dir. 91/414/EEC
Solubility in organic solvents (20°C) and identity of relevant degradation products		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	98.6% pure. All at 20° C
Dissociation constant	OECD 112 GLP	pKa = 10.7	ACD 025/014039 Comb, A.L. 2002a	(99% pure)
Viscosity		Not applicable		

Lenacil appears as light beige solid with a characteristic odour, which starts to decompose at approximately 270°C. It is very slightly volatile (vapour pressure = 1.7 x 10^{-9} Pa and Henry's law constant = 1.3 x10^{-7} Pa.m^3.mol^{-1}) and is not surface-active. Lenacil is a weak acid with a pKa of 10.7 and has a low water solubility, which does not vary very much in the pH range pH 5 to 9 (3 to 4 mg/L at 20°C).

2 MANUFACTURE AND USES

2.1 Manufacture

Lenacil is a uracil herbicide and as such it is not a requirement to specify the manufacture for the CLH proposal.

2.2 Identified uses

Agriculture

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Pesticides: The following summary information was extracted from the Draft Assessment Report Vol 3, Annex B.2 'Physical and chemical properties' and EFSA conclusion.
Whenever results from robust study summaries were available these have been reported in relevant sections of the following table.

Table 11: Summary table for relevant physico-chemical studies

METHOD	RESULTS	REMARKS	REFERENCE
FLAMMABILITY OF THE A.S. AS MANUFACTURED EEC-METHOD A10	LENACIL IS NOT CLASSIFIED AS HIGHLY FLAMMABLE	--	ACD 024/013898 COMB, A.L. 2002B
AUTO-FLAMMABILITY OF THE A.S. AS MANUFACTURED EEC-METHOD A16	LENACIL IS NOT SELF - IGNITING	--	ACD 024/013898 COMB, A.L. 2002B
EXPLOSIVE PROPERTIES OF THE A.S. AS MANUFACTURED. EEC-METHOD A14	LENACIL IS NOT EXPLOSIVE	--	ACD 024/013898 COMB, A.L. 2002B
OXIDIZING PROPERTIES OF THE A.S. AS MANUFACTURED. EEC METHOD A17 + STATEMENT	LENACIL IS NOT OXIDISING	--	ACD 024/013898 COMB, A.L. 2002B

3.1.1 Summary and discussion of physicochemical properties

None of the reported physico-chemical properties of Lenacil result in a requirement for classification using the criteria set out in either the DSD or the CLP Regulation.

3.1.2 Comparison with criteria

None of the results for Lenacil trigger a requirement for classification using the DSD or CLP criteria.

Lenacil does not meet any of the classification criteria to be considered explosive (no explosion occurred at the conditions of the thermal, shock and friction test).

Lenacil does not meet any the classification criteria to be considered an oxidising material. Preliminary test performed according to EEC-method A17 shows no burning to completion. Moreover, according to its chemical structure (statement), Lenacil is considered to have no oxidizing properties.

Lenacil does not meet any of the burning rate test classification criteria to be considered a flammable solid. The burning rate under the EEC-method A10 is 200 mm in 8 minutes and 26 seconds.

Lenacil does not meet any of the classification criteria to be considered a self heating substance. Indication is given by the result of the EEC-method A16 showing that Lenacil has no self-ignition below 400°C.

3.1.3 Conclusions on classification and labelling

On the basis of available study results, summarised in Table 10 above, lenacil (as manufactured) is not self-igniting, not highly flammable, not explosive and not oxidising and hence, it does not need to be classified for physical and chemical hazards according to CLP and DSD criteria.

RAC general comment
Lenacil is a herbicide and is not currently listed in Annex VI of the CLP Regulation (EC No 1272/2008).

RAC evaluation of physical hazards

Summary of the Dossier submitter's proposal

No classification is proposed by the Dossier Submitter (DS) for physical hazards based on the following observations:

- Lenacil did not meet any of the classification criteria to be considered explosive (no explosion occurred under the conditions of the thermal, shock and friction test).
- Lenacil did not meet any the classification criteria to be considered an oxidising material. A preliminary test performed according to EEC-method A17 showed no burning to completion. Moreover, according to its chemical structure (statement), Lenacil is therefore considered to have no oxidizing properties.
- Lenacil does not meet any of the burning rate test classification criteria to be considered a flammable solid. The burning rate under the EEC-method A10 is 200 mm in 8 minutes and 26 seconds.
- Lenacil did not meet any of the classification criteria to be considered a self-heating substance. Indication is given by the result of the EEC-method A16 showing that Lenacil has no self-ignition below 400°C.

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

RAC supported the proposal of the dossier submitter (DS) not to classify Lenacil for physical hazards.

4 HUMAN HEALTH HAZARD ASSESSMENT

This information was extracted primarily from the Draft Assessment Report Volume 3 Annex B.6, 'Toxicology and metabolism' A concise summary is also available in Volume 1, Level 2, Section 2.3 'Impact on human and animal health'.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Lenacil is rapidly absorbed following oral administration to rats at a rate of 85% at the low dose of 10 mg/kg bw, (Ghantous, 1996) although there is evidence of saturation of absorption at the high dose of 1000 mg/kg bw. This is demonstrated by the high faecal excretion of unchanged Lenacil in the high dose rats. After absorption, Lenacil is distributed, metabolised and excreted with negligible tissue residues remaining at 7 days post dosing. Highest residues were seen essentially in tissues involved in metabolism and excretion.

Metabolism of absorbed dose is important. The major biotransformation pathway was hydroxylation of either the cyclohexyl or cyclopentenyl ring, or both rings. No glucuronide or sulphate conjugates were released by glucuronidase or sulphatase.

Urine represents the main excretion route after low single or repeated dose reaching 60% of the dose. Radioactivity was mainly excreted into urine within 12-24h. There appeared no important quantitative differences between male and female rats. When the oral dose was repeatedly administered, urinary excretion was increased (72-86%) and a slight delay in excretion occurred as well, suggesting an increase in oral absorption or an increased biotransformation of Lenacil. Urinary excretion was strongly reduced to 5-8% of the dose after oral high dose administration suggesting saturation of intestinal absorption.

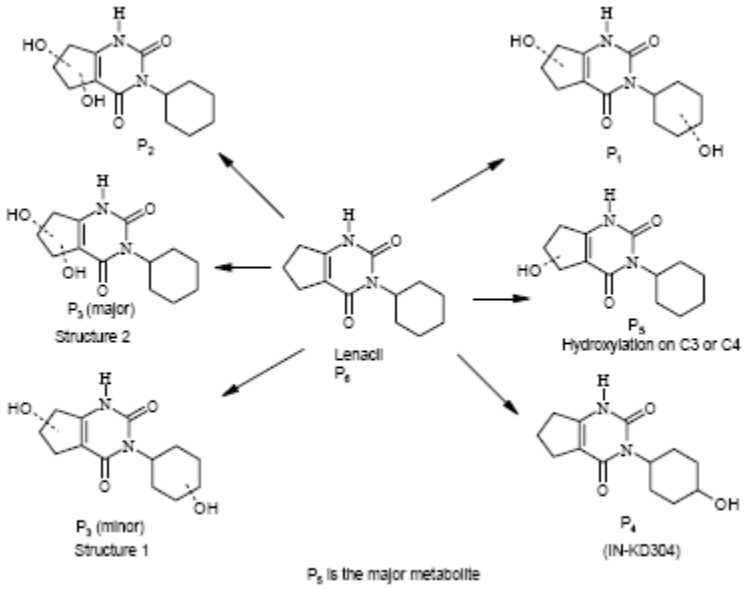
Faecal excretion represented a mean of 32% of the administered oral low dose, decreasing to a mean of 15.5% after repeated dosing but increasing to 83% after high dose. Recovery of radioactivity ranged between 92 and 100%.

The metabolic path for Lenacil is qualitatively similar in plants and animals with hydroxylation of Lenacil occurring to form the major metabolite 7-hydroxy-Lenacil (hydroxylation of cyclopentyl ring) and conjugates. Similar Lenacil metabolites were observed in hydrolysis, aqueous photolysis, water sediment, and sugar beet metabolism studies. The majority of hydroxylation and oxidation was found in cyclopentyl ring. Lenacil is rapidly degradable and at the suggested application rate, it is unlikely to accumulate in the environment.

A diagram of proposed metabolic pathway of [2-14C]-Lenacil in the rats can be found in the DAR, Volume 3 Annex B.6, page 8.

For the sake of clarity, the metabolic pathway is replicated in this CLH-report.

Figure 4.1-1: Proposed metabolic pathway of [2-^{14}C]-Lenacil in rats



4.1.2 Human information

No data available.

4.1.3 Summary and discussion on toxicokinetics

See section 4.1.

4.2 Acute toxicity

4.2.1 Non-human information

Table 12: Summary table of relevant acute toxicity studies

METHOD	RESULTS	REMARKS	REFERENCE
ACUTE ORAL TOXICITY, RAT, ACUTE TOXIC CLASS METHOD	LD_{50}> 5000MG/KG	BATCH N° 141712003; 98.6%	BLANCHARD, 2001A
ACUTE DERMAL TOXICITY, RAT, DIR EEC 92/69/EEC METHOD B.2	LD_{50}>2000 MG/KG	BATCH N° 141712003; 98.6%	BLANCHARD 2001B
INHALATION STUDY IN RATS, DIR EEC 92/69/EEC METHOD B.3	LC_{50} (4 H) >5.12 MG/LITRE AIR	BATCH N° 141712003; 98.6%	COOMBS, 2001

4.2.1.1 Acute toxicity: oral

In an acute toxic class assay there were no mortalities, no notable clinical signs of toxicity, no effects on bodyweight and no macroscopic pathological changes in female rats dosed at 5000 mg/kg bw (Blanchard, 2001a). No classification for oral toxicity is warranted on the basis of these results.

Acute oral toxicity to the rat (Acute Toxic Class Method) (Blanchard, 2001a) [ACD 004/013224/AC] (Huntingdon Life Sciences, Huntingdon, UK)

Materials and Methods:

GLP status: yes
Guideline: study is in compliance with Dir EEC 96/54/EEC Annex IV B 1ter. The test is a limit test
Material and methods:
Test substance: Lenacil technical, a light-beige powder, Batch No. 141712003, purity 98.6%.
5 fasted female rats (Sprague Dawley) received a single oral gavage dose of the test substance, formulated in 1% w/v aqueous methylcellulose, at a dose level of 5000 mg/kg bodyweight. As results at this dosage indicated the acute lethal oral dose of the test material to be greater than 5000 mg/kg bodyweight, in compliance with the study guidelines, a group of five fasted males was dosed at 5000 mg/kg to confirm results at this dosage and complete the study. No control animals were included in this study.

Findings:

Mortality: There were no deaths during the study.
Clinical signs: Clinical signs of reaction to treatment were confined to piloerection, seen in all females only approximately one hour after dosing. Recovery of rats, as judged by external appearance and behaviour, was complete by Day 2. No clinical signs of reaction to treatment were observed in any of the males throughout the study.
Body weight: All animals were considered to have achieved satisfactory bodyweight gains throughout the study.
Macroscopic examination and pathology: No abnormalities were revealed at the macroscopic examination at study termination on Day 15.

Conclusion: LD_{50} oral >5000 mg/kg bw

4.2.1.2 Acute toxicity: inhalation

In a 4 h nose only inhalation exposure study rats of both sexes were exposed to an atmosphere concentration of 5.12 mg/L air (Coombs, 2001). There were no deaths. There were no treatment related effects on bodyweight, water consumption or macroscopic pathology. Clinical signs were recorded on Day 1 only and were generally typical of effects of restraint/snout only exposure with no indication that there was any treatment association. No classification for inhalation toxicity is warranted on the basis of these results.

Lenacil technical - Acute (four-hour) Inhalation Study in Rats (Coombs, 2001)(Huntingdon Life Sciences, ACD 021/013229)

Materials and methods:

GLP status: yes

Guideline: study is not fully in compliance with Dir EEC 92/69/EEC.

Deviation from official protocol: particle diameter is 5.2 µm outside limit of acceptability (1-4) cited; respirable part (<7 µm) estimated at 62%. Clinical signs are not reported fully.

Material and methods: 5 Rats (Crl: CD(SD) IGS BR, Sprague-Dawley in origin) /sex were exposed snout-only to a mean concentration of 5.12 mg/L particulate aerosol atmosphere of Lenacil technical (Batch No. 141712003, purity 98.6%) for 4 hours. The test substance was generated using a Wright Dust Feed Mechanism, and during exposure 10 samples were taken for total Lenacil technical concentration and 2 samples for particle size determination. The Mass Median Aerodynamic Diameter (MMAD) of the Lenacil technical atmosphere was 5.2 µm and the proportion considered respirable (less than 7 µm) was 62%. A similar sized control group of rats was run concurrently with the test animals but were only 'exposed' to air.

Findings :

Mortality: There were no unscheduled deaths.
Clinical signs:

During the exposure
Exaggerated breathing was evident in a proportion of test rats from 30 minutes, and all test rats from 4 hours into exposure. Soiling of the fur with excreta was observed in all control and test group rats from 1 and 2 hours into exposure respectively and was considered to be associated with the method of restraint.

During the observation period
Exaggerated breathing was evident in all test rats immediately following exposure, persisting to at least 2 hours post exposure. Brown staining around snout/jaws was noted for a female test rat on Day 1. Soiling of the fur with excreta was noted in all control and test rats immediately following exposure. This sign was considered to be associated with the method of restraint used for exposure. All test rats were normal in appearance and behaviour from Day 2 of the observation period.

Bodyweight: Slightly increased mean bodyweight gains were evident compared with control males for male test rats throughout the 14-day observation period.
Water consumption: There were no treatment-related effects. A visual appraisal of the water bottles indicated that the amount of water consumed by test rats was similar to that of the control rats.
*Necropsy findings:*There were no treatment-related findings noted at necropsy. Lung weights were normal.

Conclusion: The LC_{50} (4-hour) for Lenacil Technical > 5.12 mg/l in air.

Remark: It could be argued whether exaggerated breathing and brown staining around snout/jaws may be relevant in relation with a potential classification for respiratory irritation (STOT-SE 3). The reviewer considers that the transient breathing pattern, which was unremarkable as soon as 2h after administration in the acute inhalation test, is insufficient to consider the substance a respiratory irritant. In addition, the necropsy did not reveal any adverse finding. In the GD, it is clearly stated:

"this special classification (respiratory tract irritation) would occur only when more severe organ effects including in the respiratory system are not observed". No information from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres on RTI was available. Therefore, the reviewer is of the opinion that no classification for RTI is warranted.

4.2.1.3 Acute toxicity: dermal

In an acute dermal toxicity study rats of both sexes were treated by single application of a 2000 mg/kg bw dose (Blanchard, 2001b). There were no mortalities, no notable clinical signs of toxicity, no indications of dermal irritation, no effects on bodyweight and no macroscopic pathological changes. No classification for acute dermal toxicity is warranted on the basis of these results.

Acute dermal toxicity to the rat (Blanchard 2001b) [ACD 005/013220/AC] (Huntingdon Life Sciences,Huntingdon, UK)

Materials and Methods:

GLP status: yes (except for stability/homogeneity/concentration of the formulation)
Guideline: study is in compliance with Dir EEC 92/69/EEC. The study is a limit test at 5000 mg/kg bw.
Material and methods: 5 rats (Hsd:Sprague-Dawley strain) /sex were treated at 5000 mg/kg bodyweight with lenacil technical, a light-beige powder, Batch No. 141712003, purity 98.6%.
One day prior to treatment, hair was removed from the dorso-lumbar region of each rat with electric clippers and an area equivalent to approximately 10% of the total body surface area was exposed. The treatment area (approximately 50 mm x 50 mm) was covered with porous gauze held in place with a non-irritating dressing, and further covered by a waterproof dressing encircled firmly around the trunk of the animal.
Treatment in this manner was performed on Day 1 (day of dosing) of the study only.
At the end of the 24 hours exposure period, skin was washed with warm water (30 - 40°C) to remove any residual test substance. The treated area was blotted dry with absorbent paper. No control animals were included in this study.

Findings:

Mortality and clinical signs: There were no deaths and no systemic response to treatment following a single dermal application of Lenacil Technical to a group of ten rats (five males and five females) at a dose level of 5000 mg/kg bodyweight.
*Dermal response***:** No dermal responses were observed for any animal throughout the study.
Body weight loss was recorded for one female and a low bodyweight gain was recorded for one further female on Day 8. All remaining animals were considered to have achieved satisfactory bodyweight gains throughout the study.
*Macroscopy***:** No abnormalities were recorded at the macroscopic examination at study termination on Day 15.

Conclusion: dermal LD_{50} of lenacil > 5000 mg/kg bw

4.2.1.4 Acute toxicity: other routes

Not applicable.

4.2.2 Human information

No acute toxicity data available.

4.2.3 Summary and discussion of acute toxicity

There were no findings in any of the acute toxicity studies to indicate adverse effects of single Lenacil exposure.

4.2.4 Comparison with criteria

The results of the various acute studies were greater than the upper levels of the Category 4 range for oral, dermal and inhalation exposure, compared to criteria as set out in annex I, 3. of the CLP regulation.

The threshold values for determining classification or hazard categories were not relevant for assessment of acute toxicity results – in each case the limit dose level proved to be non-toxic, compared to criteria as set out in DSD.

4.2.5 Conclusions on classification and labelling

No classification is warranted for acute exposure by oral, dermal or inhalation routes.

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

Acute toxicity: oral
No classification was proposed based on the absence of mortality or of any treatment-related findings including clinical signs (except transient piloerection), gross pathological findings or effects on body weight at the limit dose of 5000 mg/kg.

Acute toxicity: inhalation
Rats (5/sex) were exposed snout-only to 5.12 mg/l of Lenacil as an aerosol for four hours in a study that deviated from the OECD TG 402 in that the particle diameter of 5.2 µm was outside the range of acceptability (1-4 µm) and a full report of clinical signs was absent. No mortality, no detrimental effects on body weight gain and no adverse findings at necropsy were observed. Clinical signs consisted of exaggerated breathing during exposure and up to 2 hours post-exposure in all test animals and brown staining around the snout/jaws in one test animal. From this study, the LC_{50} of Lenacil in rat by inhalation was considered to be above 5.12 mg/l.

Acute toxicity: dermal
Rats (5/sex) were exposed to a limit dose of 5000 mg/kg in a study that was compliant with EU test method (equivalent to OECD TG 402). There were no mortality, no clinical signs of toxicity and no gross pathological changes. Transient effects on body weight gain were observed in two test females. From this study, the LD_{50} of Lenacil in rat by dermal route exceeds 5000 mg/kg.

No classification is proposed by the DS for acute toxicity.

Comments received during public consultation

No specific comments were received. Two Member State Competent Authorities (MSCA) and one company indicated their general support for the classification proposed by the DS.

Assessment and comparison with the classification criteria

Acute toxicity: oral
The LD_{50} of Lenacil in rat was above the criteria of 2000 mg/kg, below which classification for acute toxicity by oral route applies according to both CLP and DSD.

Acute toxicity: inhalation
The available study provided no evidence that the LC_{50} of Lenacil in rats is below the criteria of 5 mg/l triggering classification for acute toxicity by inhalation for aerosols under both CLP and Directive 67/548/EEC.

Acute toxicity: dermal
The LD_{50} of Lenacil in rat was above the criteria of 2000 mg/kg, below which classification for acute toxicity by dermal route applies according to both CLP and Directive 67/548/EEC.

RAC supported no classification for acute toxicity as proposed by the DS.

4.3 Specific target organ toxicity – single exposure (STOT SE)

No findings indicating any STOT-SE concerns were reported following administration by oral, dermal and inhalation routes.

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

No findings indicating any STOT-SE concerns were reported following administration by oral, dermal and inhalation routes.

4.3.2 Comparison with criteria

The guidance values set out in Table 3.8.2 of Guidance on the Application of CLP Criteria, Point 3.8.2.2.1 for oral, dermal and inhalation exposure routes do not indicate that classification as STOT-SE is required for Lenacil. There were no effects with a potential to cause adverse reaction or be potentially harmful to humans and no transient respiratory tract irritation that would have required a Cat 2 or Cat 3 STOT classification according to CLP criteria.

The transient breathing pattern, which was unremarkable 2h after administration in the acute inhalation test, is insufficient to consider the substance an respiratory irritant. In addition, the necroscopy did not reveal any adverse finding. In the GD it is clearly stated : "This special classification (respiratory tract irritation) would occur only when more severe organ effects including in the respiratory system are not observed". No information of case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centers on RTI was available. Therefore, no classification for RTI is warranted.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier submitter's proposal
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, during the

4-hour exposure and up to 2 hours post-exposure. However, it was considered insufficient by the DS 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. Hence, no classification was proposed by the DS for STOT SE.

Comments received during public consultation

No specific comments were received. Two MSCA and one company indicated their general support for the classification proposed by the DS.

Assessment and comparison with the classification criteria

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.

In conclusion, RAC supported no classification for STOT SE as proposed by the DS.

4.3.3 Conclusions on classification and labelling

No classification is required with regard to acute oral, dermal or inhalation toxicity.

4.4 Irritation

4.4.1 Skin irritation

4.4.1.1 Non-human information

Table 13: Summary table of relevant skin irritation studies

METHOD	RESULTS	REMARKS	REFERENCE
SKIN IRRITATION, RABBIT EEC METHOD B.4	NOT IRRITATING	BATCH N° 141712003; 98.6%	BLANCHARD, 2001C

Skin irritation to the rabbit (Blanchard, 2001c) (ACD 006/013201/SE Huntingdon Life Sciences, Huntingdon, UK)

Materials and methods:

GLP status: yes
Guideline: study is in compliance with Dir EEC 92/69/EEC.
Material and methods:
Approximately 24 hours prior to application of the test substance, hair was removed with electric clippers from the dorso-lumbar region of 3 female New Zealand rabbit exposing an area of skin approximately 100 mm x 100 mm. Approximately 0.5 g of Lenacil technical (Batch No. 141712003, purity 98.6%) was applied under a 2-ply 25 mm x 25 mm porous gauze pad, which had been moistened with 0.5 ml distilled water, to one intact skin site on each animal. Each treatment site was covered with elastic adhesive dressing for four hours. The animals were not restrained during the exposure period and were returned to their cages immediately after treatment. At the end of the exposure period, the semi-occlusive dressing and gauze pad were removed and the treatment site was washed with warm water (35°C) to remove any residual test substance. The treated area was blotted dry with absorbent paper.

Findings: no erythema or edema was observed in any application sites of the animals at any observation time.

<Score erythema>$_{24+48+72h}$=0

<score oedema >$_{24+48+72h}$=0

Conclusion: lenacil technical elicited no dermal irritation.

4.4.1.2 Human information

No data available.

4.4.1.3 Summary and discussion of skin irritation

In a standard three rabbit test, no erythema or oedema was observed in any application sites of the animals at any observation time.

4.4.1.4 Comparison with criteria

No responses indicative of dermal reactions that would require classification according to CLP criteria set out in Tables 3.2.1 or 3.2.2, were observed.

No responses indicative of dermal reactions that would require classification according to the DSD criteria, were observed.

According to these criteria Lenacil should not be classified for skin irritancy according to the criteria.

4.4.1.5 Conclusions on classification and labelling

No classification for skin irritation is required.

RAC evaluation of skin corrosion / irritation
Summary of the Dossier submitter's proposal In a study equivalent to OECD TG 404, Lenacil (as a powder moistened with water) was applied to the skin of three rabbits for 4 hours under semi-occlusive conditions. No irritation was observed at any time point in any animal (scores of 0). Lenacil was not irritating to the rabbit skin. No classification was proposed by the DS for skin corrosion/irritation. **Comments received during public consultation** No specific comments were received. Two MSCA and one company indicated their general support for the classification proposed by the DS. **Assessment and comparison with the classification criteria** In the absence of any irritation sign, Lenacil did not fulfil the criteria for skin irritation under CLP or DSD either in terms of severity of scores or in terms of irreversibility. It was also noted that Lenacil did not induce any effects in the acute dermal study following

a 24-hour of exposure to 5000 mg/kg Lenacil. No other study was reported by the dermal route.

In conclusion, RAC supported no classification for skin corrosion/irritation as proposed by the DS.

4.4.2 Eye irritation

Table 14: Summary table of relevant eye irritation studies

METHOD	RESULTS	REMARKS	REFERENCE
EYE IRRITATION, RABBIT EEC METHOD B.5	**NOT IRRITATING**	**BATCH N° 141712003; 98.6%**	**BLANCHARD, 2001D**

Eye irritation to the rabbit (Blanchard, 2001d) [ACD 007/013273/SE, Huntingdon Life Sciences, Huntingdon, UK)

Materials and methods:

GLP status: yes
Guideline: study is in compliance with Dir EEC 92/69/EEC.
Material and methods:
The eyes of 3 female rabbits New Zealand White were examined prior to instillation of the test substance to ensure that there was no pre-existing corneal damage, iridial or conjunctival inflammation.
Screen study - one animal - rinsed eye
One animal was treated in advance of the others, to ensure that if a severe response was produced, no further animals would be exposed. The treated eye of this animal was rinsed with distilled water approximately 30 seconds after instillation for duration of approximately 30 seconds.
Main study - three animals - unrinsed eyes
One animal was treated in advance of the other two, again to ensure that if a severe response was produced, no further animals would be exposed. A volume of 0.1 ml of lenacil technical (Batch No. 141712003, purity 98.6%) (Mean weight 70 mg) was placed in the lower reverted lid of one eye of each animal. The eyelids were then gently held together for one second before releasing. The contra lateral eye remained untreated.

Findings:

In unwashed eyes:

<Score cornea opacity>$_{24+48+72h}$ = 0/0/0

<Score iris>$_{24+48+72h}$ = 0/0/0

<Score erythema>$_{24+48+72h}$ = 0.3/0.3/0

<Score chemosis>$_{24+48+72h}$ = 0/0/0

Conclusion: Lenacil is not irritating to eyes under these experimental conditions.

4.4.2.1 Non-human information

4.4.2.2 Human information

No data available.

4.4.2.3 Summary and discussion of eye irritation

In a standard three rabbit test, the treated eyes were not rinsed after instillation of Lenacil but no irritant reactions of note were recorded. Slight conjunctival redness had resolved within 72 hours and none of the classification thresholds were exceeded.

No irritant reactions were evident in three treated rabbit eyes assessed over 72 hours following instillation.

4.4.2.4 Comparison with criteria

No responses indicative of ocular reactions that would require classification according to CLP criteria, set out in Tables 3.2.1 or 3.2.2, were observed.

No responses indicative of ocular reactions that would require classification according to the DSD criteria, were observed.

According to these criteria Lenacil should not be classified for eye irritancy according to the criteria.

4.4.2.5 Conclusions on classification and labelling

No classification for eye irritation is required.

RAC evaluation of eye corrosion / irritation
Summary of the Dossier submitter's proposal In a study equivalent to OECD TG 405, Lenacil was administered into the conjunctival sac of three rabbits. Slight conjunctival redness was observed with a mean score of 0.3 at 24 and 48 hours following instillation. Redness had resolved within 72 hours. None of the classification thresholds were exceeded and no classification was proposed by the DS for eye corrosion/irritation. **Comments received during public consultation** No specific comments were received. Two MSCA and one company indicated their general support for the classification proposed by the DS. **Assessment and comparison with the classification criteria** Signs of irritation in a guideline study were limited to slight conjunctival redness. Based on a mean score of 0.3 over 3 animals, it can be concluded that a severity of 1 was observed in a single animal 24 and 48 hours after instillation and the mean score for this animal over 24, 48 and 72 h is 0.6.

The effect was therefore reversible within 72 hours and mean severity score over 24, 48 and 72 h was in all animals below the threshold of classification of 1 according to CLP and 2 according to DSD.

In conclusion, RAC supported no classification for eye corrosion/irritation as proposed by the DS.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

No data available. There were no indications of respiratory tract irritation in the acute toxicity investigation and the repeated oral administration studies also gave no evidence of any adverse breathing response. In the absence of short term effects no repeated exposure (inhalation) data were generated.

It could be argued whether exaggerated breathing and brown staining around snout/jaws observed in the acute inhalation study may be relevant in relation with a potential classification for respiratory irritation (STOT-SE 3). The reviewer considers that the transient breathing pattern, which was unremarkable as soon as 2h after administration in the acute inhalation test, is insufficient to consider the substance a respiratory irritant. In addition, the necropsy did not reveal any adverse finding. In the GD, it is clearly stated: “this special classification (respiratory tract irritation) would occur only when more severe organ effects including in the respiratory system are not observed”. No information from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres on RTI was available. Therefore, the reviewer is of the opinion that no classification for RTI is warranted.

4.4.3.2 Human information

No data available.

4.4.3.3 Summary and discussion of respiratory tract irritation

No indications of respiratory tract irritation following inhalation exposure.

4.4.3.4 Comparison with criteria

There were no indications of respiratory tract irritation following inhalation exposure.

4.4.3.5 Conclusions on classification and labelling

No classification indicated in the absence of any respiratory tract irritation.

RAC evaluation of respiratory sensitisation
Summary of the Dossier submitter's proposal No human or experimental data are available to assess respiratory sensitisation potential and no classification was proposed by the DS for respiratory sensitisation. **Comments received during public consultation** No specific comments were received. Two MSCA and one company indicated their general support for the classification proposed by the DS. **Assessment and comparison with the classification criteria** In absence of any relevant data, RAC considered that classification was not possible due to the lack of data.

4.5 Corrosivity

4.5.1 Non-human information

There were no indications of a corrosive response in any of the reported acute studies. Lenacil is non-irritant in contact with skin, mucus membranes and eyes and is not expected to be corrosive under single or repeated exposure scenarios.

4.5.2 Human information

No data available.

4.5.3 Summary and discussion of corrosivity

No data available.

4.5.4 Comparison with criteria

No data are available for comparison.

4.5.5 Conclusions on classification and labelling

Lenacil does not require classification for corrosive properties.

4.6 Sensitisation

4.6.1 Skins s ensititsation

4.6.1.1 Non-human information

The results from a skin sensitisation test, according to the Magnusson & Kligman method, did not indicate any allergenic potential.

Table 15: Summary table of relevant skin sensitisation studies

METHOD	RESULTS	REMARKS	REFERENCE
SKIN SENSITISATION STUDY (MAXIMISATION METHOD) OECD METHOD 406	**NOT SENSITISING**	**BATCH N° 9038; 98.2%**	**ARMONDI, 1992**

Closed-Patch repeated insult dermal sensitization study (Maximization Method) with DPX-B634-91 in Guinea Pigs, (Armondi, 1992) (Du Pont HLO 34-92)

Materials and methods:

GLP status: yes (no attest of national authority)
Guideline: study is 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).
Deviation from official protocol: intradermal induction is performed with a too low concentration.
Material and methods: a preliminary range finding test was performed to determine the intradermal and topical irritation potential.
The test was performed in adult male and female Duncan Hartley albino Guinea pigs. For the main study, the intradermal induction phase was conducted in 20 guinea pigs by intradermally injecting 0.1 mL of a 1.5% (w/v) suspension of lenacil technical (Batch No. 9038, purity 98.2% (reanalysed 98.5%) with or without Freunds Complete Adjuvant. Seven days after the intradermal induction phase a topical induction was performed using patches with 0.3 mL of control, test article or positive control article. Two weeks later, a topical challenge was performed. For both, topical induction and challenge phases, the test article was dosed at a 25% concentration. 1-chloro-2, 4-dinitrobenzene was used as positive control

Findings:

Based on the results of the range finding study performed with intradermal injections of 0.1 ml at 0.5, 1.5, 3.0 and 5% suspensions of lenacil in 0.9% saline, the test article was dosed at 1.5% concentration.

In the topical range finding test, no signs of irritation were observed at 1.0, 5.0, 10 or 25% concentration in petrolatum. The test article was dosed at a 25% concentration for the topical induction and challenge.

During the challenge phase, slightly patchy mild redness was observed in one animal each in both the test and vehicle control groups. Slightly patchy mild to severe redness and swelling was observed in the positive control animals.

Conclusion: lenacil is not a sensitiser under these experimental conditions.

4.6.1.2 Human information

No data available

4.6.1.3 Summary and discussion of skin sensitisation

In a closed-patch repeated insult dermal sensitization study (Maximization Method) in Guinea Pigs, Lenacil was injected intradermally at a concentration of 1.5% in saline. For both, topical induction and challenge phases, the test article was dosed at a a 25% concentration in petrolatum. Slight patchy erythematous responses were observed in test and control groups but no reactions indicative of contact hypersensitivity were noted.

4.6.1.4 Comparison with criteria

A positive reaction in 30% of the test group is required in a maximisation test to indicate a sensitisation potential. There were no such positive reactions in the guinea pig study conducted with Lenacil.

No responses indicative of dermal reactions that would require classification according to CLP criteria, set out in Tables 3.2.1 or 3.2.2, were observed.

No responses indicative of dermal reactions that would require classification according to the DSD criteria, were observed.

According to these criteria Lenacil should not be classified for skin sensitisation according to the criteria.

4.6.1.5 Conclusions on classification and labelling

Testing for sensitising properties by the method of Magnusson & Kligman did not show an allergenic potential. No classification is required for skin sensitisation.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

In a Guinea Pig Maximization Test (GPMT) compliant with OECD TG 406, Lenacil was injected intra-dermally in 20 animals at a concentration of 1.5% in saline. For both topical induction and challenge phases, the test article was dosed at a 25% concentration in petrolatum. Slight patchy erythematous responses were observed in one animal of the test and control groups but no reactions indicative of contact hypersensitivity were noted.

Comments received during public consultation

No specific comments were received. Two MSCA and one company indicated their general support for the classification proposed by the DS.

Assessment and comparison with the classification criteria

Although it was noted by the DS 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.

On the basis of the information available, RAC therefore supported no classification for skin sensitisation.

Respiratory sensitisation

No data available

4.6.1.6 Non-human information

No data available.

4.6.1.7 Human information

No data available.

4.6.1.8 Summary and discussion of respiratory sensitisation

No data available to assess respiratory sensitisation potential.

4.6.1.9 Comparison with criteria

No data available to assess respiratory sensitisation potential. There were no indications that the classification criteria set out in Table 3.4.1 for identification of potential respiratory sensitisers were met by Lenacil.

4.6.1.10 Conclusions on classification and labelling

No classification indicated for respiratory sensitisation.

4.7 Repeated dose toxicity

4.7.1 Non-human information

Table 16: Summary table of relevant repeated dose toxicity studies

Type of test **Test species**	**Test substance purity** **doses tested : *(ppm)* and mg/kg b.w./d**	**NOAEL *(ppm)* and mg/kg b.w./day**	**LOAEL *(ppm)* and mg/kg b.w./day**	**Reference**
Preliminary study based on OECD 407 (28 day dietary study in rats)	*(wk 1-2: 0, 5000, 10000, 20000; wk 3-4: 5000, 30000, 50000 ppm)* 0, 571, 1269, 2545 (wk1-2); 0, 571, 2978, 5025 (wk3-4) mg/kg bw/d	*(30000 ppm)*	Slight increase in liver weight at 50000 ppm	Thirlwell, 2002a
Preliminary study based on OECD 407 (28 day dietary study in dogs)	*(5000, 20000, 50000 ppm)* 219, 807, 1941 mg/kg bw/d	-	Based on results from only 1 animal per sex, all doses may indicate some potential renal dysfunction	Geary, 2001
90-day oral toxicity mouse Dir.2001/59/EEC or OECD 408	Batch n° 9038; purity 98.2% *(100, 1000, 5000, 10000 ppm)* 0, 15.5, 157, 787, 1616 mg/kg bw/d	*(1000 ppm)* 157 mg/kg bw/day	*(5000 ppm)* 787 mg/kg bw/d increased liver weight in females	Malley, 1991
13 week oral toxicity study in rats with 4 week recovery Dir.2001/59/EEC or OECD 408	Batch n° 141712003; purity 98.6% *(0, 500, 5000, 50000 ppm)* 0, 40.6, 412, 4356.9 mg/kg bw/d	*(500 ppm)* 41 mg/kg bw/day	*(5000 ppm)* 412 mg/kg b.w./d leucopenia, ↑excretion urinary proteins; lipofuscin staining in thyroid follicular epithelium	Thirlwell, 2002b, 2002c
90-day oral toxicity in dogs Dir EEC 2001/59/EEC or 87/302 or OECD test guideline n° 409	Batch n° 141712003; purity: 98.6% *(0, 1000, 5000, 25000 ppm)* 0, 44.1, 221, 1121 mg/kg bw/d	*(1000 ppm)* 44 mg/kg bw/day	*(5000 ppm)* 221 mg/kg b.w./d ↑ relative liver weight in female dogs, ↑relative thyroid+ parathyroid weight, centrilobular/ midzonal hepatocyte hypertrophy	Geary, 2002

In the DAR the summary table (Table B.6.3.4-1) was proposed and accepted by PRAPeR 69, except for the mice study where the NOAEL was increased (table 17 above displays the accepted NOAELs and endpoints).

4.7.1.1 Repeated dose toxicity: oral

Toxicity Study by Dietary Administration to Rats for 13 Weeks followed by a 4 Week Recovery Period, (Thirlwell, 2002b and Thirlwell, 2002c) (Huntingdon Life Sciences ACD 002/013903, + ACD 055/024499)

Materials and methods:

GLP status: yes (no attest of competent authority)
Guideline: study is in compliance with Dir EEC2001/59/EEC.
Material and methods:
Lenacil technical (Batch No. 141712003, purity 98.6%) was incorporated into the ground diet to provide the required concentrations.
Before the commencement of treatment, homogeneity and stability investigations were carried out and confirmed for dietary concentrations at 50 and 50000 ppm. Concentration analyses were performed in weeks 1, 6 and 12 of treatment. The actual concentration average range was 97.2% (101-92.8%).
Groups of ten male and ten female Han Wistar rats received lenacil technical orally, via the diet, at concentrations of 500, 5000 or 50000 ppm for 13 weeks. A similarly constituted Control group received the basal diet only. A further five males and five females were assigned to the group receiving 50000 ppm and also to the Control group. These animals were treated for 13 weeks, followed by a four-week period without treatment to assess recovery from any treatment-related effects
Statistical analysis: Mantel test and Pair wise Fishers exact tests for comparison of control and treated groups. When Bartlett's test for variance homogeneity was not significant, then parametric analysis was applied. William test for a monotonic trend was applied. Dunnetts test was performed if F1 test was significant. For organ weight, homogeneity of variance was tested using Bartletts test. For the functional observation battery of tests, statistical analysis was performed for rearing, activity counts, grip strength and Coulbourne activity data. One way analysis was performed by Williams test. Macroscopy and microscopy were analyzed with the Fisher exact test.
The study is accepted.

Findings:

Mortality: one male rat was killed *in extremis* during week 11. The death of this animal is considered not to be related to treatment.

Clinical signs: brown staining of the tail was observed from week 7 in males and from week 8 in females at top dose. The origin of this effect was not established as there was no evidence of any change in the color of the urine in these animals. Following cessation of treatment, the incidence of this sign declined in both sexes, indicating recovery.

Body weight: Although males receiving 5000 or 50000ppm gained significantly less weight than controls, there was no evidence of dosage relationships; consequently the difference in weight gain in treated males was not considered related to treatment.

Food consumption: was slightly less in males at 500 or 5000ppm but in the absence of similar differences in males receiving 50 000ppm, these were attributed to normal biological variation.

Behavioral investigations: there were no findings at the in the hand and in the arena investigations performed during the treatment period that were attributable to treatment with lenacil. There was a slight increase in the number of male rats given 50000ppm that were seen to be walking on the toes. The number of animals showing this sign was generally low and the trend was not observed at all investigations. Consequently, this sign was not attributed to treatment.

In week 12, motor activity was apparently increased in treated females, though the magnitude of this increase was generally slight and did not follow a trend with dosage. No similar finding was observed in males. Consequently, the inter-group differences in females were attributed to normal biological variations.

At the end of the recovery period the locomotor activity of previously treated females was also higher than that of controls.

Ophthalmoscopy: no abnormalities were identified.

Hematology: lymphocyte counts in females at 5000ppm and in male and females at 50000ppm were low. Monocyte count was reduced in females at 5000 or 50000ppm. These differences resulted in a reduction of total leukocyte count in males and females at 5000 and 50000ppm, though in males at 5000 ppm this difference was not statistically significant. The cause of reduced lymphocyte numbers at 5000 and 50000 ppm in both sexes and reduced monocytes in females was not established in this study. There was no evidence of inflammatory change in any tissue, nor was there any effect of treatment upon lymphoid tissue. The company considered these effects as of uncertain toxicological significance. Monocyte counts were still slightly low at the end of the recovery period in females previously given top dose though the difference was not a great as seen at the end of the treatment period, indicating some recovery occurred.

There was complete recovery in respect of the changes in lymphocyte count.

Other differences were attributed to normal biological variations.

Clinical chemistry: low phosphorus concentrations in females at 5000ppm and in males and females receiving 50000ppm and slightly low K^+ and high creatinine in females receiving top dose. These changes showed full recovery by the end of the period of recovery. BUN was unchanged.

