Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

cymoxanil (ISO); 2-cyano-N-[(ethylamino) carbonyl]-2-(methoxyimino)acetamide

EC Number: 261-043-0
CAS Number: 57966-95-7

CLH-O-0000007044-81-01/F

Adopted

16 September 2021

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CLH-O-0000007044-81-01/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 Regulation (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:

Chemical name: **cymoxanil (ISO); 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide; [1] (2*E*)-2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide; [2]**

EC Number: **261-043-0**

CAS Number: **57966-95-7**

The proposal was submitted by **Lithuania** and received by RAC on **17 August 2020.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Lithuania has 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 **24 August 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **23 October 2020**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Ralf Stahlmann**

Advisor: **Anna Sonnenburg**

Co-Rapporteur, appointed by RAC: **Anja Menard Srpčič**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **16 September 2021** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	616-035-00-5	cymoxanil (ISO); 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide; [1] (2*E*)-2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide; [2]	261-043-0; [1]	57966-95-7; [1] 166900-80-7; [2]	Repr. 2 Acute Tox. 4 STOT RE 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H361fd H302 H373 (blood, thymus) H317 H400 H410	GHS08 GHS07 GHS09 Wng	H361fd H302 H373 (blood, thymus) H317 H410		M = 1 M = 1	
Dossier submitters proposal	616-035-00-5	cymoxanil (ISO); 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide; [1] (2*E*)-2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide; [2]	261-043-0; [1]	57966-95-7; [1] 166900-80-7; [2]	**Retain** Repr. 2 Acute Tox. 4 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1 **Modify** Skin Sens. 1A	**Retain** H361fd H302 H317 H400 H410 **Modify** H373 (blood, thymus, eyes)	**Retain** GHS08 GHS07 GHS09 Wng	**Retain** H361fd H302 H317 H410 **Modify** H373 (blood, thymus, eyes)		**Retain** M = 1 M = 1 **Add** oral: ATE = 356 mg/kg bw	
RAC opinion	616-035-00-5	cymoxanil (ISO); 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide; [1] (2*E*)-2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide; [2]	261-043-0; [1]	57966-95-7; [1] 166900-80-7; [2]	**Retain** Repr. 2 Acute Tox. 4 STOT RE 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	**Retain** H361fd H302 H317 H400 H410 **Modify** H373 (blood system, thymus, eyes)	**Retain** GHS08 GHS07 GHS09 Wng	**Retain** H361fd H302 H317 H410 **Modify** H373 (blood system, thymus, eyes)		**Retain** M = 1 M = 1 **Add** oral: ATE = 360 mg/kg bw	
Resulting Annex VI entry if agreed by COM	616-035-00-5	cymoxanil (ISO); 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide; [1] (2*E*)-2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide; [2]	261-043-0; [1]	57966-95-7; [1] 166900-80-7; [2]	Repr. 2 Acute Tox. 4 STOT RE 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H361fd H302 H373 (blood system, thymus, eyes) H317 H400 H410	GHS08 GHS07 GHS09 Wng	H361fd H302 H373 (blood system, thymus, eyes) H317 H410		oral: ATE = 360 mg/kg bw M = 1 M = 1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Cymoxanil is an agricultural, vinicultural and horticultural fungicide used in plant protection products for the control of late blight (on potatoes and tomatoes) and mildew on grapes.

It was first approved in 2008 and is now reviewed for the renewal of its approval under the AIR(IV) renewal programme.

Following a RAC opinion on Cymoxanil in 2012, it received a harmonised classification as Acute Tox. 4, H302; Skin Sens. 1, H317; STOT RE 2, H373 (blood, thymus); Repr. 2, H361fd; Aquatic Acute 1, H400, M=1; Aquatic Chronic 1, H410, M=1. In this process, all human health hazard classes except respiratory sensitisation and aspiration hazard were reviewed.

Since then, new studies have been submitted by the applicants in the renewal process, relevant for the CLH dossier: three *in vitro* comparative metabolism studies, three acute (oral, dermal and eye irritation) toxicity studies, two phototoxicity assays, eight *in vitro* and *in vivo* genotoxicity studies, one reduced one generation study, and two QSAR studies. The Dossier Submitter nevertheless chose to re-evaluate all hazard classes.

