Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Lenacil (ISO)

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

CLH-O-0000002461-82-02/F

Adopted

5 December 2013

5 December 2013

CLH-O-0000002461-82-02/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemicals name: Lenacil (ISO); 3-cyclohexyl-6,7-dihydro-1H-cyclopenta[d]pyrimidine-2,4(3H,5H)-dione

EC number: 218-499-0

CAS number: 2164-08-1

The proposal was submitted by **Belgium** and received by the RAC on **15 May 2013.**

In this opinion, all classifications are given firstly in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS) and secondly, according to the notation of 67/548/EEC, the Dangerous Substances Directive (DSD).

PROCESS FOR ADOPTION OF THE OPINION

Belgium submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **15 May 2013**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **28 June 2013**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by RAC: **Elodie Pasquier**

Co-rapporteur, appointed by RAC: **Riitta Leinonen**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation.

The RAC opinion on the proposed harmonised classification and labelling was reached on **5 December 2013** and the comments received are compiled in Annex 2.

The RAC Opinion was adopted by **consensus**.

OPINION OF THE RAC

The RAC adopted the opinion that **lenacil (ISO)** should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					Hazard Class and Category Code(s)	Hazard state ment Code	Pictogram Signal Word Code(s)	Hazard state-ment Code	Suppl. Hazard state ment Code(s)	
Current Annex VI entry	No current Annex VI entry									
Dossier submitters proposal	613-320-00-6	lenacil (ISO); 3-cyclohexyl-6,7-dihydro-1H-cyclopenta[d]pyrimidine-2,4(3H,5H)-dione	218-499-0	2164-08-1	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=10 M=10
RAC opinion		lenacil (ISO); 3-cyclohexyl-6,7-dihydro-1H-cyclopenta[d]pyrimidine-2,4(3H,5H)-dione	218-499-0	2164-08-1	Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H400 H410	GHS08 GHS09 Wng	H351 H410		M=10 M=10
Resulting Annex VI entry if agreed by COM		lenacil (ISO); 3-cyclohexyl-6,7-dihydro-1H-cyclopenta[d]pyrimidine-2,4(3H,5H)-dione	218-499-0	2164-08-1	Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H351 H400 H410	GHS08 GHS09 Wng	H351 H410		M=10 M=10

Classification and labelling in accordance with DSD

	Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits
Current Annex VI entry		No current Annex VI entry					
Dossier submitters proposal	613-320-00-6	lenacil (ISO); 3-cyclohexyl-6,7-dihydro-1H-cyclopenta[d]pyrimidine-2,4(3H,5H)-dione	218-499-0	2164-08-1	N; R50-53	N; R50/53 S: 35-57	N; R50-53: C ≥ 2.5% N; R51-53: 0.25% ≤ C < 2.5% R52-53: 0.025% ≤ C < 0.25%
RAC opinion		lenacil (ISO); 3-cyclohexyl-6,7-dihydro-1H-cyclopenta[d]pyrimidine-2,4(3H,5H)-dione	218-499-0	2164-08-1	Carc. Cat. 3; R40 N; R50-53	Xn; N R: 40-R50/53 S: (2-)36/37-60-61	N; R50-53: C ≥ 2.5% N; R51-53: 0.25% ≤ C < 2.5% R52-53: 0.025% ≤ C < 0.25%
Resulting Annex VI entry if agreed by COM		lenacil (ISO); 3-cyclohexyl-6,7-dihydro-1H-cyclopenta[d]pyrimidine-2,4(3H,5H)-dione	218-499-0	2164-08-1	Carc. Cat. 3; R40 N; R50-53	Xn; N R: 40-50/53 S: (2-)36/37-60-61	N; R50-53: C ≥ 2.5 % N; R51-53: 0.25 % ≤ C < 2.5 % R52-53: 0.025 % ≤ C < 0.25 %

SCIENTIFIC GROUNDS FOR THE OPINION

RAC general comment

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

HUMAN HEALTH HAZARD ASSESSMENT

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

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 lower 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 effects on reproductive organs or reproductive performance at any of the dietary concentrations and offspring survival was not affected by treatment. There was no effect on the physical and sexual development of the offspring. At 50000 ppm, 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 for lactation effects.