An effect upon renal function is indicated by variations of plasma electrolyte concentrations and the increased plasma creatinine concentrations and urinary specific gravity and protein content in females. There was, however, no effect upon the weight or histopathological appearance of the kidneys and these changes are considered most likely to represent an adaptive response to the excretion of the compound and/or metabolites and are not considered by the company of toxicological significance.

Urinalysis: specific gravity of males at top dose and urinary proteins were identified in males at 5000 and 50 000 ppm.

Organ weight: relative liver weight of female rats was increased at 5000 and 50000 ppm.

Histopathology: there was centrilobular hepatocyte hypertrophy in males and females given 50000ppm. This effect disappeared at the end of the 4 week recovery period. This change is considered by the company to represent enzyme induction and, as such, is considered an adaptive response to treatment. However, liver enzyme induction was not measured.

Table 16-1: 13- week dietary rat study and recovery.

Endpoints/dose	**0**		**500**		**5000**		**50000 ppm**	
Achieved dose	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
mg/kg bw/d	0	0	40.6	44.7	412	467.6	4356.9	4892.9
Clinical signs:								
Brown staining wk 13	0/15	0/15					5/14	4/15
Recovery wk 5	0/5	0/5					1/5	0/5
Body weight: wk 13			↓14%		↓17%		↓16%	↓4%

Endpoints/dose	**0**		**500**		**5000**		**50000 ppm**	
Achieved dose	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
mg/kg bw/d	0	0	40.6	44.7	412	467.6	4356.9	4892.9
Recovery wk 5							↑138%	↑60%
Food consumption wk 13			↓8%	↓5%	↓8%	↓14%	↓4%	
Recovery wk 5							↓5%	↓8%
Hematology week 13								
WBCs					↓9%*	↓27%*	↓20%*	↓27%*
Lymphocytes					(↓16%)	↓32%*	↓25%*	↓28%*
Monocytes						↓36%*		↓46%*
Large unstained cells						↓33%*		↓33%*
Eosinophils								↓25%*
Recovery wk 5								
monocytes								↓39%*
Large unstained cells								↓50%*
Clinical chemistry week 13								
phosphorus						↓22%*	↓6%*	↓18%*
Bilirubin								↓50%*
Creatinine								↑9%*
K^+								↓11%*
Recovery wk 5								creatinine, K^+ and Pi recovered
Urinalysis: week 13								
Specific gravity							↑0.6%*	
Proteins					↑24%*	(↑15%)	↑46%*	(↑15%)
Organ weight relative wk 13								
liver:				(↑2%)	(↑24%)	(↑21%)	(↑10%)	↑21%*

Endpoints/dose	**0**		**500**		**5000**		**50000 ppm**	
Achieved dose	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
mg/kg bw/d	0	0	40.6	44.7	412	467.6	4356.9	4892.9
Spleen :			(↑6%)		(↑4%)		(↑9%)	(↑10%)
kidney							(↑6%)	
Thyroid +para							(↑21%)	(↑12.5%)
Uterus+ cervix								(↑13%)
Recovery wk 5:								
Spleen :							(↑9%)	
Thyroid + para								(↑10%)
Uterus + cervix								(↑23%)
Histopathology week 13								
Hepatocyte hypertrophy, centrilobular	0/10	0/10	0/10	0/10	0/10	0/10	5/9	4/10
Recovery wk 5:								No findings

* Statistically significantly different from control; () ↑↓ not statistically significant.

Conclusion:

According to the company, oral administration, via the diet, to Han Wistar rats of Lenacil technical at concentrations up to 50000 ppm for 13 weeks did not produce any significant toxic effect. Adaptive changes in the liver occurred at 50000 ppm and reduced lymphocyte and monocyte numbers occurred at 5000 and 50000 ppm, the latter findings being of uncertain toxicological significance. All changes were shown to be fully reversible during the four week recovery period. There were no changes at 500 ppm (equivalent to 40.6 mg/kg/day in males and 44.7 mg/kg/day in females) and this is considered to represent the No-Observed-Effect Level (NOEL) in this study.

According to the RMS, NOAEL = 500 ppm (40.6 mg/kg bw/d) based on leukopenia, and the excretion of proteins in urine of males and increased relative liver weight (21-24%) occurring at 5000 ppm onwards.

From the results reported in this study, at the highest dose of 50000ppm, target organ in rats seems to be the liver as suggested by the weight increase (however not dose-related) and the centrilobular hepatocyte hypertrophy (reported at top dose). Renal dysfunction seems to occur as suggested by the alteration of electrolytes excretion as well as the increased urinary protein at 5000ppm onwards. Effects on white blood cells which were not explained were observed at the two high doses. RMS considers that there is no reason to disregard these different effects.

- Subchronic oral toxicity: 90 day study with DPX-B634-91 (Lenacil) Feeding study in mice (HLR293-91) (Malley, 1991)

Materials and Methods

GLP status: yes (no attest of competent authority)
Guideline: study is not fully in compliance with Dir 2001/59/EC or 87/302 or OECD test guideline n° 408 (1998-81).
Deviation from official protocol: coagulation time was not measured; epididimydes, thymus, uterus and ovary were not weighed. Salivary glands, stomach and urinary bladder not examined for histopathology (OK for 87/302); blood chemistry limited to proteins. Duration of treatment and sacrifice time not clearly reported.
Material and methods:
Lenacil technical (Code DPX-B634-91, batch no. 9038, purity 98.2%) was incorporated into the ground diet to provide the required concentrations. Before the commencement of treatment, concentration, homogeneity and stability investigations were carried out. A repeat homogeneity analysis was carried out from samples collected on day 46. The actual concentration average range was between 103 and 116 % from nominal values. The stability in diet was confirmed over 14 days. 10 CrL: CD-1(ICR) BR mice/sex/dose received lenacil technical orally, via the diet, at concentrations of 0, 100, 1000, 5000 and 10000 ppm. Body weight and food consumption were determined weekly. Evaluation of haematology parameters was performed at 45 and 90 days. At termination, all mice were sacrificed, selected organs were weighed and tissues examined microscopically.
Statistical analysis: one way analysis of variance for bw, bw gain, organ weight, clinical laboratory; Dunnetts test for comparison between test and control; incidence of clinical signs was evaluated by the Fisher exact test with a Bonferroni correction and Cochran-Armitage test for trend. The Bartletts test for homogeneity of variance was performed on organ weight and clinical laboratory data if significant.
The study is accepted.

Findings:

Mortality: did not occur during the course of the study.

Body weight: no effects were reported on body weight or body weight gain

Food consumption: was not affected and food efficiency was not altered.

Clinical signs: a compound-related effect on the incidence of clinical signs was not evident.

Ophthalmoscopy: all of the mice examined were normal.

Hematology: male mice had decreased mean total leucocytes at 1000ppm onwards and this effect was related to decreased neutrophils, lymphocytes and monocytes (affected at 45-day sampling). A similar trend was observed in 1000, 5000 and 10000ppm females at 45-day sampling period, although differences were not statistically significant. At the 90-day sampling period, the neutrophil count was lower for the 10000ppm females. The leucopenia observed in males and females at 1000, 5000, 10000ppm was initially considered to be compound related. At the 45-day evaluation period, male mice administered 1000 ppm onwards had significantly increased RBCcounts. In addition, Hb was significantly higher at 1000 and 10000ppm males and 100, 1000, 5000, 10000ppm males had higher hematocrit values compared to controls. At the 90-day evaluation, 1000, 5000, 10000 ppm females had significantly higher hematocrit values and mean corpuscular Hb values, which were however within the range of biological variations and not considered to be biologically significant. During peer review, the WBC effects were finally disregarded for the establishment of the mouse subchronic NOAEL.

Clinical chemistry: plasma proteins were slightly increased in males at 5000 and 10000 ppm. Other parameters were not measured.

Organ weight: relative liver weight was increased in females at top dose.

Histopathological findings: a higher incidence of extramedullary hematopoiesis was seen in females at top dose in liver and spleen.

Table 16-2 13- week dietary mice study: results at week 13.

Endpoints/dose	**0**		**100**		**1000**		**5000**		**10000 ppm**	
Achieved dose	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
mg/kg bw/d			15.5	20.2	157	207	787	1127	1616	2150
Clinical signs:										
Alopecia	1	0	0	1	0	1	4	1	2	1
Ruffled fur	0	0	1	0	2	0	2	1	2	1
Sore	1	0	4	0	6	0	2	1	0	3
Body weight			No compound related effect							
Bw gain			No compound related effect							
Food consumption			No compound related effect							
Hematology: wk 6-7										
WBCs					↓27%*		↓32%*		↓30%*	
Lymphocytes						(↓20%)		(↓25%)		(↓28%)
Neutrophils					↓52%*	(↓38%)	↓40%*	(↓40%)	↓56%*	(↓44%)
Monocytes					↓43%*		↓49%*		↓49%*	
RBCs					↑13%*		↑9%*		↑12%*	
Hb					↑11%*	↑6.7%*	↑6.6%*		↑11.3%*	
Ht					↑11*	↑9%*	↑7%*		↑11%*	
Platelets					↑25%*	(↓19%)	↑11%*	(↓16%)	↑30%*	(↓16%)
Hematology: wk 13										
WBCs					↓31%*		↓38%*		↓34%*	
Lymphocytes					↓30%*		↓37%*		↓27%*	
Neutrophils					↓40%*	(↓6%)	↓37%*	(↓12%)	↓64%*	(↓31%)
Ht						↑10%*		↑10%*		↑10%*
Platelets						(↓3%)		(↓13%)	(↓20%)	(↓15%)
Clinical chemistry										
Plasma proteins							↑6.8%*		↑6.7%*	
Organ weight: relative										
liver						(↑5%)	(↑2%)	(↑14.4%)	(↑6%)	↑18%*

Endpoints/dose	**0**		**100**		**1000**		**5000**		**10000 ppm**	
Achieved dose	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
mg/kg bw/d			15.5	20.2	157	207	787	1127	1616	2150
spleen										(↑36%)
Histopathology:										
Liver extramedul. hematopoiesis	1/10	0/9	0	0	0	0	0	0	2/10	4/10
Single cell necrosis	0	0	0	0	0	0	0	0	0	1/10
Spleen lymphoid cell hyperplasia	0/10	0/9	0	0	0	0	0	0	2/10	0/10
Extra.hematopoiesis	0/10	2/9	0	0	0	0	0	0	1/10	5/10

*↑↓ statistically significant at 5% level; (↑↓) not statistically significant.

Conclusion: Proposal from the company: Oral administration, via the diet, to CrL: CD-1 mice of lenacil technical at concentrations up to 10000ppm for 13 weeks did not produce any significant toxic effect. Adaptive changes in the liver occurred at 5000 and 10000ppm (increased organ weight without concomitant histopathological changes) and reduced neutrophilic granulocytes, lymphocyte and monocyte numbers occurred from and including 1000ppm onwards, the latter findings being of uncertain toxicological significance, because these findings were not dose-related. Therefore these haematological findings are not considered to be of toxicological importance. Due to liver weight changes, the NOEL can be set at 1000ppm, (equivalent to 157 mg/kg/day in males and 207 mg/kg/day in females).

According to the company, since all statistically significant changes in haematology and organ weight determinations were considered due to an adaptive effect rather than a significant toxicological effect, 10000ppm could be classified as the highest NOAEL in this study, equivalent to 1616 mg/kg/bw/day for males and 2150 mg/kg/bw/day for the females, respectively.

Further comment from the company: The company conclusions presented above are based on a lack of dose relationship for the majority of haematological findings, combined with an absence of consistency between weeks 6 and 13 and the absence of any increasing effect with repeated administration of lenacil. This combined with the adaptive response in liver weight at the 1000 ppm and 5000 ppm level, demonstrates that 100 ppm can be clearly stated to be an NOEL but the level at which non-adverse findings are detected is clearly higher than the NOEL. The toxicological and biological significance of the high dose findings in mice, when extrapolated to man may be debated, particularly since similar effects were not recorded in the rat when similarly exposed, at doses of less than 5000 ppm. In the opinion of the notifier, 1000 ppm is the NOAEL for this study.

During the peer review, the relevant NOAEL from the 90-mice study was discussed and agreed to be 1000 ppm (157 mg/kg bw/day) based on increase liver weight in females treated at dose level of 5000 ppm (787 mg/kg bw/day).

- Toxicity study by dietary administration to beagle dogs for 13 weeks (Geary, 2002) (Huntingdon Life Sciences, ACD 022/014297).

Materials and methods:

GLP status: yes
Guideline: study is in compliance with Dir EEC 2001/59/EEC or 87/302 or OECD test guideline n° 409 (1998-81).
Material and methods:
Lenacil technical (Batch No. 141712003, purity 98.6%) was incorporated into the ground diet to provide the required concentrations. The homogeneity and stability of Lenacil technical in diet formulations were assessed analytically in trial formulations, at concentrations of 50 and 50000 ppm. Each formulation achieved an accuracy within 3% of the nominal concentration and a precision, measured by the coefficient of variation, of <1.5%. The mean analyzed concentrations remained very close to the Day 0 values (±1%) after ambient temperature storage for 22 days. The mean concentrations of Lenacil technical in formulations, prepared for dosing during Weeks 1, 6 and 12 of treatment of the study ranged from 98.4% to 99.9% of nominal concentrations and were considered satisfactory. Three groups of pure-bred beagle dogs (four males and four female animals per group) received Lenacil technical, by dietary administration at dosages of 1000, 5000, or 25000 ppm for 13 weeks. A further group of pure-bred beagle dogs (four male and four female animals) was held as concurrent control receiving basal diet alone. Laboratory examinations were performed prior to the start of the study and at weeks 6 and 13. At terminal autopsy, macroscopic findings and organ weights were recorded and a broad spectrum of organs was subjected to histopathological examination from all animals.
Statistical analysis:
All statistical analyses were carried out separately for males and females. The individual animals are the basic experimental unit. Bodyweight data were analysed using weight gains. Food consumption data could not be analysed statistically due to the small group size (1 cage/sex/group). Organ weight data were analysed as absolute and adjusted for terminal bodyweight, where appropriate.
Bodyweight, haematology, blood chemistry, urinalysis and organ weight data: frequency analysis was applied. Treatment groups were compared using a Mantel test for a trend in proportions and also pairwise Fisher's Exact tests for each dose group against the control. If Bartlett's test for variance homogeneity was not significant at the 1% level, then parametric analysis was applied. If the F1 test for monotonicity of dose-response was not significant at the 1% level, Williams' test for a monotonic trend was applied. If the F1 test was significant, suggesting that the dose-response was not monotone, Dunnett's test (Dunnett 1955, 1964) was performed instead.
If Bartlett's test was significant at the 1% level, then logarithmic and square-root transformations were tried. If Bartlett's test was still significant, then non-parametric tests were applied. If the H1 test for monotonicity of dose-response was not significant at the 1% level, Shirley's test for a monotonic trend was applied. If the H1 test was significant, suggesting that the dose-response was not monotone, Dunn's test was performed instead.
Where appropriate, analysis of covariance was used in place of analysis of variance.
For organ weight data, analysis of variance was performed using terminal bodyweight as covariate when the within group relationship between organ weight and bodyweight was significant at the 10% level in an attempt to allow for differences in bodyweight which might influence the organ weights.
Significant differences between control and treated groups were expressed at the 5% ($p<0.05$), or 1% ($p<0.01$) level.
The study is accepted.

Findings:

Mortality: there were no unscheduled deaths.

Clinical signs: there were no signs of ill health, behavioral change or reaction to treatment.

Body weight: bw gain was slightly reduced at top dose and in males at 5000ppm. However, the statistical significance was not attained and the differences from controls were not considered to represent an effect of treatment. Lower mean bw gain was also noted for females at 1000ppm and this effect was attributable to 1 female.

Food consumption: was near maximal and was similar to that of the controls for all groups.

Behavioural investigations:

Ophthalmoscopy: there were no treatment-related changes.

Hematology: there were no differences from controls thought to be related to treatment.

Clinical chemistry: during week 6 and 13, higher mean alkaline phosphatase was seen at top dose for both sexes. There were no other differences from controls thought to be related to treatment as they tended to reflect pre-dose trends and/or were minor in magnitude and did not follow dosage relationships when noted in more than one treatment group.

Urinalysis: no differences from controls.

Organ weight: mean liver weight for all treated groups was increased in comparison with controls, the differences being dose-related, though statistical significance was not attained.

At top dose, thyroid weights were higher for males and as the individual values showed some degree of overlap with the control values and in the absence of corroborative macroscopic or microscopic finding, this is not considered to be of toxicological importance.

Thymus weight was reduced for all male dogs in comparison with controls, with a dose related effect at top dose, though statistical significance was not achieved and some degree of overlap of individual values between treated dogs and controls was evident.

Histopathological findings: treatment related microscopic changes were noted in the liver for both sexes at 25000ppm and males only at 5000ppm and was characterized as centrilobular and midzonal hepatocyte hypertrophy.

Marginally increased incidences of involution/atrophy in the thymus were seen in all male treated groups, when compared with controls. This finding was associated with lower thymus weight at 5000 and 25000ppm. This finding was low in incidence and severity, and the toxicological importance was equivocal.

Table 16-3: 13-week dietary dog study.

Endpoint/dose	**0**		**1000**		**5000**		**25000ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Achieved intake mg/kg bw/d	0	0	44.07	45.77	221.19	224.85	1120.67	1101.92
Bw gain wk 0-13 (kg)(% of control)	3.7	3.3	3.5 (↓5%)	2.5 (↓25%)	3.4 (↓8%)	3.0 (↓9%)	3.2 (↓13%)	2.8 (↓15%)
Food consumption			No compound related effect					
Hematology:								
Reticulocytes wk 6					↓47%*		↓30%*	
Eosinophils wk 6				↑107%*		↑107%*		↑61%*
monocytes wk 6								↑49%*
Eosinophils wk13						↑87%*		↑81%*
APTT week 13							↑22%*	
Blood chemistry:								
Alkaline phosphatase wk 6							↑29%*	(↑21%)
phosphates wk 6								↓14%*
Total protein wk 6			↓3%*		↓3%*		↓3%*	

Endpoint/dose	**0**		**1000**		**5000**		**25000ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Alkaline phosphatase wk 13							↑53%*	↑31%*
Na^+ wk 13							↑1.3%*	↓15%*
Total protein wk 13								↓7%*
Urinalysis:								
pH wk 6	6.2	6.0	5.9	6.5	6.1	6.1	5.8	5.8
Proteins wk 6								(↑↑)
Organ weight:								
Liver relative			(↑5.4%)	(↑6%)	(↑6.6%)	(↑15%)	(↑15%)	(↑13%)
Thymus relative				(↓9%)			(↓38%)	(↓16%)
Thyroid + paras			(↑7%)		(↑15 %)	(↑21%)	↑23%*	(↑33 %)
Histopatology:								
Liver hepatocyte								
Centrilobular hypertrophy minimal	0	0	0	0	2	0	3	1
midzonal hypertrophy minimal	0	0	0	0	2	0	3	1
Vacuolation focal								1
Parenchymal inflammatory cell foci	1	0	0	2	1	0	1	2
Thymus involution/atrophy								
Minimal/slight/total	0/0/0	1/0/1	1/0/1	0/1/1	0/1/1	1/0/1	2/0/2	0/0/0

*↑↓ statistically significant $p<0.05$; (↑↓) not statistically significant.

Conclusion:

The company considered that: based on the results above the No Effect Level (NOEL) on this study was considered to be 1000 ppm (corresponding to a daily intake of 44 mg/kg in the males and 46 mg/kg/day in the females) based on adaptive histopathological findings in the liver. The highest No Adverse Effect Level (NOAEL) was 25000 ppm (equivalent to 1121 mg/kg/day for males and 1102 mg/kg/day for the females).

RMS considers that the NOAEL = 1000 ppm (44 mg/kg bw/d) taking into account the increased relative liver weight in female dogs, the increased relative thyroid+ parathyroid weight in male and female dogs. Liver centrilobular/midzonal hepatocyte hypertrophy was reported in male dogs at 5000ppm.

Notifier comment:

Taking the two studies (28 day dog study and 90 day dog study) together it is apparent that considerable background variation occurs in a number of parameters following low dose administration of lenacil, without adverse effect on the animals over 4 or 13 weeks. The liver, rather than the kidney, is the target organ and at high doses this organ responds adaptively to the challenge of metabolizing lenacil. The test material is extensively metabolized following oral administration and so the functional liver changes are not unexpected.

Hence the low dose levels can reasonably be assumed to reflect biological variation and the high dose findings indicate an adaptive liver response. Based on these findings, the notifier disagrees with the RMS conclusion and respectfully requests reconsideration of an NOAEL of 25000 ppm.

-Additional histopathological investigation to a toxicity study by dietary administration to Han Wistar rats for 13 weeks followed by a 4 week recovery period (Thirlwell, 2004c) report No ACD 055/024499, Huntingdon Life Sciences Limited.

Material and methods: The thyroids of all animals of groups 1, 2, 3 and 4, sacrificed after completion of the 13-week treatment period, and five male and 5 female rats of Group 1 and 4 sacrificed on completion of the 4 week recovery period, were subjected to histopathological evaluation. In the original study (ACD/002, see under Point 5.3.2.1), the thyroids of all males and females from Group 1 (control) and 4 (high dose), killed after completing the 13 weeks of treatment were examined.

This additional study was intended to re-assess these tissues in all animals of these groups, together with those from females of the low and intermediate dose groups and recovery phase animals, in the light of changes seen in other toxicity studies performed for Lenacil technical. The thyroids of all animals were originally fixed in the original study (ACD/022) in 10% neutral buffered formalin. Appropriate samples of the thyroid including, where possible, the parathyroid sections, were dehydrated, embedded in paraffin wax, sectioned at approximately four to five micron thickness. The section were stained with haematoxylin and eosin and Schmorl's stain (Schmorl's positive staining can indicate the presence of a variety of materials including thyroid colloid, bile pigments, melatonin or lipofucscin specific stain for lipofuscin) and subjected to light microscopy examination.

The effect of Lenacil technical on the thyroid function to female rats, as reflected in the capacity of the thyroid to take up and "organify" 125Iodide was assessed over a period of 20 weeks. Previous studies with the test material had revealed a darkening of the thyroid gland and the purpose of this study was to specifically investigate the action of Lenacil technical on thyroid function.

Findings:

The objective of this study was to perform an additional histopathological examination of the thyroid from a 13-week study in order to assist in the further interpretation of thyroid changes reported in other studies.

In the multigeneration study, thyroids in some treated animals were macroscopically dark and microscopically demonstrated increased pigmentation when stained with haematoxylin and eosin. The thyroids were examined further on the reproduction study. Those thyroids stained with Schmorls reagent showed an increased incidence of Schmorls positive reaction, even in animals where no pigment deposition has been detected with haematoxylin and eosin staining procedure. In view of these observations the decision was taken to perform additional histopathological investigations, by the application of Schmorls stain, on the thyroids taken from the 13 week rat study.

Schmorls staining of the thyroids revealed a background level of positive staining in all groups, particularly in males. Positive staining is indicative of lipofuschin in the follicular epithelium. Lipofuscin pigment is associated with the degradation of the cell membrane and could suggest the presence of persistent chronic injury. Lipofuscin is reported where there is atrophic change, though in this study, examination of haematoxylin and eosin stained sections of the thyroid did not identify any evidence of atrophy. This change may be related to an increased rate of thyroid metabolism as a consequence of hypertrophic change in the liver which was reported in the original study at 50000ppm and was attributed to the induction of hepatic enzymes.

In females, there was a treatment-related increase in the incidence and severity of Schmorls positive staining at 50000ppm and a slight increase in the severity of this finding in males at 50000ppm. The slightly increased incidence of staining in females at 5000ppm was within the background incidence.

At the end of the recovery period, the incidence and severity of staining was higher than controls in females at top dose and in males the severity was marginally higher than controls.

Lipofuscin is an insoluble endogenous formed pigment which represents the indigestible residue of autophagic vacuoles within cells formed during aging or atrophy. The pigment appears to be composed of polymers of lipids and phospholipids complexed with protein.

The following is the manner in which lipofuscin is formed:

During atrophy and aging, degenerating cellular organelles are enclosed in autophagic vacuoles. Subsequently, lysosomes discharge their hydrolytic enzymes into these membrane bounded vacuoles and the cellular organelles are digested by autophagy. However, some of the organelle components may resist digestion or be incompletely digested. Lipoproteins and other lipids make up most of the indigestible debris and their accumulation reflects the lack of sufficient quantities of lipase in most lysosomes. When organelles are not digested completely, the debris persists as membrane-bounded residual bodies. Some of these residual bodies may be extruded from the cytoplasm, or may be eventually digested. However, in some instances, the residual bodies persist in the cytoplasm of atrophic or aging cells. Microscopically, lipofuscin pigment appears as minute yellow-brown granules. Grossly, the lipofuscin pigment may impart a brownish discoloration to tissues when present in sufficient amounts (brown atrophy). Lipofuscin itself is not injurious to the cell or to its function. Lipofuscin occurs in a variety of organs and tissues, but it is especially prominent in the brain neurons, myocardial cells and in the adrenal and thyroid glands.

Comment from RMS: accumulation of lipofuscin in thyroid could then suggest that atrophy occurred and that membranes of destroyed organelles are converted within the lysosomes to lipid-containing lipofuscin. Lipofuscin in itself is not injurious to the cell but it presence could suggest that something adverse occurred.

Conclusion:

RMS considers that the NOAEL is 500ppm taking into account the slight increased incidence of staining of lipofuscin in the follicular epithelium of thyroids of females at 5000ppm. The effects of lenacil on the thyroid are not clear and could result 1) from an effect on hypothalamic/thyroid axis resulting from the enzyme inducing effect; however this was not demonstrated but could also result 2) from an atrophic change, which was not evident from this study. Black thyroid is rare and pigment accumulation in normal tissue is thought to occur by inhibition of thyroid peroxidase.

According to the company:It is concluded that oral administration, via the diet, to Han Wistar rats of Lenacil technical at a concentration of 50000 ppm caused an increase in the incidence and

severity of Schmorl's-positive staining in females and a slight increase in the severity of this finding in males. In view of the nature of the staining reaction applied in this highly specific study, it was not possible to establish evidence for any significant recovery after four weeks respite from treatment. The no-observed-effect level (NOEL) for changes in the thyroid as identified by this study was 5000 ppm.
The notifier disagrees with RMS in relation to the interpretation of the effect of lenacil on the thyroid. While it is possible that the effects of lenacil at 50000 ppm were evident in terms of lipofuscin staining, there are no findings in the study to support the postulated causes of minor thyroid changes. The report author and the notifier consider it is reasonable to assume, in the absence of any such evidence, that the slight changes noted at 5000 ppm were not adverse and that 5000 ppm is a valid choice of NOAEL.

- Lenacil technical – Investigation into potential effects on thyroid function after 20 weeks of treatment in female HAN Wistar rats using the "Perchlorate Discharge Test". (Whittaker, 2004) (ACD 060/033946, 28 June 2004, Huntingdon Life Sciences Limited)

Materials and methods:

GLP status: yes

Guideline: no EU or OECD guidelines correspond to this study.

Material and methods: 2 groups of 18 female rats received Lenacil technical (Batch No. 141712003, purity 98.6%) by the dietary route at dosages of 250 or 50000 ppm over an entire period of 20 weeks. A similarly constituted negative (untreated) Control and positive Control received Propylthiouracil (Batch No. 32K2526, purity 99%) at a dosage of 200 mg/kg/day by gavage for 2 weeks only (weeks 19 and 20).

During the study, clinical condition, detailed physical observation, bodyweight, food consumption, blood chemistry, organ weight and macropathology investigations were undertaken in addition to the terminal metabolic investigations of the perchlorate discharge test. The accuracy of the test formulations was confirmed by periodic chemical analysis of the diets prepared for administration.

Findings:

Lenacil treated rats: There were no unscheduled deaths.
Clinical signs: a higher incidence of hairloss, poor grooming and brown stained tails was recorded at top dose.
Body weight: mean body weight gain was marginally lower at top dose without attaining statistical significance. Food intake was unaffected.
Blood chemistry: T4 was lower than that of controls for animals given either 250 or 50000ppm lenacil in week 10 and were then higher in week 19 than in week 10. T3 and TSH values were similar to those of controls throughout the study. Lower rT3 values seen for rats receiving 250 or 50000ppm lenacil during week 19.

Notifier comment: The lower rT3 values seen for rats receiving 250 or 50000ppm lenacil during week 19 are not considered to be toxicologically significant since rT3 is biologically inactive. No biological importance attaches to this finding. No disruption of rT3 occurred following administration of the positive control.

T3 and T4 levels:

At 250 ppm, mean T4 was statistically lower in week 10. This change was not accompanied by lower T3 or rise in TSH values and was no longer evident in week 19.

At 50000 ppm, mean T4 was statistically lower in week 10. This change was not accompanied by lower T3 or rise in TSH values and was no longer evident in week 19.

Thyroid weights: Mean thyroid weight was increased.

^{125}Iodide uptake: There was no clear reduction in the ability of the thyroid to take up and accumulate ^{125}Iodide.

^{125}Iodide displacement: The ability of thyroid peroxidases to convert the ^{125}Iodide to organic compounds was unaffected by treatment.

Propylthiouracil treated rats

Clinical signs: Rats had salivation with paddling of forepaws. Irritable behavior was noted in rats during the treatment periods of weeks 19-20.
Body weight: of rats was not affected.
Food intake was unaffected.

Propylthiouracil is a compound that exerts a direct toxic effect on the thyroid by inhibition of the thyroidal peroxidase enzymes and is used here as the positive control.

Typical and statistically significant differences from control rats were as follows:

*T3 and T4 levels:*There was a large reduction in circulating T3 and T4 levels (attributable to the direct effect of propylthiouracil on the thyroid leading to decreased production of T3 and T4) accompanied by marked elevation of mean TSH levels (due to the resulting negative feedback).
Thyroid weights: A large increase in mean thyroid weight was noted, consistent with TSH-mediated hypertrophy.
^{125}Iodide uptake: The ability of the thyroid to take up and accumulate ^{125}Iodide was reduced.
^{125}Iodide displacement: About 80% of thyroid radioactivity was displaced by perchlorate, when propylthiouracil/saline treated rats were compared with propylthiouracil/perchlorate treated animals. The large amount of free ^{125}Iodide present in the thyroids of propylthiouracil treated animals is a consequence of the inhibition by propylthiouracil of the thyroid peroxidases that would normally convert the ^{125}Iodide to organic compounds. This is in contrast to the control rats, were little free ^{125}Iodide was present.

Thyroid: blood concentration ratio:

The reduced ability of the thyroid to take up and metabolise ^{125}Iodide was further demonstrated by the much lower thyroid: blood concentrations ratio in propylthiouracil treated animals. Lenacil did not disrupt iodide organification in the thyroid.

Conclusion: There was no evidence to suggest that Lenacil technical at dosages of up to 50000 ppm was affecting the ability of the thyroid to take-up and organify ^{125}Iodide. Measurements of T3 made during the study also indicate that the test substance is not acting as an inhibitor of the deiodinase which convert T4 into T3.

Overall, the results of the study show that Lenacil technical was not directly toxic to the thyroid.

Comment from RMS:

The effects of lenacil on thyroid function can be summarized as follows: slight reduction of T4 and rT3 while TSH is not altered, at high doses in females. From the ADME studies it appeared that radioactivity was identified in the thyroid. Lenacil does not to act through deiodinase or peroxidase inhibition. In females, there was a treatment-related increase in the incidence and severity of Schmorls positive staining and a slight increase in the severity of this finding in males at 50000ppm. At the end of the recovery period, the incidence and severity of staining was higher than controls in females at top dose and in males the severity was marginally higher than controls. Thyroid hypertrophy was reported.

Changes in serum concentrations of thyroid hormone can be caused by chemicals that inhibit thyroid hormone synthesis, release, and transport, and by chemicals that increase metabolism of various thyroid hormones (e.g.deiodinases, UDPGTs). In the case of lenacil, no sufficient information is provided for interpreting changes in hormone levels in term of mechanisms of toxicant action or potential adverse effects. The reason for the observation of black thyroids is not clear. Therefore, RMS considers that these effects should be taken into account for setting of NOAELs.

General conclusions:

-From the first study (Thirlwell, 2004c) it is concluded that oral administration, via the diet, to Han Wistar rats of Lenacil technical at a concentration of 50000 ppm caused an increase in the incidence and severity of Schmorl's-positive staining in females and a slight increase in the severity of this finding in males. In view of the nature of the staining reaction applied in this highly specific study, it was not possible to establish evidence for any significant recovery after four weeks respite from treatment. Black thyroid is rare and pigment accumulation in normal tissue is thought to occur by inhibition of thyroid peroxidase. The NOAEL was proposed to be 500 ppm taking into account the slight increased incidence of staining of lipofuscin in the follicular epithelium of thyroids of females at 5000 ppm. The possible effect on rat thyroid function did not affect the classification of Lenacil.

-From the second study (Whittaker, 2004) it appeared that Lenacil technical at dosages of up to 50000 ppm was not affecting the ability of the thyroid to take-up and organify ^{125}Iodide. Measurements of T3 made during the study also indicate that Lenacil does not acting as an inhibitor of the deiodinase which converts T4 into T3.

Changes in serum concentrations of thyroid hormone can be caused by chemicals that inhibit thyroid hormone synthesis, release, and transport, and by chemicals that increase metabolism of various thyroid hormones (e.g.deiodinases, UDPGTs). In the case of Lenacil, no sufficient information is provided for interpreting changes in hormone levels in term of mechanisms of toxicant action or potential adverse effects. The reason for the observation of black thyroids is not clear.

4.7.1.2 Repeated dose toxicity: inhalation

No data available.

4.7.1.3 Repeated dose toxicity: dermal

No data available.

4.7.1.4 Repeated dose toxicity: other routes

No data available.

4.7.1.5 Human information

No data available.

4.7.1.6 Other relevant information

No data available.

4.7.1.7 Summary and discussion of repeated dose 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 100-400 mg/kg bw/d, WBC count was decreased, without evidence of inflammatory change in any tissue, or any effect in lymphoid tissues (Malley, 1991, Thirlwell, 2002b, 2002c, Geary, 2002)

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 the high dose. Some other organ weights were altered at the high 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 5000ppm onwards, but without any evidence of organ atrophy. After a 4 week rest, the rats showed good recovery.

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 in rat and dogs (resp. 41 mg/kg b.w./d and 44 mg/kg b.w./d).

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

See 4.7.1.7 summary above. No classification for long term or repeated exposure was necessary based on the NOAEL values identified in sub-chronic exposure studies. None of the effects observed in the toxicity studies required classification for Lenacil according to DSD criteria.

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

The criteria for classification according to the DSD are set out in Commission Directive 2001/59/EEC and subsequent amendments, Annex VI, section 3.2.2. According to these criteria Lenacil should not be classified for repeated administration toxicity. There were no effects observed in sub acute, sub-chronic or chronic exposure studies to indicate a risk of serious damage, death, clear functional disturbance or morphological changes. Effects observed at high doses included primarily renal dysfunction and possible thymic changes and an adaptive response in the liver involving increased metabolic activity and associated cellular changes. None of the effects

were apparent at lower doses, the NOAEL in rats and dogs was circa 45 mg/kg bw/day and the lowest NOAEL in mice, was 157 mg/kg bw/day. None of these values trigger classification with R48 according to DSD criteria.

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

None of the effects were apparent at lower doses, the no effect level in rats and dogs was circa 45 mg/kg bw/day and the lowest NOAEL, in mice, was 157 mg/kg bw/day. Neither of these values trigger classification with R48 according to DSD criteria.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

The effects observed in the battery of repeated administration tests completed for Lenacil were limited to indications of renal dysfunction, thymus changes at very high doses particularly in dogs, minor effects on rat thyroids and a general increase in liver weight that was attributed to an adaptive response to an increased metabolic workload. None of the observed changes were significantly or severely adverse and none triggered the STOT-RE classification.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

•*an effect on WBC was reported in rat and mice at doses of 100-400 mg/kg whereas NOAEL/LOAEL reported in Table 16 doesn't reflect effects occurring at a potential dose of 100 mg/kg in a 90-day study.* Following clarification was given by the RMS:

- 90 d rat study:

o Lymphocyte counts in females at 5000ppm (412-468 mg/kg bw/d) and in male and females at 50000ppm (4357-4893 mg/kg bw/d) were low. Monocyte count was reduced in females at 5000 or 50000ppm. These differences resulted in a reduction of total leukocyte count in males and females at 5000 and 50000ppm, though in males at 5000 ppm this difference was not statistically significant. The cause of reduced lymphocyte numbers at 5000 and 50000 ppm in both sexes and reduced monocytes in females was not established in this study. There was no evidence of inflammatory change in any tissue, nor was there any effect of treatment upon lymphoid tissue. The company considered these effects as of uncertain toxicological significance. Monocyte counts were still slightly low at the end of the recovery period in females previously given top dose though the difference was not as large as seen at the end of the treatment period, indicating some recovery occurred.

o During the 2 year (week 26 interim bleeding), a number of altered haematologic findings were observed, some of which attained statistical significance when compared with controls. These differences were minor or lacking dose-relationship and were attributed to normal biological

variation by the company. In the blood smears however, decreased WBC differential counts (lymphocytes: 4% wk52, 8.5% wk104; monocytes: 33% wk52, 75% wk104) were observed in the top-dose males (25000 ppm, 1223 mg/kg bw/d). For the company, some counts attained statistical significance, but overall haematological effects were considered fortuitous. In contrast, RMS considered that the effects observed in blood smears which are reported at week 52 and are increased at week 104 at top dose are probably related to treatment as such effects were also reported in short term studies.

- 90d mouse study:

o Male mice had decreased mean total leucocytes at 1000ppm (157-207 mg/kg bw/d) onwards and this effect was related to decreased neutrophils, lymphocytes and monocytes (affected at 45-day sampling). A similar trend was observed in 1000, 5000 and 10000ppm females at 45-day sampling period, although differences were not statistically significant. At the 90-day sampling period, the neutrophil count was lower for the 10000ppm females. The leucopenia observed in males and females was finally considered to be not compound related by the RMS, in the absence of a proper dose-dependency in the males (in the females the differences were not statistically significant).

o During the 18 months mouse study, occasional statistically significant hematology findings such as decreases in platelet, total leukocyte, neutrophil, or lymphocyte counts in male and or female mice were observed but were not dose- or time related, and were considered not toxicologically important for this reason.

o It was questionable whether the WBC effects in the mouse in the subchronic study were to be considered compound-related, as the effect was not replicated in the chronic mouse study, and in the absence of dose-responsiveness.. During PRAPeR tox expert consultation, it was considered that the leucopenia effects in mice at dose levels of 1000 ppm and above were of doubtful toxicological relevance on the basis of a lacking dose response. The NOAEL was rather seen at 1000 ppm (157 mg/kg bw/d) than at 100 ppm (15.5 mg/kg bw/d). EFSA concluded therefore with the experts that the subchronic toxicity NOAEL should be established at 1000 ppm (157 mg/kg bw/d), based upon the liver weight increase in the females at the next-higher dose.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

There were no changes observed in any of the test species that indicated effects considered to be clear functional disturbance, serious or significant toxic changes to specific organs. The changes observed in liver, thymus, thyroid and kidneys were addressed according to criteria for hazardous properties and a suitable NOAEL identified. None of the target organs were affected at sub-toxic doses and none of the effects warrants classification as STOT-RE.

Subsequent to a question for clarification on the relevance of the rodent 90d haematological findings for the STOT-RE classification, following conclusion was proposed:

o Contrarily to the opinion of the company, decreased lymphocyte counts in the subchronic rat study at 412 mg/kg bw and above, were considered substance-related by the RMS, since subtle decreases in the differential WBC count in the top-dose males were also observed in the chronic toxicity study (albeit at a dose >1000 mg/kg bw/d). Therefore, the effect could not be disregarded and was taken into account for the determination of the lowest relevant subchronic toxicity NOAEL.

o The leucopenia observed in the mouse studies were agreed to be of doubtful toxicological relevance in the absence of a proper dose-responsiveness.

o No mode of action could be deduced from the studies, but no immunotoxic effect, secondary to a possible drop of the WBC was observed in any study either, and the toxicological implication of the finding remained unexplained. In any case, no such effect was observed at a dose lower or equal than 100 mg/kg bw/d. Therefore, the LOAEL being detected at a dose superior to the guidance value of 100 mg/kg bw/d for a 90d oral study, RMS considered that STOT-RE classification was not triggered.

RAC evaluation of repeated dose toxicity (DSD) and specific target organ toxicity (CLP) – repeated exposure (STOT RE)

Summary of the Dossier submitter's proposal

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 100-400 mg/kg bw/d, white blood cell (WBC) count was decreased, without evidence of inflammatory change in any tissue, or any effect in lymphoid tissues (Malley, 1991, Thirlwell, 2002b, 2002c, Geary, 2002)

In rats, at dose levels ranging from 400 to 4000 mg/kg bw/d, some blood electrolytes were altered and protein in urine was increased suggesting a loss of the kidneys' ability to filter adequately blood. However, there were no effects upon kidney weight and histopathological examinations of kidneys revealed nothing abnormal. At these dose levels, liver weight was increased and hepatocyte centrilobular hypertrophy was noted at the highest dose. Some other organ weights were altered at the highest dose in rats without histological findings to support an adverse effect in these organs except in the thyroid where thyroid follicular epithelium staining indicative of lipofuscin was observed at 412/467 mg/kg bw/d (5000 ppm) onwards, but without any evidence of organ atrophy. After a 4 week rest, the rats showed good recovery.