Toxicokinetic data showed a rapid absorption after oral administration and extensive metabolism that didn't qualitatively differ between species. Most of the applied oral doses were excreted via urine (up to 80%) and faeces (up to 30%) within the first 48 hours after administration. No potential for bioaccumulation was shown.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The DS proposed no classification for all physical hazards, based on test results and the results of the screening procedure relevant for each hazard class.

Comments received during consultation

One comment was received from a Member State Competent Authority (MSCA) about typos, an obsolete CAS number and a request to state the purity of the test substance.

Assessment and comparison with the classification criteria

As cymoxanil is a solid, hazard classes for gases and liquids do not apply.

Three tests according to EEC method A.14 showed cymoxanil not to be explosive. However, these are not sufficient for classification according to the CLP Regulation, which requires the use of the relevant UN RTDG test methods. Cymoxanil does not contain structural features related to explosive properties as laid out in table A6.1 of Annex 6 of UN RTDG.

Four tests according to EEC method A.10 were negative for flammability. When negative, these tests are equivalent to UN RTDG N.1 tests.

Cymoxanil does not contain any molecular structures associated with self-reactive properties and no peroxide or acidic moieties. Thus, it does not fulfil criteria for self-reactive substances, organic peroxides, and corrosive to metals.

According to two EEC A.16 and one OECD TG 113 tests, cymoxanil is not a self-heating substance.

Based on long-term handling experience, cymoxanil does not emit flammable gases upon contact and does not react with water.

Five EEC method A.17 tests concluded that cymoxanil was not an oxidising solid. One test according to this method showing oxidising properties was deemed unreliable, since no information were provided regarding composition of the mixtures used. The results from these tests are not sufficient for classification. Cymoxanil contains one methoxyimino group, thus an oxygen atom that is bound to nitrogen (and not exclusively to carbon or hydrogen as is required by the CLP Regulation for substances that are considered not oxidising). However, there are no indications that cymoxanil has oxidising properties.

Thus, RAC agrees in line with its previous opinion (RAC, 2012) and with the assessment of the DS and therefore proposes **no classification** for the physical hazards.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

No cases of acute intoxication with cymoxanil have been reported in workers of three manufacturing plants.

Oral

The DS summarised two acute oral toxicity studies. Both were performed in rats according to OECD TG 401 and following GLP standards. In one, the combined LD_{50} for both sexes was 960 mg/kg bw (760 mg/kg bw for males and 1200 mg/kg bw for females). In the other study, the combined LD_{50} was 485 mg/kg bw (538 mg/kg bw for males and 356 mg/kg bw for females).

Clinical signs comprised lethargy, ataxia, low, hunched, or prostrate posture, loss of righting reflex, incoordination, laboured respiration and decreased respiratory rate, dry red nasal and/or ocular discharge, and isolated incidents of exophthalmos and tremors. Clinical signs resolved within one to seven days after exposure. No gross pathological findings were observed in the first study. In the second study, haemorrhagic lungs and gastric mucosa, dark liver and kidneys were reported in animals that died during the study.

Dermal

The DS summarised two acute dermal toxicity studies performed in rats according to OECD TG 402 (limit test) and under GLP conditions. No mortalities occurred at the dose of 2000 mg/kg bw. No dermal irritation or clinical signs, and no gross pathological abnormalities were observed.

Inhalation

One acute inhalation toxicity in rats was available. It was conducted according to OECD TG 403 (1981) and GLP standards. Concentrations of 3.21, 4.98, and 5.06 mg/L dust containing 98.2% of the pure substance were applied nose only for four hours.

One male rat died in the mid dose group. No other mortalities occurred. Therefore, the LC_{50} for this study was > 5.06 mg/L. One male from the high dose group showed tremors on day 7-15 of the observation period. Clinical signs observed immediately after exposure were abnormal gait,

lethargy, irregular respiration, ocular, nasal, or oral discharges, vocalization, hunched posture, diarrhoea, and stained fur.