The PRAPeR 69 (EFSA) meeting concluded that considering the very high dose level applied in the study (4300 mg/kg bw/d which exceeded the 1000mg/kg bw/d limit for reproduction toxicity studies), the decrease in offspring weight gain during lactation was insufficient to justify classification for lactation effects and did not consider the effects on sexual function or fertility as toxic effects on the offspring.

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

Overall, no classification was proposed by the DS for reproductive toxicity.

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

Fertility

No effects on fertility were observed in a rat two-generation study. Some isolated effects were reported in the uterus or vagina of female rats. These were observed at high doses (significant effects only at doses >1000 mg/kg) and the toxicological relevance of effects observed in the uterus is uncertain considering the absence of histological findings or functional effect on the female reproductive function.

Overall, RAC agreed with the DS that a classification of Lenacil for fertility was not warranted.

Developmental toxicity and effects on/via lactation

No developmental effects were reported in the rat or the rabbit in prenatal developmental studies. In the two-generation study, no effect was reported on foetus or offspring survival. The initial birth weight of the F1 and F2 offspring was unaffected by treatment but there was a reduction of weight gain at 50000 ppm (approx. 4 279 mg/kg bw/d) that occurred from day 7 of age for the F1 offspring (-6%) and from day 4 of age for the F2 offspring (-11%). No effect was noted on behavioural or developmental landmarks assessed prior to and after weaning. Bodyweights of the pups selected for the F1 generation were not different from controls at the start of the pre-mating period and the effect is considered as transient.

No effect on body weight of the dams was reported during lactation but significant decreases in dam body weight were reported during gestation (-7% in F0 and -9% in F1 dams). Although a direct link between maternal toxicity and effect on offspring weight gain cannot be established, a role cannot be excluded and the data does not provide sufficient evidence that developing animals are more sensitive than adults to Lenacil toxicity at high dose and classification is not justified on this basis. The effect occurred before offspring begin to consume solid food suggesting that the effect may also be related to the presence of Lenacil or its metabolite in the milk at toxic levels or to an effect on lactation. However, no information was available on the possible presence of Lenacil or its metabolite in the milk or the quality or quantity of milk produced. Considering also the high dose triggering the effect on offspring body weight, it is not considered to justify a classification for effect on/via lactation.

It is also noted that effects on relative spleen, thymus and pituitary weights were seen only in animals exposed from the foetal stage and not in F0 and may suggest an increased sensitivity of developing organs. But this effect was seen only at the very high dose of 50000 ppm (approx.

4279 mg/kg bw/d) and was not accompanied by histological effects. Effects on the liver and the thyroid were noted in this study: an increased incidence of dark thyroid was observed from the mid-dose in F1 animals and not in F0 but histopathological findings in the thyroid were observed with a similar incidence and severity in F0 and F1 animals at the two high doses. Overall, these findings are considered consistent with the toxicity of Lenacil identified in the repeated dose toxicity studies. They can be explained by the dose level and the duration of exposure of the different generations and do not indicate an increased sensitivity when animals are exposed from the foetal stage.

Overall, RAC agreed that classification of Lenacil for developmental toxicity or effects on or via lactation was not warranted.

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.

ENVIRONMENTAL HAZARD ASSESSMENT

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 ≥ 2.5%
N; R51/53: 0.25% ≤ C < 2.5%
R52/53: 0.025% ≤ C ≤ 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.

ANNEXES:

Annex 1 Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the dossier submitter; the evaluation performed by RAC is contained in RAC boxes.

Annex 2 Comments received on the CLH report, response to comments provided by the dossier submitter and rapporteurs' comments (excl. confidential information).

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