In mice at the highest 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 a dose of 220 mg/kg bw/d onwards, liver weight was increased and centrilobular/midzonal hepatocyte hypertrophy was observed. At the highest dose, some dogs had thymus involution/atrophy.

The lowest NOAELs were found in rats and dogs respectively 41 mg/kg bw/d and 44 mg/kg bw/d.

Overall, the DS concluded that there were no effects observed in subacute, subchronic or chronic exposure studies to indicate a risk of serious damage, death, clear functional disturbance or morphological changes. Effects observed at high doses included primarily renal dysfunction and possible thymic changes and an adaptive response in the liver involving increased metabolic activity and associated cellular changes. None of the effects were seen below the guidance values triggering classification.

Comments received during public consultation

No specific comments were received. Two MSCA and one company indicated their general support for the classification proposed by the DS.

Assessment and comparison with the classification criteria

In rats, the main target organs were the liver, the thyroid and the kidneys. Some effects were also identified in the uterus and on the thymus. In the 90-day study, leucopenia and an effect on the spleen were also reported. These effects were generally observed at doses above the guidance values triggering classification. At exposure levels below the guidance values, only decreased levels of the thyroid hormones T4 and reverse T3 (rT3) were observed at 250 ppm (21 mg/kg) in the 20-week study investigating thyroid function. No effect was observed on T3 and TSH levels and no macroscopic or microscopic findings were noted at this dose. The thyroid weight was increased but not significantly. Thyroid effects were observed only at doses above the classification threshold in the 90-day or 2-year studies (including levels of T3, T4 and TSH at week 52 in the 2-year study). No functional, morphological or histological effect related to disturbance of thyroid was therefore identified at a dose relevant for classification.

In mice, the main target organs were the liver and the kidney in a 90-day and an 18-month study. Leucopenia (in the 90-day study) and effects on the spleen were also observed. These effects were generally observed at doses above the threshold for classification. At doses below the threshold, the effects were limited to a decrease in the relative kidney (-12%) and spleen (-16%) weight in females dosed with 100 ppm Lenacil (20 mg/kg bw/d) in the 18-month study. Although this effect was also observed in females at the two highest doses and was most probably linked to treatment, it was not significant, no histopathological findings were reported at this dose in the respective organs and it is not considered to indicate significant organ toxicity sufficient to trigger classification.

In dogs, indications of potential kidney dysfunction were reported in a 28-day study at all doses. This study was not described in detail and it was not possible to assess the relevance and severity of this finding. It was based on only one animal per dose and per sex and no effects were reported in the 90-day study on the kidney weight or its histological examination. In this 90-day study, effects were identified on the liver, thymus and thyroid.

At the dose relevant for classification (1000 ppm or 44/46 mg/kg bw/d) it is noted that:

- the increase in liver weight was slight and non-significant (+5.4% and +6% in males and females) and was not considered indicative of significant toxicity. Histological examination reports only two females with parenchymal foci of inflammatory cells. Considering that this finding is present in one control male and that the incidence is not significantly increased at higher doses, the interpretation of this finding is uncertain. It is therefore not considered to justify classification.
- the decrease in thymus weight was restricted to females, non-significant (-9%), and not observed at 5000 ppm. Microscopically, one male had minimal and one females slight involution/atrophy of the thymus. Considering that this finding is present in one control female and that the incidence is not significantly increased at higher doses, the interpretation of this finding is uncertain. It is therefore not considered to justify classification.
- the increase in thyroid weight (+7%) was restricted to males, slight and non-significant. In absence of any histological findings in the thyroid it was not considered to justify classification.

It is also noted that liver and thyroid effects were reported in the available two-generation study in rats (see description in the reproductive toxicity section) that are consistent with the effects observed in the repeated dose toxicity studies in rats and no effect are observed at doses relevant for STOT RE classification.

RAC therefore agrees with the DS that classification is not justified for repeated toxicity under both CLP and Directive 67/548/EEC.

Supplemental information - In depth analyses by RAC

All available studies were conducted by oral route.

28-day studies (to be compared with classification criteria range of 30-300 mg/kg for category 2 under CLP and 15-150 mg/kg for Xn; R48 under DSD)

Treatment-related toxicity was limited to a slight increase in liver weight at 50000 ppm (5025 mg/kg) in rats in a 28-day study (Thirlwell, 2002a) that was not further detailed in the CLH report.

A preliminary 28-day study (Geary, 2001) perfomed on Beagle dogs with only 1 animal per sex and per dose was also very briefly mentioned in the CLH report with the conclusion that all doses (219, 807 and 1941 mg/kg) may indicate some potential renal dysfunction.

90-day studies (to be compared with classification criteria range of 10-100 mg/kg for category 2 under CLP and 5-50 mg/kg for Xn; R48 under DSD)

In a 90-day study (Thirlwell 2002b), Wistar rats were exposed through diet to 500, 5000 or 50000 ppm (corresponding to 41/45, 412/467 or 4357/4893 mg/kg in males/females, respectively). Significantly decreased WBC, lymphocytes and monocytes counts, decreased phosphorus and increased urinary proteins were observed in females and/or males from 5000 ppm. At 50000 ppm, significantly decreased eosinophils counts in females, and increased specific urine gravity in males were observed.
Effects on relative organ weight consisted of increased liver weight in males and females from 5000 ppm (significant in high dose females only: +21%) and at the highest dose in increased spleen weight (+9% in males and +10% in females), increased kidney weight in males (+6%), increased thyroid+parathyroid weight (+21% in males and +12.5% in females) and increased uterus+cervix weight in females (+13%). Histopathology findings were restricted to liver centrilobular hypertrophy in 5/9 males and 4/10 females at the highest dose. This effect was not observed in animals examined after a 5-week recovery period.

In a subsequent report (Thirlwell, 2002c), additional histopathological examination of the thyroid tissues was performed. In females, there was a treatment-related increase in the incidence and severity of Schmorls positive staining at 50000 ppm and a slight increase in the severity of this finding in males at 50000 ppm. The slightly increased incidence of staining in females at 5 000 ppm was within the background incidence. Positive staining is indicative of lipofuscin in the follicular epithelium. Lipofuscin pigment is associated with the degradation of the cell membrane and could suggest the presence of persistent chronic injury.

An additional study (Whittaker, 2004) was performed to investigate thyroid function. Wistar rats (18 females/dose) were administered 250 or 50000 ppm Lenacil (21 or 4 421 mg/kg bw/d) in diet for 20 weeks. Mean T4 concentration was significantly decreased in both test groups after 10 weeks of treatment (21 and 20 nmol/L at 250 and 50000 ppm vs. 32 in controls) but not after 19 weeks. Mean reverse T3 (rT3) concentration was significantly decreased in both test groups after 19 weeks of treatment (0.19 and 0.19 nmol/L at 250 and 50000 ppm vs. 0.23 in controls). No change was observed on T3 and TSH levels. Thyroid weight was increased to the same level in both dose groups and it was significant only at the high dose (0.0159 g vs. 0.0129 g in controls). Macroscopic examination revealed 6 animals of the 50000 ppm group with dark thyroid. There was no clear reduction in the ability of the thyroid to take up and accumulate ^{125}Iodide. The ability of thyroid peroxidases to convert the ^{125}Iodide to organic compounds was unaffected by treatment.

In a 90-day study (Malley, 1991), CD-1 mice were exposed through diet to 100, 1000, 5000 or 10 000 ppm (corresponding to 15/20, 157/207, 787/1 127 or 1 616/2 150 mg/kg in males/females, respectively). Significantly decreased white blood cell, neutrophils and monocytes counts in male mice and increased hematocrite in females from 1000 ppm were reported. Non-significant decreases in neutrophil count and in platelets were also observed in females from 5000 ppm. Effect on relative organ weight consisted in increased liver weight in females from 1000 ppm (significant in high dose females only: +18%) and in males from 1000 ppm and increased spleen weight in high dose females. Histopathology findings were observed only at the highest dose in the liver (extramedullar hematopoiesis in 2/10 males and 4/10 females and single cell necrosis in 1/10 female) and in the spleen (lymphoid cell hyperplasia in 2/10 males and extramedullar hematopoiesis in 1/10 males and 5/10 females).

In a 90-day study (Geary, 2002), Beagle dogs (n=4/sex/dose) were exposed through diet to 1000, 5000 or 25000 ppm. It corresponds to 44/46, 221/225 or 1121/1102 mg/kg in males/females, respectively. Decreases of body weight gain were observed at all doses (up to 13% in male and 15% in females at the highest dose) but statistical significance was not attained and it was not considered to represent an effect of the treatment. Significantly decreased eosinophils counts in females from 5000 ppm, increased activated partial thromboplastin time (APPT) in males at the highest dose (+22%) and decreased blood protein concentration in female at the highest dose (-7%) were observed and in both sexes at the highest dose, increased alkaline phosphatase (+ 53% in males and 31% in females) and variations in sodium (+1.3% in males and -15% in females).

Effect on relative organ weight consisted in a non-significant increase in liver relative weight in males and females from 1000 ppm, a non-significant decrease in thymus relative weight in females at 1000 ppm and in males and females at the highest dose (-38% in males and -16% in females) and an increase in thyroid+parathyroid weight from 1000 ppm in males and 5000 ppm in females that was significant only in males at 25000 ppm (-23%).
Histopathology findings were observed in the liver and in the thymus as reported in Table 1 below.

Table 1 – Main findings in the 90-day dog study

Dose	0		1000 ppm		5000 ppm		10 000 ppm	
Sex	M	F	M	F	M	F	M	F
Organ weight:								
Liver relative			(↑5.4%)	(↑6%)	(↑6.6%)	(↑15%)	(↑15%)	(↑13%)
Thymus relative				(↓9%)			(↓38%)	(↓16%)
Thyroid + parathyroid			(↑7%)		(↑15 %)	(↑21%)	↑23%*	(↑33 %)
Histopathology:								
Liver hepatocyte								
Centrilobular hypertrophy minimal	0	0	0	0	2	0	3	1
Midzonal hypertrophy minimal	0	0	0	0	2	0	3	1
Vacuolation focal	0	0	0	0	0	0	0	1
Parenchymal inflammatory cell foci	1	0	0	2	1	0	1	2
Thymus involution/atrophy								
Minimal/slight/total	0/0/0	1/0/1	1/0/1	0/1/1	0/1/1	1/0/1	2/0/2	0/0/0

*p<0.05

<u>18-month study (to be compared with classification criteria range of 2-20 mg/kg for category 2 under CLP and 1-10 mg/kg for Xn; R48 under DSD)</u>

In an 18-month study (Malek, 1994), CD-1 mice (n=80/sex/dose) were exposed through diet to 100, 2 500 or 7 000 ppm (corresponding to 14/20, 332/482 or 977/1358 mg/kg bw/d in males/females, respectively). No significant effect related to treatment was reported on body weight or hematology. Relative liver weight was increased at the highest dose (+16% in males and +6.7% in females) and reached significance in females. Decreases in relative kidney and spleen weight were observed from 100 ppm in females only and was not statistically significant. Histopathological findings were reported in the liver in high dose males and consisted of centrilobular hypertrophy in 7/80 animals (0 in controls).
Neoplastic effects are described in the carcinogenicity section.

2-year study (to be compared with classification criteria range of 1.25-12.5 mg/kg for category 2 under CLP and 0.65-6.50 mg/kg for Xn; R48 under DSD)

In a 2-year study (Thirlwell, 2004), Wistar rats were exposed through diet to 250, 2 500 or 25000 ppm (corresponding to 14/19, 139/188 or 1446/1894 mg/kg bw/d in males/females, respectively) for 52 (n=20/sex/dose) or 104 weeks (n=50/sex/dose). Significant change on body weight was restricted to a decrease in female at the highest dose (-9%) at week 104. Changes in haematology and blood chemistry were transient and without dose-response and were considered as normal biological variations. At week 52, levels of T3 and T4 were not affected by treatment. An increase in TSH was noted in 5 males and 3 females at the highest dose without reaching statistical significance (+33 and +27% respectively). At week 104, relative heart, brain, thyroid (+40% in males and +49% in females, non-significant), kidney and liver weight were increased in both sexes at the highest dose.
At the interim 52-week sacrifice, macroscopic examination revealed 5/20 males and 10/19 females with dark thyroid at the highest dose.

After 104 weeks, an increased incidence of dark thyroid was observed only in the female at the highest dose (12/50). Other significant findings were increased incidence of thymus with dark area in females at 2 500 ppm (8/50 vs. 1/80 in controls), uterus with fluid distension in females at the highest dose (6/50 vs. 0/50 in controls) and masses in the mammary area in females at 250 ppm (18/50 vs. 7/50 in controls). Non-neoplastic histopathology findings were observed in the eye, the liver and the thyroid as reported in Table 2 below. Neoplastic effects are described in the carcinogenicity section.

Table 2 – Histological findings in the 2-year rat study

Dose	**0 ppm**		**250 ppm**		**2500 ppm**		**25000 ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Eyes: unilateral lenticular degeneration	4/50	1/50	3/15	1/18	1/5	2/11	2/50	7*/49
Retina loss of outer nuclear layer bilateral	3/50	0/50	1/15	0/18	1/5	0/11	7*/50	1/49
Liver :								
Centrilobular vacuolation hepatocytes	16/50	2/50	21/50	4/50	18/50	2/50	28*/50	2/50
Centrilobular hypertrophy hepatocytes	11/50	1/50	11/50	0/50	15/50	1/50	26*/50	4/50
Thyroid								

Increased luminal concretions	11/50	5/50	16/50	6/50	17/49	10/50	33*/50	32*/49

*p<0.05

4.9 Germ cell mutagenicity (Mutagenicity)

4.9.1 Non-human information

The data set, prepared and submitted in the dossier in accordance with the requirements of Directive 91/414/EEC, has been reviewed at Member State level and by the EFSA.

Table 17: Summary table of relevant in vitro and in vivo mutagenicity studies

Type of test Cell/Test species	Conditions	Tested batch; Purity	Results	References
In vitro tests				
Salmonella typhimurium, strains TA1535, TA1537, TA98 and TA100, and *Escherichia coli*, strain WP2uvrA/pKM101 (CM891) OECD test guideline n° 471	2 tests performed with or w/o S9 mix. *First test* : standard plate incorporation assay; *Second test* involved a 30 minute pre-incubation stage. Concentrations of Lenacil technical 5 to 5000 µg/plate in DMSO	Batch No. 141712003, purity 98.6%	Negative	May, 2001
Mouse lymphoma L5178Y cells OECD test guideline n° 476	Lenacil technical suspended in culture medium at concentrations up to 5000 µg/mL for 3 hours w/ or w/o S9 mix and for 24 hours in the absence of S9 mix	Batch No. 141712003, purity 98.6%	Negative	Clare, 2003
Unscheduled DNA synthesis assay using adult rat hepatocyte Dir 87/302/EEC Annex V B	0.078 µg/mL to 10µg/mL were tested. Two independent assays. DMSO was used as solvent	Batch no. 8903, purity not stated in report	Negative	Mohammed and Riach, 1989
Chromosome Aberration test in Human Lymphocytes Dir 2000/32/EEC Annex IV A	3 hour exposure + 17 hour recovery period w or w/o S9 mix rat liver. *First test*: 39.06, 78.13, 156.05, 312.5, 625, 1250, 2500 and 5000 µg/ml. *Second test*: 312.5, 625, 1250, 2500 and 5000 µg/ml in the absence of S9 mix, and at 625, 1250, 2500 and 5000 µg/ml in the presence of S9 mix.	Batch No. 141712003, purity 98.6%	Positive w/o S9, negative with S9	Allais, 2001
In vivo tests				
Bone marrow micronucleus test OECD test guideline n° 474	Mice, gavage, 500, 1000, 2000 mg/kg bw/d	Batch n° 141712003, purity 98.6%	Negative	Mehmood, 2001

4.9.1.1 In vitro data

In vitro bacterial cell gene mutation studies

- Lenacil technical: Bacterial Mutation Assay (May, 2001) Huntingdon Life Sciences, report n° ACD 016/013217

Material and methods:

GLP status: yes
Guideline: study is in compliance with Dir EEC 2000/32/EEC Annex 4D.
Salmonella typhimurium, strains TA1535, TA1537, TA98 and TA100, and Escherichia coli, strain WP2uvrA/pKM101 (CM891), were exposed to Lenacil technical (Batch No. 141712003, purity 98.6%) diluted in DMSO. Two independent mutation tests were performed in the presence and absence of liver preparations from Aroclor 1254-treated rats (S9 mix). The first test was a standard plate incorporation assay; the second involved a 30 minute pre-incubation stage. Concentrations of Lenacil technical up to 5000 µg/plate were tested. Mixtures of the test dilution, positive control or negative control, S9 mix or phosphate buffer and bacterial culture were added to agar containing a trace of histidine and tryptophan and overlaid onto Petri dishes containing minimal agar. All plates were incubated at 37°C for ca 72 hours. After this period, the appearance of the background bacterial lawn was examined and revertant colonies counted. Positive controls were sodium azide, 9-aminoacridine, 2-nitrofluorene, AF-2, 2-aminoanthracene, benzopyrene and gave the expected results.
The study is accepted.

Findings:

No substantial increases in revertant colony numbers over control counts were obtained with any of the tester strains following exposure to Lenacil technical at any concentration in either the presence or absence of S9 mix. No cytotoxicity was observed.

Conclusion: Lenacil technical showed no evidence of mutagenic activity in this bacterial system under the test conditions employed.

- Mutagenicity testing of IN E 1512-2 in the Salmonella Typhimurium plate incorporation assay (Reynolds, 1989) DuPont USA, HLR 550-89.

Material and methods:

GLP status: yes (no attest of competent authority)
Guideline: study is 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).
Deviation from official protocol: strain TA 102 and E.coli WP2uvrA were not included in the study. In tier 2, the tested compound seems not to be lenacil
Lenacil technical synonyms: IN E1512-2= Haskell No. 17980, purity 99.4%.
Doses of Lenacil technical were selected on the basis of the test article to tester strain S. typhimurium TA98. Toxicity was observed at 5000 µg/plate without, but not with activation. Therefore doses of up to 4000 µg/plate were chosen in the plate incorporation assay for the test with activation and up to 5000 mg/plate without activation. Tester strains chosen were TA1535, TA97, TA98, and TA100. Negative control was DMSO and a number of positive control articles were included (2 aminoanthracene, 2-nitrofluorene, sodium azide, acridine) to demonstrate the sensitivity of the test system.
The study is accepted.

Findings:

Lenacil technical did not produce a positive response in any of the tester strains with and without metabolic activation.

The positive control articles demonstrated the sensitivity of the test system.

Conclusion: Lenacil technical was non-mutagenic in the reverse mutation assay with and without metabolic activation

- Mutagenic Activity of Uracil, 3-Cyclohexyl-5,6-Trimethylene in the Salmonella/Microsome Assay, DuPont USA, HLR 601-77, (Russell, 1977)

Material and methods:

GLP status: no
Guideline: study is 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).
Deviation from official protocol: 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.
Lenacil technical (Code: INB-634-50), purity not specified.

Findings and conclusions:
Lenacil technical did not produce a positive response in any of the tester strains with and without metabolic activation.
Doses of Lenacil technical were selected on the basis of the test article to tester strain *S. typhimurium* TA1535.
Therefore doses of up to 500µg/plate were chosen for the test with activation and also up to 500 mg/plate without activation. Tester strains chosen were TA1535, TA1537, TA1538T, TA98 and TA100. Positive control substance was 2-aminoanthracene.
The study is used to provide additional information.

- Mutagenicity testing of DPX-B634-107 (Lenacil) in the Salmonella Typhimurium plate incorporation assay. DuPont USA, HLR 413-94 (D'Amico, 1994)

Material and methods:

GLP status: no
Guideline: study is 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).
Deviation from official protocol: strain TA 102 and E.coli WP2uvrA were not included in the study. Experimental protocol not described.
Lenacil technical (DPX-B634-107), purity: not specified. Doses of Lenacil technical were selected on the basis of the test article to tester strain S. typhimurium TA98. Dose levels of up to 5000 µg/plate were chosen for the test with activation and also up to 5000 mg/plate without activation. Tester strains chosen were TA1535, TA97, TA98, and TA100.
The study is accepted to provide additional information.

Findings: Lenacil technical did not produce a positive response in any of the tester strains with and without metabolic activation.

Conclusion: Lenacil technical was non-mutagenic in the reverse mutation assay with and without metabolic activation

Published study:

- Lack of genotoxic and cytotoxic effects of the herbicide lenacil on mouse tumor cells and on some Salmonella typhimurium strains(Grancharov K, Gorneva G, Mladenova J, Norpoth K, Golovinsky E (Arzneimittelforschung, 1986, 36(11), 1660-1663.)

Findings and conclusions:

The effects of (lenacil) on macromolecular synthesis, thymidilate synthetase activity, and viability and cell cycle progression were studied using Friend leukemia (FL). P388 and Ehrlich ascites tumor cells in suspension, and its cytogenetic effects were studied in a Salmonella/mammalian microsome assay using both frameshift and base-substitution tester strains. At a concentration of 0.5mmol/l lenacil inhibited 45 to 70% thymidine incorporation into DNA fraction, while incorporations of uridine into RNA and leucine into protein were less affected. Thymidilate synthetase activity in P388 cells as assayed by the release of tritiated water from 5-3H-deoxyuridine was inhibited by the compound to about 20%. Lenacil neither showed an in vivo inhibitory action on thymidine incorporation into acid-insoluble material in P388 cells, nor on thymidilate synthetase activity after a 24 or 48 h treatment. The compound did not change the melting temperature of isolated DNA. Studies of lenacil's effect on cell cycle kinetics of FL cells demonstrated that 48 h treatment increased the percentage of S-phase cells. Lenacil exerted a weak cytotoxic effect on FL cells. At concentrations above 0.1 mmol/l it inhibited cell growth the effect being nonlethal. Cytogenetic studies of lenacil revealed no indication of its mutagenicity against Salmonella typhimurium TA97, TA98, TA100 and TA102.

In vitro mammalian cell gene mutation studies

- Lenacil technical; In Vitro Mammalian Cell Gene Mutation Test (Clare, 2003) (ACD 053/023530] Huntingdon Life Sciences, Huntingdon, UK)

Material and methods:

GLP status: yes
Guideline: study is not fully in compliance with Dir EEC 2000/32/EEC Annex 4E or 87/302 or OECD test guideline n° 476 (1997-84).
Deviation from official protocol: diameter of colonies was not measured for control cells (OK for 87/302).
Cultures of mouse lymphoma L5178Y cells were exposed to Lenacil technical(Batch No. 141712003, purity 98.6%) suspended in culture medium at concentrations up to 5000 µg/mL for 3 hours in both the absence and presence of supplemented Aroclor-induced rat liver fraction (S9 mix) and for 24 hours in the absence of S9 mix. The cells were washed and resuspended. Aliquots were diluted and plated for determination of Day0 survival. Further aliquots were diluted to 2 x 105 cells/ml and incubated for 48 hours, with readjustment of cell density after 24 hours. Using 96-well plates, cloning efficiency was assessed by plating at 1.6 cells/well, incubating at 37°C in an atmosphere of 5% CO_2 in air for at least 7 days, and counting empty wells. Cells were also plated at 2 x 103 cells/well in selective medium containing trifluorothymidine (lethal to TK-/- mutants) and incubated for 10-14 days. Mutant frequency (forward mutation to the homozygous TK-/- form) was calculated relative to survival. 3MC, and MMS were used as positive controls and induced significant increases in mutant frequency.
The study is accepted.

Findings:

There were no significant increases in mutant frequency in either the presence or absence of S9 mix.

Conclusion: Lenacil technical did not demonstrate mutagenic potential in this *in vitro* cell mutation assay, under the experimental conditions described.

In vitro mammalian cytogenetic test studies

- Lenacil technical, In vitro Mammalian Chromosome Aberration Test in Human Lymphocytes (Allais, 2001) Huntingdon Life Sciences [report n° ACD 017/013707]

Material and methods:

GLP status: yes
Guideline: study is in compliance with Dir EEC 2000-32/EEC Annex 4A.
Human blood was collected aseptically from two healthy non-smoking male donors, pooled and diluted with RPMI tissue culture medium supplemented with foetal calf serum, heparin, glutamine and antibiotics.
Lenacil technical (Batch No. 141712003, purity 98.6%) was tested as a suspension in culture medium at the highest final concentration of 5000 µg/ml. The study was performed on two separate occasions and on duplicate cultures. A three hour exposure followed by a 17 hour recovery period was used in both tests and in both the absence and presence of S9 mix derived from rat liver. In the first test, cultures were exposed to the test substance at final concentrations of 39.06, 78.13, 156.05, 312.5, 625, 1250, 2500 and 5000 µg/ml. In the second test, cultures were exposed to the test substance at 312.5, 625, 1250, 2500 and 5000 µg/ml in the absence of S9 mix, and at 625, 1250, 2500 and 5000 µg/ml in the presence of S9 mix. Vehicle and positive controls were also prepared.
Two hours before the cells were harvested; mitotic activity was arrested by addition of Colcemid. After two hours incubation, the cells were treated with a hypotonic solution and fixed. Slides were then prepared and stained with Giemsa.
One hundred metaphase figures were examined, where possible, from each culture. The incidence of polyploid metaphase cells, out of 500 metaphase cells, was determined quantitatively for negative control cultures and cultures treated with the highest dose level of the test substance used in the analysis for chromosomal aberrations. The number of aberrant metaphase cells in each treatment group was compared with the solvent control value using Fisher's test. Criteria for evaluation of the results are well defined.
The study is accepted.

Findings:

On the basis of the mitotic index data, the following concentrations were selected for metaphase analysis:

First test, without S9 mix: 625, 1250, 2500 and 5000 µg/mL.

First test with S9 mix: 1250, 2500 and 5000 µg/mL.

Second test, without S9 mix: 625, 2500 and 5000 µg/mL.

Second test with S9 mix: 1250, 2500 and 5000 µg/mL.

In the absence of S9 mix, Lenacil technical caused statistically significant increases in the proportion of metaphase figures containing chromosomal aberrations, at 5000 µg/ml in the first test ($P<0.001$), and at 2500 and 5000 µg/mL in the second test ($P<0.01$ and $P<0.001$, respectively), when compared with the solvent control.

In the presence of S9 mix, Lenacil technical caused no statistically significant increases in the proportion of metaphase figures containing chromosomal aberrations at any dose level, in either test.

No increases in the proportion of polyploid cells were seen in either test.

All positive control compounds caused large, statistically significant increases in the proportion of aberrant cells, demonstrating the sensitivity of the assay.

Table 17-1: Summary of results of chromosomal aberrations in human lymphocytes (Test 1)

Exposure period/ S9 mix	**Chroma-tid type**		**Chromo-some type**		**Concentration of Lenacil technical**	**Cells with aberrations Excluding gaps**			**Cells with aberrations Including gaps**			**Relative Mitotic**
-S9 mix	**ctb %**	**cte %**	**csb**	**cse**	**(µg/ml)**	**Individual values (%)**		**Mean (%)**	**Individual values (%)**		**Mean (%)**	**Index (%)**
3 hours	1 3	1			0 (Culture medium)	1	2	1.5	1	2	1.5	100
	1 1				625	1	1	1.0	1	1	1.0	82
	1 1				1250	1	1	1.0	1	1	1.0	82
	2 6				2500	2	4	3.0	2	4	3.0	68
	10 23				5000	7	16	11.5**	7	16	11.5**	54
	12 12	4 2	1 1		0.2 (Mitomycin C)	17	12	14.5**	17	12	14.5**	-
+ S9 mix												

Exposure period/ S9 mix	**Chroma-tid type**		**Chromo-some type**		**Concentration of Lenacil technical**	**Cells with aberrations Excluding gaps**			**Cells with aberrations Including gaps**			**Relative Mitotic**
-S9 mix	**ctb %**	**cte %**	**csb**	**cse**	**(µg/ml)**	**Individual values (%)**		**Mean (%)**	**Individual values (%)**		**Mean (%)**	**Index (%)**
	1				0 (Culture medium)	1	0	0.5	1	0	0.5	100
					1250	0	0	0.0	1	0	0.5	90
	3				2500	2	0	1.0	2	0	1.0	84
	1	1			5000	1	0	0.5	1	0	0.5	75
	10 11	1 3	2 2		6 (Cyclophosphamide)	12	13	12.5**	12	13	12.5**	-

Statistically significant at **p<0.001; *: p<0.01

Ctb/csb= chromatid /chromosome break

Cte/cse= chromatid/chromosome exchange

Table 17-2: Summary of results of chromosomal aberrations in human lymphocytes (Test 2)

Exposure period/ S9mix	**Chroma-tid type**		**Chromo-some**		**Concentration of Lenacil technical**	**Cells with aberrations Excluding gaps**			**Cells with aberrations Including gaps**			**Relative Mitotic**
- S9mix	**ctb %**	**cte %**	**csb %**	**cse%**	**(µg/ml)**	**Individual values (%)**		**Mean (%)**	**Individual values (%)**		**Mean (%)**	**Index (%)**
3hours	1 1				0 (Culture medium)	1	1	1.0	1	1	1.0	100
	1				625	0	1	0.5	0	1	0.5	124
	5 6				2500	5	6	5.5*	5	6	5.5*	61
	25 14		1		5000	16	11	13.5**	16	11	13.5**	39
	10 11	4 2			0.1 (Mitomycin C)	13	11	12.0**	13	11	12.0**	-
+ S9mix												
3hours	1				0 (Culture medium)	0	1	0.5	0	1	0.5	100
			1		1250	1	0	0.5	1	0	0.5	79
	1		1		2500	2	2	2.0	2	2	2.0	58

	2		3									
	1 2		1		5000	1	1	1.0	1	1	1.0	56
	9 8	1	1 3	Other 1 1	6 (Cyclophosphamide)	11	11	11.0**	11	11	11.0**	-

Statistically significant at **p<0.001; *: p<0.01

Ctb/csb= chromatid /chromosome break

Cte/cse= chromatid/chromosome exchange

Conclusion:

It is concluded that Lenacil technical has shown evidence of clastogenic activity, in this in vitro cytogenetic test system, in the absence of S9 mix only, under the experimental conditions described. No clastogenic activity was observed in the presence of S9 mix.

- Lenacil: Assessment of genotoxicity in an unscheduled DNA synthesis assay using adult rat hepatocyte primary cultures, Inveresk Research, IRI 6135, (Mohammed and Riach, 1989)

Material and methods:

GLP status: yes (no attest of competent authority)

Guideline: study is in compliance with Dir EEC 87/302/EEC Annex VB.

Lenacil (batch no. 8903, purity not stated in report) in DMSO was tested for its ability to induce unscheduled DNA synthesis (UDS) in primary cultures of adult rat hepatocytes as measured by silver grain counts in photographic emulsion formed by radiation from [6-3H]-thymidine taken up by the cells. Cultures were established with cells derived from the collagenase-perfused liver of Fischer 344 rats. Eight one-half decreasing concentrations of Lenacil from 0.078 µg/mL-1 to 10 µg/mL-1 were tested. Two independent assays were performed. Vehicle controls were treated with DMSO only. Positive control substance 2AAF and Michler's ketone demonstrated the sensitivity of the test system. Criteria for a positive test were well defined.

The study is accepted.

Findings:

No significant evidence of unscheduled DNA synthesis was obtained at any test concentration of Lenacil, in either of the 2 independent experiments. Direct and indirect acting positive control compounds demonstrated the sensitivity of the test system.

Conclusion:

Lenacil technical did not induce unscheduled DNA synthesis in cultures of primary rat hepatocytes when tested at concentrations extending into the toxic range.

Conclusion on *in-vitro* mutagenicity evaluation:

Lenacil was tested in a battery of *in vitro* studies including a bacterial reverse mutation assay, a mouse lymphoma cell mutation assay, a cytogenetic test for clastogenicity in human lymphocytes and a test for unscheduled DNA synthesis in rat primary hepatocytes. The results were all negative for mutagenic potential, with or without metabolic activation, except for the positive indication of clastogenicity, without S-9, in human lymphocytes but the overall assessment was that Lenacil is not genotoxic and this was confirmed by the *in vivo* response.

4.9.1.2 In vivo data

- Lenacil technical – Mouse micronucleus test (Mehmood, 2001) ACD 018/013472, Huntingdon Life Sciences.

Material and methods:

GLP status: yes

Guideline: study is not fully in compliance with Dir EEC 2000/32/EEC Annex 4C or 92/69-84/449/EEC or OECD test guideline n° 474 (1997-83).

Deviation from official protocol: females were not included in the test; oral route was used although it was not demonstrated that lenacil reached bone marrow. However, from the ADME studies it appeared that marrow was reached. Results are reported w/o standard deviation. Only 7 mice were tested.

Mice were treated with a single oral administration of Lenacil technical in 0.5% methylcellulose (Batch No. 141712003, purity 98.6%) at dose levels of 500, 1000 and 2000 mg/kg bodyweight. A preliminary toxicity test had previously shown that a dose of 2000 mg/kg (the standard limit dose for the micronucleus test) was tolerated. This level was therefore selected as an appropriate maximum for use in the micronucleus test.

The test substance, negative and positive control groups were administered orally by intragastric gavage. The negative control group received the vehicle, 0.5% w/v methylcellulose and the positive control group received mitomycin C at 12 mg/kg bodyweight. Following the preliminary toxicity test, no substantial differences in toxicity were observed between the sexes, in line with current guidelines, the micronucleus test was performed using male animals only. Bone marrow smears were obtained from 7 male animals in the negative control, each of the test substance groups and 5 male animals in the positive control group 24 hours after dosing. In addition bone marrow smears were obtained from 7 male animals in the negative control and high level treatment groups 48 hours after dosing. One smear from each animal was examined for the presence of micronuclei in 2000 immature erythrocytes. The proportion of immature erythrocytes was assessed by examination of at least 1000 erythrocytes from each animal. A record of the incidence of micro-nucleated mature erythrocytes was also kept. Criteria for positive test are clearly reported and acceptable.

The study is accepted.

Findings:

Following the preliminary toxicity test performed at the limit dose of 2000mg/kg bw with males and females, no substantial difference in toxicity were observed between sexes and the main test was performed using males only.

No statistically significant increases in the frequency of micronucleated immature erythrocytes and no substantial decreases in the proportion of immature erythrocytes were observed in mice treated with Lenacil technical and killed 24 or 48 hours later, compared to vehicle control values ($P>0.01$ in each case).

The positive control compound, mitomycin C, produced significant increases in the frequency of micronucleated immature erythrocytes ($P<0.01$).

Conclusion:

Lenacil technical did not show any evidence of causing chromosome damage or bone marrow cell toxicity when administered orally by intra-gastric gavage in this in vivo test procedure.

4.9.2 Human information

No data available.

4.9.3 Other relevant information

No other data available.

4.9.4 Summary and discussion of mutagenicity

Lenacil technical showed no evidence of mutagenic activity in the *Salmonella typhimurium* bacterial system under the test conditions employed.

Lenacil technical did not demonstrate mutagenic potential in the *in vitro* mouse lymphoma cell mutation assay, under the experimental conditions described.

Lenacil technical has shown evidence of clastogenic activity, in human lymphocytes *in vitro* cytogenetic test system, in the absence of S9 mix only. No clastogenic activity was observed in the presence of S9 mix.

Lenacil technical did not induce unscheduled DNA synthesis in cultures of primary rat hepatocytes when tested at concentrations extending into the toxic range.

Lenacil technical did not show any evidence of causing chromosome damage or bone marrow cell toxicity when administered orally to mice *in vivo*.

Overall, it can be concluded that Lenacil is not genotoxic.

4.9.5 Comparison with criteria

Lenacil was tested in a battery of *in vitro* and *in vivo* assays without displaying any signs of mutagenic activity. There was a positive response in the *in-vitro* clastogenicity assay in, the absence of S9, but not in the presence of S9. In addition, the *in-vivo* study was negative.
Based on the results and the abovementioned criteria, no classification for mutagenicity is required.

Lenacil was not found to give a clearly positive response in any of the *in vitro* or *in vivo* tests conducted, and as such does not meet the DSD criteria for classification as a Category 1, 2 or 3 mutagen.

4.9.6 Conclusions on classification and labelling

Lenacil was concluded to be non-genotoxic, and consequently no classification for mutagenic hazard is required.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

Lenacil technical showed no evidence of mutagenic activity *in vitro*, in the *Salmonella typhimurium* bacterial system, no mutagenic potential in the *in vitro* mouse lymphoma cell mutation assay and did not induce unscheduled DNA synthesis in cultures of primary rat hepatocytes when tested at concentrations extending into the toxic range. However, Lenacil technical has shown evidence of clastogenic activity in human lymphocytes in *in vitro* cytogenetic test system, in the absence of S9 mix only. No clastogenic activity was observed in the presence of S9 mix.
Lenacil technical did not show any evidence *in vivo*, of causing chromosome damage or bone marrow cell toxicity when administered orally to mice.
Overall, the DS concluded that Lenacil is not genotoxic and no classification is proposed for mutagenicity.

Comments received during public consultation

No specific comments were received. Two MSCA and one company indicated their general support for the no classification proposed by the DS.

Assessment and comparison with the classification criteria

Considering the negative outcome of the available *in vivo* bone marrow micronucleus test that was performed according to OECD TG 474 up to the limit dose of 2000 mg/kg, Lenacil is considered to be non-mutagenic *in vivo*.

Therefore, RAC agreed with the DS that classification for mutagenicity is not warranted.

4.10 Carcinogenicity

The data in the DAR of 2007 were Peer reviewed in April-May 2009 in a series of scientific meetings with member State experts. A final discussion of the outcome of the consultation of experts took place during a written procedure with the Member States in July 2009. Taking into account the incidence of mammary gland and lung tumours, the classification Carc. cat. 3, R40 'Limited evidence of a carcinogenic effect' was initially proposed.

The company proposed in a position paper prepared by Andrew in 2011 that:

"*The oncogenic relevance of the tumours observed at high dose levels was compared with various historical databases and it was concluded that Lenacil is unlikely to induce any treatment related increase in either of these tumour types*".

In the DAR, tumour incidence was compared with historical control data provided by the company at that time and related to ten studies initiated at the test laboratory in 1999 or earlier and reported in the original study report. In the Position Paper of the company (2011), updated historical control data are provided covering a time period when the study was performed. This updated database is related to nineteen studies performed at the test laboratory from 2001-2006 where the background incidences of mammary adenocarcinoma is much higher (0.02%-22%) than that reported in the historical control data, background range original report (0.0%-6.7%) making the conclusion of RMS and EFSA inappropriate.

Table 18: Summary table of relevant carcinogenicity studies

Test type Doses tested (ppm) and mg/kg b.w./d	Species	NOAEL (ppm) and mg/kg b.w./d	LOAEL (ppm) and mg/kg b.w./d Findings	Reference
Rat, oral (diet), *(0, 250, 2500, 25000 ppm)* 0, 14.3, 139.1, 1446 mg/kg bw/d 12 months toxicity phase of combined toxicity and carcinogenicity OECD N° 453	Wistar rat	*(2500 ppm)* 139.1 mg/kg bw	*(25000 ppm)* 1446 mg/kg bw/d organ weight effects, thyroid discolorations, hepatic hypertrophy, urinary protein excretion, some eye effects	Thirlwell, (2004)
Rat, oral (diet), *(0, 250, 2500, 25000 ppm)* 0, 12, 118.4, 1223.2 mg/kg bw/d 24 months carcinogenicity phase	Wistar rat	*(250 ppm)* 12 mg/kg bw/d	*(2500 ppm)* 118 mg/kg bw reduced weight gains, reduced motor activity, organ weight effects, thyroid discolorations, increased thyroidal luminal concretions, centrilobular hepatocyte hypertrophy and vacuolation, mammary gland tumours	Thirlwell, (2004)
2007: *Mammary adenocarcinoma:* incidence of malignant mammary adenocarcinoma in female rats at top dose (10%) and at intermediate dose (12%) were slightly outside the historical controls of the laboratory (6.7%) and within the data of Charles River laboratories (13.33%), the incidence is considered an equivocal finding.				
Mouse, oral (diet) *(100, 2500, 7000 ppm)* 0, 13.8, 332, 977 mg/kg bw/d carcinogenicity study	CD-mice	*2500 ppm in males* 332 mg/kg bw/d, and *7000 ppm in females* 1358 mg/kg bw/d	*(7000 ppm)* 977 mg/kg bw/d hepatocellular adenomas, lung alveolar tumours	Malek, (1994)
2007: number of any type *lung alveolar neoplasms* in males receiving 7000 ppm is slightly increased (26/80, 32%) compared to the concurrent untreated control (18/80, 22.5%), it is statistically significant ($p<0.05$) and is outside the range of the historical controls at the testing facility (18-21%). However, because this increase is small, and did not demonstrate decreased latency compared to controls, it is considered to be of equivocal toxicological significance.				