Conclusion on classification

Based on two oral acute toxicity studies that yielded LD_{50} values in the range for Cat. 4 (300 < ATE ≤ 2000 mg/kg bw) the DS proposed to classify cymoxanil as Acute Tox. 4 (H302), using the lowest LD_{50} value reported for female rats (356 mg/kg bw) as the ATE.

For acute dermal and inhalation toxicity, based on the fact that no treatment related mortalities were observed above the cut-off values for Category 4 (2000 mg/kg bw for acute dermal toxicity, and 5 mg/L for acute inhalation toxicity), the DS proposed no classification for these two hazard classes.

Comments received during consultation

One MSCA commented on this hazard class and supported the proposed classification as Acute Tox. 4, H302 with an ATE of 356 mg/kg bw.

Assessment and comparison with the classification criteria

Two reliable **acute oral toxicity** studies performed according to the version of OECD TG 401 current at the time both yielded LD_{50} values that were within the boundaries for Cat. 4 categorisation for acute oral toxicity. Therefore, RAC concurs with the DS that classification for cymoxanil as **Acute Tox 4, H302** is warranted. However, RAC proposes to round the ATE value based on the most sensitive species (female rats) to 360 mg/kg bw.

RAC also concurs with the DS that **no classification for acute dermal toxicity** is warranted based on guideline and GLP compliant studies presented in the CLH dossier, in which no mortalities were observed at the cut-off value for classification.

For **acute inhalation toxicity**, one guideline and GLP compliant study using milled pure substance with (guideline compliant) mean mass aerodynamic diameters of 2.6 to 3.1 µm and a 4-hour nose only exposure in rats was presented. One death occurred in the mid dose group but none in the high dose group. Thus, the LC_{50} for this study was above the highest concentration tested (5.06 mg/L) and above the cut-off value for classification (5 mg/L). Therefore, RAC concurs with the DS that **no classification** for this hazard class is warranted.

These recommendations are in line with RAC's previous evaluation (RAC, 2012) of Cymoxanil.

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

Summary of the Dossier Submitter's proposal

In the absence of human data and in the absence of any effects (clinical signs or pathology) considered to constitute significant or severe effects in the acute oral, dermal or inhalation toxicity studies, the DS stated that classification of cymoxanil in Category 1 or 2 for STOT SE is not required. With regard to Category 3, the DS argues that clinical signs following inhalation exposure to cymoxanil were indicative of non-specific, general toxicity.

As there was no evidence of toxic effects on a specific target organ or tissue, and since there were no signs of respiratory tract irritation or narcotic effects, no classification for specific target organ toxicity (single exposure) was proposed by the DS.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

There are two studies on acute oral toxicity, which lead to a classification as Acute Tox. 4, H302, two studies on acute dermal toxicity and one study on acute inhalation toxicity (see Acute Toxicity). No human data on STOT SE are available. No new information regarding STOT SE was received for renewal.

As no specific target organ toxicity was observed in acute toxicity studies with cymoxanil, RAC agrees with the DS that no classification for STOT SE 1 or 2 is warranted.

However, some clinical signs were observed in three acute toxicity studies with oral and inhalation exposure, in one in vivo micronucleus test in mice (1993) within 1 hour after gavage dosing and in one bone marrow cytogenetic assay (1982) with a single oral gavage dose. These may be relevant for STOT SE 3 (narcotic effects) classification: lethargic behaviour, low, depressed or prostrate posture, incoordination, abnormal gait, irregular respiration, tremors, ataxia with signs of decreased respiratory rate, and isolated incidents of loss of righting reflex (oral exposure), and abnormal gait or mobility, low gait, and lethargy (exposure via inhalation). RAC notes that these signs occurred in all dose groups but that in the oral toxicity studies these doses (from 250 mg/kg bw in one study with mortality in 2/10 males, and from 300 mg/kg bw in the second study with mortality in 0/5 males and 2/5 females) were also associated with mortality. Nevertheless, only one male of the mid dose group (4.98 mg/L) died in the inhalation toxicity study (none in the low and high dose groups at 3.21 mg/L or 5.06 mg/L, respectively), and clinical signs were also observed in surviving animals of the two oral toxicity studies. In these animals, effects resolved by day 7 at the latest. In the micronucleus test, transient lethargy and/or abnormal gait was observed also in the mid dose group (225 mg/kg bw/d) along with ruffled fur but no other clinical signs. In this study, although 6/18 females died in the high dose group (450 mg/kg bw/d for males and 350 mg/kg bw/d for females), none of the males died but the males exhibited the same clinical signs as females. In the cytogenetic assay, some animals of the low and mid doses (50 and 100 mg/kg bw/d, respectively) were described as slightly depressed but there was no mortality. This effect in itself is not relevant for classification but adds to the overall picture.