Position of the company (Andrew D, 2011), attached to this report.

4.10.1 Non-human information

Based on test results of the studies with Lenacil, no classification or risk phrases are appropriate for human health. The EFSA conclusion indicates Lenacil is not classified as Toxic or Very Toxic. No classification is required for Lenacil regarding developmental or reproductive effects. The parent molecule was extensively evaluated to determine whether a Cat 3; R40 classification for carcinogenicity, based on a slight increase in mammary gland tumour – adenocarcinoma in rats, was required (although lung alveolar tumours and hepatocellular adenoma were apparent in mice at very slightly greater incidence than in historic controls, these were considered species specific effects having no relevance to the human hazard assessment). The conclusion was that no carcinogenic category was appropriate and classification is not proposed.

Lenacil technical was non-mutagenic in four reverse mutation assays (with or without metabolic activation). An assessment of genotoxicity in an unscheduled DNA synthesis assay in rats indicated Lenacil did not induce unscheduled DNA synthesis. An *in vitro* mammalian cell gene mutation assay was completed in cultured mouse lymphoma L5178Y cells. Lenacil showed no mutagenic potential in this study. An *in vitro* mammalian chromosomal aberration test in human lymphocytes showed some evidence for clastogenicity in the absence of S9 but not in the presence of metabolic activation and it was concluded that overall Lenacil was not clastogenic. Despite the overall negative response to the battery of *in vitro* mutagenicity assays, the slight possibility of clastogenicity was indicated and an *in vivo* assay was completed to further investigate this possibility. The mouse micronucleus test showed no increase in the frequency of micronucleated cells and it was concluded that Lenacil showed no evidence of causing chromosomal damage or bone marrow cell toxicity in mice, confirming the conclusion of no *in vitro* clastogenic response.

Evidence for a carcinogenic potential for Lenacil is equivocal and no mechanism of oncogenicity was established. Data from carcinogenicity studies in rats and mice, together with background incidence rates derived from various historical databases, support the proposition that lenacil administration is not associated with a toxicologically significant increase in mammary tumour incidence. Similarly pulmonary tumours in male mice were also shown to fall within historical ranges and no clear evidence of a treatment-association with Lenacil was established.

4.10.1.1 Carcinogenicity: oral

Testing was performed in Wistar rats and CD-mice to assess carcinogenic potential of Lenacil.

Full description of the evaluation follows:

1: Combined chronic toxicity / carcinogenicity study

- Combined Chronic Toxicity and Carcinogenicity Study by Dietary Administration to Han Wistar Rats over 104 Weeks (Thirlwell, 2004) (Huntingdon Life Sciences, ACD 045/024288)

The toxicity phase of the study was completed after 52 weeks and the carcinogenicity phase after 104 weeks. The results of the carcinogenicity phase are reported under point 2.

Material and methods: see below point 2

Findings:

Mortality: 2 rats assigned to the toxicity phase died or were killed during the 52 week treatment period. One male had a large ventral mass and 1 female had ocular damage. These deaths were considered unrelated to treatment.

During the 104-week treatment period a total of 43 male and 50 female rats died or were killed prematurely. The distribution of deaths was considered unaffected by treatment.

Clinical signs: in females receiving 25000ppm the incidence of exfoliation on the tail and yellow staining in the peri-genital region was higher than the control, but the number affected animals was small. There were no signs observed at the physical examinations and arena investigations that were clearly attributable to lenacil, nor was there any treatment-related effect upon the group distribution, multiplicity and mean time of onset of palpable swellings.

There was no evidence of neurotoxicity from arena observations or assessment of sensory reactivity or grip strength. Motor activity in week 50 in males receiving 2500 or 25000ppm was lower than controls at certain time points in the 60-minute assessment period, resulting in low total motor activity scores but in the absence of any other indications of reduced motor activity, these findings were not considered toxicologically significant for the company. Females were not affected.

Body weight: overall bodyweight gain during the 104-week treatment period was low in comparison with the controls in females receiving 25000ppm. The overall weight gain of females at top dose was also slightly lower than control. Body weight gain was decreased without reaching statistical significance.

Food consumption was not affected by treatment. There was no effect on food conversion efficiency.

Haematology: according to the company, number of differences occurred, some of which attained statistical significance when compared with controls. These differences were minor or lacking dose-relationship and were attributed to normal biological variation. These changes also included the small variations of prothrombin and activated partial thromboplastin times at week 78 and 104.

Blood smears did not indicate any differences attributed to treatment. Minor variations occurred, some of which attained statistical significance, but they were considered fortuitous.

RMS considers that the effects observed in blood smears which are reported at week 52 and are increased at week 104 at top dose are probably related to treatment as such effects were also reported in short term studies.

Blood chemistry: there were no changes in the blood plasma that were attributed to treatment according to the company. Changes such as transiently reduced plasma urea, creatinine and glucose in week 26 in females and minor differences in plasma protein and electrolytes were considered as normal biological variations. In 5 males and 3 females at top dose, TSH was increased without reaching statistical significance. T3 and T4 were not changed. The company concluded that thyroid hormone levels were not affected by treatment.

Urinalysis: slightly high protein concentration was noted in week 12 and 51 in males at top dose.

Organ weight: relative heart, brain, thyroid, kidney and liver weight were increased in both sexes at top dose.

Macroscopy: dark colouration of thyroid was seen in male and female rats at top dose after 52 week, affecting females solely after 104 week.

Histopathology: changes were evident in liver of male rats at top dose where there was an increased incidence of centrilobular hepatocyte hypertrophy and increased vacuolation accumulation. Vacuolation is considered a toxic change and normally represents fat accumulation, suggesting that the compound influences the uptake, intracellular fat metabolism or fat release by the hepatocyte. However, in this case, there was no evidence of any effect upon plasma cholesterol and triglycerides as a result of the fatty vacuolation in the liver. Females were not affected.

In thyroids, an increased incidence of luminal concretions was seen in males and females at top dose. These findings are considered to be a common background change which is exaggerated by treatment at top dose.

A slight increase in the incidence of luminal dilatation was seen in uterus of rats given 25000ppm. As this finding is commonly seen in animals of this age, this is considered to be an exaggeration over the background level and is not attributed to treatment.

All other changes observed in this study were of the types normally encountered in Han Wistar rats at these laboratories.

Table 18-1: chronic study in rats treated by gavage with Lenacil

	0 ppm		**250 ppm**		**2500 ppm**		**25000 ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Achieved dose: mg/kg bw/d								
Week 1-52			14.3	18.8	139.1	188.5	1446	1894
Week 1-104			12	15.9	118.4	160.2	1223.2	1699.2
Mortality week 1-52 tox phase			1/20					1/20
Mortality weeks 1-104 carcino phase	14/50	9/50	15/50	17/50	5/50	9/50	9/50	15/50
Body weight:								
Week 52			↓3%	↓2%			↓2%	↓3%
Week 104			↓2%		↓4%		↓6%	↓9%*
Bw gain								
week 52			↓3%	↓2%			↓2%	↓5%
week 104			↓3%		↓6%	↓2%	↓8%	↓13%
Food consumption								
Week 52			↓2%	↓4%	↓3%		↓1%	↓3%
week 104			↓2%		↓5%	↓2%	↓2%	↓1%
Haematology:								
Week 13								
Large Unstained Cells					↓37%*		↓12%*	
Lymphocytes						↓23%*		↓19%*
Week 26								
Lymphocytes				↓32%*		↓25%*		↓16%*
WBCs				↓28%*		↓20%*		↓10%*

	0 ppm		250 ppm		2500 ppm		25000 ppm	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
PT wk 52								↓5%*
PT wk 78				↑9%*	↓16%*	↑11%*	↓20%*	↑6%*
APTT wk 78					↓23%*		↓12%*	
Hct wk 78								↓4%*
APTT wk 104			↓12%*		↓16%*		↓16%*	
Blood smears:								
Neutrophils wk 52							↑13%*	
Neutrophils wk 104							↑24%*	
Lymphocytes wk 52							↓4%*	
Lymphocytes wk 104							↓8.5%*	
Monocytes wk 52							↓33%*	
Monocytes wk 104							↓75%*	
Blood chemistry								
Week 26								
Ca^{2+}							↓1%*	
Phosphate							↓8%*	
Na^{+}				↓8%*		↓1.4%*		↓0.7%*
Urea				↓24%*		↓10%*		↓27%*
Creatinine				↓8%*		↓6%*		↓6%*
A/G ratio				↓6%*		↓9%*		↓11%*
Week 52								
glucose					↑14%*		↑14%*	
triglycerides					↑27%*		↑36%*	
Total proteins							↑3%*	
Albumin							↑5.5%*	
CPK								↓50%*
A/G ratio								↓6%*
Free T3, T4	No compound related effect							
TSH							↑33%	↑27%

	0 ppm		250 ppm		2500 ppm		25000 ppm	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Week 78								
CPK						↑22%		↑94%*
Week 104								
Ca^{2+}			↓3%*	↑2.9%*	↓4%*	↑3.6%*	↓1.5%*	↑0.35%*
A/G ratio								↓8%*
Urinalysis								
Week 12								
Volume			↓45%*		↓48%*		↓42%*	↓30%
SG					↑1.1%*		↑1.1%*	
Proteins							↑32%*	
Week 25								
pH	7.4		6.9*		6.9*		6.9*	
Week 51								
volume							↓29%*	
Proteins							↑50%*	↑68%*
Organ weight								
Week 52								
Kidney relative								↑8%*
Liver							↑9%*	↑6%
Thyroid +para							↑23%*	↑20%*
Week 104								
Kidney relative							↑9%*	↑12%*
Liver							↑14%*	↑10%*
Thyroid +para							↑40%	↑49%
Brain							↑7%*	↑8%
Heart							↑8%*	↑9%*

*significant when compared with control group at a < 0.05% level

Table 18-2: chronic study in rats treated by gavage with lenacil-macroscopy

	0 ppm		**250 ppm**		**2500 ppm**		**25000 ppm**	
Achieved dose: mg/kg bw/d	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
	20	20	19	20	20	20	20	19
Week 52								
Macroscopy:								
Thyroid dark							5*	10*
Carcinogenicity phase:								
Macroscopy:								
Rats killed/dying during study								
Liver: pale area							3/9	1/15
Lung: pale area	4/14	6/9	6/15	6/17	2/5	4/9	6/9	10/15
Thyroid dark area	0/14	0/9	0/15	0/17	0/5	0/9	1/9	1/15
Thyroid dark	0/14	0/9	0/15	0/17	0/5	0/9	1/9	2/15
Uterus:								
Fluid distension		0/9		0/17		1/9		3/15
cysts		0		5		1		4
thickened		0		1		1		2
Skin scabs	1/14	0/9	1/15	0/17	0/5	2/9	3/9	3/15
Rats killed after 104 weeks								
Kidneys depression		2/41		2/33		0/41		4/35
Liver dark depression	1/36	2/41	0/35	2/33	3/45	4/41	3/41	4/35
Lung dark area	9/36	7/41	8/35	8/33	14/45	10/41	13/41	10/35
Spleen swollen	5/36	1/41	3/45	1/33	1/41	1/41	1/41	5/35
Testes subcapsular fluid	1/36		3/35		1/45		4/41	
Thymus dark area	5/36	1/41	2/35	3/33	6/45	8*/41	6/41	4/35
Thyroid dark	0/36	0/41	0/35	0/33	0/45	0/41	0/41	10*/35
Enlarged	0/36	1/41	0/35	0/33	2/45	0/41	5/41	1/35
All animals:								

	0 ppm		250 ppm		2500 ppm		25000 ppm	
Achieved dose: mg/kg bw/d	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Spleen swollen	6/50	3/50	7/50	3/50	3/50	2/50	2/50	7/50
Testes subcapsular fluid	1/50		3/50		1/50		5/50	
Unilaterally small	1/50		2/50		1/50		5/50	
Thymus dark area	6/50	1/50	2/50	3/50	6/50	8*/50	7/50	4/50
Thyroid dark	0/50	0/50	0/50	0/50	0/50	0/50	1/50	12*/50
Enlarged	0/50	1/50	0/50	0/50	2/50	0/50	5/50	1/50
Uterus fluid distension		0/50		0/50		3/50		6*/50
Mammary area masses		7/50		18*/50		14/50		14/50

Table 18-3: chronic rat study by gavage with lenacil- histopathology- non neoplastic findings for all rats.

	0 ppm		250 ppm		2500 ppm		25000 ppm	
Achieved dose: mg/kg bw/d	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
	50	50	50	50	50	50	50	50
Adrenals prominent accessory adrenocortical tissue	5/50	7/50	6/35	5/45	4/29	5/45	3/50	12/50
Eyes: unilateral lenticular degeneration	4/50	1/50	3/15	1/18	1/5	2/11	2/50	7*/49
Retina loss of outer nuclear layer bilateral	3/50	0/50	1/15	0/18	1/5	0/11	7*/50	1/49
Liver :								
Centrilobular vacuolation hepatocytes	16/50	2/50	21/50	4/50	18/50	2/50	28*/50	2/50
Centrilobular hypertrophy hepatocytes	11/50	1/50	11/50	0/50	15/50	1/50	26*/50	4/50
Ovary : atrophy		5/50 10%		6/23 26%		7/17 41%		10/50 20%

	0 ppm		250 ppm		2500 ppm		25000 ppm	
Achieved dose: mg/kg bw/d	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
	50	50	50	50	50	50	50	50
Absent corpora lutea		5/50 10%		1/23 26%		5/17 29%		12/50 24%
Thyroid								
Increased luminal concretions	11/50	5/50	16/50	6/50	17/49	10/50	33*****/50	32*/49
Uterus								
Glandular dilatation		2/50		4/37		6/34		5/50
Endometrial gland hyperplasia		2/50		1/37		2/34		6/50
Luminal dilatation		17/50		20/37		19/34		27/50
Skin scabs	3/23	0/11	1/20	0/7	0/20	4/14	8/24	3/10

Conclusion from the RMS: from the toxicity study, a NOAEL is proposed at 2500ppm (139-188mg/kg bw/d) taking into account the effects reported at 25000ppm on:

- The thyroid gland (relative weight increase, increased TSH and luminal concretions)

- The liver effects (an increased weight and hepatocellular hypertrophy/vacuolation in both sexes)

At top dose, some effects were reported in the eyes of males (loss of outer nuclear layer bilateral) and females (unilateral lenticular degeneration) and kidney weight and urinary protein excretion were increased and male rats had abnormal blood smears.

The company concluded that the administration of Lenacil technical to Han Wistar rats, via the diet, at concentrations up to 25000ppm for 104 weeks caused non-specific toxicity in females at 25000ppm and adaptive and toxic change in the liver in males at 25000ppm.

Notifier comment:

Notifier also concluded that the NOAEL for rats dosed in a one/two year long term toxicity study is 2500 ppm.

2: Carcinogenicity study in the rat

- Lenacil technical – Combined chronic toxicity and carcinogenicity study by dietary administration to HAN Wistar rats over 104 weeks (Thirlwill, P.M. (2004d) ACD 045/0242214, Huntingdon Life Sciences Limited

Materials and methods:

The results reported here are limited to the carcinogenicity findings of the study reported under point 1.

GLP status: yes
Guideline: study is in compliance with Dir EEC 87/302/EEC Annex V B or OECD test guideline n° 453 (1981).
Lenacil technical (Batch No. 141712003, purity 98.6%) was administered via dietary admixture into the powdered diet. At specified intervals, (weeks 1, 13, 26 and 52) during the toxicity phase, prepared dietary formulations were sampled and analysed for concentration. The homogeneity and stability of Lenacil, conducted as part of an earlier study, were confirmed at nominal concentrations of 50 ppm and 50000 ppm during ambient temperature storage for 22 days. The mean concentrations of Lenacil technical in test formulations during the Toxicity phase of the study were between – 4.8 and + 2.0% of intended, which were within the acceptable limits of -15% to 10%, confirming the accuracy of formulation.
Three groups of 50 male and 50 female rats HsdBrl Han:Wist (Han Wistar) are receiving Lenacil technical orally, via the diet, at concentrations of 250, 2500 or 25000 ppm. Together with a similarly constituted control group receiving the vehicle, untreated diet, these animals comprise the carcinogenicity phase of the study. A further 20 male and 20 female rats were allocated to each group. These animals comprised the toxicity phase of the study and were sacrificed after the completion of 52 weeks of treatment.
Animals were observed daily for evidence of a reaction to treatment. During the study detailed physical and arena observations, sensory reactivity and grip strength, motor activity, bodyweight, food consumption, ophthalmic examination, haematology, blood chemistry, urinalysis, organ weight, macroscopic and microscopic pathology investigations were undertaken.
Statistics: were carried out separately for males and females using the individual rat as unit. For categorical data, including pathological findings, the proportion of rats were analyzed using Fisher exact test for each group compared to control. For continuous data, Bartlett test was applied to test homogeneity of variance. When statistically different a Behrens-Fisher test was used to perform pair wise comparisons otherwise a Dunnett test. Intergroup differences in mortality and tumor incidence were performed using the Peto approach.
The study is accepted.

Neoplastic findings:

In males, no statistically significant results were found.

In females:

Thyroids: for benign follicular cell adenoma the trend test was found to be statistically significant when taking the top dose into account. Pair wise comparison control and top dose was statistically significant.

When follicular cell adenoma and malignant follicular cell carcinoma were combined the trend test was statistically significant when the top dose was included.

According to the company the thyroid follicular cell adenomas and carcinomas occurred to some extent in all groups. The percentage incidence of follicular cell adenomas in treated groups was well within the back ground range for both sexes. In addition, the group distribution, and lack of clear dosage relationship indicates that these particular tumors are not related to the administration of Lenacil and are not considered to be toxicologically significant. The incidence of follicular cell adenomas was not associated with follicular cell carcinomas. The group incidence of other non neoplastic proliferative lesions such as follicular cell hyperplasia did not show any effects of treatment.

RMS considers those thyroid follicular cell adenomas are within historical control data of the laboratory. The laboratory background incidence of follicular cell carcinoma is not reported.

An increased incidence of C-cell adenoma was seen in females at 25000 and 2500ppm. The incidences observed however, were either within background range or marginally outside. There was, however, no dose-relation in the occurrence of these tumors which are hence considered unrelated to treatment.

The finding C – cell carcinoma was seen in females at 25000ppm. Although the incidence of C-cell carcinoma in females that had received 25000ppm was higher than the background range in females, the incidence (4%) was within the male background range for this finding. The pair wise comparison between the control and the top dose treated group was found to be statistically significant. The company considers that C-cell carcinomas of the thyroid in two high dose females are considered to have arisen incidentally and the etiology probably related to age.

A position paper was provided by the company (Gopinath was author) in which it was concluded that C-cell tumors are spontaneous age-related lesions with a widely variable incidence in laboratory rats. The carcinogenicity study in Wistar rats reported an increased incidence of C cell adenomas in females receiving 2500 or 25000ppm lenacil technical. The incidences reported were only marginally greater than the historical control rats from Huntingdon Life Sciences laboratories. The incidence of C-cell carcinoma was well within the control range; Male rats did not reveal similar changes. C-cell lesions including C-cell tumors are seldom observed as treatment related end points. There was no treatment related C-cell hyperplasia in the study. The overall proliferative lesions of C-cells did not show any intergroup differences from controls. The examination of clinical biochemical parameters did not reveal any evidence of disturbance of calcium homeostasis to suggest any C-cell involvement.

The review of 2 other short term studies using higher dosages up to 50000ppm did not show any treatment related changes in C-cells or any indications for disturbances in calcium/phosphorus levels. The 2 studies reviewed revealed a few minor changes in follicular epithelium of thyroid, such as increased Schmorl's positive pigment and or follicular cell hypertrophy at high dosages. These changes have no connection or impact on C-cell lesions. In view of the above mentioned facts, the minor increased incidence reported of C-cell adenoma in the female rats receiving 2500 or 25000ppm in this study is considered incidental and of no toxicological importance.

According to the open literature, in many rat strains, C-cell hyperplasia occurs in an age-dependent manner and is often associated with multifocal C-cell carcinoma. The incidence of C-cell hyperplasia shows a significant increase with age ($P<0.001$) and is much higher in female rats than in male rats ($P<0.05$). From 3 to 24 months of life, 27.5% of female rats showed a normal C-cell pattern, 55.0% showed C-cell hyperplasia, and 17.5% showed C-cell tumors; while 57.5% of male rats showed a normal C-cell pattern, 32.5% showed C-cell hyperplasia, and 10% showed C-cell tumors. Although the overall frequency of C-cell neoplasms in females was nearly double that in males, these data are not statistically significant. However, the number of C-cell tumors showed a significant increase with age ($P<0.05$) (Lacave et al., 1999).

Therefore, RMS accepts that the significant differences in the incidence of the total spectrum of C-cell proliferative abnormalities in the thyroid gland of Wistar rats are both age-dependent and gender-dependent.

Mammary tissue

For benign mammary adenoma the trend test was found to be statistically significant. Upon exclusion of the top dose the trend test was no longer statistically significant. For malignant mammary adenocarcinoma, the pairwise comparison between the control and the top dose treated group and the control and the 2500ppm were both found to be statistically significant. For benign mammary adenoma, benign mammary fibroadenoma and malignant mammary adenocarcinoma combined the pairwise comparison between control and the 250 ppm treated group was found to be statistically significant.

According to the company, the incidence of mammary fibroadenoma was well within background range in all female groups. Mammary adenocarcinomas were seen in treated females; the incidences seen in females at 25000 and 2500 ppm were higher in comparison with the background historical data.

Although the control incidence of mammary adenocarcinoma in this study was the same as the lowest recorded background incidence (0.0%), it is considered atypical as out of 10 compatible back ground studies examined, only one had the mammary adenocarcinoma incidence of 0.0%. An increase in mammary adenocarcinomas would normally be associated with an increase in mammary fibroadenomas and acinar hyperplasia (Boorman et al, 1990). Although there is an increased incidence of the mammary adenocarcinomas over background range in the intermediate and high dose females in this study, in the absence of a similar increase in mammary fibroadenomas and acinar hyperplasia, and in the absence of dosage relationship, the increase in adenocarcinomas is not considered to be associated with the administration of Lenacil.

RMS considers that the incidence of malignant mammary adenocarcinoma in females at top dose (10%) and at intermediate dose (12%) were slightly outside the historical controls of the laboratory (6.7%) and within the data of Charles River laboratories (13.33%), the incidence represents an equivocal finding.

Table 18-3: chronic study in rats treated by gavage with lenacil- tumor incidence /laboratory or published background incidence.

	0 ppm		**250 ppm**		**2500 ppm**		**25000 ppm**	
Achieved dose: mg/kg bw/d	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
	50	50	50	50	50	50	50	50
Adrenals:								
benign adenoma + malignant carcinoma cortical	2	2					3	5
Benign + malignant pheochromocytoma	1						2	
Liver: Hepatocellular adenoma/carcinoma	3/0	2/0	0/0	0/0	1/1	2/0	3/1	0/0
Pancreas Benign islet cell adenoma	3						5	

	0 ppm		250 ppm		2500 ppm		25000 ppm	
Achieved dose: mg/kg bw/d	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
	50	50	50	50	50	50	50	50
Pituitary: benign adenoma *pars distalis*	10	32					8	25
Leydig cell adenoma	0						2	
Thyroid:								
follicular cells								
benign adenoma	3	1	2	0	2	1	5 10%	4*** 8%
Laboratory background incidence:	Male: 0.0%-16% Female: 0.0% -11.7%							
Malignant carcinoma	0	2	0	0	1	2	1	4 8%
background incidence (Poteracki and Walsch, 1998):	Male: 0.0%-1.7% Female: 0.0% -3.3%							
Charles River data Wistar Han rats, 2003	Male: 1.67-3.64% Female: 1.82-3.64%							
C-cell								
Adenoma/carcinoma	4/0	2/0	3/0	2/2	5/0	8*/0	5/0 10%/0 %	7/2*** 14%/4%
Laboratory background incidence: adenoma/carcinoma	Male: no data/0-5.1% Female: 0-13.6%/0-1.7%							
Charles River data Wistar Han rats, 2003	Male: 3.64-18.33/1.82-5.45% Female: 3.64-21.82%/1.82-1.82%							
Uterus: Endometer								
polyps benign/ adenocarcinoma		5/2						5/5
Mammary gland								
Benign adenoma		0		1		0		3**
Fibroadenoma benign		7		12		8		8

	0 ppm		250 ppm		2500 ppm		25000 ppm	
Achieved dose: mg/kg bw/d	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
	50	50	50	50	50	50	50	50
Laboratory background incidence	Females: 6.7%-32%							
Malignant adenocarcinoma		0		2		6** 12%		5** 10%
Laboratory background incidence	Females: 0.0%-6.7%							
Charles River data Wistar Han rats, 2003	Females: 1.82%-13.33%							

* Statistically significant pair wise comparison ** trend test statistically significant; (Poteracki and Walsch 1998)

Conclusion: the company concluded that the administration of Lenacil technical to Han Wistar rats, via the diet, at concentrations up to 25000ppm for 104 weeks caused non-specific toxicity in females at 25000ppm and adaptive and toxic change in the liver in males at 25000ppm. Lenacil technical was not associated with the occurrence of any of the tumours observed in the study. The no-observed-effect level (NOEL) in this study was 250ppm (equivalent to 12.0 mg/kg/day in males and 15.9 mg/kg/day in females) due to slightly reduced motor activity in males at 2500 ppm.

The no-observed-adverse –effect Level (NOAEL) is considered to be 2500ppm, (equivalent to 118 mg/kg/day for males and 160 mg/kg/day for females).

According to the RMS, a NOAEL for oncogenicity should be set at 250ppm (16 mg/kg bw/d) taking into account the increased incidence of for mammary gland malignant adenocarcinoma at 2500ppm (160 mg/kg bw/d).

Comment from notifier:

The Notifier suggests that the data support the proposition that the administration of lenacil is not associated with mammary tumour incidence, since the incidence at high dose levels is less than that in background data. The Notifier proposes that the same information is used to set a NOAEL for oncogenicity, where, if lenacil is not associated with induction of any of the tumours observed, as concluded by Notifier and supported by RMS in text above, then 2500 ppm is the appropriate NOAEL

Carcinogenicity study in the mouse

- Oncogenicity study with Lenacil eighteen-month feeding study in mice (Malek, 1994)(Dupont USA, HLR-336-93)

Materials and methods:

GLP status: yes (no attest of competent authority)
Guideline: study is not fully in compliance with Dir EEC 87/302/EEC Annex V B or OECD test guideline n° 451 (1981).
Deviation from official protocol: 2 doses are without adverse effects in males and 3 doses are without adverse effects in females. For a combined test there is one dose lacking as well as clinical chemistry (except blood proteins, platelets and ovary weight)
Material and methods:
Four groups of each 80 male and 80 female CRL-CD®-1(ICR)BR were fed diets containing 0, 100, 2500 or 7000 ppm of Lenacil technical (synonyms DPX-B634-91 (B634-91) DPX-B634; IN B634-91(Batch No. 9038, purity 98.2% (reanalysis 98.5%) administered via dietary admixture into the powdered diet. The technical material was analysed for stability at the beginning, in the middle and at the end of the study. On test day –1, samples were collected from each dietary concentration to verify concentration, homogeneity and stability. At approximately three-month intervals throughout the study, feed samples were collected for concentration analyses. Measured concentrations ranged from 86.8 to 104% of nominal and appeared to be stable in the diet. The homogeneity was confirmed.
Body weight and food consumption were measured and clinical signs conducted weekly (first three months) or bi-weekly during the remainder of the study. Ophthalmoscopic examinations were performed during pre-test and at study end. Haematology and clinical chemistry analyses were conducted after 3, 6, 12 and 18 months. After 18 months, all survivors were sacrificed, selected organs were weighed and tissues examined for the presence of gross or microscopic lesions.
Statistical analyses: bw, bw gain, organ weight, clinical pathology were analyzed by analysis of variance. Pairwise comparison between test and control were made with the Dunnett's test. Clinical observations were evaluated by the Fisher exact test with a Bonferroni correction and if significant followed by the Cochran Armitage test for trend. The incidence of all primary neoplastic hyperplastic and compound related non neoplastic lesions and survival among groups observed microscopically were evaluated by the Cochran Armitage test for trend and or the Fisher exact test. The Barletts test for homogeneity of variances was performed on the organ weight and clinical laboratory data.
The study is accepted.

Findings:

Mortality: no compound-related mortality was observed.

Clinical signs: no signs were attributed to the dietary administration of lenacil.

Body weight: mean bw and bw gains of male and female mice were comparable to controls at all dose levels.

Food consumption and efficiency were comparable with controls at all dose levels.

Ophthalmoscopy: at the end of the study the most common ocular findings were unilateral or bilateral central corneal opacities which were not considered to be compound-related.

Hematology: occasional statistically significant findings such as decreases in platelet, total leukocyte, neutrophil, or lymphocyte counts in male and or female mice were not dose- or time related, nor were they toxicologically important.

Organ weight: relative liver weight was increased in males at top dose. This effect was considered to represent a normal physiological response of the liver to xenobiotic administration.

Kidney weight was decreased in females at all dose levels but did not correlate with any microscopic lesions and was considered by the company to be unrelated to lenacil.

Macroscopic findings: in male mice at top dose, there was an increased incidence of lung masses which was not considered compound related. Liver masses were considered attributable to a toxicologically significant increase in hepatocellular adenomas.

Microscopy:

Liver: centrilobular hypertrophy was observed in male livers and the incidence was low. This effect was considered by the company to be the result of the induction of smooth endoplasmic reticulum and an increase in SER-associated enzymes but this was not demonstrated, or measured. The centrilobular hypertrophy observed in male mice was not considered as adverse by the company.

Lung: there was no significant statistical increase in the incidence of pulmonary alveolar adenomas or adeno-carcinoma. However, there was a borderline increase in the combined incidence of alveolar adenomas and adeno-carcinoma observed in male mice at top dose. Although this increase was significant by Cochran-Armitage trend test, the increase was not significant by the Fisher exact test. The incidence of various alveolar tumors observed in the concurrent control males was similar to those of historical controls in this laboratory, except at top dose. However, it was not considered compound related based on the following reasons:

1. Incidences of adenoma and adenocarcinoma, taken separately, were not statistically increased.
2. There was no statistical significance with the Fisher exact test at p=0.05 for any dose group.
3. There was no decrease in alveolar tumor latency; most tumors were observed in mice killed at terminal sacrifice.
4. There was no increase in focal hyperplasia of type II alveolar cells.
5. There was no shift in tumor cell anaplasia.

Comment from RMS on the microscopy: the company did not provide the laboratory historical control data for liver tumors and RMS used historical control data published by Charles River laboratories for Crl:CD-1 BR mice, 1995. The incidence of liver cell adenoma multiple reported in males at top dose (16%) is within the maximum range of historical control data at Charles River Laboratories (19%).

The incidence of 17/80 (21%) lung alveolar adenomas for males at 7000 ppm is slightly above the maximum range of historical control data at the testing laboratory (16%) and at Charles River Laboratories (12%). The incidence of 8/80 (10%) alveolar carcinomas in males at 7000 ppm is above the maximum range of historical control data at the testing facility (0%) but inside Charles River Laboratories (21%) and not statistically significant.

The number of any type lung alveolar neoplasms in males receiving 7000 ppm is also slightly increased (26/80, 32%) compared to the concurrent untreated control (18/80, 22.5%), it is statistically significant ($p<0.05$) and is outside the range of the historical controls at the testing facility (18-21%). However, because this increase is small, and did not demonstrate decreased latency compared to controls, it is considered to represent only equivocal toxicologic significance.

Table 18-4: 18-month mice study with lenacil.

Endpoints/dose	**0**		**100**		**2500**		**7000 ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mortality	25	24	23	9	15	18	23	15
Compound intake mg/kg bw/d	0	0	13.8	19.6	332	482	977	1358
Ocular opacity %	21	31	14	21	21	22	19	29

Endpoints/dose	0		100		2500		7000 ppm	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mortality	25	24	23	9	15	18	23	15
Organ weight								
Liver relative			↑7%		↑6%		↑16%	↑6.7%*
Kidney relative				↓12%		↓13%		↓16%
Kidney absolute				↓13%		↓14%		↓17%
Spleen relative				↓16%		↓31%		↓35%
Macroscopy:								
Lung masses	6	3	3	3	6	1	13	2
Kidney cyst	8	3	12	4	9	5	13	2
Kidney discoloration	3	7	6	9	8	8	4	4
Eyes discoloration	1	3	2	3	3	3	5	1
Exophthalmus	0	0	0	0	0	0	3	0
Harderian gland masses	0	0	0	0	0	3	4	0
Histopathology:								
N° examined animals	80	78	79	79	80	79	80	80
Kidney cysts tubular	15		21		22		25	
Pleural fibrosis focal	2	1	6	0	5	1	8	1
Lung alveolar histiocytosis	6	6	7	8	12	5	12	5
Lung alveolitis focal	1	2	4	2	9	6	5	4
Testes hyperplasia Leydig cell	7	-	0	-	3	-	12	-
Pituitary cysts	2	1	0	-	0	-	6	1
Harderian gland adenoma	6	-	2	-	2	3	9	1
Liver: Hepatocellular								
Centrilobular hypertrophy	-	-	-	-	-	-	7*	-
Karyomegaly	2	-	2	-	4	-	5	-

Endpoints/dose	0		100		2500		7000 ppm	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mortality	25	24	23	9	15	18	23	15
Adenoma single	11	2	10	0	10	0	11	1
Adenoma multiple	0	0	5	0	4	0	13* ** 16%	0
Published historical control data for adenoma			Male:0-19% Female: 0.0-2%					
carcinoma	5	0	3	0	3	0	2	0
Lung alveolar								
Adenoma single	14 17%	5	9	5	15	4	17 21%	6
Laboratory historical control (2 studies)			7-10 male mice/60 11.6-16%					
Adenoma multiple	1	1	2	0	0	2	3	0
Laboratory historical control (2 studies)			1-3 male mice/60					
carcinoma single	3 3%	3	4	4	4	2	8 10%	2
Carcinoma multiple	1	1	0	0	2	0	0	0
Laboratory historical control (2 studies)			0-0 male mice/60					
Any type	18 22.5%	10	15	8	18	7	26* 32%	8
Laboratory historical control (2 studies)			11-13 male mice/60 18-21%					
Published historical control data:								
Bronchiolar/alveolar adenoma			Male: 1.92-12% Female: 0-15.38%					
Bronchiolar/alveolar carcinoma			Male: 0-21% Female: 0-9.62%					

*p<0.05 for Cochran Armitage trend test and for ** Fisher exact test; Historical control data from laboratory and Published historical control data from Charles River laboratories, 1995, Crl: CD-1 BR mouse.

Conclusion: a NOAEL for systemic toxicity is proposed at 2500ppm (332 mg/kg bw/d) taking into account the increased liver weight associated with centrilobular hypertrophy.

NOAEL oncogenicity can be set at 2500ppm (332 mg/kg bw/d) taking into account the increased incidence of alveolar tumors in lung, and multiple adenomas in liver.

Discussion of the neoplastic incidences:

Rats:

In a combined chronic toxicity/carcinogenicity study in rats (Thirlwell, (2004), dietary concentrations of 250, 2500 and 25000 ppm were used. In the 12-month chronic toxicity part, Lenacil showed higher kidney, liver and thyroid weights and a discolouration of thyroids. With regard to the thyroids, there was a slight not statistically significant increase in TSH. Liver weight increase was combined with centrilobular hypertrophy. There was an increase in kidney weights and occasional proteinuria and abnormal blood smears at top dose level.

Due to the changes reported at 25000 ppm, the NOAEL was set at 2500 ppm corresponding to a daily intake of 139.1 mg/kg bw in males and 188.5 mg/kg bw in the females.

In the rat carcinogenicity study, the main target organ was the liver, affecting over 50% of the high dose males (250000 ppm) and characterized by centrilobular hepatocyte hypertrophy and vacuolation as well as increase in liver weights. Liver enzymes were not increased in the blood plasma. Hepatocellular hypertrophy is considered by the company to represent an induction of hepatocellular enzymes in response to the administration of a xenobiotic and, as such, is an adaptive response to treatment. Xenobiotic liver metabolizing enzymes were not measured to support this assumption.

The other target organ in rats was the thyroid. There was an increase in thyroid weight in male and females at 25000 ppm and the thyroids were macroscopically darker than normal and concretions were observed in the follicle lumen.

The incidences of C-cell tumours (adenomas) in female rats treated at 2500 and 25000 ppm of Lenacil were considered to be age and gender-dependent.

The incidence of malignant mammary adenocarcinoma in females at top dose (10%) and at intermediate dose (12%) was slightly outside the historical controls of the laboratory (6.7%) and within the data of Charles River laboratories (13.33%). The incidence was concluded in the DAR as representing an equivocal finding.

The "*Lenacil: Review of carcinogenicity and proposed R40 classification paper*" (Dr D Andrew, TSGE, 2012) documents the assessment of tumour incidence against a range of historical background control incidences and concludes that the data do not indicate any relationship to treatment with Lenacil for the mammary gland tumour findings in female rats in this study. The incidence of macroscopically observed masses was significantly higher in the low dose group only. Incidences of all tumours (adenoma, fibroadenoma and adenocarcinoma) lie within the range of published historical control data. Statistically significant increases in the incidence of adenocarcinoma are additionally not considered to be treatment-related due to the absence of a dose-response relationship; their association with an unusually low concurrent control value and the absence of correlative findings (described in further detail below).

Rat - Mammary adenocarcinoma

Table 19: Rat carcinogenicity study: mammary gland findings (females)

Finding	Timepoint	Dose level			
		Control	250 ppm	2500 ppm	25000 ppm
Mammary masses	12 months	1/20	0/20	0/20	0/20
	Decedent	3/9	7/17	4/9	6/15
	24 months	4/41	11/33	10/41	8/35
	Total	7/50	18/50*	14/50	14/50
		14%	**36%**	**28%**	**22%**
Acinar hyperplasia	12 months	5/9	7/17	3/9	7/15
	Decedent	0/20	0/20	0/20	0/19
	24 months	17/41	18/33	23/41	21/35
	Total	22/50	25/50	26/50	28/50
		44%	**50%**	**52%**	**56%**
Mammary adenoma	12 months	0/20	0/20	0/20	0/20
	Decedent	0/9	1/17	0/9	1/15
	24 months	0/41	0/33	0/41	2/35
	Total	0/50	1/50	0/50	3/50
		0%	**2%**	**0%**	**6%**
Mammary fibroadenoma	12 months	0/20	0/20	0/20	0/19
	Decedent	3/9	3/17	0/9	3/15
	24 months	4/41	9/33*	8/41	5/35
	Total	7/50	12/50	8/50	8/50
		14%	**24%**	**16%**	**16%**
Mammary adenocarcinoma	12 months	1/20	0/20	0/20	0/19
	Decedent	0/9	2/17	3/9	3/15
	24 months	0/41	0/33	3/41	2/35
	Total	0/50	2/50	6/50*	5/50*
		0%	**4%**	**12%**	**10%**
Total mammary tumours	Total	7/50	15/50*	13/50	10/50
		14.0%	30.0%	26.0%	20.0%

**significantly different to controls (p<0.05)*

The incidences of mammary tumours (mammary adenoma, mammary fibroadenoma, mammary adenocarcinoma and the combined tumour incidence) are discussed and data compared against a number of sources of background (historical control) data:

- Ten studies initiated at the test laboratory during 1996-2001 (i.e. immediately prior to the Thirlwell study), referred to in the original study report.
- An updated database of nineteen studies performed at the test laboratory from 2001-2006.
- Published data for HsdRCCHan (Wistar Hannover) rats from 50 carcinogenicity studies performed at RCC (Switzerland) between 1981-2006.
- Published data for Wistar Han Rats from Charles River Laboratories (10 studies terminated in 1999 or earlier).
- Data compiled from reviews of tumour incidence in Wistar rats (Poteracki & Walsh, 1998).

Female rat: mammary adenocarcinoma

The incidences of mammary adenocarcinoma in this study in the 2500 ppm and 25000 ppm dose groups of 6/50 (12%) and 5/50 (10%) respectively are significantly increased when compared to the concurrent control incidence of 0/50 (0%), but without any relationship to dose level. However, the absence of findings in the concurrent control is unusual and was seen only in one of the 19 studies constituting the updated laboratory historical data. The statistical significance of the findings at 2500 and 25000 ppm is therefore attributable to an unusually low concurrent control incidence. The incidences of this tumour type in the 2500 ppm and 25000 ppm dose groups lie within the laboratory's updated historical control range (0-22%) and are also clearly within the background range when compared to the RCC and Charles River data. Poteracki & Walsh (1998) also report a relatively high incidence of mammary adenocarcinoma (1.7-12.4%; mean 6.7%) in female Wistar rats.

Table 20: Background incidences of mammary adenocarcinoma

Data source	Tumour incidence			
	Total	Study mean	Minimum	Maximum
Laboratory (1)	-	3.6%	0.0%	6.7%
Laboratory (2)	4.81%	Not Reported	0.0%	22.0%
RCC	5.35%	5.63%	0.0%	18.0%
Charles River	5.49%	NR	1.82%	13.33%
Poteracki & Walsh	6.7%	NR	1.7%	12.4%

The development of malignant mammary adenocarcinoma is usually associated with a concurrent increase in the incidence of benign mammary fibroadenoma and acinar hyperplasia; such an effect was not observed in this study. In addition to the absence of associated findings and an incidence that fell within the historical background range, the absence of a dose-response relationship is also notable for this tumour type. Despite a 10-fold increase in the dose level between the intermediate and high dose groups, there is no associated increase in tumour incidence. This pattern of response clearly does not indicate an effect of treatment.

It is therefore concluded that there is no treatment-related increase in the incidence of mammary adenocarcinoma in the Lenacil study.

Female rat - Mammary adenoma

The incidence of mammary adenoma was highest in 25000 ppm females (6%) in this study; this value was not statistically significant using a pair-wise comparison but attained statistical significance ($p = 0.028$) using the trend test of Poteracki & Walsh. The incidence of this benign tumour at 25000 ppm (6%) very marginally exceeds the laboratory's historical control range (0-5.5%), however the tumour incidence is clearly within the background range when compared to the RCC data. Additional data published by Poteracki & Walsh (1998) report an incidence of mammary adenoma of 2.0-6.7% in female Wistar rats.

Table 21: Background incidences of mammary adenoma

Data source	Tumour incidence			
	Total	Study mean	Minimum	Maximum
Laboratory (1)				
Laboratory (2)	1.96%	Not Reported	0.0%	5.5%
RCC	1.43%	1.51%	0.0%	14.0%
Charles River	1.42%	NR	1.82%	3.64%
Poteracki & Walsh	3.9%	NR	2.0%	6.7%

(1) background range original report
(2) background range updated

It is therefore concluded that there is no clear treatment-related increase in the incidence of benign mammary adenoma in female rats in the Lenacil study.

Female rat - Mammary fibroadenoma

The incidences of benign mammary fibroadenoma (14-24%) in this study are below the laboratory's historical control range in some groups. The highest tumour incidence of 24% was observed in the low dose group; incidences in the intermediate and high dose groups are comparable to the concurrent control value. The tumour incidence in all groups is within the background range when compared to the laboratory, RCC and Charles River data. Poteracki & Walsh (1998) also report a high incidence of mammary fibroadenoma (18.0-45.0%; mean 36.1%) in female Wistar rats.

Table 22: Background incidences of mammary fibroadenoma

Data source	Tumour incidence			
	Total	Study mean	Minimum	Maximum
Laboratory (1)	NR	Not Reported	16.7%	33.3%
Laboratory (2)	23.45%	Not Reported	10.9%	34.0%
RCC	28.3%	28.9%	6.0%	60.0%
Charles River	22.12%	NR	10.91%	33.85%
Poteracki & Walsh	36.1%	NR	18.0%	45.0%

(1) background range original report
(2) background range updated

It is therefore concluded that there is no treatment-related increase in the incidence of mammary fibroadenoma in the Lenacil study.

Female rat – combined mammary tumours

No historical control data are available for the combined incidence of mammary gland tumours, however the clear absence of a dose-response relationship for this finding and an incidence in the highest dose group close to the concurrent control incidence does not indicate any effect of treatment with Lenacil. The data therefore do not indicate any relationship to treatment with Lenacil for the mammary gland findings in female rats observed in this study. The incidence of macroscopically observed masses was significantly higher in the low dose group only. Incidences

of all tumours (adenoma, fibroadenoma and adenocarcinoma) lie within the range of published historical control data. Statistically significant increases in the incidence of adenocarcinoma are additionally not considered to be treatment-related due to their association with an unusually low concurrent control value, the absence of correlative findings and in the absence of a dose-response relationship.

Complementary questions from RMS on the submitted historical control incidences in the rat post-PRAPeR:

(i) RMS requested some clarification on the timeframe of the HCD. In the initial DAR, RMS considered the incidence of mammary adenocarcinoma (10-12%) observed at the highest doses relevant, as it exceeded the HCD provided at that time. In the references, it is found that the Thirlwell study was from 2002. It was stated that these HCD referred to studies in that lab. It was unclear to the CLH reviewer to which time-frame this really referred, as these dates were not explicitly stated in the TSGE appendix. The company responded as follows:

"*The rat carcinogenicity of Thirlwell et al (2004) was performed [in-life phase] from 24th September 2001-3rd October 2003. The original historical control dataset of 10 studies reported in the original study represents studies performed immediately prior to the Thirlwell study and commencing between 1996 and 2001. Please note that there is some overlap between this dataset and the updated laboratory background data of 19 studies. Studies 7-10 from the original study report correspond to studies 1-4 from the updated laboratory historical range.*"

(ii) Further, clarification was requested on the validity of some HCD.
In the 'updated' laboratory HCD (CLH reviewer supposes in-house HCD, 2001-2006), 19 studies were presented having spontaneous adenocarcinoma varying from 0-22%. However, 18/19 studies exhibit an incidence rate up to maximally 8%. There was only one study, exhibiting 22% of adenocarcinoma. It was questioned what weight should be attributed to one outlier in the HCD. On what grounds does this value enter into the HCD? Was there an explanation for this unusually high background incidence rate? Could you precise in what year this study was conducted? The company responded as follows:
"The study with the highest incidence of mammary adenocarcinoma commenced in March 2006. Incidences of other mammary tumours in this study are consistent with the other 18 studies in the dataset, therefore there is no indication that the mammary adenocarcinoma incidence in this study was skewed, for example by observer bias or altered diagnostic criteria. While the incidence of 22% in this study is somewhat higher than other studies performed at the laboratory, it is not inconsistent with other sources of data from other suppliers and from published references. The evidence therefore indicates that the incidence in this individual study should not be excluded from the historical control dataset."

(iii) Finally, a question on the use of the extra set of HCD was asked:
In a further investigation, a HCD compilation was made for Han-Wistar rats, for the period 1983-2006, in RCC Switzerland. In this database, 6/50 studies have a background of adenocarcinoma >10%. However, how do these data relate to those obtained at HLS (as RCC data are obviously not obtained at HLS). The company responded as follows:
"*The RCC data are for carcinogenicity studies performed using HsdRCC Han:WIST (Wistar Hannover) rats, which were supplied by RCC (now Harlan) in Switzerland. The study of Thirlwell et al (2004) was performed using HsdBrl Han:WIST (Wistar Hannover) rats supplied by Harlan UK. Harlan state that the background data for the HsdRCC Han:WIST are equally relevant to the HsdBrl Han:WIST rat, as both sub-lines derive from the same original (BRL-RCC) source.*"

RMS conclusion: the statistically significant increased incidence of the mammary adenocarcinoma study is probably a result of the unusually low study control incidence. In the light of the reported HCD, notwithstanding a high value in one single study, the notifier's case is accepted.

Mice:

The results from a carcinogenicity study in mice (Malek, 1994) using dietary concentrations of 100, 2500 and 7000 ppm indicated increased incidences and multiplicity of hepatic adenomas in male mice given 7000 ppm. Liver weight was increased at this concentration in both sexes and was related in males to centrilobular hepatocyte hypertrophy. The hepatocyte hypertrophy could be indicative of induction of mixed function oxidase systems, but as this was not demonstrated or measured, the effect could be an adaptive physiological response to increased metabolic workload.

Historical control data published by Charles River laboratories for Crl: CD-1 BR mice, (1995) were used by the RMS to evaluate the significance of tumour incidence; the incidence of multiple liver cell adenoma reported at top dose in males (16%) was within the maximum range of historical control data at Charles River Laboratories (19%). The incidence of lung alveolar neoplasms in males receiving 7000 ppm is slightly increased (26/80, 32%) compared to the concurrent untreated control (18/80, 22.5%), is statistically significant ($p<0.05$) and is outside the range of the historical controls at the testing facility (18-21%). However, because the increase is small, and did not demonstrate decreased latency compared to controls, this effect is considered to represent a finding of equivocal toxicological significance.

The "*Lenacil: Review of carcinogenicity and proposed R40 classification paper*" (Dr D Andrew, TSGE, 2012) documents further assessments looking at other historical databases and also concludes the hepatocellular and alveolar effects observed in male mice treated at the highest dose were not applicable to the human health hazard assessment. The incidences of total (i.e. single or multiple) adenomas in the Lenacil study of 13.8-25.0% are comparable to the laboratory's original limited historical control data of 13.6-21.7% and the updated range of 1.8-21.7%. The incidence at the highest dose level therefore marginally exceeds the laboratory's range but does not represent a statistically significant increase compared to the concurrent control.

More extensive published historical control data for male CD-1 mice from Charles River report adenoma incidences of 0.0-26.0%; the adenoma incidence in all groups of male mice in the Lenacil study therefore lies within the background range. It can therefore be concluded that the adenoma incidence in the Lenacil study is not related to treatment. The relative effects of high background incidences and large background control ranges on the interpretation of the study data are discussed more extensively below.

The very high spontaneous occurrence of this tumour type in CD-1 mice is well known and means that they should not be used as a basis for classification of carcinogenicity. This conclusion is supported by the fact there were no statistically significant increases in the individual tumour types (i.e., single or multiple, adenoma or adenocarcinoma) or when total alveolar tumours were evaluated alone by the Fisher's exact test. Further, there was no decrease in tumour latency as most tumours were observed in animals at the end of the eighteen-month exposure period. There was no increase in focal hyperplasia of type II alveolar cells and no shift in tumour cell anaplasia. Finally, there was no treatment-related tumour response in females.

Mouse – Alveolar tumours

In the mouse oral toxicity study performed in 1991-93, groups of Crl:CD-1(ICR)BR mice obtained from Charles River (Quebec) were administered lenacil in the diet at concentrations of 0, 100, 2500 or 7000 ppm. No treatment-related mortality or clinical signs were observed; mean bodyweights and weight gains were unaffected by treatment with Lenacil. Lung tumour incidence was highlighted among male mice dosed orally – only at the high dose level and females were

unaffected in any way. The review of tumour incidence among male mice compared study data with historical control incidence and concluded that the data do not indicate any treatment-related increase in the incidence of bronchoalveolar tumours in male CD-1 mice. The very high spontaneous occurrence of this tumour type in CD-1 mice is well known and means that they should not be used as a basis for classification.

The data are summarised in the position paper prepared to address possible classification of Lenacil as R40, Cat 3. The information is summarised below.

A higher incidence of alveolar tumours was observed in male mice at the highest dose level of 7000 ppm; similar findings were not apparent in females, therefore female mice are not considered further. In male mice, the incidence of alveolar tumours at the highest dose level did not attain statistical significance for individual tumour types (i.e. single or multiple, adenoma or adenocarcinoma) but attained statistical significance when all these tumour types were considered in total. Since this increase was significant when analysed using the Cochrane-Armitage trend test (p =0.0441) but not when analysed using Fisher's exact test (p =0.1075) it is considered to be only of borderline statistical significance. The increase is additionally not considered to be related to treatment with Lenacil in the absence of any significant increase in the incidence of any individual tumour type, any decrease in tumour latency, any increase in the incidence of focal hyperplasia of Type II cells or any shift in tumour cell anaplasia.

Table 23: Mouse carcinogenicity study: incidence of alveolar tumours (males)

Tumour type	**Dose level** (ppm)			
	0	**100**	**2500**	**7000**
Single adenoma	14/80	9/80	15/80	17/80
	17.5%	**11.3%**	**18.8%**	**21.3%**
Multiple adenoma	1/80	2/80	0/80	3/80
	1.3%	**2.5%**	**0.0%**	**3.8%**
Adenoma (single or multiple)	15/80	11/80	15/80	20/80
	18.8%	**13.8%**	**18.8%**	**25.0%**
Single adenocarcinoma	3/80	4/80	4/80	8/80
	3.8%	**5.0%**	**5.0%**	**10.0%**
Multiple adenocarcinoma	1/80	0/80	2/80	0/80
	1.3%	**0.0%**	**2.5%**	**0.0%**
Adenocarcinoma combined	4/80	4/80	6/80	8/80
	5.0%	**5.0%**	**7.5%**	**10.0%**
Alveolar tumours (total)	18/80	15/80	18/80	26/80*
	22.5%	**18.8%**	**22.5%**	**32.5%**

**statistically significant according to the Cochran-Armitage trend test (p<0.05)*

Lung tumours are known to occur in CD-1 mice (and particularly in male CD-1 mice) with a high spontaneous incidence. The relevance of the alveolar tumours seen in the Lenacil study was therefore compared against three sources of historical control data:

- Data from two studies performed by the test laboratory and presented in the original study report.
- More extensive background data for the test laboratory (16 studies initiated between 1983-2000)
- Published data for CD-1 mice from Charles River Laboratories (25 studies performed from 1988-1995).

Male mouse - Single alveolar adenomas

The incidences of single adenomas in the lenacil study of 11.3-21.3% are comparable to the laboratory's very limited historical control data of 11.9-16.7%. Although it is noted that the tumour incidences in males at 2500 ppm (18.8%) and 7000 ppm (21.3%) lie outside the historical range, the fact that the laboratory's background incidence is derived from only two studies and that the range is only slightly exceeded in the lenacil study does not provide a strong indication that the tumours are treatment-related. It is also notable that the concurrent control incidence of 17.5% exceeds the historical range. More extensive historical control data from the performing laboratory give a background range of 5.0-17.5%. Published historical control data from Charles River do not distinguish between animals with single and multiple tumours, therefore a relevant comparison cannot be made. However, comparison can be made for the total adenoma incidence of up to 26.0%. The marginal increase in the incidence of tumours seen in the Lenacil study at dose levels of 2500 ppm (18.8%) and 7000 ppm (21.3%) compared to that in the concurrent control group (17.5%) cannot be considered to be treatment-related in the absence of statistical significance, the high background incidence of this tumour type and the occurrence of a 'spike' in the background incidence at the time of the study.

Table 24: Incidence of single alveolar adenomas in male mice and comparison to historical data

Source	Tumour incidence			
	0 ppm	**100 ppm**	**2500 ppm**	**7000 ppm**
Lenacil study	17.5%	11.3%	18.8%	21.3%
Laboratory background range (original report)	11.9-16.7%			
Laboratory background range (updated)	5.0-17.5%			
Charles River background range	NA			

Male mouse - multiple alveolar adenomas

The incidences of multiple adenomas in the Lenacil study of 0-3.8% are comparable to (and do not exceed) the laboratory's original very limited historical control data of 1.7-5.0%. Incidences also lie within the background range of 0-6.7%, based on the more extensive laboratory data. Findings are therefore clearly not considered to be related to treatment with Lenacil. Published historical control data from Charles River do not distinguish between animals with single and multiple tumours, therefore a relevant comparison cannot be made. However, comparison can be made for the total adenoma incidence.

Table 25: Incidence of multiple adenomas in male mice and comparison to historical data

Source	Tumour incidence			
	0 ppm	**100 ppm**	**2500 ppm**	**7000 ppm**
Lenacil study	1.3%	2.5%	0.0%	3.8%
Laboratory background range (original report)	1.7-5.0%			
Laboratory background range (updated)	0.0-6.7%			
Charles River background range	NA			

Male mouse - Total alveolar adenomas

The incidences of total (i.e. single or multiple) adenomas in the Lenacil study of 13.8-25.0% are comparable to the laboratory's original limited historical control data of 13.6-21.7% and the updated range of 1.8-21.7%. The incidence at the highest dose level therefore marginally exceeds the laboratory's range but does not represent a statistically significant increase compared to the concurrent control.

More extensive published historical control data for male CD-1 mice from Charles River report adenoma incidences of 0.0-26.0%; the adenoma incidence in all groups of male mice in the Lenacil study therefore lies within the background range. It can therefore be concluded that the adenoma incidence is not related to treatment.

Table 26: Incidence of total adenomas in male mice and comparison to historical data

Source	Tumour incidence			
	0 ppm	**100 ppm**	**2500 ppm**	**7000 ppm**
Lenacil study	18.8%	13.8%	18.8%	25.0%
Laboratory background range (original report)	13.6-21.7%			
Laboratory background range (updated)	1.8-21.7%			
Charles River background range	0.0-26.0%			

Male mouse - Single alveolar adenocarcinomas

The incidences of single adenocarcinomas in the Lenacil study were 3.8-10.0%; the incidence was highest at the highest dose level of 7000 ppm and this exceeded the laboratory's historical control range (0.0-5.1%), however the value of this data is severely limited by the fact that it is based on two studies only. The more extensive historical control data for the laboratory gives a range of 2.5-11.3%; the tumour incidences in the Lenacil study are therefore clearly within the background range and cannot be considered to be treatment-related. Published historical control data from Charles River do not distinguish between animals with single and multiple tumours, therefore a relevant comparison cannot be made, however comparison can be made with the total adenocarcinoma incidence of up to 23.2%.

Table 27: Incidence of single adenocarcinomas in male mice and comparison to historical data

Source	Tumour incidence			
	0 ppm	**100 ppm**	**2500 ppm**	**7000 ppm**
Lenacil study	3.8%	5.0%	5.0%	10.0%
Laboratory background range (original report)	0.0-5.1%			
Laboratory background range (updated)	2.5-11.3%			
Charles River background range	NA			

Male mouse - Multiple alveolar adenocarcinomas

The incidences of multiple adenocarcinomas in the Lenacil study were 0.0-2.5%; no animals with multiple tumours are noted in the laboratory's historical control data in the study report, however the value of this data is severely limited by the fact that it is based on two studies only. The more extensive historical data from the laboratory gives a background range of 0-2.5%; the tumour incidences in the Lenacil study are therefore within the background range and cannot be considered to be treatment-related. It is also notable that there is no dose-response relationship for the number of animals exhibiting multiple tumours; the incidence was highest in the intermediate dose group and no animals with multiple tumours were noted in the high dose group. Findings are therefore clearly not related to treatment with Lenacil. Published historical control data from Charles River do not distinguish between animals with single and multiple tumours, therefore a relevant comparison cannot be made, however comparison can be made with the total adenocarcinoma incidence.

Table 28: Incidence of multiple adenocarcinomas in male mice and comparison to historical data

Source	Tumour incidence			
Lenacil study	**0 ppm**	**100 ppm**	**2500 ppm**	**7000 ppm**
	1.3%	0.0%	2.5%	0.0%
Laboratory background range (original report)	0.0%			
Laboratory background range (updated)	0.0-2.5%			
Charles River background range	NA			

Male mouse - Total adenocarcinomas

The incidences of total (i.e. single or multiple) adenocarcinomas in the Lenacil study are 3.8-10.0%. Although it is noted the tumour incidence at 7000 ppm (10.0%) lies outside the laboratory's original historical range (0-5.1%) reported in the study report, the incidence is clearly within the range (0.0-12.5%) based on the more extensive laboratory data.

More extensive published historical control data for male CD-1 mice from Charles River report adenocarcinoma incidences of 0.0-23.2%; the adenocarcinoma incidence in all groups of male mice in the lenacil study therefore clearly lies within the background range. It can therefore be concluded that the adenocarcinoma incidence in the Lenacil study is not related to treatment.

Table 29: Incidence of total adenocarcinomas in male mice and comparison to historical data

Source	Tumour incidence			
Lenacil study	**0 ppm**	**100 ppm**	**2500 ppm**	**7000 ppm**
	5.0%	5.0%	7.5%	10.0%
Laboratory background range (original report)	0.0-5.1%			
Laboratory background range (updated)	0.0-12.5%			
Charles River background range	0.0-23.2%			

Male mouse - Total alveolar tumours

The incidences of total alveolar tumours (i.e. single or multiple; adenomas or adenocarcinomas) in the Lenacil study are 18.8-32.5%. Although it is noted the tumour incidences in males at 7000 ppm (32.5%) lies outside the laboratory's historical range (18.6-21.7%), the fact that the background incidence is only derived from only two studies does not provide a strong indication that these tumours are treatment-related. The more extensive laboratory data report a background incidence of 3.8-25.0%. Published historical control data from Charles River do not distinguish between animals with single and multiple tumours and do not include figures for animals with combined tumours, therefore a direct comparison cannot be made with the Lenacil study. However the high background incidence of both tumour types in male CD-1 mice (for example incidences of 21.7% for adenoma and 23.2% for adenocarcinoma incidence reported in one study in the Charles River data) clearly indicates that the total tumour incidence of 32.5% in the 7000 ppm Lenacil group is very likely to be within the background range, even allowing for a fact that a small number of animals may exhibit both tumour types.

Additional information on the background incidence of lung tumours in CD-1 mice is provided by literature data. Manenti *et al* (2003) report total lung tumour incidences of up to 61.1% in male CD-1 mice (range 8.8-61.1%); Fox *et al* (2007) also report total lung tumour incidences of up to 43% in male CD-1 mice. Maita *et al* (1988) report a mean incidence of 33.4% for total lung tumours in male CD-1 mice based on data from eleven carcinogenicity studies, with a range of 21.3-43.8%.

Table 30: Incidence of total alveolar tumours in male mice and comparison to historical data

Source	Tumour incidence			
	0 ppm	**100 ppm**	**2500 ppm**	**7000 ppm**
Lenacil study	22.5%	18.8%	22.5%	32.5%*
Laboratory background range (original report)	18.6-21.7%			
Laboratory background range (updated)	3.8-25.0%			
Charles River background range	NA			

Therefore, there is insufficient evidence to indicate that Lenacil induces bronchoalveloar tumours in CD-1 mice. The very high spontaneous occurrence of this tumour type in CD-1 mice is well known and means that they should not be used as a basis for classification. This conclusion is supported by the fact there were no statistically significant increases in the individual tumour types (i.e., single or multiple, adenoma or adenocarcinoma) or when total alveolar tumours were evaluated alone by the Fisher's exact test. Further, there was no decrease in tumour latency as most tumours were observed in animals at the end of the eighteen-month exposure period. There was no increase in focal hyperplasia of type II alveolar cells and no shift in tumour cell anaplasia. Finally, there was no treatment-related tumour response in females.

Conclusion

The available data show that the incidence of mammary gland tumours in females in the rat carcinogenicity study and the incidence of lung tumours in males in the mouse carcinogenicity study performed with Lenacil are not related to treatment. In the absence of any evidence of treated-related carcinogenicity in animal studies, Lenacil does not fulfil the criteria for classification

with ‘R40’ ‘Limited evidence of a carcinogenic effect’ (Category 3 carcinogen) under Directive 67/548/EEC) and therefore also does not fulfil the criteria for classification as a Category 2 carcinogen under the CLP Regulation (EC 1272/2008).

The classification with R40 (DSD) or H351 (CLP), as a carcinogen, is therefore not required for Lenacil according to the Dangerous Substances Directive or the CLP Regulation

4.10.1.2 Carcinogenicity: inhalation

No study data are available for exposure via the inhalation route.

4.10.1.3 Carcinogenicity: dermal

No study data are available for exposure via the dermal route.

4.10.2 Human information

No data available

4.10.3 Other relevant information

none

4.10.4 Summary and discussion of carcinogenicity

The EFSA Conclusion on the peer review of Lenacil (2009) noted an increased incidence of malignant mammary adenocarcinoma in the rat carcinogenicity study and considered these to be of relevance for humans. In the mouse carcinogenicity study, increased incidences of lung single alveolar tumours (adenoma and carcinoma) and multiple liver adenomas were observed and were considered to be of equivocal relevance for humans. Based on the findings of mammary gland tumours in female rats and lung tumours in male mice, the EFSA conclusion proposes the classification (R40) ‘Limited evidence of a carcinogenic effect’ [Category 3 carcinogen] for Lenacil. The relevant findings from the rat carcinogenicity study (mammary gland tumours in females) and the mouse carcinogenicity study (lung tumours in males) performed with Lenacil are summarised in 4.10.1.1 above. The significance of the findings is considered in light of more extensive historical control data, and the implications of the findings for the classification of Lenacil as a carcinogen are discussed.

In conclusion there are no data to support any necessity to classify Lenacil for tumorigenicity.

4.10.5 Comparison with criteria

These various relevant factors have been evaluated in the position paper (“*Lenacil: Review of Carcinogenicity and Proposed R40 Classification. Report No. TSGE 19-10-05*”, Andrew, D. TSGE, 2012). Based on the available study data and historical control information, it is concluded that classification in accordance with DSD and CLP criteria is not warranted for Lenacil in respect of carcinogenicity.

Factors for additional consideration :

(a) tumour type and background incidence;

both rat-liver and mouse-lung tumour incidence were within the historical control incidence. Although it is noted the mouse lung tumour incidence at 7000 ppm (10.0%) lies outside the laboratory's original historical range (0-5.1%) reported in the study report, the incidence is clearly within the range (0.0-12.5%) based on the more extensive laboratory data.

(b) multi-site responses;

Increase of other tumour types were not observed: only mammary tumour in the rat and lung tumours in the mouse

(c) progression of lesions to malignancy;

both for mammary and lung tumour, there was no indication that treatment-related increase of preneoplastic or hyperplastic events occured

(d) reduced tumour latency;

the latency time was not reduced, neither for the mammary tumours, nor for the lung tumours

(e) whether responses are in single or both sexes;

the mammary tumours are confined to the female rat; the apparent increase of alveolar tumours is restricted to the male mouse

(f) whether responses are in a single species or several species;

mammary tumours were found in the rat but not in the mouse, and conversely lung alveolar tumours were found in the mouse but not in the rat.

(g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity;

no mammary nor lung tumours were observed in other known uracil herbicides

(h) routes of exposure;

only relevant for the oral route; there is no need to investigate other routes of entry

(i) comparison of absorption, distribution, metabolism and excretion between test animals and humans;

there is no experimental information concerning comparative toxcokinetic or metabolic behaviour between species. As far as the test animals are concerned, there is no indication of a meaningful difference of sensitivity between species.

(j) the possibility of a confounding effect of excessive toxicity at test doses;

the carcinogenesis studies were performed up to doses >1000 mg/kg bw/d; no excessive toxicity was observed

(k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity."

as the incidences were within HCD, no mechanistic study was performed. However, lenacil is not mutagenic, induces no cell division in any particular organ, and didsplays no immunotoxic action.

4.10.6 Conclusions on classification and labelling

The available data show that the incidence of mammary gland tumours in females in the rat carcinogenicity study and the incidence of lung tumours in males in the mouse carcinogenicity performed with Lenacil are not considered related to treatment, due to high historical control incidence, and very low study control value. In the absence of any evidence of carcinogenicity in animal studies, Lenacil does not fulfil the criteria for classification with (R40) '*Limited evidence of a carcinogenic effect*' (Category 3 carcinogen) under Directive 67/548/EEC) and does not fulfil the criteria for classification as a Category 2 carcinogen under the CLP Regulation (EC 1272/2008).

No classification as a carcinogen is therefore required for Lenacil, according to the Dangerous Substances Directive or the CLP Regulation.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

The DS reported the EFSA Conclusion on the peer review of Lenacil (2009) in which an increased incidence of malignant mammary adenocarcinoma in the rat carcinogenicity study was considered of relevance for humans. In the mouse carcinogenicity study, increased incidences of single, alveolar lung tumours (adenoma and carcinoma) and multiple liver adenomas were observed and were considered to be of equivocal relevance for humans. Based on the findings of mammary gland tumours in female rats and lung tumours in male mice, EFSA proposed classification as a Category 3 carcinogen under DSD (R40; 'Limited evidence of a carcinogenic effect') for Lenacil.

The significance of these findings was considered in the CLH report by the DS in the light of more extensive historical control data. According to the DS, evidence of the carcinogenic potential of Lenacil is equivocal and no mechanism of oncogenicity was established. Data from carcinogenicity studies in rats and mice, together with background incidence rates derived from various historical databases, supported the conclusion that Lenacil administration was not associated with a toxicologically significant increase in mammary tumour incidence. Similarly, pulmonary tumours in male mice were also shown to fall within historical ranges and no clear evidence of a treatment-association with Lenacil was established.

Overall, the DS concluded that Lenacil was not carcinogenic and no classification was proposed for carcinogenicity.

Comments received during public consultation

One MSCA and one company indicated their general support for the no classification proposed by the DS. One MSCA specifically mentioned its support for no carcinogenicity classification in contrast to the EFSA conclusion when considering the additional information provided on historical control values.

Assessment and comparison with the classification criteria

Various tumour types are induced by Lenacil in both rats (females and males) and male mice. They are discussed separately below:

- Induction of thyroid tumours in female rats.
  - The incidence of follicular cell adenoma was significantly increased in high-dose females but remained within the historical control data (HCD) for the laboratory. The incidence of carcinomas was not elevated at any dose when compared to the

controls. The incidence of combined adenomas and carcinomas was within the HCD for adenomas only and there was no evidence that Lenacil induced follicular cell tumours.

- An increased incidence of C-cell adenomas was observed in females, which was not (although borderline) statistically significant at mid-dose (p=0.051). The incidence exceeds the laboratory HCD at the two highest doses but without clear dose-response relationship. Two females in the high dose group had C-cell carcinomas. This incidence is above available HCD. A dose-response was observed for the incidence of combined C-cell tumours.
- Thyroid is a target organ of Lenacil in rats. The effects consist mainly of dark appearance of the thyroid. Microscopically, lipofuscin staining of the follicular epithelium indicates membrane degradation and in follicular cell hypertrophy. However, no microscopic treatment-related effects were reported in C-cells. The primary function of C-cells is to secrete calcitonin that reduces the blood calcium level. No effect was reported on calcium homeostasis in the 90-day study in the rat studies. Calcium levels were significantly decreased in males but significantly increased in females at the end of the carcinogenicity study at all doses but without a dose-response so, a link to treatment was unclear.
- Contrary to humans in which there is no great change in C cells with age, laboratory rats show an age-related increase in the number of C-cells and this may correlate with the fact that tumours of the C cells are relatively common findings in aged rats (Thomas & Williams, 1999), in particular in females as stated in the CLH report.
- Overall, considering that the incidence of C-cell tumours in female rats was marginally above HCD, there is equivoqual evidence of carcinogenicity of Lenacil on the thyroid in the rat.

- Induction of mammary gland tumours in female rats.
  - The incidence of mammary adenoma was elevated in high-dose females (6%); this value was not statistically significant using a pair-wise comparison but attained statistical significance (p =0.028) using a trend test. The incidence of this benign tumour very marginally exceeds the laboratory's historical control range (0-5.5%).
  - The incidence of benign fibroadenoma was not increased significantly or above HCD.
  - The incidences of mammary adenocarcinoma in the mid- and high-dose groups of 6/50 (12%) and 5/50 (10%) respectively are significantly increased when compared to the concurrent control incidence of 0/50 (0%), but without a clear relationship to dose level. However, the absence of findings in the concurrent control is unusual and was seen only in one of the 19 studies constituting the updated laboratory historical data (mean HCD incidence 4.81%). The statistical significance of the findings at 2500 and 25000 ppm is therefore attributable to an unusually low concurrent control incidence. The incidences of this tumour type in the 2500 ppm and 25000 ppm dose groups lie within the laboratory's overall, updated historical control range (0-22%). However, detailed analysis of the distribution of HCD shows that the upper incidence of 22% was observed in a single study out of 19 and the maximum value in the 18 other studies was 8%. After exclusion of this outlier, the incidences of mammary adenocarcinomas were slightly above HCD at the mid- and high doses.
  - The incidence of combined mammary adenomas and adenocarcinomas was not provided but a dose-response is likely for combined tumours (although this calculation may overestimate cumulative incidences as some animals may bear both adenomas and adenocarcinomas, addition of adenomas and adenocarcinomas incidences result in incidences of 0, 6, 12 and 16% in females exposed to 0, 250, 2500 or 25000 ppm).
  - Combined incidences for all mammary tumour types (fibroadenomas, adenomas

and adenocarcinomas revealed no dose-response relationship, with a statistical significant increase of tumours only at the low dose that is mainly due to fibroadenomas, but within HCD (and below mean HC incidence) for this tumour type alone.

- Overall, the incidence of adenocarcinomas in the mammary gland is significantly increased and elevated compared to expected incidence based on the analysis of HCD at the mid- and high-dose. With the support of an elevated incidence of adenomas at the highest dose and an apparent dose-response when adenomas and adenocarcinomas are added, there is some evidence of carcinogenicity of Lenacil on the mammary gland in the rat.

- Induction of liver adenomas in male mice.
  - No increase of liver single adenomas was observed. The incidence was similar in controls and high dose males.
  - A statistically significant increase of multiple adenomas was observed in high dose males.
  - Laboratory historical control data were not provided. Although of lower relevance, historical control data at Charles River Laboratories were considered but the incidence of liver cell multiple adenoma reported in males at the highest dose (16%) is within the maximum range of historical control data at Charles River Laboratories (28%, single or multiple type not specified). Cumulative incidence of single and multiple adenomas at the high dose (30%) is slightly above this HCD.
  - No increase of liver carcinomas was observed.
  - Incidence and historical control data for combined hepatocellular adenomas and carcinomas were not provided and no conclusion is possible on a combined analysis of tumours.
  - Considering the lack of effect observed on hepatic single adenomas and carcinomas and that only benign tumours were increased, the significance of the isolated increase of multiple adenomas is unclear. There is equivoqual evidence of carcinogenicity of Lenacil in the mouse liver.

- Induction of lung alveolar tumours in male mice.
  - The incidences of single adenomas (11.3-21.3%) are comparable to the laboratory's very limited historical control data of 11.9-16.7%. Although it is noted that the tumour incidences in males at 2500 ppm (18.8%) and 7000 ppm (21.3%) lie outside the historical range, the fact that the laboratory's background incidence is derived from only two studies and that the range is only slightly exceeded in the Lenacil study does not provide a strong indication that the tumours are treatment-related. It is also notable that the concurrent control incidence of 17.5% exceeds the historical range. More extensive historical control data from the performing laboratory provided a background range of 5.0-17.5%. The marginal increase in the incidence of tumours seen in the Lenacil study at dose levels of 2500 ppm (18.8%) and 7000 ppm (21.3%) compared to that in the concurrent control group (17.5%) cannot be considered to be treatment-related in the absence of statistical significance and considering that the incidence in controls is also at the upper limit of the background incidence of this tumour type. The incidence of multiple alveolar adenomas was not significantly increased and was below the laboratory HCD.
  - The incidences of total (i.e. single or multiple) adenocarcinomas in the Lenacil study are 3.8-10.0%. Although it is noted the tumour incidence at 7000 ppm (10.0%) lies outside the laboratory's original historical range (0-5.1%) reported in the study report, the incidence is clearly within the range (0.0-12.5%) based on the more extensive laboratory data.
  - Overall, a significantly increased incidence of alveolar tumours is observed in male mice at the highest dose. The incidence is above laboratory historical control data. However, several studies in the literature provide evidence of the high incidence of

bronchoalvealoar tumours in CD-1 male mice, up to 61.1% (Manenti, 2003), 43% (Fox, 2007) and 33.4% (Maita, 1988).

- Besides, it is noted that lung is not a target organ of Lenacil toxicity and that the observed increase was restricted to males.
- The link between the induction of bronchoalveolar tumours and Lenacil is therefore uncertain

Overall, RAC considered that the classification of Lenacil in category 2 for carcinogenicity under CLP (Carc 2 – H351) and carcinogenicity 3 under DSD (Carc. cat. 3; R40) was warranted, based on some evidence of induction of mammary gland tumours in female rats.

Supplemental information - In depth analyses by RAC

Carcinogenicity of Lenacil has been investigated in a 2-year rat study and in an 18-month mouse study.

In a 2-year study (Thirlwell, 2004), Wistar rats were exposed through diet to 250, 2 500 or 25000 ppm (corresponding to 14/19, 139/188 or 1446/1894 mg/kg bw/d in males/females, respectively) for 52 (n=20/sex/dose) or 104 weeks (n=50/sex/dose). Toxicity findings are reported in the repeated toxicity section here above.
Increased incidence of thyroid and mammary gland tumours were observed as reported in Table 4 below.

Table 4 – significant neoplastic findings in the rat 2-year study

Dose	**0 ppm**		**250 ppm**		**2500 ppm**		**25000 ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Thyroid:								
Follicular cells								
Adenoma	6%	2%	4%	0	4%	2%	10%	8%** *
Laboratory HCD[a]	Male: 0.0%-16% Female: 0.0% -11.7%							
Carcinoma	0	2%	0	0	2%	2%	2%	0
Combined adeno/carc.	6%	4%	4%	0	6%	4%	10%	8% **
C-cell								
Adenoma	8%	4%	6%	4%	10%	16%	10%	14%
Laboratory HCD[a]	Male: no data Female: 0-13.6%							
Carcinoma	0	0	0	0%	0	0	0	4%
Laboratory HCD[a]	Male: 0-5.1% Female: 0-1.7%							
Combined adeno/carc.	8%	4%	6%	4%	10%	16%	10%	18%* **
Mammary gland:								
Adenoma		0		2%		0		6%**
Laboratory HCD[a]	Females: 0-2%							
Updated laboratory HCD[d]	Females: 0-5.50%							
Fibroadenoma benign		14%		24%		16%		16%
Laboratory HCD[a]	Females: 16.7%-33.3%							
Malignant adenocarcinoma		0		4%		12%*		10%*
Laboratory HCD[a]	Females: 0.0%-6.7%							
Updated lab. HCD[d]	Females: 0-22.0%							
Combined mamm. tumours		14%		30%*		26%		20%

* Statistically significant pair wise comparison ** trend test statistically significant
[a] historical control data; from 10 studies initiated at the test laboratory during 1996-2001, referred to in the original study report
[b] data compiled from reviews of tumour incidence in Wistar rats (Poteracki & Walsh, 1998)
[c] Published data for Wistar Han Rats from Charles River Laboratories (10 studies terminated in 1999 or earlier)
[d] updated database of 19 studies performed at the test laboratory from 2001-2006

Detailed information of the updated laboratory historical control data (HCD) for mammary tumours and its distribution (see Table 5 below) is available and shows that the upper incidence reported in HCD for adenocarcinomas is unusual and after exclusion of this isolated finding, the upper limit for HCD is 8%.

Table 5 – number of studies from HCD by category of incidence (out of 19 studies)

Incidence range	0	>0-5%	>5-10%	>10-15%	>15-20%	22%	Range	Mean
Adenocarcinoma	2	9	7	0	0	1	0 - 22%	4.81%
After exclusion of the single upper value							0 - 8.0%	

In an 18-month study (Malek, 1994), CD-1 mice (n=80/sex/dose) were exposed through diet to 100, 2 500 or 7 000 ppm (corresponding to 14/20, 332/482 or 977/1358 mg/kg bw/d in males/females, respectively). Toxicity findings are reported in the repeated toxicity section here above. Increased incidence of liver and lung tumours were observed as reported in Table 6 below.

Table 6 – significant neoplastic findings in the mouse 18-month study

Endpoints/dose	**0**		**100**		**2500**		**7000 ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Liver: hepatocellular tumours								
Adenoma single	14%	2.5%	12%	0	12%	0	14%	1%
Adenoma multiple	0	0	6%	0	5%	0	16%* **	0
Published HCD	Male: 0-28%, Female 0-7.84%							
Carcinoma	6%	0	4%	0	4%	0	2.5%	0
Lung alveolar:								
Adenoma single	17%	6%	11%	6%	19%	5%	21%	7.5%
Laboratory HCD	Male: 11.6-16%							
Updated laboratory HCD	Male: 5.0-17.5%							
Adenoma multiple	1%	1%	2.5%	0	0	2.5%	4%	0
Laboratory HCD	Male: 1.6-5%							
Adenoma total	19%		14%		19%		25%	
Laboratory HCD	Male: 13.6-21.7%							
Updated laboratory HCD	Male: 1.8-21.7%							
Carcinoma single	4%	4%	5%	5%	5%	2.5%	10%	2.5%
Laboratory HCD	Male: 0-5.1%							
Updated laboratory HCD	Male: 2.5-11.3%							
Carcinoma multiple	1%	1%	0	0	2.5%	0	0	0
Laboratory HCD	Male: 0							
Updated laboratory HCD	Male: 0-2.5%							
Carcinoma total	5%		5%		7.5%		10%	
Laboratory HCD	Male: 0-5.1%							
Updated lab. HCD	Male: 0-12.5%							
Combined alveolar	22.5%	12.5%	19%	10%	22.5%	9%	32%*	10%
Laboratory HCD	Male: 18-21%							

*p<0.05 for Cochran Armitage trend test and for ** Fisher exact test;
Laboratory HCD from 2 studies, updated laboratory HCD from 16 studies initiated between 1983-2000 and Published HCD for liver adenomas or bronchio/alveolar tumours from Charles River laboratories, 2000, Crl: CD-1 BR mouse (from 46 studies performed between 1987 and 1996).