Overall, in light of the proposed ATE of 360 mg/kg bw for acute oral toxicity, and the overall non-specific clinical signs, RAC considers these effects do not warrant classification.

This recommendation is in line with RAC's previous evaluation (RAC, 2012) of Cymoxanil.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

No human data are available on skin corrosion or irritation.

The DS summarised one OECD TG 404 (1981) study conducted according to GLP standards with cymoxanil at a dose of 0.5 g on an area of 6.25 cm2. No signs of erythema or oedema were

observed in any of the three rabbits at any of the observation points. No clinical signs were reported.

Conclusion on classification

Since no signs of toxicity or dermal irritation were observed in a reliable OECD TG 404 study, the DS concluded that no classification was warranted for skin corrosion/irritation.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

One OECD TG 404 study was summarised in the CLH report. Cymoxanil technical grade (purity 97.6%) moistened with water was applied to the clipped backs of three female New Zealand White (NZW) rabbits at a dose of 0.5 g on an area of 6.25 cm2 (equating to a concentration of 0.08 g/cm2) under semi-occlusive dressing for 4 hours. No irritation or corrosion responses were observed at any of the readings post-exposure. Mean scores for erythema and oedema were 0 for all animals.

Thus, RAC concurs with the DS that in accordance with the Guidance on the Application of the CLP Criteria (CLP guidance) **no classification** for skin corrosion/irritation is warranted.

This recommendation is in line with RAC's previous evaluation (RAC, 2012) of Cymoxanil.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

No human data are available on eye irritation or damage.

The DS summarised two OECD TG 405 (1987) studies that were conducted according to GLP standards.

The first study was already evaluated in the first DAR for cymoxanil (2007) and (presumably) used to evaluate the eye irritation hazard in the 2012 RAC opinion on cymoxanil. In this study, slight redness (grade 1) of the eye was observed in all animals one hour after exposure (1 animal was applied 12 mg of the substance and 3 animals were applied 60 mg of the substance). Chemosis (grade 1) was observed in the animal receiving the lower dose and one animal of the high dose group at the one-hour reading. All effects were resolved by the 24-hour reading. Thus, mean values for conjunctival redness, conjunctival chemosis, iritis, and corneal opacity were 0 in this study.

In the second study, three NZW rabbits (one male, two females) were instilled with 46 mg of cymoxanil to the conjunctival sac of one eye. Grade 1 or 2 redness, discharge, chemosis, and iritis was observed in all animals at the one-hour reading. At the 24-hour reading, effects had resolved in all but the male animal which showed grade 1 redness and chemosis. Thus, mean grades for conjunctival redness and conjunctival chemosis were 0.33 for 1/3 animals, and 0 for iritis and corneal opacity in all animals.

Conclusion on classification

Since mean scores for conjunctival redness, conjunctival chemosis, iritis, and corneal opacity were below guidance values in both OECD TG 405 studies presented, the DS concluded that no classification was warranted for eye damage/eye irritation.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Two OECD TG 405 studies were summarised in the CLH report. Based on mean scores of 0 in the first study, RAC in 2012 proposed no classification for eye damage/eye irritation for cymoxanil.

In the second study presented in the CLH report that was submitted by the applicant in the current renewal process for cymoxanil a lower dose of the substance was instilled into one eye of three rabbits. Only one of these animals (the only male) showed slight redness and slight chemosis (both grade 1) at the 24-hour observation yielding a mean score for these eye irritation endpoints of 0.33 for this animal. No such effects were observed in the remaining two rabbits at the 24, 48, and 72-hour readings.