4.11 Toxicity for reproduction

Table 31: Summary table of relevant reproductive toxicity studies

Method **Tested doses *(ppm)* and mg/kg b.w./d**	**Results at doses *(ppm)* and mg/kg b.w./d**	**Remarks**	**Reference**
Preliminary study of reproductive performance in rats.	Slightly low bodyweight gains for F_0 females at 50000 ppm prior to pairing and low bodyweights generally for treated females (10,000, 20,000 or 50,000 ppm) during middle phase of lactation.	Doses up to 50000 ppm were well tolerated and considered suitable for the main study investigation	Patten, 2002
Two-generation reproductive performance study in rats, diet *(0, 1000, 10000 or 50000 ppm)* 0, 81.9, 817, 4279 mg/kg bwb/d	NOAEL systemic: *(1000 ppm)* 81.9 mg/kg bw/d	LOAEL systemic: *(10000 ppm)* 817 mg/kg bw/d Thyroid toxicity	Patten, 2003
	NOAEL offspring: *(1000 ppm)* 89.7 mg/kg bw/d	LOAEL offspring: *(10000 ppm)* 817 mg/kg bw/d Decreased body weight gain during lactation	
	NOAEL reproduction: *(10000 ppm)* 1727 mg/kg bw/d	LOAEL reproduction: *(50000 ppm)* 4279 mg/kg bwb/d Altered lactation at top dose R64 classification was initially considered but is not proposed by EFSA	
Preliminary embryotoxicity investigation in rats	NOEL *(5000 ppm)* 485.7 mg/kg bw/day or	No effects observed on dams or offspring	Smith, 1978
Developmental toxicity study in rats, oral (gavage) 0, 100, 300, 1000 mg/kg b.w./d	Maternal and developmental NOAEL >1000 mg/kg bw/d	No effects observed on dams or offspring at 1000 mg/kg bw/d	Patten, 2003
Developmental toxicity study in rabbits, oral (gavage) 0, 50, 200, 1000, 4000 mg/kg b.w./d	NOAEL Maternal: 1000 mg/kg bw/d	Reduced bodyweight gain for dams at 1000 mg/kg bw/d	Hurtt, 1991
	NOAEL Developmental: >4000 mg/kg bw/d	No effects on offspring up to 4000 mg/kg bw/day	

4.11.1 Effects on fertility

4.11.1.1 Non-human information

In a two-generation study (Patten, 2003), dietary administration of Lenacil to Han Wistar rats at concentrations of 1000, 10000 or 50000 ppm was associated with effects at 50000 ppm on maternal bodyweight change (10%, $p<0.05$) during gestation and lactation, and body weight performance for the resultant progeny. At 10000 and 50000 ppm there was evidence of altered thyroid and liver metabolism in parental animals. There was, however, no effect on reproductive organs or reproductive performance at any of the dietary concentrations and offspring survival was unaffected by treatment. In addition, there was no effect upon the physical and sexual development of the offspring. Additional thyroid function tests undertaken in response to the findings in this study showed Lenacil is not directly toxic to the thyroid at dose levels up to 50000 ppm in the rat

Thus the reproductive no-observed-effect-level (NOEL) in this study was 50,000 ppm (equivalent to mean dosages in the region of 4278.8 to 5312.8 mg/kg bw/day for males and 4787.6 to 8839.8 mg/kg bw/day for females. The systemic no-observed-adverse-effect-level (NOAEL) in this study was 10,000 ppm (equivalent to mean dosages in the region of 817 to 1013 mg/kg bw/day for males and 935 to 1734 mg/kg bw/day for females).and the NOEL was 1000 ppm (equivalent to mean dosages in the region of 81.9 to 99.5 mg/kg bw/day for males and 92.5 to 166.6 mg/kg bw/day for females).

The results of this study confirmed the absence of any effect on reproductive organs or reproductive performance, offspring survival or physical and sexual development of the offspring.

2-generation study

- Study of Reproductive Performance in Han Wistar Rats treated continuously through two successive Generations by Dietary Administration, Huntingdon Life Sciences, ACD 020/023865 (Pattern, 2003a)

Material and methods

GLP status: yes
Guideline: study is in compliance with Dir EEC 87/302/EEC Annex V B or OECD test guideline n° 416 (2001-1983)..
Reproductive function and fertility was assessed in a preliminary study in sexually mature male and female rats of the Hsd Brl Han Wistar strain. Lenacil technical (Batch No. 141712003, purity 98.6%) was administered continuously via the diet through two successive generations at levels of 10000, 25000 or 50000 ppm. A fourth group received the basal diet without the test material and served as the Control. The F0 generation comprised 8 males and 8 females per group, which were treated for 14 days prior to pairing, throughout pairing, during gestation and lactation and up to termination. Selected F1 animals, 12 males and 12 females in each group, received the treated diet from weaning up to completion of physical sexual maturation. The mean concentrations of lenacil technical in formulations prepared for dosing during weeks 1 and 12 of the study ranged from 95.2 to 103% of nominal concentrations.
In the main study, the F0 generation comprised 28 male and 28 female rats, received the diet for 10 weeks before pairing, throughout pairing, gestation and lactation, until termination; F0males were terminated after 17 weeks of treatment and the F0 females were terminated on day 28 post partum and the unselected F1 offspring were terminated at day 30 of age. Selected F1 rats, comprising 24 males and 24 females were exposed to diet from weaning until they were paired for mating at approximately 14 weeks of age.
Batches of the test diets were prepared and issued each week. The stability and homogeneity of the dietary formulations had been assessed and confirmed by a trial preparation prior to the study start. The stability was confirmed over 21 days. Concentration analyses were performed throughout the study at weeks 1, 11, 18, 28 and 32 and satisfactory levels were obtained (average –0.5%: range 5.0 to –4.4%)
The study is accepted.

Findings:

Parental data:

Mortality was not considered to be treatment related.

Clinical signs: F0 rats did not show signs attributed to treatment.

In F1 at top dose, male showed an increased incidence of hair loss from the dorsal body surface from week 3, with females being similarly affected up to week 8.

Body weight:

- Before mating F0 rats were unaffected by treatment. At the start of the F1 generation, week 0, weight was not affected. The overall bw for F1 males was unaffected by treatment. Females receiving top dose showed slightly lower weight gain for the 10-week period prior to pairing.

- During gestation, F0 females at 10000 and 50000ppm and F1 females at 50000ppm lost slightly weight.

- At 50000ppm, during the lactation period, maternal body weight gain tended to be superior to the controls and did not show the weight loss that is generally seen as the offspring become more independent and the lactation demand is reduced. This suggests that the lactation demand at this dietary concentration was not as high a in the controls and, as consequence, there was no major impact on maternal weight gain as the offspring started to consume the diet.

- The initial birth weight of the F1 and F2 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 F1 offspring and from day 4 of age for the F2 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.

This effect could have triggered a labelling of lenacil with **R64**. However, this proposal was discussed and during the EFSA peer-review, it was considered that the effect was insufficient to warrant classification.

Food consumption and food conversion efficiency of F0 animals was unaffected during the first 10 weeks of treatment.

The overall food efficiency of F1 rats was slightly low during the 10-week period prior to pairing for mating and for animals receiving 50000ppm.

Reproduction performance: oestrus cycle, mating performance, fertility, gestation index and lenght, litter size, sex ratio and offspring survival were unaffected.

Lenacil did not delay the return to normal oestrus cycle of the F0 and F1 females, with all females showing oestrus before termination on day 28 post partum (PP). Sperm motility, morphology and concentration were unaffected by treatment.

Organ weight: liver weight was high in F0 and F1 parental males rats at 10000 and 50000ppm and for F1 rats at 50000ppm and thyroid weight was high at 50000ppm. At top dose, there was centrilobular hypertrophy in some rats.

The F1 females at top dose had low uterine weight on day 28 post partum. A comparison of the individual uterine weights with the oestrus cycle classification at termination showed a correlation between stage of the oestrus cycle on the morning of termination and the uterine weight at

termination. Rats at pro-oestrus tended to have the highest uterine weights, whilst those at metoestrus tended to have the lowest uterine weights. The apparent decrease in uterus weight at top dose may therefore be simply related to the stage of oestrus rather than a result of treatment because a high proportion of control females were at pro-estrus prior to termination, whilst a high proportion of females given 50000ppm were at metoestrus.

Macroscopic findings: F0 males or F1 offspring did not reveal any findings that could be attributed to treatment. On day 28 PP, the majority of females that received 50000ppm had dark thyroids, wtih one female given 10000ppm being similarly affected in F0. Discoloration of the thyroid gland has been reported as a treatment related effect of administration of a variety of compounds and can be attributed either to an accumulation of the chemical/metabolite, or to increased cellular lipid oxidation.

Histopathology:

Examination of the thyroid sections stained with hematoxylin and eosin revealed a minimal or slight accumulation of pigment in the follicular epithelium of some animals at top dose.

In the F0 and F1 females at 10000 and 50000ppm, there was an increased incidence and severity of Schmorl's positive pigment whilst in males F0 and F1 given 50000ppm there was an increased severity of this change. A slight increased incidence of follicular cell hypertrophy was observed in some animals, which may indicate hyperactivity of the thyroid. Follicular cell debris was present in the colloid of a few rats given 10000ppm and in rats given 50000ppm and was generally associated with the Schmorl's positive pigment. The presence of cellular debris in the follicles of a few animals is indicative of increased follicular cell turnover as a consequence of an increase in metabolic activity. A follicular cell adenoma was observed in a F1 male given the top dose and, in view of this treatment related changes observed in the thyroids, involvement of treatment in this finding cannot be excluded. Additional investigations were performed on thyroids to clarify the toxicological significance of the thyroid findings. The further thyroid tests concluded that there was no evidence to suggest that lenacil affected the ability of the thyroid to take up and organify iodide and lenacil dose not act as an inhibitor of the deiodinase which converts T4 toT3.

Litter data:

Pre-weaning surface and air righting reflex were unaffected and all F1 offspring displayed normal auditory and visual responses. Physical sexual maturation of the selected F1 rats, as assessed by the age and bw at completion of balano-preputial separation and vaginal opening, was unaffected by treatment.

Table B.31-1: 2 generation rat study with lenacil:

Endpoints/dose	**0**		**1000ppm**		**10000ppm**		**50000ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Mortality						1 F0 day 24 PP		1 F0 week 2
Compound intake mg/kg bw/d								
Prior pairing F0			82	92.5	817	935	4279	4787
F1			99.5	107	1013	1115	5312	5762
Gestation F0				92		919		4839
F1				90		965.6		5060
Lactation F0				166		1727		8659
F1				164		1733		8839
Clinical signs:								
Hair loss F0	2	7	1	3	3	6	5	10
Hairloss F1	1	6	1	4	5	5	12	10
Body weight:								
Prior pairing F0						↓4%	↓5%	↓4%
F1			↓5%	↓1%	↓2%	↓6%	↓4%	↓9%
During gestation F0 d 0-20						↓10%*		↓7%*
d 0-20 F1								↓9%*
Bw change Offspring F1 Day1-21							↓6%*	↓6%*
Bw change Offspring F2 Day1-21							↓11%*	↓11%*
Food conversion efficiency: F0						↓5%	↓7%	↓7%
F1							↓8%	↓11%
Organ weight absolute:								
Liver F0							↑8.5%	↑13%*
Liver F1							↑9%*	
Thyroid + Para F0							↑17%*	
Spleen F1								↓9%*
Spleen F1 offspring							↓14%*	↓20%*

Endpoints/dose	**0**		**1000ppm**		**10000ppm**		**50000ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Spleen F2 offspring			↓12%*		↓9%*		↓14%*	↓14%*
Thymus F1								↓21%*
Thymus F1 offspring							↓13%*	↓14%*
Thymus F2 offspring					↓7%*	↓11%*	↓18%*	↓13%*
Pituitary F1							↑28%*	
Uterus & cervix F1								↓22%*
Relative organ weight:								
Liver F0					↑4%*	↑5%*	↑12%*	↑16%*
Liver F1					↑7%*		↑12%*	↑16%*
Thyroid + Para F0							↑19%*	↑12%*
Thyroid+para F1							↑16%*	↑14%*
Spleen F1 offspring							↓15%*	
Thymus F2 offspring							↓10%*	
Thymus F1								↓17%*
Pituitary F1					↑26%*		↑40%*	
Macroscopy:								
Thyroid dark F0					0	1	1	25*
Thyroid dark F1					5*	8*	23*	22*
Histopathology:								
Thyroid: follicular cells								
Debris: F0					6*	5*	15*	25*
F1					1	2	5	15*
Schmorl positive pigment:								
F0 Minimal/slight/moderate	6/7/1	7/0/0	8/5/1	8/1/0	7/6/3	12/6/2	* 4/5/10	* 4/12/8
F1Minimal/slight/moderate/marked	8/5/1/0	0/0/0/0	6/7/2/0	2/0/0/0	9/7/2/0	* 11/2/0/0	* 2/5/11/5	* 5/10/5/0
Hypertrophy:								
F0	1	0	0	0	3	0	4	9*

Endpoints/dose	**0**		**1000ppm**		**10000ppm**		**50000ppm**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
F1	0	0	0	0	0	0	2	2
Haemorrhage: F0							2	
Epithelium pigment:								
F0								2
F1	1	0	0	0	0	0	4	2
Adenoma: F1							1	
Liver: Centrilobular hepatocyte hypertrophy								
F0							1	
F1	0	0	0	0	0	0	3	0
Vagina acute inflammatory infiltration epithelium F1		1		0		0		6

* Fisher exact test $p<0.05$

Conclusion: at 10000ppm and 50000ppm, maternal body weight was altered and there was evidence of altered thyroid metabolism. Reproductive organs and reproductive preformance and offspring survival were unaffected by treatment. Physical and sexual development of the offsprings were not altered. At top dose, body weight of offsprings were reduced during lactation. Lenacil is suspected to be secreted into the breast milk at toxic levels and should be labeled R64 “may cause harm to breastfed babies”. This proposal should be discussed.

NOAELreproduction toxicity = 10000ppm (1727 mg/kg bw/d) taking into account the effects on lactation reported at top dose.

Systemic parental NOAEL = 1000ppm (81.9-99.5 mg/kg bw/d) taking into account the effects observed in thyroid at 10000ppm.

NOAEL offspring toxicity= 10000ppm taking into account the decreased weight gain of F1 and F2 offsprings after birth.

Notifier comment:

The company proposes to set a NOAELreproduction toxicity = 50000ppm (4278-5312mg/kg bw/d for males and 4787-8839mg/kg bw/d for females).

The notifier disagrees with the RMS proposal for a systemic parental NOAEL of 1000 ppm since this does not appear to take account of the additional thyroid investigations with the conclusion that lenacil is not directly toxic to thyroid function. The notifier has also submitted argumentation (see previous notifier comment) relating to the effects on offspring weight gain, which if accepted as non-adverse in the context of this study, will affect the derived NOAEL.

The Notifier disagreed with the original proposal of the RMS to classify the active substance lenacil with R64.

The relevant legislation is Council Directive 67/548/EEC, as amended by Commission Directive 2001/59/EC, Annex 6 (Annex VI) Section3 2.8 and 4.2.3.3.

It is accepted that offspring bodyweights were slightly lower than controls in the F0F1 (by 6%) and F1F2 (by 11%) during the lactation period, but offspring survival was not adversely affected, and the bodyweights of the F0F1 pups selected for the F1 generation were not different from controls at the start of the pre-mating maturation period. Also, the behavioural and developmental landmarks assessed prior to and after weaning were not adversely affected by either maternal treatment or by direct intake of the test material. Any marginal bodyweight effects on offspring prior to weaning are considered transient, and insufficient evidence for adverse effects via maternal milk.

During the peer review, it was concluded that considering the very high dose level applied in the study (4300 mg/kg bw/d which exceeds the 1000 mg/kg bw/d limit dose for reproductive toxicity studies) the decrease in offspring weight gain during lactation was deemed insufficient to justify R64 and did not consider the effects as reproductive but offspring toxic effects. Therefore, the offspring and reproductive NOAELs were considered to be 1727 and 4300 mg/kg bw/day, respectively.

4.11.1.2 Human information

No data available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

No developmental toxicity (teratogenicity) was observed in rats and rabbits up to and including doses which proved to have a slight effect to the dam's body weights(circa 1000 mg/kg bw/day).

Consideration of the requirement to classify Lenacil in respect of potential reproductive effects is presented below.

4.11.2.2 Human information

No data available.

4.11.3 Other relevant information

The evidence from metabolism studies is that neither Lenacil nor its metabolites would be excreted in the milk. The effects observed on the offspring are minor, transient and there is no indication of impaired development or reduced survival. Finally, there is no evidence in humans. In conclusion, Lenacil should not be classified with R64.

The proposal to classify Lenacil as R64 was countered in a position paper prepared as a response to RMS in March 2009. It was noted that conclusions drawn were made in the absence of other studies with lactating mammals since such studies were not a requirement for Lenacil, no data were available. It was indicated the R64 position is predicated on a slight bodyweight change at a very high dose level which was maternally toxic and so insufficient evidence is available to conclude an independent effect on the neonate as a result of Lenacil present in breast milk.

Section 3.2.8 states the criteria for R64 as:
For substances and preparations which are absorbed by women and may interfere with lactation or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child.

In rat metabolism studies, Lenacil is primarily excreted via urine as water-soluble hydroxyl metabolites. It is generally considered that the high fat content of milk may lead to fat-soluble substances and fat-soluble metabolites being present in the milk rather than water-soluble metabolites. Urinary excretion was rapid 12-24 hours for circa 60% of a single dose with higher amounts excreted after repeated administration (72-86% albeit with a slight delay but still within 24 hours) and as doses increased there was a switch from urinary excretion of parent and metabolites to increasing (up to 83%) direct excretion of unchanged parent in faeces. These metabolic pathways are inconsistent with the excretion of parent or metabolites in milk.

The test compound intake at the high dose level in the multi-generation study was circa 5000-9000 mg/kg bw/d, which induced signs of maternal toxicity including reduced maternal bodyweight gain and reduced food conversion efficiency. Effects on F1 and F2 pups bodyweight became apparent from approximately 4-7 days after birth and prior to consumption of treated diet. The implication of R64 classification is that the effects on pup weight are due to toxic levels of Lenacil absorbed from milk but the metabolic pathway would suggest this is highly unlikely for Lenacil and a more reasonable assumption is that effects are secondary to maternal toxicity at this very high dose level. There were no other effects on pup maturation and the reduced weight gains were transient. The achieved maternal intake was some 35,000 fold higher than the proposed ADI. The NOAEL(offspring) in the reproductive toxicity study is circa 650 fold greater than the ADI and it is considered that the margin of safety is sufficient to conclude that no toxicologically significant levels of Lenacil are likely to be present in human breast milk following exposure to the plant protection product at levels below the ADI. One criterion for R64 classification includes the words 'in amounts sufficient to cause concern' – this clearly cannot be the case for Lenacil.

Criteria for classification in Section 4.1.3.3 state that 'For the purpose of classification, toxic effects on offspring resulting only from exposure via the breast milk, or toxic effects resulting from direct exposure of children will not be regarded as Toxic to reproduction, unless such effects result in impaired development of the offspring'.

It is accepted that offspring bodyweights were slightly lower than controls in the F_1 generation (by 6%) and in the F_2 generation (by 11%) during the lactation period at massively high maternal exposure levels, but offspring survival was not adversely affected, and the bodyweights of the pups selected for the F1 generation were not different from controls at the start of the pre-mating period. Also, the behavioural and developmental landmarks assessed prior to and after weaning were not adversely affected by either maternal treatment or by direct intake of the test material. Any marginal bodyweight effects on offspring prior to weaning are considered transient, and insufficient evidence for adverse effects via maternal milk. The effects on maternal bodyweight at these toxic levels are considered more relevant to the early growth of the pups.

R64 may also be appropriate for substances which affect the quantity or quality of the milk'. Where there is an effect on quantity of the milk, there is usually evidence from the immediate post-partum

period. The body wall of the newborn rat is translucent, and the study technicians can see the presence of milk in the pups' stomach as a whitish crescent in the abdomen. Absence of this crescent is recorded in the data for the study as an indication that the dam is not nursing the pups. It is frequently accompanied by high post natal mortality in pups. Neither finding was made in this study. However, the bodyweight effect was not detected until almost one week post-partum and itis quite probable that the dams, with their own bodyweight affected, may have been producing poorer quality milk as the lactation phase progressed.

In conclusion, Lenacil should not be classified with R64 under DSD.

4.11.4 Summary and discussion of reproductive toxicity

In a preliminary reproduction study, dietary administration 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 F0 females at 50000 ppm and for treated females during mid-lactation. Mating performance, fertility and development of subsequent F1 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 to rats at concentrations of 1000, 10000 or 50000 ppm was associated with effects at 50000 ppm on maternal bodyweight change during gestation and lactation, and bodyweight performance for the resultant progeny. At 10000 and 50000 ppm there was evidence of altered thyroid and liver metabolism. 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 50000ppm, the body weight gain for offspring was reduced during lactation from post partum day 7 for the F1 offspring and from post-partum day 4 for the F2 offspring. It was not possible to conclude positively that any reduction in milk production or quality could be attributed to treatment nor whether the offspring were actually exposed to Lenacil via milk. Since these criteria cannot be ascertained from the study data, it is not reasonable to propose that Lenacil should be classified with the risk phrase R64 "may cause harm to breastfed babies".

PRAPeR 69 (EFSA) conclusion: the meeting concluded that considering the very high dose level applied in the study (4300 mg/kg bw/d which exceeds the 1000mg/kg bw/d limit for reproduction toxicity studies) the decrease in offspring weight gain during lactation was deemed insufficient to justify R64 and did not consider the effects as reproductive but offspring toxic effects. Therefore, the offspring and reproductive NOAEL were considered to be 1727 and 4300 mg/kg bw/d, respectively.

Oral administration of Lenacil technical to rats at 100, 300 or 1000 mg/kg bw/d did not affect maternal or foetal parameters at any of the doses tested. Therefore, both the maternal and foetal NOAEL was at 1000 mg/kg body weight/day.

Oral administration of Lenacil technical to rabbits at doses of 50, 200, 1000, or 4000 mg/kg bw/day did not affect foetal parameters at any of the doses tested. Maternal toxicity was evident at a daily dose of 4000 mg/kg/day. Therefore, the NOAEL was 1000 mg/kg/day for the dam and greater than 4000 mg/kg/day for the conceptus.

No evidence was adduced from the available reproductive toxicity data to support classification of Lenacil with the risk phrase R64.

4.11.5 Comparison with criteria

Lenacil did not meet the CLP or DSD criteria classification for fertility toxicity, developmental toxicity or toxicity via lactation.

4.11.6 Conclusions on classification and labelling

Lenacil is not considered a reproduction or a developmental toxicant. It was not possible to determine from the available study data whether treatment with Lenacil caused a reduction in milk production or quality by the dams or whether the offspring were exposed to Lenacil via breast milk. Nor could it be determined whether there was any significant concentration of Lenacil in the milk, nor was it established whether any Lenacil in milk had any adverse effects on the offspring.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Lenacil is a uracil type herbicide. This class of compounds is devoid of any neurotoxic effects and in addition, the chemical structure of Lenacil has no structural relationships with any known neurotoxicants.

Review of the toxicity studies completed for the submission under Directive 91/414/EEC showed no evidence of clinical signs indicative of neurotoxicity in the acute, sub-acute, subchronic (90-day) or long term toxicity studies, even when administered up to international regulatory limit dose levels. Similarly no neuropathological changes were observed in this data set. In the two generation reproduction toxicity study, no clinical signs were seen in either the F_1 or F_2 offspring or their parents.

Acute, subchronic or developmental neurotoxicity studies were not required or conducted.

Based on the available information, no classification is required for Lenacil neurotoxicity.

4.12.1.2 Immunotoxicity

No available data.

4.12.1.3 Specific investigations: other studies

No available data.

4.12.1.4 Human information

No available data.

4.12.2 Summary and discussion

See 4.12.1.3

4.12.3 Comparison with criteria

No relevant criteria available for comparison in either the CLP Regulation or the DSD.

4.12.4 Conclusions on classification and labelling

The findings of the special investigations and 'other' studies did not affect the proposed classification for Lenacil.

RAC evaluation of aspiration toxicity
Summary of the Dossier submitter's proposal The DS did not provide data on aspiration toxicity. However, no classification is proposed in table 3 of the CLH report. **Comments received during public consultation** No specific comments were received. Two MSCA and one company indicated their general support for the classification proposed by the DS. **Assessment and comparison with the classification criteria** Lenacil is a solid and classification for aspiration toxicity is not relevant for solid substances according to section 3.10.1.6.2 *bis* of the CLP regulation. RAC therefore supported no classification for aspiration toxicity.

5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental fate properties assessment for Lenacil is based on the Draft Assessment Report, the Addendum to the Draft Assessment Report and the EFSA Scientific Report on the peer review of Lenacil.

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.

5.1 Degradation

Table 32: Summary of relevant information on degradation

Property	Method	Results	Reference	Remarks
Stability				
Hydrolysis	EEC-Method C7 GLP	pH 4: stable pH 7: stable pH 9: stable	ACD 046/013764 Caldwell, E, 2002	Purity > 97%
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	FAO revised guideline GLP	pH 5: stable	ACD 047/022138 Millais, A., 2002	Purity > 98%
Soil photolysis	SETAC 'procedures for Assessing the Environmental Fate and Ecotoxicology of Pesticides GLP	DT_{50} = 67.6 days	ACD 041/023429 Millais, A., 2002	Purity > 97%
Biodegradation				
Ready biodegradability	EE-Method C5 GLP	Not biodegradable according to the criteria of OECD 301 B	ACD037/013644 Barnes, S.P., 2001	Purity > 98.6%
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	DT_{50} whole system = 103 days – 122 days	A&M00-078 Theis, M., 2002	Purity ≥ 98.5%
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 GLP	DT_{50} = 15 days	A&M00-077 Theis, M., 2003	Purity > 97%

Property	Method	Results	Reference	Remarks
Aerobic soil degradation in laboratory conditions	SETAC 'Procedures for Assessing the Environmental Fate and Ecotoxicology of Pesticides', March 1995 GLP	DT_{50} = 18, 14, 15 and 11 days	ACD 042/023664 Girkin, R., 2003	Purity > 97%
Field soil dissipation	IVA guideline for residue trials ; BBA guidelines ; SETAC 'Procedures for Assessing the Environmental Fate and Ecotoxicology of Pesticides', March 1995 GLP	DT_{50} = 25, 28, 18 and 88 days	20011048/E1-FSD Pollmann, B., 2003	Purity : VENZAR 80% WP product containing 816 g/kg lenacil

5.1.1 Stability

Hydrolysis
The 'preliminary test' at 50°C 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.

Dissociation constant
Lenacil is a weak acid with a pKa of 10.7

Water photolysis
The measured photolytic degradation of Lenacil in aqueous buffer at pH5 was negligible. The lifetimes for the photodegradation in the environment (calculated using the GCSOLAR Program) indicate photolysis is unlikely to be a significant route of degradation of Lenacil as the values of DT_{50} and DT_{90} are >1 year. The quantum yield (φ) for Lenacil in pH 5.0 aqueous buffer was 2.62×10^{-7}.

Soil photolysis study
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.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

Ready biodegradability
The Assessment of Ready Biodegradability in a Modified Sturm Test 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.

5.1.2.3 Simulation tests

Water/sediment systems
A study describing the biodegradation of Lenacil in water/sediment system is available. 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 Rűckhaltebecken (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 Rűckhaltebecken 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 Rűckhaltebecken 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 Rűckhaltebecken system and in the Schaephysen system.

Distribution of lenacil in water and sediment phases in both systems accounted for as following. In the Rűckhaltebecken 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, M20.5 (5-oxo-Lenacil, also known as IN-KF313). 5-oxo-Lenacil peaked in the sediment phase on day 120 reaching the maximum levels of 10.7% AR in the sediment phase of one of the systems. In the water phase, 5-oxo-Lenacil 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.

The rate of degradation observed in this study was re-calculated in a modelling study by Shaw, D. (2004) using non-linear first-order regression performed by the ModelMaker programme. The result obtained gave Lenacil whole system DT_{50} values of 122 days in the Ruckhaltebecken system and 103 days in the Schaephysen system. Corresponding DT_{90} values were 405 and 342 days.

Insufficient data were available to calculate separate degradation rates for the water phase and sediment phase and for the major water sediment metabolite IN-KF313.

Aerobic soil metabolism studies
Five soil experiments treated with lenacil were carried out under aerobic conditions in the laboratory (20°C, 40% maximum water holding capacity (MWHC)) in the dark. The formation of residues not extracted were a sink for the applied [4,7a-14C2]-lenacil (19.4-25.8% of the applied radioactivity (AR) after 120 days). Volatile compounds including presumably mainly carbon dioxide, accounted for 47.6-61.1% AR after 120 days. The major (>10% AR) extractable breakdown products presented were metabolite IN-KE 121 (maximum occurrence 9.2-13.9% AR at 14-30 days), metabolite IN-KF 313 (maximum occurrence 8.5-14.7% AR at 7-14 days) and the unidentified metabolite „Polar B" (maximum occurrence 6.8-14.6% AR at 60-91 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.

One experiment was repeated at 10 ºC 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 24.4% AR after 120 d; at the end of this experiment.

Single first order (SFO) soil DT_{50} values under aerobic conditions at 20°C and 40% maximum water holding capacity (MWHC) were calculated to be 11-25 days (number of soils considered was 5). After normalization of these values to FOCUS reference conditions (20°C and pF2 soil moisture content), the range became 11-18 days, with a geometric mean of 14.4 days.

Single first order soil DT_{50} values were also calculated for the metabolite IN-KF 313. The soil DT_{50} were calculated to be between 3-350 days (at 20°C or 25°C and 40% MWHC or pF2.5 soil moisture content, n=8). After normalisation to FOCUS reference conditions (20°C and pF2 soil moisture content) this range of single first order DT_{50} became 3-444 days, with a geometric mean of 41 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. Single first order (SFO) soil DT_{50} values at 20°C were calculated to be 4-12 days (number of soils considered were 5). After normalization of these values to FOCUS reference conditions (20°C and pF2 soil moisture content), the range became 4-11 days, with a geometric mean of 6.4 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, however it is noted that the pH of the soils investigated for aerobic degradation was limited (pH ranges from 5.4 to 6.4 ; $CaCl_2$ method).

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

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.

5.1.3 Summary and discussion of degradation

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. DT_{50} values of lenacil at 20°C and 40% maximum water holding capacity (MWHC) were calculated to be 11-18 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%). Based upon the above information, Lenacil and IN-KF313 should be defined as the relevant residue in water. DT_{50} values of lenacil for the whole system were calculated to be 103–122 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.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Adsorption coefficients
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, (median 83 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-KE 121 and IN-KF 313 was investigated in three soils. Calculated adsorption KFoc for IN-KE 121 varied from 30.5-43.5 mL/g (mean 38 mL/g) and the 1/n values ranged from 0.92 – 0.96 (mean 0.95). There was no indication of any relationship between adsorption and any soil characteristic including pH. Calculated adsorption KFoc for IN-KF 313 varied from 79 - 824 mL/g (mean 557 mL/g) and the 1/n values ranged from 0.67 – 1.0 (mean 0.89). pH dependency cannot be established nor excluded based on the available data with this narrow pH range.

Freundlich adsorption constants for IN-KE121 were in the range 31 to 44 for the 3 test soils. The mean Kfoc was 38 and the mean value of 1/n was 0.94.

5.2.2 Volatilisation

The low vapour pressure of 1.7 x 10^{-9} Pascals 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 x10^{-7} Pa.m^3.mol^{-1}) calculated from the water solubility value of 3 mg/L and vapour pressure 1.7 x 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.

5.2.3 Distribution modelling

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5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

The estimation of bioaccumulation potential in fish is based on the partition coefficient n-octanol/water (log Pow) of the active substance.
In the section on physico-chemical properties different values for the log Pow pending on the pH were measured.

Table 33: Summary of relevant physicochemical properties for aquatic bioaccumulation

METHOD	RESULTS	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**	**99 % PURE. ALL AT 25°C**	**ACD 025/014039** **COMB, A.L. 2002A**

The log P_{ow} values are then compared with the threshold values for bioaccumulation, threshold DSD ≥ 3 and threshold CLP ≥ 4. Since, the log P_{ow} of lenacil is lower than both threshold values, the potential risk for bioaccumulation in tissues of aquatic organisms is low.

5.3.1.2 Measured bioaccumulation data

No data available and not required (see 5.3.1.1).

5.3.2 Summary and discussion of aquatic bioaccumulation

The measured log P_{ow} values for lenacil were all below the threshold value for bioaccumulation, i.e. threshold DSD ≥ 3 and threshold CLP ≥ 4. Therefore, no experimental bioaccumulation data are required. The potential risk for bioaccumulation of lenacil in tissues of aquatic organisms is considered low.

5.4 Aquatic toxicity

Table 34: Summary table of relevant aquatic toxicity data

Type of test **Test species**	**Test substance purity, batchn°** **Test concentrations (mg a.s./L)**	**Test system**	**Endpoints**	**Reference**
acute fish study based on OECD 203 and US EPA 72-1 GLP *Oncorhynchus mykiss*	lenacil, purity: 98.2%, batch n°: 9038 nominal: control; solvent control (dimethylformamide); 0.26; 0.44; 0.72; 1.2; 2.0 mg a.s./L mean measured: 0.00; 0.00; 0.48; 0.51; 0.80; 1.3; 2.0 mg a.s./L	96 h static fingerlings 10 fish/replicate 1 replicate/treatment	**LC_{50}> 2.0 mg a.s./L** (mean measured)	Hutton D.G., 1991a
acute fish study based on OECD 203 and US EPA 72-1 GLP *Pimephales promelas*	lenacil, purity: 98.2%, batch n°: 9038 nominal: control; solvent control (dimethylformamide); 0.26; 0.44; 0.72; 1.2; 2.0 mg a.s./L mean measured: 0.00; 0.00; 0.38; 0.48; 0.80; 1.2; 2.0 mg a.s./L	96 h static juveniles 10 fish/replicate 1 replicate/treatment	**LC_{50}> 2.0 mg a.s./L** (mean measured)	Hutton D.G., 1991b

Type of test **Test species**	**Test substance purity, batchn°** **Test concentrations (mg a.s./L)**	**Test system**	**Endpoints**	**Reference**
acute fish study based on OECD 203, 92/69/EEC method C.1 and draft US EPA OPPTS 850.1075 GLP *Cyprinus carpio*	lenacil, purity: 98.6%, batch n°: 141712003 nominal: control; solvent control (dimethylformamide); 3.0 mg a.s./L mean measured: 0.00; 0.00; 3.0 – 3.1 mg a.s./L	96 h semi-static mean weight: 1.26 g mean standard length: 4.3 cm 10 fish/replicate 3 replicates/treatment	LC_{50}> 3.1 mg a.s./L (mean measured)	Flatman D., 2003a
chronic fish juvenile growth study based on OECD 204 GLP *Oncorhynchus mykiss*	lenacil, purity: 98.2%, batch n°: 9038 nominal: control; solvent control (dimethylformamide); 0.29; 0.58; 1.2; 2.3 mg a.s./L mean measured: 0.00; 0.00; 0.33; 0.65; 1.1; 2.3 mg a.s./L	21 d flow-through fingerlings 5 fish/replicate 2 replicates/treatment	NOEC = 2.3 mg a.s/L (mean measured) based on mortality and growth	Hutton D.G., 1991c
chronic fish early life stage study based on OECD 210 GLP *Oncorhynchus mykiss*	lenacil, purity: 98.5%, batchn°: 9038 nominal: control; solvent control (dimethylformamide); 0.020; 0.050; 0.130; 0.320; 0.800; 2.000 mg a.s./L mean measured: 0.00; 0.00; 0.031; 0.053; 0.160; 0.280; 0.640; 1.600 mg a.s./L	90 d flow-through 20 embryos/cup 2 embryo cups/replicate 2 replicates/treatment	**NOEC = 0.160 mg a.s./L** (mean measured) based on mean standard length	Kreamer G.-L.C., 1996
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 nominal: control; no solvent control (dimethylformamide); 50; 67; 89; 119; 158; 211; 281; 375; 500 mg a.s./L measured after 48 h: 0.00; -; 4.3; 4.8; 4.7; 6.0; 5.5; 4.6; 5.2; 5.3; 8.4 mg a.s./L	48 h static 5 daphnids/replicate 4 replicates/treatment	**EC_{50}> 8.4 mg a.s./L** (measured after 48 h)	Hutton D.G., 1989a
chronic daphnia study based on OECD 202 part II GLP *Daphnia magna*	lenacil, purity: 95.1%, blended batch n°s: 8802 and 8805 nominal: control; no solvent control (dimethylformamide); 0.15; 0.30; 0.6; 1.2; 2.5; 5.0 mg a.s./L mean measured: 0.00; -; 0.08; 0.13; 0.28; 0.48; 0.97; 1.7 mg a.s./L	21 d semi-static 4 daphnids/replicate 10 replicates/treatment	**NOEC = 0.48 mg a.s./L** (mean measured) based on adult survival and total numbers of offspring	Hutton D.G., 1989b
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 nominal: control; no solvent control (dimethylformamide); 0.01057; 0.02124; 0.04695; 0.1075; 0.2116; 0.4764 mg a.s./L mean measured: 0.0000; 0.0000; 0.011; 0.022; 0.047; 0.105; 0.219;	72 h static initial cell count: 1 x 10^4/mL 6 replicates for control 3 replicates/treatment	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)	Flatman D., 2003b

Type of test Test species	Test substance purity, batchn° Test concentrations (mg a.s./L)	Test system	Endpoints	Reference
	0.468 mg a.s./L			
algal growth inhibition study based on OECD 201, 92/69/EEC method C.3 and draft US EPA OPPTS 850.5400 GLP *Pseudokirchneriella subcapitata*	lenacil, purity: 98.6%, batch n°: 141712003 nominal: control; no solvent control (dimethylformamide); 0.0004127; 0.0008678; 0.001453; 0.003962; 0.008234; 0.01652; 0.03488 mg a.s./L mean measured: 0.0000; 0.0000; 0.00041; 0.00079; 0.0015; 0.0034; 0.0081; 0.017; 0.036 mg a.s./L	96 h static initial cell count: 1 x 10^4/mL 6 replicates for control and solvent control 3 replicates/treatment	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)	Flatman D., 2003c
Algistatic activity based on OECD GLP *Pseudokirchneriella subcapitata*	lenacil, purity: 95.4%, batch n°: D231 20193 nominal : control; 0.01, 0.02, 0.04, 0.08, 0.16 mg a.s./L mean measured : exposure was not verified analytically		Study not considered valid	
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 nominal: control; no solvent control (dimethylformamide); 0.003610; 0.009059; 0.01560; 0.02360; 0.07019 mg a.s./L mean measured: 0.0000; 0.0000; 0.0037; 0.0088; 0.015; 0.024; 0.071 mg a.s./L	7 d semi-static inoculation with 4 plants bearing 3 fronds 3 replicates for control, solvent control and per treatment	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)	Flatman D., 2003d

The endpoints from the key studies are highlighted in bold.

The most sensitive species for this herbicide were the algae and the aquatic plants, with endpoints EC_{50}/NOEC down to <0.01 mg/L, thus well in the water solubility range (around 3mg/l). In contrast, fish and daphnia were far less sensitive, with EC_{50}/NOEC values several orders of magnitude higher (about 2-3 mg/L, still beyond or comparable to the water solubility limit). Even in the acute tests with these relatively insensitive indicator organisms, the mean measured concentrations of Lenacil in the water was >=80%, and often >=100% of nominal. Since in these acute assays, Lenacil was tested up to water solubility limit and no mortalities (fish) and no immobilisation (Daphnia) was observed, the expression of the L(E)C_{50}-values as ">" than the top concentration was deemed justified.

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Information extracted from DAR Volume 3, Annex B, Section B.9.2 'Effects on aquatic organisms'.

Three short-term toxicity studies to fish are available for lenacil:

Static, Acute, 96-hour LC_{50} of DPX-B634-91 (Lenacil) to rainbow trout (*Oncorhynchus mykiss*). (Hutton D.G., 1991a).

Guidelines :
OECD 203 (1984), US EPA 72-1 (1985)
GLP :
Yes
Material and Methods :
*Test substance :*lenacil, chemical purity : 98.2 %, batch n^{o} : 9038
Test species :Oncorhynchus mykiss (rainbow trout)
Number of organisms, age, weight, length : 10 fish per replicate, 1 replicate per treatment, fingerlings, weight : 0.2 - 0.99 g (mean weight : 0.5 g), standard length : 2.4 - 4 cm (mean standard length : 3.3 cm), total length : 2.9 - 4.7 cm (mean total length : 3.8 cm)
Type of test : 96-hour static toxicity test
Biological loading : 0.33 g biomass/L
Applied and measured concentrations :
nominal : control; solvent control (dimethylformamide); 0.26, 0.44, 0.72, 1.2, 2.0 mg a.s./L
mean measured : 0.00; 0.00; 0.48, 0.51, 0.80, 1.3, 2.0 mg a.s./L (100 - 185 % of nominal concentrations)
Test conditions :
temperature : 12.0 - 12.6 ºC
pH : 6.6 - 7.8
dissolved oxygen : 75 – 85 % O_2 saturation (8.1 - 9.2 mg/L O_2)
total hardness : 75 mg/L $CaCO_3$
photoperiod : 16/8 hours light/dark cycle
light intensity : 247 lux
Analytical methods : lenacil concentrations were measured by HPLC/UV
Findings :
Mortality : No mortalities occurred in the controls or at any treatment level.
Behavioural observations : No unusual behaviour or signs of intoxication were observed at any treatment level.
Conclusions :
The study is acceptable.
Endpoints :
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)

Static, Acute, 96-hour LC_{50} of DPX-B634-91 (Lenacil) to fathead minnows (*Pimephales promelas*). (Hutton D.G., 1991b).

Guidelines :
OECD 203 (1984), US EPA 72-1(1985)
GLP :
Yes
Material and Methods :
*Test substance :*lenacil, chemical purity : 98.2 %, batch n^{o} : 9038
Test species :Pimephales promelas (fathead minnow)
Number of organisms, age, weight, length : 10 fish per replicate, 1 replicate per treatment, juveniles, 188-194 days old, weight : 0.34 - 0.74 g (mean weight : 0.63 g), standard length : 3.2 - 3.9 cm (mean standard length : 3.7 cm), total length : 3.8 - 4.7 cm (mean total length : 4.4 cm)
Type of test : 96-hour static toxicity test
Biological loading : 0.42 g biomass/L
Applied and measured concentrations :
nominal : control; solvent control (dimethylformamide); 0.26, 0.44, 0.72, 1.2, 2.0 mg a.s./L
mean measured : 0.00; 0.00; 0.38, 0.48, 0.80, 1.2, 2.0 mg a.s./L (100 – 146 % of nominal concentrations)
Test conditions :
temperature : 12.0 - 12.6 ºC
pH : 7.0 - 7.6
dissolved oxygen : 68 – 98 % O_2 saturation (6.0 - 8.6 mg/L O_2)
total hardness : 72 mg/L $CaCO_3$
photoperiod : 16/8 hours light/dark cycle
light intensity : 387 lux
Analytical methods : lenacil concentrations were measured by HPLC/UV

Findings :
Mortality : No mortalities occurred in the controls or at any treatment level.
Behavioural observations : No unusual behaviour or signs of intoxication were observed at any treatment level.
Conclusions :
The study is acceptable.
Endpoints :
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)

Lenacil technical, acute toxicity to fish (Cyprinus carpio). (Flatman D., 2003a).

Guidelines :
92/69/EEC, method C.1 (1992),OECD 203 (1984), draft US EPA OPPTS 850.1075 (1996)
GLP :
Yes
Material and Methods :
*Test substance :*lenacil, chemical purity: 98.6 %, batch n°: 141712003
Test species :Cyprinus carpio (common carp)
Number of organisms, age, weight, length : 10 fish per replicate, 3 replicates per treatment, age not stated,
mean weight : 1.26 g, mean standard length : 4.3 cm
Type of test : 96-hour semi-static toxicity test, limit test
Biological loading : 0.63 g biomass/L
Applied and measured concentrations :
nominal : control; solvent control (dimethylformamide); 3.0 mg a.s./L
mean measured : 0.0; 3.0 - 3.1 mg a.s./L (100 – 103 % of nominal concentrations)

Table: Measured concentrations of lenacil during a 96-hour acute toxicity test with *Cyprinus carpio* under semi-static conditions

Nominal concentration (mg lenacil/L)	Measured concentration (mg lenacil/L)				
	0 h (fresh)	24 h (expired)	72 h (fresh)	96 h (expired)	Mean
solvent control 1	< lod	< lod	< lod	< lod	< lod
solvent control 2	< lod	< lod	< lod	< lod	< lod
solvent control 3	< lod	< lod	< lod	< lod	< lod
100 rep. 1	3.398	3.247	2.883	2.677	3.1
100 rep. 2	3.173	2.662	3.164	2.924	3.0
100 rep. 3	3.316	3.196	3.120	2.593	3.1

lod: limit of detection (0.04 mg a.s./L)

Test conditions :
temperature : 23 ºC
pH : 7.4 - 7.6
dissolved oxygen : 84 – 86 % O_2 saturation (7.3 - 7.5 mg/L O_2)
total hardness : 152 – 170 mg/L $CaCO_3$
photoperiod : 16/8 hours light/dark cycle
light intensity : 503 - 615 lux
Analytical methods : lenacil was measured by HPLC/UV
Findings :
Mortality : A single mortality occurred after 24 hours in one replicate of the untreated control treatment. There were no mortalities in the solvent control group or at any of the treatment levels.

Behavioural observations : No unusual behaviour or signs of intoxication were observed at any treatment level.
Conclusions :
The study is acceptable.
Endpoints :
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)

5.4.1.2 Long-term toxicity to fish

Information extracted from DAR Volume 3, Annex B, Section B.9.2 'Effects on aquatic organisms'.

Two long-term toxicity studies to fish are available for lenacil:

Flow-through, 21-day toxicity of DPX-B634-91 (Lenacil) to rainbow trout (*Oncorhynchus mykiss*). (Hutton D.G., 1991c).

Guidelines :
OECD 204
GLP :
Yes
Material and Methods :
*Test substance :*lenacil, chemical purity : 98.2 %, batch n° : 9038
Test species :Oncorhynchus mykiss (rainbow trout)
Number of organisms, age, weight, length : 5 fish per replicate, 2 replicates per treatment, fingerlings, mean weight : 1.07 g, mean standard length : 3.8 cm
Type of test : 21-day flow-through toxicity test, five volume changes per vessel per day
Biological loading : 0.77 g biomass/L
Applied and measured concentrations :
nominal : control; solvent control (dimethylformamide); 0.29, 0.58, 1.2, 2.3 mg a.s./L
mean measured : 0.00; 0.00; 0.33, 0.65, 1.1, 2.3 mg a.s./L (92 – 117 % of nominal concentrations)

Table: Measured concentrations of lenacil during a 21-day juvenile growth toxicity test with *Oncorhynchus mykiss* under flow-through conditions

Nominal concentration (% of stock dispersion)	Measured concentration (mg lenacil/L)								
	day 0		day 7		day 14		day 21		Overall mean
	rep. A	rep. B	rep. A	rep. B	rep. A	rep. B	rep. A	rep. B	
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
DMF control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12.5	0.32	0.32	0.34	0.34	0.32	0.35	0.34	0.34	0.33
25	0.64	0.64	0.67	0.67	0.63	0.64	0.67	0.65	0.65
50	1.07	1.07	1.21	1.21	1.11	1.06	1.14	1.17	1.1
100	2.13	2.19	2.51	2.51	2.19	2.19	2.31	2.28	2.3

Feeding : once per day with *Artemia* sp.
Test conditions :
temperature : 12.5 - 13.6 °C
pH : 6.9 - 7.4
dissolved oxygen : 85 – 98 % O_2 saturation (9.0 - 10.4 mg/L O_2)
mean total hardness : 74 mg/L $CaCO_3$

photoperiod : 16/8 hours light/dark cycle
light intensity : 54 - 86 lux
Analytical methods : lenacil concentrations were measured by HPLC/UV
Findings :
Mortality : No mortalities were observed in either of the control groups, or at the treatment levels of 0.33, 1.1 and 2.3 mg a.s./L. Four fish died at the treatment level of 0.65 mg a.s./L, between days 14 and 15, but these mortalities were not treatment-related and were all confined to a single replicate vessel where cannibalisation by the lone survivor may have been the cause of death.
Behavioural observations : Fish appeared normal at all treatment levels throughout the study, except in the single vessel of the 0.65 mg a.s./L treatment group where mortalities occurred and where survivors were described as 'discoloured' on day 14.
Growth : At the end of the test there were no statistically significant differences in terms of mean length and mean weight between the solvent control and the untreated control, or between the solvent control and any of the treatments with lenacil.

Table 35: Summary of effects of lenacil during the fish juvenile growth test with Oncorhynchus mykiss

Evaluation criteria	Control	Solvent control	Mean measured test concentration (mg a.s./L)			
			0.33	0.65	1.1	2.3
Cumulative % mortality after 21 d	0	0	0	40	0	0
Mean body weight at day 21 (g)	0.989	1.070	0.963	1.318	1.047	1.022
Mean body length at day 21 (cm)	3.9	3.8	3.6	4.0	3.7	3.7

Conclusions :
The study is acceptable.
Endpoints :
NOEC (*Oncorhynchus mykiss*, 21 d) = 2.3 mg a.s./L (mean measured) based on mortality and growth

Early life–stage toxicity of DPX-B634-91 (lenacil) to rainbow trout, *Oncorhynchus mykiss*. (Kreamer G.-L.C., 1996).

Guidelines :
OECD 210
GLP :
Yes
Material and Methods :
*Test substance :*lenacil, chemical purity : 98.5 %, batch n^{o} : 9038
Test species :Oncorhynchus mykiss (rainbow trout)
Number of organisms : 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.
Type of test : 90-day flow-through toxicity test, six volume changes per vessel per day
Biological loading : 0.181 g fish/L/day at test end
Applied and measured concentrations :
nominal : control; solvent control (dimethylformamide); 20, 50, 130, 320, 800, 2000 µg a.s./L
mean measured : 0; 0; 31, 53, 160, 280, 640, 1600 µg a.s./L (80 – 155 % of nominal concentrations)
Test conditions :
temperature : 10.6 - 11.7 ºC
pH : 7.2 - 7.6
dissolved oxygen : 77 – 103 % O_2 saturation (8.5 - 11.4 mg/L O_2)
total hardness : 78 - 85 mg/L $CaCO_3$
photoperiod : relative darkness until hatch was completed, 16/8 hours light/dark cycle from day 40 onwards
light intensity : 43 - 65 lux from day 40 onwards
Analytical methods : lenacil concentrations were measured by HPLC/UV
Findings :
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 to be significant at 640 and 1600 µg a.s./L. Statistical significant effects on length of surviving fingerlings at test end were found at 280, 640 and 1600 µg a.s./L.

Table 36: Summary of hatching, survival, abnormalities, swim-up and growth for the 90-day early life-stage test with Oncorhynchus mykiss

Mean measured test concentration µg a.s./L	Mean hatching day[a]		% hatch[a]	Hatch to thinning [b]				Mean first day of swim-up[b]
				Survival: number alive/total (%)		Abnormalities: number affected/number alive (%)		
	Start	End						
Water 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.
[b] Based on four replicates per concentration, last observation was made on day 45.
* Significantly different from combined control ($p<0.05$).

Table 36 continued: Summary of hatching, survival, abnormalities, swim-up and growth for the 90-day early life-stage test with Oncorhynchus mykiss

Mean measured test concentration µg a.s./L	Thinning to test-end				Standard length, cm		Wet weight, g	
	Survival: number alive/total (%)		Abnormalities: number affected/number alive (%)		Mean (Std dev)		Mean (Std dev)	
Water 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)[a]	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)[b]	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)*

[a] One fish dead, another missing, presumed dead.
[b] Four fish missing, presumed dead.
* Significantly different from solvent control ($p<0.05$).

Conclusions :
The study is acceptable.
Endpoints :
NOEC (*Oncorhynchus mykiss*, 90 d) = 0.160 mg a.s./L (mean measured), based on mean standard length

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Information extracted from DAR Volume 3, Annex B, Section B.9.2 'Effects on aquatic organisms'.

A single short-term toxicity study to aquatic invertebrates is available for lenacil:

Static Acute 48-hour EC_{50} of DPX-B634-84 to fed Daphnia magna. (Hutton D.G., 1989a).

Guidelines :
OECD 202 Part I (1984), US EPA 72-2 (1985)
GLP :
Yes
Material and Methods :
*Test substance :*lenacil, chemical purity : 95.1 %, blended batch n°s : 8802 and 8805
Test species :Daphnia magna
Number of organisms, age : 5 daphnids per replicate, 4 replicates per treatment (20 daphnids per treatment), juveniles (less than 24 hours old at test initiation)
Type of test : 48-hour static toxicity test
Applied and measured concentrations :
nominal : control; no solvent control; 50, 67, 89, 119, 158, 211, 281, 375, 500 mg a.s./L (all >> solubility limit).
measured 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

Table: Measured concentrations of lenacil during an acute toxicity test with *Daphnia magna* under static conditions

Nominal concentration (mg lenacil/L)	Measured lenacil concentration (mg a.s./L)		
	0 hours	48 hours	Mean
Control	0.0	0.0	0.0
50	8.3	4.3	6.3
67	12.9	4.8	8.9
89	11.0	4.7	7.9
119	19.7	6.0	13
158	27.6	5.5	17
211	31.9	4.6	18
281	42.7	5.2	24
375	49.2	5.3	27
500	69.9	8.4	39

In the report, there is no mention of particular worries on the possible side-effect of non-dissolved Lenacil on the test outcome. 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, considered, on the basis of the day 2 analytical results to be in the range of 4 to 8 mg/L. For this reason, the day 2 measured concentrations were considered to be the more representative of true exposure concentrations. Dissolved

oxygen concentrations dropped below 60 % of saturation in all treatments during the test, with no apparent effect on the daphnids. All other test parameters were within acceptable ranges for this study.

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 : lenacil concentrations were measured by HPLC/UV
Findings :
Immobility : No immobilization occurred in the control or at any treatment level.
Conclusions :
Dissolved oxygen concentrations fell below 60 % of ASV during the test, but there was no evidence of adverse impact on the test organisms. The study is acceptable.
Endpoints :
EC_{50} (*Daphnia magna*, 48 h) > 8.4 mg a.s./L (measured after 48 h)

5.4.2.2 Long-term toxicity to aquatic invertebrates

Information extracted from DAR Volume 3, Annex B, Section B.9.2 'Effects on aquatic organisms'.

A single long-term toxicity study to aquatic invertebrates is available for lenacil:

Chronic toxicity of DPX-B634-84 (Lenacil) to *Daphnia magna*. (Hutton D.G., 1989b).

Guidelines :
OECD 202 Part II.
GLP :
Yes
Material and Methods :
*Test substance :*lenacil, chemical purity : 95.1 %, blended batch n^{o}s : 8802 and 8805
Test species :Daphnia magna
Number of organisms, age : 4 daphnids per replicate, 10 replicates per treatment (40 daphnids per treatment), juveniles (less than 24 hours old at test initiation)
Type of test : 21-day semi-static toxicity test (3 renewals per week)
Applied and measured concentrations :
nominal : control; 0.15, 0.30, 0.6, 1.2, 2.5, 5.0 mg a.s./L
mean measured : 0.00; 0.08, 0.13, 0.28, 0.48, 0.97, 1.7 mg a.s./L (34 – 53 % of nominal concentrations)

In Hutton D.G. 1989b the oxygen saturation has been very poor at some point. Dissolved oxygen concentrations dropped below 60 % of saturation in *all* treatments during the test, with no apparent effect on the daphnids. All other test parameters were within acceptable ranges for this study. RMS believes that in any case, this study was not critical for C&L, since a 21d *Daphnia magna* assay was conducted, and a valid IC_{50}-value could be calculated from the dose-response curve. The EC_{50} for immobilisation was calculated to be 1.2 mg/L Lenacil at test termination. Thus, 50% of Daphnia magna will be expected to die if they are exposed to Lenacil at a concentration of 1.2 mg/L for a continuous period of 21 days. Taking this value as a worst-case estimation, it is inferred that the 48h-IC_{50} could not be lower than this value.

Table: Measured concentrations during a chronic toxicity test with *Daphnia magna* exposed to lenacil under semi-static conditions

Nominal concentration (mg lenacil/L)	Measured lenacil concentration (mg a.s./L)								
	day 0 fresh	day 2 expired	day 5 expired	day 7 expired	day 7 fresh	day 14 expired	day 14 fresh	day 21 expired	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

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
Analytical methods : lenacil concentrations were measured by HPLC/UV

Findings :
Statistically significant ($p < 0.05$) reductions in total numbers of juveniles and juveniles per adult occurred at the treatment levels of 0.08, 0.97 and 1.7 mg a.s./L, and at treatment levels of 0.08 and 1.7 mg a.s./L respectively, relative to the control. 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.
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 37: 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 :
The study is acceptable.
Endpoints :

NOEC (*Daphnia magna*, 21 d) = 0.48 mg a.s./L (mean measured), based on adult survival and total numbers of offspring

5.4.3 Algae and aquatic plants

Information extracted from DAR Volume 3, Annex B, Section B.9.2 'Effects on aquatic organisms'.

Three toxicity studies to algae are available for lenacil, only two were considered valid.

Lenacil technical, algal growth inhibition assay, *Navicula pelliculosa*. (Flatman D., 2003b).

Guidelines :
92/69/EEC, method C.3 (1992),OECD 201 (1984)
GLP :
Yes
Material and Methods :
*Test substance :*lenacil, chemical purity : 98.6 %, batch n^{o} : 141712003
Test species :Navicula pelliculosa (freshwater diatom)
Number of replicates, initial cell density : 6 replicates for the control and the solvent control; 3 replicates per treatment, initial cell count : 1×10^4/mL
Type of test : 72-hour static toxicity test
Applied and measured concentrations :
nominal : 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, equivalent to 10.57, 21.24, 46.95, 107.5, 211.6, 476.4 µg a.s./L
mean measured : 0.00; 0.00; 11, 22, 47, 105, 219, 468 µg a.s./L, corresponding to 98 - 104 % of nominal concentrations

Table: Measured concentrations of lenacil during a toxicity test with *Navicula pelliculosa*

Nominal concentration[a]	Measured lenacil concentration (µg a.s./L)		
	0 hours	72 hours	Mean
DMF control	< lod	< lod	< lod
1.94	10.57	10.74 10.19[b]	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	460.0 518.2[b]	468

[a] Expressed as percentage of an aqueous solution of lenacil.
[b] Medium containing no algae.
lod Limit of detection (0.7 µg lenacil/L)

Test conditions :
temperature : 22 ± 1ºC.
pH : 7.7 - 7.8 (initial), 7.5 - 7.8 (final)
light regime : continuous illumination
light intensity : 8180 lux
Analytical methods : lenacil concentrations were measured by HPLC/UV
Findings :

Table 38: Percentage inhibition of growth of algae exposed for 72 hours to lenacil

Parameter	Solvent control	Mean measured test concentration (µg a.s./L)					
		11	22	47	105	219	468
0-72 h area under curve	-	-25	31*	68*	88*	91*	96*
0-72 h growth rate	-	-4.2	9.2*	31*	55*	73*	85*

* statistically significantly different from control ($p < 0.01$)
Negative value denotes an increase when compared to the solvent control

No signs of morphological abnormalities were detected at any treatment level.
Conclusions :
The study is acceptable.
Endpoints :
E_bC_{50} (*Navicula pelliculosa*, 72 h) = 0.036 mg a.s./L (mean measured)
E_rC_{50} (*Navicula pelliculosa*, 72 h) = 0.096 mg a.s./L (mean measured)
NOEC (*Navicula pelliculosa*, 72 h) = 0.011 mg a.s./L (mean measured)

Lenacil technical, algal growth inhibition assay, *Selenastrum capricornutum*. (Flatman D., 2003c).

Guidelines :
92/69/EEC, method C.3 (1992),OECD 201 (1984), draft US EPA OPPTS 850.5400 (1996)
GLP :
Yes
Material and Methods :
*Test substance :*lenacil, chemical purity : 98.6 %, batch n° : 141712003
Test species :Pseudokirchneriella subcapitata (formerly known as *Selenastrum capricornutum*), unicellular freshwater green alga
Number of replicates, initial cell density : 6 replicates for the control and the solvent control, 3 replicates per treatment, initial cell count : 1×10^4/mL
Type of test : 96-hour static toxicity test
Applied and measured concentrations :
nominal : 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 0.4127, 0.8678, 1.453, 3.962, 8.234, 16.52, 34.88 µg a.s./L
mean measured : 0.00; 0.00; 0.41, 0.79, 1.5, 3.4, 8.1, 17, 36 µg a.s./L, corresponding to 86 - 103 % of nominal concentrations.

Under 91/414 evaluation, RMS are consistently checking all quality criteria to declare aquatox assays acceptable. RMS verified all cell densities during all time points both for the assays on the active substances and for the metabolites. The most sensitive assay was re-inspected, and it was confirmed that exponential growth conditions were respected during the test. Culture conditions in the most critical assay (Flatman, 2003c) : Conical flasks (250 mL) each containing 100 mL of test or control culture were loosely stoppered and placed without conscious bias in a Gallenkamp illuminated orbital incubator. The cultures were incubated, without medium renewal, for 96 hours under continuous illumination of 4210 to 4740 lux provided by fluorescent tubes.

The temperature was maintained at 22 – 24°C. Gaseous exchange and suspension of the algal cells were ensured by the action of the orbital shaker, oscillating at 140 cycles per minute. 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. Cell counts were used to determine growth inhibition, based on specific growth rates and on integrated biomass (areas beneath growth curves).

Table: Cell counts of *S. capricornutum* following 96-hour exposure to Lenacil (Flatman, 2003c):

Mean measured concentration (µg lenacil/L)	Mean[a] cell density (cells/mL)				
	0 h	24 h	48 h	72 h	96 h
H_2O control	11197	46563	202282	870280	3155000
DMF control	11791	47719	207775	853687	3096900
0.41	11524	50641	216617	917360	3139267
0.79	12292	52980	226540	924280	3441400
1.5	11421	50527	219563	904053	3232800
3.4	12102	47252	195387	780280	2818000
8.1	11954	37925	126990	306413	742080
17	11665	27379	40208	82499	153907
36	11382	20457	22592	26268	25444

[a]*Means for three replicates per Lenacil treatment and six replicates for the two control groups at each timepoint.*

From these data, the QC criterium may easily be verified. Water control culture shows an increase (0h-72h) of about 78x, while the solvent control increase amounts to about 72x the initial t0.

Table: Measured concentrations of Lenacil during a toxicity test with *Selenastrum capricornutum*

Nominal concentration[a]	Measured Lenacil concentration (µg a.s./L)		
	0 hours	96 hours	Mean
DMF control	< lod	< lod	< lod
0.010	0.4127	0.3989	0.41
0.022	0.8678	0.7084	0.79
0.046	1.453	1.501	1.5
0.10	3.962 4.068[b]	2.803 3.522[b]	3.4 3.8
0.22	8.234	8.056	8.1
0.46	16.52	17.07	17
1.0	34.88	38.00	36

[a] Expressed as percentage of an aqueous solution of lenacil.
[b] Medium containing no algae.
lod Limit of detection (0.16 µg Lenacil/L)

Test conditions :
temperature : 22 – 24 ºC
pH : 7.1 - 7.4 (initial), 7.6 - 7.8 (final)
light regime : continuous illumination

light intensity : 4210 - 4740 lux
Analytical methods : lenacil concentrations were measured by HPLC/UV

Findings :

Table 39: Percentage inhibition of growth of algae exposed for 72 hours to lenacil

Parameter	Solvent control	Mean measured test concentration (µg a.s./L)						
		0.41	0.79	1.5	3.4	8.1	17	36
0-72 h area under curve	-	-6.8	-8.9	-6.2	7.7	56*	88*	96*
0-72 h growth rate	-	-2.3	-1.1	-2.3	2.5	25*	55*	80*

* statistically significantly different from the control ($p < 0.01$)
Negative value denotes an increase when compared to the solvent control

Table 40: Percentage inhibition of growth of algae exposed for 96 hours to lenacil

Parameter	Solvent control	Mean measured test concentration (µg a.s./L)						
		0.41	0.79	1.5	3.4	8.1	17	36
0-72 h area under curve	-	-3.7	-7.5	-5.1	8.7	69*	93*	98*
0-72 h growth rate	-	-0.68	-0.48	-1.4	2.1	26*	54*	86*

* statistically significantly different from the control ($p < 0.01$)
Negative value denotes an increase when compared to the solvent control

No signs of morphological abnormalities were detected at any treatment level.

Recovery check : 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 study is acceptable.
Endpoints :
E_bC_{50} (*Pseudokirchneriella subcapitata*, 72 h) = 0.0077 mg a.s./L (mean measured)
E_bC_{50} (*Pseudokirchneriella subcapitata*, 96 h) = 0.0065 mg a.s./L (mean measured)
E_rC_{50} (*Pseudokirchneriella subcapitata*, 72 h) = 0.016 mg a.s./L (mean measured)
E_rC_{50} (*Pseudokirchneriella subcapitata*, 96 h) = 0.015 mg a.s./L (mean measured)
NOEC (*Pseudokirchneriella subcapitata*, 96 h) = 0.0034 mg a.s./L (mean measured)

The algistatic activity of lenacil technical. (Douglas M.T. and Handley J.W., 1988).

Guidelines :
OECD 201 (1984), US EPA 122-2
GLP :
Yes
Material and Methods :
*Test substance :*lenacil, chemical purity : 95.4 %, batch n° : D231 206193
Test species :Pseudokirchneriella subcapitata (formerly known as *Selenastrum capricornutum*), unicellular freshwater green alga
Number of replicates, initial cell density : 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 : control; 0.01, 0.02, 0.04, 0.08, 0.16 mg a.s./L
mean measured : exposure was not verified analytically
Test conditions :
temperature : 24 ± 1ºC
pH : 7.6 - 7.7 (initial), 7.6 - 7.8 (final)
light regime : continuous illumination
light intensity : 7000 lux

Analytical methods : not performed

This study on the algistatic activity of lenacil technical (Douglas M.T. and Handley J.W., 1998) was not considered valid due to absence of any analytical confirmation of the exposure concentrations. No analysis was performed to confirm initial exposure levels or to confirm stability during the test. Therefore, RMS was unable to propose EC50 values. Since two other acceptable algae studies were available, this non-accepted study was not added in the overview table. The study was reliable for the establishment of endpoints, and was only reported for the sake of completeness. It is not believed that the mention of this study in table 34, where no one is in a position to establish valid endpoint, has any added value in the environmental hazard assessment of Lenacil.

Findings :

Table 41: Percentage inhibition of growth of algae

Parameter	Control	Nominal test concentration (mg a.s./L)				
		0.01	**0.02**	**0.04**	**0.08**	**0.16**
area under curve at 72 h	-	2	88	103	108	109
area under curve at 120 h	-	2	80	102	103	104
growth rate (24-48 h)	-	-1	76	108	118	121

Negative value denotes an increase when compared to the solvent control

No abnormalities were observed in the control or at the treatment levels of 0.01, 0.02 and 0.04 mg a.s./L. Colourless and deformed cells were observed at the treatment levels of 0.08 and 0.16 mg a.s./L.
Recovery check : 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 :
The study was not considered valid due to absence of any analytical confirmation of the exposure concentrations. No analysis was performed to confirm initial exposure levels or to confirm stability during the test.

One toxicity study to aquatic plants is available for Lenacil:

Lenacil technical higher plant (Lemna) growth inhibition test. (Flatman D., 2003d).

Guidelines :
OECD draft (2000), US EPA draft OPPTS 850.4400 (1996)
GLP :
Yes
Material and Methods :
*Test substance :*lenacil, chemical purity : 98.6 %, batch n^{o} : 141712003
Test species :Lemna gibba (common duckweed)
Number of replicates, inoculum : 3 replicates for the control, the solvent control and per treatment,
each inoculated with four plants bearing three fronds (12 fronds total)
Type of test : 7-day semi-static toxicity test (media renewal at 48 to 72-hour intervals)
Applied and measured concentrations :
nominal : control; solvent control (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 3.610, 9.059, 15.60, 23.60, 70.19 µg a.s./L
mean measured : 0.0; 0.0; 3.7, 8.8, 15, 24, 71 µg a.s./L, corresponding to 96 – 102 % of nominal concentrations
Test conditions :
temperature : 23.5 - 26.2 °C
pH : 7.6 - 7.7 (fresh media), 7.9 - 8.6 (old media)
light regime : continuous illumination
light intensity : 4870 - 5610 lux
Analytical methods : lenacil concentrations were measured by HPLC/UV

For the test to be valid, the doubling time of frond number in the control must be less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275 d-1. In the most critical study (Flatman, 2003d), it may be verified that this QC is met:

Table: L. gibba frond counts and dry weights following 7-day exposure to Lenacil

Mean measured concentration (µg lenacil/L)	Mean[a] frond counts			% inhibition		Mean[a] dry weight/frond on day 7 (mg)	% inhibition
	day 2	day 5	day 7	0-7 day growth rate	0-7 day integrated growth		
Control	25	71	136	4.21	4.56	0.11	8.41
DMF Control	23	73	151	-	-	0.12	-
3.7	21	69	153	-0.61[b]	3.20	0.12	5.54
8.8	23	67	146	1.31	5.66	0.098	20.11
15	20	46	86	22.67**	43.74**	0.082	32.95*
24	20	37	59	37.51**	59.56**	0.10	18.84
71	15	16	15	90.33**	93.45**	0.15	-18.97

[a]Means for three replicates per treatment at each timepoint.
[b]Negative values indicate stimulated growth relative to solvent control.
*Significantly different ($p < 0.05$) from solvent control (Dunnett's test).
**Significantly different ($p < 0.01$) from solvent control (Williams' test).

Findings :

Table 42: Summary of effects on Lemna gibba after 7 days of exposure to lenacil

Evaluation criteria	Control	Solvent control	Mean measured test concentration (µg a.s./L)				
			3.7	8.8	15	24	71
Mean number of fronds	136	151	153	146	86	59	15
Mean dry weight of fronds (mg)	0.11	0.12	0.12	0.098	0.082	0.10	0.15

Frond counts : 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 weights : Frond dry weights showed a more variable response after exposure to lenacil : dry weight was significantly reduced at the treatment level of 15 µg a.s./L, but no significant differences in frond dry weights were observed between the solvent control group and plants exposed to higher concentrations of lenacil.
Growth : No visible effects after 7 days of exposure were observed on growth of fronds exposed to lenacil at the treatment levels of 3.7, 8.8 and 15 µg a.s./L. 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.
Conclusions :
The study is acceptable.
Endpoints:
E_bC_{50} (*Lemna gibba*, 7 d) = 0.019 mg a.s./L (mean measured)
E_rC_{50} (*Lemna gibba*, 7 d) = 0.029 mg a.s./L (mean measured)
NOEC (*Lemna gibba*, 7 d) = 0.0088 mg a.s./L (mean measured)

Two more studies were available on soil degradates IN-KE 121 and IN KF 313

Soil breakdown Products

IN-KE 121, algal growth inhibition assay. (Jenkins C.A., 2004a).

Guidelines :

92/69/EEC, method C.3 (1992), OECD 201 (1984)

GLP :

Yes

Material and Methods :

Test substance : IN-KE 121 (metabolite of lenacil), chemical purity : 96.7 %, batch n° : 7X-0245

Test species : Pseudokirchneriella subcapitata (formerly known as *Selenastrum capricornutum*), unicellular freshwater green alga

Number of replicates, initial cell density : 6 replicates for the control, 3 replicates per treatment,

initial cell count : 1×10^4/mL

Type of test : 72-hour static toxicity test

Applied and measured concentrations :

nominal : control; 1.94, 4.27, 9.39, 20.7, 45.5, 100 mg IN-KE 121/L

mean measured : 0.00; 1.36, 4.26, 10.1, 23.2, 50.4, 111 mg IN-KE 121/L (70 – 111 % of nominal concentrations)

At the start of the test, measured IN-KE 121 concentrations ranged between 102 and 113 % of their nominal values. At 9.39 to 100 mg a.s./L (nominal), measured concentrations were between 90 and 105 % of their initial values after 72 hours. At 1.94 and 4.27 mg a.s./L, measured concentrations were reduced to 48 and 78 % of initial values, respectively, after 72 hours. The loss of the test substance at the two lowest concentrations was due to the presence of the algal cells.

Table 43: Measured concentrations of lenacil metabolite IN-KE 121 during a toxicity test with *Selenastrum capricornutum*

Nominal concentration (mg/L)	Measured IN-KE 121 concentrations (mg/L)					Overall Mean [#]
	0 hours	%N	72 hours	%N	%ti	
Control	nd	-	nd	-	-	-
1.94	1.97	102	0.938	48	48	1.36
1.94A	-	-	1.99	103	101	
4.27	4.82	113	3.76	88	78	4.26
9.39	10.6	113	9.53	102	90	10.1
20.7	23.3	113	23.1	112	99	23.2
45.5	49.8	110	51.0	112	102	50.4
100	109	109	114	114	105	111
100A	-	-	110	110	101	

nd : none detected (<0.007 mg/L).
%N : measured concentration expressed as a percentage of the nominal concentration (calculated using unrounded values but expressed to 3 significant figures).
%ti : measured concentration after 72 hours expressed as a percentage of the starting concentration.
A : culture medium incubated under test conditions without algal cells.
[#] : geometric mean.

Test conditions :

temperature : 22.9 – 24.3 ºC

pH : 7.89 - 8.00 (initial), 7.62 - 9.48 (final)

light regime : continuous illumination

light intensity : 8100 - 8950 lux

Analytical methods : IN-KE 121 concentrations were measured by HPLC/UV

Findings :

Table 44 : Percentage inhibition of growth of algae exposed for 72 hours to the metabolite IN-KE 121

Parameter	**Control**	**Mean measured test concentration (mg a.s./L)**					
		1.36	**4.26**	**10.1**	**23.2**	**50.4**	**111**
area under curve at 72 h	-	12	15	46	81	99	99
growth rate (0-72 h)	-	1	3	12	35	92	96

No microscopic abnormalities of the cells were detected.

Recovery check : Following transfer to unamended control medium, regrowth was observed after 5 and 6 days, respectively, for cultures previously inhibited by exposure to 50.4 and 111 mg IN-KE 121/L during the definitive test. Consequently, the effect of IN-KE 121 was algistatic at these concentrations.

Conclusions :

The study is acceptable.

Endpoints :

E_bC_{50} (*Pseudokirchneriella subcapitata*, 72 h) = 10.7 mg IN-KE 121/L (mean measured)

E_rC_{50} (*Pseudokirchneriella subcapitata*, 72 h) = 27.8 mg IN-KE 121/L (mean measured)

NOEC (*Pseudokirchneriella subcapitata*, 72 h) = 1.36 mg IN-KE 121/L (mean measured) based on biomass

NOEC (*Pseudokirchneriella subcapitata*, 72 h) = 4.26 mg IN-KE 121/L (mean measured) based on growth rate

IN-KF 313, algal growth inhibition assay. (Jenkins C.A., 2004b).

Guidelines :

92/69/EEC, method C.3 (1992), OECD 201 (1984)

GLP :

Yes

Material and Methods :

Test substance : IN-KF 313 (metabolite of lenacil), chemical purity : 99.6 %, batch n° : IY-0622

Test species : Pseudokirchneriella subcapitata (formerly known as *Selenastrum capricornutum*), unicellular freshwater green alga

Number of replicates, initial cell density : 6 replicates for the control, 3 replicates per treatment,

initial cell count : 1×10^4/mL

Type of test : 72-hour static toxicity test

Applied and measured concentrations :

nominal : control; 0.625, 1.25, 2.5, 5, 10 mg IN-KF 313/L

mean measured : 0.00; 0.601, 1.26, 2.52, 5.15, 10.9 mg IN-KF 313/L (96 – 109 % of nominal concentrations)

At the start of the test, measured IN-KF 313 concentrations ranged between 95 and 108 % of their nominal values. After 72 hours measured concentrations were 97 and 109 % of nominals.

Table 45: Measured concentrations of lenacil metabolite IN-KF 313 during a toxicity test with *Selenastrum capricornutum*

Nominal concentration (mg/L)	Measured IN-KF 313 concentrations (mg/L)					Overall Mean #
	0 hours	%N	72 hours	%N	%ti	
Control	nd	-	nd	-	-	-
0.625	0.595	95	0.607	97	102	0.601
0.625A	-	-	0.659	105	111	
1.25	1.26	101	1.26	101	100	1.26
2.50	2.55	102	2.49	100	98	2.52
5.00	5.16	103	5.14	103	100	5.15
10.0	10.8	108	10.9	109	101	10.9
10.0A	-	-	10.2	102	94	

nd : none detected (< 0.002 mg/L).
%N : measured concentration expressed as a percentage of the nominal concentration (calculated using unrounded values but expressed to 3 significant figures).
%ti : measured concentration after 72 hours expressed as a percentage of the starting concentration.
A : culture medium incubated under test conditions without algal cells.
: arithmetic mean.

Test conditions :

temperature : 22.8 – 24.8 ºC

pH : 7.60 - 7.65 (initial), 7.90 - 9.64 (final)

light regime : continuous illumination

light intensity : 7750 - 7910 lux

Analytical methods : IN-KF 313 concentrations were measured by HPLC/UV

Findings :

Table 46 : Percentage inhibition of growth of algae exposed for 72 hours to the metabolite IN-KF 313

Parameter	**Control**	**Mean measured test concentration (mg a.s./L)**

		0.601	1.26	2.52	5.15	10.9
area under curve at 72 h	-	8	19	55	93	98
growth rate (0-72 h)	-	2	6	16	62	95

No microscopic abnormalities of the cells were detected.

Recovery check : Following transfer to unamended control medium, regrowth was observed after 5 days, respectively, for cultures previously inhibited by exposure to 5.15 and 10.9 mg IN-KF 313/L during the definitive test. Consequently, the effect of IN-KF 313 was algistatic at these concentrations.

Conclusions :

The study is acceptable.

Endpoints :

E_bC_{50} (*Pseudokirchneriella subcapitata*, 72 h) = 2.10 mg IN-KF 313/L (mean measured)

E_rC_{50} (*Pseudokirchneriella subcapitata*, 72 h) = 4.27 mg IN-KF 313/L (mean measured)

NOEC (*Pseudokirchneriella subcapitata*, 72 h) = 0.601 mg IN-KF 313/L (mean measured), based on biomass

NOEC (*Pseudokirchneriella subcapitata*, 72 h) = 1.26 mg IN-KF 313/L (mean measured), based on growth rate

Conclusion: both the acute and the chronic endpoints of the soil metabolites IN-KE121 an IN-KF313 were an order of magnitude higher than the parent compoubnd Lenacil itself.

5.4.4 Other aquatic organisms(including sediment)

To prevent unnecessary testing with substances of low toxicity to aquatic invertebrates, the NOEC in the chronic *Daphnia* test must be < 0.1 mg/L for testing on sediment-dwelling organisms to be warranted (SANCO/3268/2001). For Lenacil, the chronic NOEC for *Daphnia magna* is 480 µg a.s./L.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Degradation

As conclusion concerning the classification of the substance, the results of the ready degradability 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.

Aquatic bioaccumulation

The measured log P_{ow} values for lenacil (1.25 – 1.70) were all below the threshold value for bioaccumulation, i.e. threshold DSD ≥ 3 and threshold CLP ≥ 4. The potential risk for bioaccumulation of lenacil in tissues of aquatic organisms is considered low.

Aquatic toxicity

Both acute and chronic toxicity studies were conducted for the three trophic levels.
The 96 hour acute LC_{50} for fish is higher than 2.0 mg a.s./L and the 90 day chronic NOEC is 0.160 mg a.s./L. The 48 hour EC_{50} for aquatic invertebrates is higher than 8.4 mg a.s./L and the 21 d chronic NOEC is 0.480 mg a.s./L.
The most sensitive species are the algae with 72 h E_rC_{50} of 0.016 mg a.s./L and 96 h NOEC of 0.0034 mg a.s./L.
The 7 day E_rC_{50} for aquatic plants is 0.029 mg a.s./L and the 7 day NOEC is 0.0088 mg a.s./L.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

In aquatic toxicity studies the algae were identified as the most sensitive species with E_rC_{50} of 0.016 mg a.s./L and NOEC of 0.0034 mg a.s./L. Lenacil is not rapidly degradable and the potential for aquatic bioaccumulation is low.

Proposal for classification and labelling of lenacil according to DSD:

Classification:
N; R50/53
SCL concentration Cn in %:

N R50/53	Cn ≥2.5
N R51/53	0.25≤Cn<2.5
R52/53	0.025≤Cn<0.25

Labelling

Indication of danger:	N
R phrases:	R50/53 Dangerous for the environment; Very toxic to aquatic organisms, may cause long term adverse effects in the aquatic environment
S phrases:	S35 This material and its container must be disposed of in a safe way S57 Use appropriate containment to avoid environmental contamination

Proposal for classification and labelling of lenacil according to CLP and 2nd ATP:

Classification:
Aquatic Acute category 1 (based on E_rC_{50} algae and aquatic plants ≤ 1 mg/L)
H400
M-factor = 10 (based on 0.01 mg/L < $L(E)C_{50}$ ≤ 0.1 mg/L)

Aquatic Chronic category 1 (based on NOEC algae and aquatic plants ≤ 0.1 mg/L)
H410
M-factor = 10 (based on NRD and 0.001 < 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

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

The DS proposed to classify the substance as Aquatic Acute Category 1: H400, M=10; Aquatic Chronic Category 1, H410, M=10 (DSD: N, R50-53; SCLs: N; R50/53: C ≥ 2.5%, N; R51/53: 0.25% ≤ C < 2.5%, R52/53: 0.025% ≤ C ≤ 0.25%). The classification was based on the substance being not readily/not rapidly degradable, non-bioaccumulative and very toxic to aquatic organisms. The lowest acute toxicity value was ErC50 of 0.016 mg/l for algae and the lowest chronic toxicity value was NOEC of 0.0034 mg/l for algae.

Degradation

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

Lenacil is a weak acid with a pKa of 10.7. The preliminary hydrolysis test at 50°C shows that Lenacil is hydrolytically stable within the pH range of 4 to 9 (EEC-Method C7). The DT_{50} at 25°C can be estimated to be greater than 1 year.

The measured photolytic degradation of Lenacil in aqueous buffer at pH 5 was negligible. The calculated (GCSOLAR Program) DT_{50} and DT_{90} are greater than 1 year indicating that photolysis in unlikely to be a significant route of degradation. The photodegradation rate of Lenacil in soil at 20°C is equivalent to 67.6 days.

Mean cumulative CO_2 production by aqueous 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 in a Modified Sturm Tests (EEC-method C5). This shows that the substance is not readily biodegradable.

A study describing biodegradation of Lenacil in water/sediment system is available. The study was carried out with two independent water/sediment systems.

Lenacil	**Rückhaltebecken**	**Schaephysen**
Water, 0 days	92.8% AR	90.6% AR
Water, 120 days	24.5% AR	5.5% AR
Sediment max	58 days: 30.6% AR	30 days: 51.8 % AR
Sediment, 120 days	25.2% AR	41.9% AR
Whole system after 120 days	49.8% AR	46.4% AR
Evaluation of CO_2 after 120 days	3.8% AR	4.8% AR
Bound residue after 120 days	16.5% AR	10.6% AR

Calculated whole system DT_{50} values were 122 days in the Rückhaltebecken system and 103 days in the Schaephysen system. Corresponding DT_{90} values were 405 and 342 days. Insufficient data were available to calculate separate degradation rates for the water phase and sediment phase and for the major water sediment metabolite 5-oxo-Lenacil. In both systems there was only one significant metabolite which accounted for > 10% AR, M20.5 (5-oxo-Lenacil, also known as IN-KF313). 5-oxo-Lenacil peaked in the sediment phase on day 120 reaching the maximum levels of 10.7% AR in the sediment phase of one of the systems. In the water phase 5-oxo-Lenacil reached the maximum 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.

Five experiments in soil treated with Lenacil were carried out under aerobic conditions in the laboratory (20°C, 40% maximum water holding capacity (MWHC)) in the dark. DT_{50} values were calculated to be 11-18 days.

Hydrolysis and photolysis are of minor importance for its degradation in the environment. In the ready biodegradability test, CO_2 production by mixtures containing Lenacil technical was negligible. In the water/sediment study, Lenacil remained at 49.3% AR in the water phase at day 30 in one of the water/sediment systems. As conclusion, the substance is not readily/rapidly degradable.

Bioaccumulation

No measured bioaccumulation data are available. Measured (EEC-Method A8) log P values are 1.70 (pH4), 1.70 (pH7) and 1.25 (pH9). Thus the potential risk for bioaccumulation of Lenacil in tissues of aquatic organisms is considered low.

Aquatic toxicity

Table 1. Lowest acute aquatic toxicity data available

Species	**Test Guideline**	**Test type and duration**	**Result**
Oncorhynchus mykiss	OECD 203; US EPA 72-1 (GLP)	96h static	LC50 > 2.0 mg a.s./L (mean measured 100-180% of nom.)
Daphnia magna	OECD 202; US EPA 72-2 (GLP)	48h static	EC50 > 8.4 mg a.s./L (measured after 48 h)
Pseudokirchneriella subcapitata	OECD 201; 92/69/EEC C.3; draft US EPA OPPTS 850.5400 (GLP)	96h static	72h ErC50=0.016 mg a.s./L (mean measured 86-103% of nom.)
Lemna gibba	OECD draft; US EPA draft OPPTS 850.4400 (GLP)	7d semi-static	ErC50=0.029 mg a.s./L (mean measured 96-102% of nom.)

Table 2. Lowest chronic aquatic toxicity data available

Species	**Test Guideline**	**Test type and duration**	**Result**
Oncorhynchus mykiss	OECD 210 (GLP)	90d flow-through	NOEC=0.160 mg a.s./L based on mean standard length (mean measured 80-155% of nom.)
Daphnia magna	OECD 202 part II (GLP)	21d semi-static	NOEC=0.48 mg a.s./L based on adult survival and total numbers of offspring (mean measured 34-53% of nom.)
Pseudokirchneriella subcapitata	OECD 201; 92/69/EEC C.3; draft US EPA OPPTS 850.5400 (GLP)	96h static	96h NOEC=0.0034 mg a.s./L (mean measured 86-103% of nom.)

Lemna gibba	OECD draft; US EPA draft OPPTS 850.4400 (GLP)	7d semi-static	NOEC=0.0088 mg a.s./L (mean measured 96-102% of nom.)

All the studies in tables 1 and 2 are considered valid by the DS. There were, however, problems with the only available acute and chronic Daphnia tests. In the acute test the nominal concentrations were all considerably over (50-500 mg/l) the water solubility limit (~3 mg/l). In both acute and chronic tests dissolved oxygen concentration dropped below 60% of saturation in all treatments during the test having no apparent effect on the daphnids.

The lowest acute toxicity values for Lenacil are ErC_{50} values of 0.016 mg/l and 0.029 mg/l for algae and aquatic plant, respectively. The lowest chronic toxicity values are NOEC values 0.0034 mg/l and 0.0088 mg/l for algae and aquatic plant, respectively. A 72-hour NOEC value for algae would have been preferred but since it is not available a 96-hour NOEC value is used for classification purposes.

Table 3. Acute toxicity values available for degradation products

Algae, Pseudokirchneriella subcapitata	**IN-KE 121**	**IN-KF 313**
ErC50 72h	27.8 mg/l	4.27 mg/l
NOErC 72h	4.26 mg/l	1.26 mg/l
mean measured concentrations		

Comments received during public consultation

Four MSCAs and one company agreed with the classification proposal made by the DS.

Assessment and comparison with the classification criteria

Degradation

RAC agreed with the DS proposal to consider Lenacil as not readily/not rapidly degradable. The substance was hydrolytically stable and not readily degradable in a Modified Sturm test performed with aqueous mixtures containing technical Lenacil. This was confirmed by the calculated DT_{50} values of 103 and 122 days for the whole system in a water/sediment test.

Bioaccumulation

RAC agreed that Lenacil has a low potential to bioaccumulate based on the log P values 1.70 (pH4), 1.70 (pH7) and 1.25 (pH9).

Aquatic toxicity

There are adequate acute and chronic toxicity data available on fish, daphnia, algae and aquatic plant Lemna. The lowest acute toxicity value was ErC_{50} of 0.016 mg/l for algae and the lowest chronic toxicity NOEC value was of 0.0034 mg/l for algae.

Conclusion on classification

RAC agreed with the DS proposal to classify Lenacil as:
Aquatic Acute Category 1: H400, M=10 and
Aquatic Chronic Category 1, H410, M=10 according to the CLP and
N, R50-53 with the following concentration limits
N; R50/53: $C \geq 2.5\%$
N; R51/53: $0.25\% \leq C < 2.5\%$
R52/53: $0.025\% \leq C \leq 0.25\%$

according to the DSD.

The classification was based on the substance being not readily/rapidly degradable, non-bioaccumulative and very toxic to aquatic organisms.

Supplemental information - In depth analyses by RAC

Photodegradation in soil

The photodegradation rate of Lenacil in 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% applied radioactivity (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 one of the degradation products H1 reached a maximum of 7.6% AR. Thin layer chromatography (TLC) indicated that this radioactivity was associated with more than one component.

Degradation in soil

Five experiments in soil treated with Lenacil were carried out under aerobic conditions in the laboratory (20°C, 40% maximum water holding capacity (MWHC)) in the dark. The test was characterised by the following:

- Unextractable residues: 19.4-25.8% AR after 120 days
- Volatile compounds (presumably mainly CO_2): 47.6-61.1% AR after 120 days
- Major extractable breakdown products:
  - IN-KE 121 (7-oxo-Lenacil) max 9.2-13.9 % AR at 14-30 days.
  - IN-KF 313 max 8.5-14.7% AR at 7-14 days.
- Unidentified "Polar B" max 6.8-14.6% AR at 60-91 days.
- In one soil: unidentified "M15.0" more than 5% AR at two consecutive sampling times (later characterized as an oxo-isomer of Lenacil, conformity with IN-KE 121 could not be fully confirmed. However, expert agreed that the exposure assessment for IN-KE 121 would probably cover the assessment for "M15.0" even with respect to degradation).

One experiment was repeated at 10°C:

- Unextractable residues: 20.9% AR after 120 days.
- Volatile compounds (presumably mainly CO_2): 24.4% AR after 120 days.
- IN-KE121: 7.8% AR at 30 days.
- IN-KF 313: 9.4% AR at 60 days.
- "Polars": max 12.5% at 120 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, however it is noted that the pH of the soils investigated for aerobic degradation was limited (pH ranges 5.4 to 6.4; CaCl2 method).

No anaerobic soil degradation study was available.

Field soil dissipation studies were provided from 4 sites in Europe where spray applications of Lenacil were made in June and 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 DT50 were between 18-88 days. Small residues of the major soil metabolite IN-KF 313 were detected only in few cases in the top 10 cm layer, no decline kinetics were calculated for this metabolite.

The following table summarizes the results obtained in the aerobic soil degradation

studies, including the values obtained after normalization to FOCUS reference conditions.

	Lenacil	**IN-KE 121**	**IN-KF 313**
Single first order soil DT50	11-25 days (aerobic, 20°C, 40% MWHC, n=5)	4-12 days (aerobic) (estimated, n=5)	3-350 days (aerobic, 20 or 25 °C, 40% MWHC or pF2.5, soil moisture content, n=8)
FOCUS Single first order soil DT50 (20°C. pF2 soil moisture content)	11-18 days (geometric mean 14.4 days)	4-11 days (geometric mean 6.4 days)	3-444 days (geometric mean 41 days)

Environmental distribution

Freundlich adsorption constant (KFoc) values for Lenacil varied from 75 to 254 mL/g (median 83 mL/g) indicating that Lenacil is rather slightly mobile in soil. Calculated adsorption KFoc values for metabolite IN-KE 121 varied from 30.5-43.5 mL/g (mean 38 mL/g). There was no indication of any relationship between adsorption and any soil characteristic including pH. Calculated adsorption KFoc for IN-KF 313 varied from 79-824 mL/g (mean 557). pH dependency cannot be established nor excluded based on the available data with this narrow pH range.

The low vapour pressure of 1.7×10^{-9} Pa at 25°C indicates little potential for volatilisation of the active substance. The Henry's law constant 1.3×10^{-7} $Pa.m^3.mol^{-1}$ indicates that Lenacil is very slightly volatile from water.

6 OTHER INFORMATION

No other data available for consideration in determining the classification of Lenacil.

7 REFERENCES

Data point / Reference number	Author(s)	Year	Title Source (where different from notifier) Company, Report No GLP or GEP status (where relevant), Published or not	Data protection Y/N	Owner
IIA 5.5	Andrew , D.	2012	Lenacil: Review of Carcinogenicity and Proposed R40 Classification. Report No. TSGE 19-10-05 Expert statement, Non-GLP, Unpublished	N	DuPont
EFSA conclusion report	EFSA	2009	European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance lenacil on request form the European Commission. EFSA Journal 2009; 7(9):1326	N	Public domain
DAR	RMS Belgium	2007	Draft Assessment Report, November 2007, Lenacil	N	Public domain
Addendum to DAR	RMS Belgium	2009	Volume 3, Annex B, Toxicology and Metabolism – B.6 Toxicology and Metabolism Addendum. February 2009	N	Public domain

7.1 Physical and chemical properties of the active substance

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA, 2.6/02	Bell, A.	2005	Water solubility of Lenacil, CEM analytical services study number CEMS-2787. GLP, Unpublished	Yes	DuPont
IIA, 2.1.1/01	Comb, A.L.	2002a	Lenacil (Pure Grade) Physico-Chemical Properties, Huntingdon, ACD025/014039. GLP, Unpublished	Yes	DuPont
IIA, 2.1.2/01	Comb, A.L.	2002a	Lenacil (Pure Grade) Physico-Chemical Properties, Huntingdon, ACD025/014039. GLP, Unpublished	Yes	DuPont
IIA, 2.1.3/01	Comb, A.L.	2002a	Lenacil (Pure Grade) Physico-Chemical Properties, Huntingdon, ACD025/014039. GLP, Unpublished	Yes	DuPont
IIA, 2.2/01	Comb, A.L.	2002	Lenacil (Pure Grade) Physico-Chemical Properties, Huntingdon, ACD025/014039. GLP, Unpublished	Yes	DuPont
IIA, 2.3.1/01	Comb, A.L.	2002a	Lenacil (Pure Grade) Physico-Chemical Properties, Huntingdon, ACD025/014039. GLP, Unpublished	Yes	DuPont
IIA, 2.3.2/01	Comb, A.L.	2002a	Lenacil (Pure Grade) Physico-Chemical Properties, Huntingdon, ACD025/014039. GLP, Unpublished	Yes	DuPont
IIA, 2.4.1	Comb, A.L.	2002a	Lenacil (Pure Grade) Physico-Chemical Properties, Huntingdon, ACD025/014039. GLP, Unpublished	Yes	DuPont

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
IIA, 2.4.2	Comb, A.L.	2002a	Lenacil (Pure Grade) Physico-Chemical Properties, Huntingdon, ACD025/014039. GLP, Unpublished	Yes	DuPont
IIA, 2.5.1/01	Comb, A.L.	2002a	Lenacil (Pure Grade) Physico-Chemical Properties, Huntingdon, ACD025/014039. GLP, Unpublished	Yes	DuPont
IIA, 2.8/01	Comb, A.L.	2002a	Lenacil (Pure Grade) Physico-Chemical Properties, Huntingdon, ACD025/014039. GLP, Unpublished	Yes	DuPont
IIA, 2.9.4/03	Comb, A.L.	2002a	Lenacil (Pure Grade) Physico-Chemical Properties, Huntingdon, ACD025/014039. GLP, Unpublished	Yes	DuPont
IIA, 2.11.1/01	Comb, A.L.	2002 b	Lenacil Technical Physico-Chemical Properties, Huntingdon, ACD024/013898. GLP, Unpublished	Yes	DuPont
IIA, 2.11.2/01	Comb, A.L.	2002 b	Lenacil Technical Physico-Chemical Properties, Huntingdon, ACD024/013898. GLP, Unpublished	Yes	DuPont
IIA, 2.13/01	Comb, A.L.	2002 b	Lenacil Technical Physico-Chemical Properties, Huntingdon, ACD024/013898. GLP, Unpublished	Yes	DuPont
IIA, 2.14/01	Comb, A.L.	2002a	Lenacil (Pure Grade) Physico-Chemical Properties, Huntingdon, ACD025/014039. GLP, Unpublished	Yes	DuPont
2.15/01	Comb, A.L.	2002 b	Lenacil Technical Physico-Chemical Properties, Huntingdon, ACD024/013898. GLP, Unpublished	Yes	DuPont
IIA, 2.4.1/01	Hamroll, K.	2003	Description of the physical state, colour and odour of Lenacil technical, Schirm GmbH, No. not stated. Not GLP, Unpublished	Yes	DuPont
IIA, 2.4.2/01	Hamroll, K.	2003	Description of the physical state, colour and odour of Lenacil technical, Schirm GmbH, No. not stated. Not GLP, Unpublished	Yes	DuPont
IIA, 2.7/01	McQuage ,J. D.	1992	Unpublished Solubility of Lenacil in Organic Solvents, DuPont AMR 2377-92. GLP, Unpublished	No	DuPont

7.2 Toxicology and metabolism of the active substance

Data point / Reference number	Author(s)	Year	Title Source (where different from notifier) Company, Report No GLP or GEP status (where relevant), Published or not	Data protection Y/N	Owner

Data point / Reference number	Author(s)	Year	Title Source (where different from notifier) Company, Report No GLP or GEP status (where relevant), Published or not	Data protection Y/N	Owner
Annex II, 5.4.1.3/01	Allais, L.	2001	Lenacil technical ; In vitro Mammalian Chromosome Aberration Test in Human Lymphocytes, [redacted], ACD 017/013707. GLP, Unpublished.	Yes	DuPont
Annex II, 5.2.6.1/01	Armondi , S.	1992	Closed-Patch repeated insult dermal sensitization study (Maximization Method) with DPX-B634-91 in Guinea Pigs, Du Pont HLO 34-92. GLP, Unpublished.	No	DuPont
Annex II, 5.2.1/02	Blanchard , E. L.	2001a	Acute oral toxicity to the rat (Acute Toxic Class Method), [redacted], ACD 004/013224/AC. GLP, Unpublished.	Yes	DuPont
Annex II, 5.2.2/01	Blanchard , E. L.	2001 b	Acute dermal toxicity to the rat, [redacted], ACD 005/013220/AC. GLP, Unpublished.	Yes	DuPont
Annex II, 5.2.4/01	Blanchard , E. L.	2001c	Skin irritation to the rabbit, [redacted], ACD 006/013201/SE. GLP, Unpublished.	Yes	DuPont
Annex II, 5.2.5/01	Blanchard , E. L.	2001 d	Eye irritation to the rabbit, [redacted] ACD 007/013273/SE. GLP, Unpublished.	Yes	DuPont
Annex II, 5.4.1.4/01	Clare , M. G.	2003	Lenacil technical; In Vitro Mammalian Cell Gene Mutation Test, Huntingdon Life Sciences, ACD 053/023530. GLP, Unpublished.	Yes	DuPont
Annex II, 5.2.3/01	Coombs, D. W	2001	Lenacil technical - Acute (four-hour) Inhalation Study in Rats, [redacted], ACD 021/013229. GLP, Unpublished.	Yes	DuPont
Annex II, 5.4.1.1/03	D'Amicoi, S.W.	1994	Mutagenicity testing of DPX-B634-107 (Lenacil) in the Salmonella Typhimurium plate incorporation assay. DuPont USA, HLR 413-94. GLP, Unpublished.	No	DuPont
Annex II 5.3.1.2/01	Geary ,M.	2001	Preliminary toxicity study by dietary administration to beagle dogs for 4 weeks, [redacted], ACD 003/013230. GLP, Unpublished.	Yes	DuPont
Annex II, 5.3.2.3/01	Geary ,M.	2002	Toxicity study by dietary administration to beagle dogs for 13 weeks, [redacted], ACD 022/014297. GLP, Unpublished.	Yes	DuPont
Annex II, 5.1.1/01	Ghantous, H. N.	1996	Adsorption, Distribution, Metabolism, and Excretion of [2-^{14}C]-Lenacil ([2-^{14}C]-DPX-B634) in the rat, DuPont HLR 62-94. GLP, Unpublished.	No	DuPont
Annex II, 5.6.2.2/01	Hurtt, M.E.	1991	Teratogenicity Study of DPX-B634-91 in Rabbits, [redacted], HLR626-91. GLP, Unpublished.	No	DuPont
Annex II 5.9.1/01	Klotzbach, K.	2003	Medical expertise for the Lenacil production. Unpublished letter report, B·A·D Gesundheitsvorsorge und Sicherheitstechnick GmbH, not detailed. Not GLP, Unpublished.	Yes	DuPont

Data point / Reference number	Author(s)	Year	Title Source (where different from notifier) Company, Report No GLP or GEP status (where relevant), Published or not	Data protection Y/N	Owner
Annex II, 5.5.2/01	Malek, D. E.	1994	Oncogenicity study with DPX-B634-91 (Lenacil) eighteen-month feeding study in mice, [redacted] HLR-336-93. GLP, Unpublished.	No	DuPont
Annex II, 5.3.2.2/01	Malley, L. A.	1991	Subchronic oral toxicity: 90 day study with DPX-B634-91 (Lenacil) Feeding study in mice, [redacted], HLR293-91. GLP, Unpublished.	No	DuPont
Annex II, 5.4.1.1/04	May , K.	2001	Lenacil technical: Bacterial Mutation Assay, Huntingdon Life Sciences, ACD 016/013217. GLP, Unpublished.	Yes	DuPont
Annex II, 5.4.2.1/01	Mehmood, Z.	2001	Lenacil technical – Mouse micronucleus test, [redacted], ACD 018/013472. GLP, Unpublished.	Yes	DuPont
Annex II, 5.4.1.2/01	Mohammed, R; Riach, C G	1989	Lenacil: Assessment of genotoxicity in an unscheduled DNA synthesis assay using adult rat hepatocyte primary cultures, Inveresk Research, IRI 6135. GLP, Unpublished.	No	DuPont
Annex II 5.6.2.1/02	Munley ,S. M.	1996	DPX-B634 (Lenacil): Pilot Developmental Toxicity Study in Rats[redacted], HLR996-96. Not GLP, Unpublished.	No	DuPont
Annex II, 5.6.1.1/01	Patten, R.	2002	Lenacil technical: preliminary study of effects on reproductive performance in Han Wistar rats by dietary administration, [redacted], ACD 019/010186. GLP, Unpublished.	Yes	DuPont
Annex II, 5.6.1.2/01	Patten, R.	2003a	Study of Reproductive Performance in Han Wistar Rats treated continuously through two successive Generations by Dietary Administration, [redacted], ACD 020/023865. GLP, Unpublished.	Yes	DuPont
Annex II, 5.6.2.1/03	Patten, R.	2003 b	Lenacil technical: Preliminary study of effects on embryo-fetal development in CD rats treated by oral gavage administration, [redacted], ACD 057/030001. Not GLP, Unpublished.	Yes	DuPont
Annex II, 5.6.2.1/04	Patten, R.	2003c	Study of effects on embryo-fetal development in CD rats treated by oral gavage Administration, [redacted] ACD 058/032316. GLP, Unpublished.	Yes	DuPont
Annex II, 5.4.1.1/02	Reynolds, V. L.	1989	Mutagenicity testing of IN E 1512-2 in the Salmonella Typhimurium plate incorporation assay, DuPont USA, HLR 550-89. GLP, Unpublished.	No	DuPont
Annex II, 5.4.1.1/01	Russell, J. F., Jr.	1977	Mutagenic Activity of Uracil, 3-Cyclohexyl-5,6-Trimethylene in the Salmonella/Microsome Assay, [redacted] HLR 601-77. Not GLP, Unpublished.	No	DuPont

Data point / Reference number	**Author(s)**	**Year**	**Title** **Source (where different from notifier)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data protection Y/N**	**Owner**
Annex II, 5.2.1/01	Sarver, J. W.	1989	Approximate lethal dose (ALD) of IN E1512-2 in rats, HLR564-89. GLP, Unpublished.	No	DuPont
Annex II, 5.6.2.1/01	Smith, L.W.	1978	Embryotoxic and teratogenic study in rats with Lenacil (INB-634), [redacted], HLR 405-78. Not GLP, Unpublished.	No	DuPont
Annex II 5.5.1.1/01	Thirlwell, P.	2004c	Combined Chronic Toxicity and Carcinogenicity Study by Dietary Administration to Han Wistar Rats over 104 Weeks, [redacted], ACD 045/042214. GLP, Unpublished.	Yes	DuPont
Annex II 5.5.1.2/01	Thirlwell, P.	2004c	Combined Chronic Toxicity and Carcinogenicity Study by Dietary Administration to Han Wistar Rats over 104 Weeks, [redacted], ACD 045/042214. GLP, Unpublished.	Yes	DuPont
Annex II, 5.3.1.1/01	Thirlwell, P. M.	2002a	Lenacil technical: preliminary study by dietary administration to Han Wistar rats for 4 weeks, [redacted], ACD 001/010098. GLP, Unpublished.	Yes	DuPont
Annex II, 5.3.2.1/01	Thirlwell, P. M.	2002 b	Toxicity Study by Dietary Administration to Han Wistar Rats for 13 Weeks followed by a 4 Week Recovery Period, [redacted]s ACD 002/013903. GLP, Unpublished.	Yes	DuPont
Annex II, 5.3.2.1.1/01	Thirlwell, P. M.	2004c	Lenacil technical – Additional histopathological investigation to a toxicity study by dietary administration toxicity study by dietary administration to Han Wistar rats for 13 weeks followed by a 4 week recovery period, [redacted] ACD/055 024499. GLP, Unpublished.	Yes	DuPont
Annex II 5.8.2.1/01	Whittaker, R.	2004	Lenacil technical – Investigation into potential effects on thyroid function after 20 weeks of treatment in female HAN Wistar rats using the "Perchlorate Discharge Test"., [redacted], ACD 060/033946. GLP, Unpublished.	Yes	DuPont

7.3 Additional information used in the DAR by the RMS

Data point / Reference number	Author(s)	Year	Title Source (where different from notifier) Company, Report No GLP or GEP status (where relevant), Published or not	Data protection Y/N	Owner
Annex II, 5.5.2	Boorman et al.	1990	Pathology of the Fischer Rat: Reference and Atlas by Gary A. Boorman (Editor), Scot L. Eustis (Editor), Michael R. Elwell, Charles Montgomery (Editor) Publisher: Academic Press; ISBN: 0121156400; (November 1990) Chapter 19, Mammary Gland.	-	-
Annex II, 5.5.2	Charles River Laboratories	2003	Spontaneous neoplasms and survival in Wistar Han rats: compilation of control data.	-	-
Annex II, 5.5.2	Charles River laboratories	1995	Spontaneous neoplastic lesions in the Crl:CD-1 BR mouse.	-	-
Annex II, 5.4.1	Grancharov K, Gorneva G, Mladenova J, Norpoth K, Golovinsky E	1986	Lack of genotoxic and cytotoxic effects of the herbicide Lenacil on mouse tumor cells and on some *Salmonella typhimurium* strains Arzneimittelforschung, 36(11), 1660-1663.)	-	-
Annex II, 5.5.2	Lacave et al	1999	Correlation between gender and spontaneous C-cell tumors in the thyroid gland of the Wistar rat. Cell and tissue research, 297, 3, 451-457.	-	-
Annex II, 5.5.2	Poteracki and Walsch	1998	Spontaneous neoplasms in control Wistar rats: a comparison of reviews Toxicological Sciences, 45,1-8	-	-
Annex II, 5.1	Zhang et al	1999	Lenacil degradation in the environment and its metabolism in the sugar beets: J.Agric. Food Chem, 47, 3843-3849	-	-

7.4 Environmental fate and behaviour of the active substance

Data point / Reference number	Author(s)	Year	Title Source (where different from notifier) Company, Report No GLP or GEP status (where relevant), Published or not	Data protection Y/N	Owner
IIA 7.2.1.3.1/01	Barnes, S.	2001	Lenacil Technical –Assessment of Ready Biodegradability : Modified Sturm Test, Huntingdon Life Sciences, ACD037/013644. GLP, Unpublished	Yes	DuPont
IIA, 7.1.1.2.1.1/ 01	Berg, D. S.	1994a	Degradation Rate of ^{14}C-Lenacil in Soil, E.I. Du Pont de Nemours, AMR2400-92. GLP, Unpublished	No	DuPont
IIA, 7.1.1.2.1.3/ 01	Berg, D. S.	1994 b	Degradation rate on IN-KF313 in three soils, E.I. du Pont de Nemours, AMR 2545-92. GLP, Unpublished	No	DuPont
IIA, 7.1.2./03	Berg, D. S.	1996c	Batch equilibrium (adsorption/desorption) study with IN-K 313, E.I. du Pont de Nemours AMR2948-94. GLP, Unpublished	No	DuPont
IIA 7.2.1.1/01	Caldwell, E.	2002	14C-Lenacil; Hydrolysis under Laboratory Conditions, Huntingdon Life Sciences Ltd. ACD046/013764. GLP, Unpublished	Yes	DuPont
IIA, 7.2.2/01	Comb, A.L.	2002a	Lenacil pure grade: Physico-chemical properties, Huntingdon Life Sciences Ltd, ACD025/014039. GLP, Unpublished	Yes	DuPont
IIA, 7.1.1.2.1.1/ 02 IIA, 7.1.1.2.1.2	Girkin, R.	2003	Lenacil Aerobic Rate of Degradation in one Soil Type at 10°C and in four Soils at 20°C, Huntingdon Life Sciences Ltd, ACD 042/023664. GLP, Unpublished	Yes	DuPont
IIA, 7.1.2 /02	Girkin, R.	2002a	Lenacil; Adsorption/Desorption on Soil, Huntingdon Life Sciences Ltd., ACD 044/022152. GLP, Unpublished	Yes	DuPont
IIA, 7.1.2/04	Kane, T.	2004	IN-KE 121, Adsorption / Desorption on soil, Huntingdon Life Sciences Ltd, ACD 063/042264. GLP, Unpublished	Yes	DuPont
IIA 7.2.1.2/01	Millais, A.	2002 b	Lenacil quantum yield of direct phototransformation, Huntingdon Life Sciences Ltd, ACD047/022138. GLP, Unpublished	Yes	DuPont
IIA, 7.1.1.1.2.2/ 01	Millais, A.J.	2002a	Lenacil; Photodegradation on Soil, Huntingdon Life Sciences Ltd., ACD 041/023429. GLP, Unpublished	Yes	DuPont
IIA, 7.3	Pollard-Langford, A.	2004	Lenacil Definition of the residue in plants and soil, Huntingdon Life Sciences Ltd, Not GLP, Unpublished	Yes	DuPont
IIA, 7.1.1.2.2/01 IIA, 7.1.1.2.3/01	Pollmann, B.	2003	Venzar 80 % WP (containing 80% Lenacil) Related Soil Dissipation on Bare Soil, four Sites in Europe, 2001, GAB, 20011048/E1-FSD. GLP, Unpublished	Yes	DuPont
IIA 7.1.3.3/01	Schnöder, F.	2004	Lysimeter Study with (14C)-Lenacil Revised Final Report, Covance, CLE Study No. 550-022, (AMR3498-95). GLP, Unpublished + Position Paper	Yes	DuPont

Data point / Reference number	Author(s)	Year	Title Source (where different from notifier) Company, Report No GLP or GEP status (where relevant), Published or not	Data protection Y/N	Owner
IIA, 7.1.2/01	Sheftic, G. D., Priester, T. M.	1992	Batch equilibrium (adsorption/desorption) study with [2-^{14}C] Lenacil, E.I. du Pont de Nemours, AMR 2332-92. GLP, Unpublished	No	DuPont
IIA, 7.1.1.1.1/01	Theis, M.	2003	Lenacil –Fate and behaviour in soil, A&M Labor GmbH, A&M00-077. GLP, Unpublished	Yes	DuPont
IIA 7.2.1.3.2/01	Theis, M.	2002a	Lenacil Fate and behaviour in Water-sediment, A&M Labor, A&M00-078. GLP, Unpublished	Yes	DuPont

7.5 Ecotoxicology of the active substance

Data point / Reference number	Author(s)	Year	Title Source (where different from notifier) Company, Report No GLP or GEP status (where relevant), Published or not	Data protection Y/N	Owner
IIA, 8.7/01	Barnes, S.P.	2001	Lenacil technical – activated sludge : respiration inhibition test, Huntingdon Life Sciences, ACD 038/013510, GLP, Unpublished	Yes	DuPont
IIA, 8.5/01	Carter, J.N.	2002	Lenacil technical; Effects on soil non-target micro-organisms: nitrogen transformation, carbon transformation, Huntingdon Life Sciences, ACD 026/014045, GLP, Unpublished	Yes	DuPont
IIA 8.2.6/03	Douglas M.T., Handley, J.W.	1988	The algistatic activity of Lenacil technical, Huntingdon Research Centre, DPT171(k)/88189, GLP, Unpublished	No	DuPont
IIA, 8.2.1/03	Flatman, D.	2003a	Lenacil technical; acute toxicity to fish (*Cyprinus carpio*), [redacted] ACD 035/022512, GLP, Unpublished	Yes	DuPont
IIA 8.2.6/01	Flatman, D.	2003 b	Lenacil technical; algal growth inhibition assay *Navicula pelliculosa*, Huntingdon Life Sciences, ACD 036/024694, GLP, Unpublished	Yes	DuPont
IIA 8.2.6/02	Flatman, D.	2003c	Lenacil technical; algal growth inhibition assay *Selenastrum capricornutum*, Huntingdon Life Sciences, ACD 034/022511, GLP, Unpublished	Yes	DuPont
IIA, 8.2.8/01	Flatman, D.	2003 d	Lenacil technical; higher plant (*Lemna*) growth inhibition test, Huntingdon Life Sciences, ACD 039/023827, GLP, Unpublished	Yes	DuPont
IIA, 8.1.3/01	Gallagher, S.P.; Stence, M., Beavers, J.B., Jaber M.	1996	DPX-B634-91 (Lenacil): A reproduction study with the northern bobwhite (*Colinus virginianus*), [redacted] AMR 3419-95; GLP, Unpublished	No	DuPont

Data point / Reference number	Author(s)	Year	Title Source (where different from notifier) Company, Report No GLP or GEP status (where relevant), Published or not	Data protection Y/N	Owner
IIA 8.3.1.1/01	Hoxter K.A.; Bernard, W. L.; Beavers, J. B.	1994a	H-18,759: A dietary LC_{50} toxicity study with the honey bee, Wildlife International, HLO 404-93, amended, GLP, Unpublished	No	DuPont
IIA 8.3.1.1/02	Hoxter K.A.; Bernard, W.L.; Beavers, J.B.	1994 b	H-18,759: An acute contact toxicity study with the honey bee, Wildlife International, HLO 405-93, amended, GLP, Unpublished	No	DuPont
IIA, 8.2.1/01	Hutton, D.G.	1991a	Static, acute, 96-hour LC_{50} of DPX-B634-91 (Lenacil) to rainbow trout (*Oncorhynchus mykiss*), [redacted], HLR 199-91, GLP, Unpublished	No	DuPont
IIA, 8.2.1/02	Hutton, D.G.	1991 b	Static, acute, 96-hour LC_{50} of DPX-B634-91 (Lenacil) to fathead minnows (*Pimephales promelas*), [redacted], HLR 198-91, GLP, Unpublished	No	DuPont
IIA, 8.2.2.1/01	Hutton, D.G.	1991c	Flow-through, 21 day toxicity of DPX-B634-91 (Lenacil) to rainbow trout (*Oncorhynchus mykiss*), [redacted], HLR-200-91, GLP, Unpublished	No	DuPont
IIA 8.2.4/01	Hutton, D.G.	1989a	Static acute 48-hour EC_{50} of DPX-B634-84 to fed *Daphnia magna*, Du Pont, HLR 86-89, GLP, Unpublished	No	DuPont
IIA 8.2.5/01	Hutton, D.G.	1989 b	Chronic toxicity of DPX-B634-84 (Lenacil) to *Daphnia magna*, DuPont, HLR 130-89, GLP, Unpublished	No	DuPont
IIA, 8.2.6/04	Jenkins, C.A.	2004a	IN-KE 121 algal growth inhibition assay, Huntingdon Life Sciences, ACD 064/042730, GLP, Unpublished	Yes	DuPont
IIA, 8.2.6/05	Jenkins, C.A.	2004 b	IN-KF 313 algal growth inhibition assay *Selenastrum capricornutum*, Huntingdon Life Sciences, ACD 066/042848, GLP, Unpublished	Yes	DuPont
IIA, 8.2.2.2/01	Kreamer, G.-L.C.	1996	Early life-stage toxicity of DPX-B634-91 (Lenacil) to rainbow trout (*Oncorhynchus mykiss*), [redacted] HLR-235-96, GLP, Unpublished	No	DuPont
IIA, 8.1.1/01	Rodgers, M.H.	2002a	Lenacil technical acute oral toxicity (LD50) to the mallard duck, [redacted] ACD048/022425, GLP, Unpublished	Yes	DuPont
IIA, 8.1.1/02	Rodgers, M.H.	2002 b	Lenacil technical acute oral toxicity (LD_{50}) to the bobwhite quail, [redacted], ACD049/022426, GLP, Unpublished	Yes	DuPont
IIA, 8.1.2/01	Rodgers, M.H.	2004a	Lenacil technical, dietary toxicity (LC_{50}) to the bobwhite quail, [redacted], DPT 637/033931, GLP, Unpublished	Yes	DuPont
IIA, 8.4.1/01	Rodgers, M.H.	2002c	Lenacil technical: Acute toxicity (LC_{50}) to the earthworm, Huntingdon Life Sciences, ACD 027/014409, GLP, Unpublished	Yes	DuPont

Data point / Reference number	**Author(s)**	**Year**	**Title** **Source (where different from notifier)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data protection Y/N**	**Owner**
IIA, 8.4.1/02	Rodgers, M.H.	2004 b	IN-KF 313 Acute Toxicity (LC_{50}) to the Earthworm, Huntingdon Life Sciences, ACD 062/043039, GLP, Unpublished	Yes	DuPont
IIA, 8.4.1/03	Rodgers, M.H.	2004c	IN-KE 121 Acute Toxicity (LC_{50}) to the earthworm, Huntingdon Life Sciences, ACD 061/043033, GLP, Unpublished	Yes	DuPont
IIA, 8.3.2/01	Wainwright, M.J.	2002 b	Venzar 80% WP: Acute toxicity to *Aphidius rhopalosiphi* in the laboratory, Huntingdon Life Sciences, ACD 028/013631, GLP, Unpublished	Yes	DuPont
IIA, 8.3.2/02	Wainwright, M.J.	2002c	Venzar 80% WP: Acute toxicity to *Typhlodromus pyri* in the laboratory, Huntingdon Life Sciences, ACD029/013961, GLP, Unpublished	Yes	DuPont
IIA, 8.3.2/03	Wainwright, M.J.	2002 d	Venzar 80% WP: Evaluation of the effect on the rove beetle *Aleochara bilineata* in the laboratory, Huntingdon Life Sciences, ACD 030/013462, GLP, Unpublished	Yes	DuPont
IIA, 8.3.2/04	Wainwright, M.J.	2002e	Venzar 80% WP: Evaluation of the effects of pesticides on the green lacewing *Chrysoperla carnea* in the laboratory, Huntingdon Life Sciences, ACD 031/022547, GLP, Unpublished	Yes	DuPont

7.6 Ecotoxicology of the formulation

Data point / Reference number	Author(s)	Year	Title Source (where different from notifier) Company, Report No GLP or GEP status (where relevant), Published or not	Data protection Y/N	Owner
IIIA, 10.2.1/03	Douglas, M.T., Halls, R.W.S.	1993	Venzar® (Lenacil, 80 % WP): Algal growth inhibition. Huntingdon Research Centre, Ltd., DPC 16(n)/920443. GLP, Unpublished	Yes	DuPont
IIIA, 10.8/01	Fiebig, S.	2001	Venzar 80% WP: Terrestrial plants toxicity, vegetative vigour, Tier II. Dr U. Noack für angewandte Biologie, TNW77232. GLP, Unpublished	Yes	DuPont
IIIA, 10.8/02	Goßmann, A., Meinerling, M.	2006	Effects of Venzar 500 SC on terrestrial (non-target) plants: seedling emergence and seedling growth test. Institut für biologische Analytik und Consulting IBACON GmbH, 26803086. GLP, Unpublished	Yes	DuPont
IIIA, 10.6.1.2/02	Gottrup, O.	1985	Toxicity of Lenacil to earthworm. Lenacil formulated as Venzar., Agrolab A/S, Report no 17-85-08-01 and 17-85-09-1. Not GLP, Unpublished	No	DuPont
IIIA, 10.2.2/02	Jenkins, C.A.	2005	Lenacil (Venzar 80% WP) Effects on primary productivity and macrophyte biomass in field-based microcosms. Huntingdon Life Sciences, Ltd., ACD 072/043691. GLP, Unpublished	Yes	DuPont
IIIA, 10.6.1.2/01	Rodgers, M.H.	2002 d	Venzar 80% WP: to determine the effects on reproduction and growth of the earthworm, *Eisenia fetida*, Huntingdon Life Sciences, Ltd., ACD 032/023270. GLP, Unpublished	Yes	DuPont
IIIA, 10.2.2/01	Taylor, S.A.	2004	Venzar 80% WP: A Laboratory assessment of the impact on macrophyte biomass following simulated spray drift contamination, Huntingdon Life Sciences, Ltd., ACD 070/043195. GLP, Unpublished	Yes	DuPont
IIIA, 10.4.1/01	Wainwright, M.J.	2002a	Venzar 80% WP: Acute toxicity to honey bees (*Apis mellifera*), Huntingdon Life Sciences, Ltd., ACD033/013732. GLP, Unpublished	Yes	DuPont
IIIA, 10.2.1/01	Ward, T.J., Kowalski, P.L., Boeri, R.L.	1995a	Acute toxicity of DPX-B634-106 (Venzar® 80 WP) to the rainbow trout, *Oncorhynchus mykiss*, [redacted] HLO 150-95, (Revision No.1) . GLP, Unpublished	Yes	DuPont
Annex III, 10.2.1/02	Ward, T.J., Kowalski, P.L., Boeri, R.L.	1995 b	Acute toxicity of DPX-B-634-106 (Venzar® 80 WP) to the daphnid, *Daphnia magna*, T.R. Wilbury Laboratories, Inc., HLO 149-95, (Revision No. 1) . GLP, Unpublished	Yes	DuPont

8 ANNEXES

See CLH report – Confidential Annex