According to CLP guidance, a substance is placed in category 2 for eye irritation when it produces in at least in 2 of 3 tested animals, a positive response of: (a) corneal opacity ≥ 1 and/or (b) iritis ≥ 1, and/or (c) conjunctival redness ≥ 2 and/or (d) conjunctival oedema (chemosis) ≥ 2. None of these criteria were fulfilled for cymoxanil in either study.

Thus, RAC concurs with the DS that in accordance with CLP guidance **no classification** for eye damage/eye irritation is warranted.

This recommendation is in line with RAC's previous evaluation (RAC, 2012) of Cymoxanil.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

No animal or human data are available on respiratory sensitisation for cymoxanil.

Therefore, the DS concluded that **no classification** was warranted for respiratory sensitisation due to lack of data.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Since no data on this endpoint are available, RAC concurs with the DS that **no classification** of cymoxanil for respiratory sensitisation is warranted.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

No human data are available on skin sensitisation.

The DS summarised three OECD TG 406 guinea pig maximisation tests (GPMT, also known as the Magnusson and Kligman test), two of which followed the test guideline as adopted 1981, and one that followed the amended guideline from 1992. All three studies were conducted according to GLP standards and used cymoxanil of purities between 97.6% and 99.4%. In two of the studies using intradermal induction concentrations of 1% in 0.5% carboxymethylcellulose and 3% in 0.9% saline, cymoxanil was deemed non-sensitising, when 25% cymoxanil in paraffin oil or petrolatum, respectively, was employed for the topical challenge. The remaining study with an induction concentration of 1% in Alembicol-D yielded positive results in 90% (challenge with 20% cymoxanil) and 100% (challenge with 40% cymoxanil) of the tested animals.

Conclusion on classification

In the 2012 RAC opinion, cymoxanil was classified as Skin Sens. 1, H317 without sub-categorisation. RAC argued that all three GPMT studies were of similar reliability and no factors could have been identified that may have explained the different results. Therefore, the data were not sufficient for sub-categorisation. In contrast to this, the DS argued that the topical induction concentration in the two negative studies was too low (since it didn't induce mild irritation as required by the test guideline), and the choice of vehicles was not sufficiently justified. Therefore, the DS deemed the third study most reliable and based their classification proposal on the positive result yielded in this study (> 60% animals reacting to an intradermal induction concentration of 1%). Thus, they concluded that **Skin Sens. 1A, H317** was warranted.

The DS further concluded that according to the CLP Guidance, Table 3.7, and the results of this last GPMT, cymoxanil is a strong sensitiser (more than 60% of animals reacting to a 1% intradermal induction concentration). Therefore, the GCL for the strong potency group (0.1%) applies.

Comments received during consultation

Two MSCAs commented during consultation. Both supported Skin Sens. 1A classification. One MSCA supported the DS in their assessment of the reliability of the studies but pointed out that no irritation was observed also in the third study using 40% cymoxanil in Alembicol-D for topical induction. The DS responded that technically the third study also failed to meet the requirements of OECD TG 406.

Assessment and comparison with the classification criteria

Three OECD TG 406 studies were summarised in the CLH report.

Concerning the reliability of results, RAC notes that there are several points to consider:

a) Two of the studies gave negative results with *none* of the ten treated animals reacting, and one gave positive results with *all* of the treated animals reacting. However, this was also the study using the lowest test substance purity.
b) The two studies with negative results used 25% in the respective vehicle as topical induction and challenge concentrations. The study with positive results used 40% cymoxanil for topical induction and 20% or 40% for challenge. Nine or ten of the 10 treated animals in each group reacted to both of the challenge concentrations, respectively, with generally stronger

reactions with the higher concentration. Some of the animals in the high concentration group even showed necrotic patches at the treatment site.

c) None of the topical induction concentrations used in any of the studies induced irritation. However, all three of the studies did indeed follow the guideline in employing a topical treatment with 10% SLS prior to topical induction to create skin irritation as is required by point 19 of the TG (OECD TG 406) for non-irritating test substances.
d) Concerning the vehicle used, the guideline requires a "suitable vehicle" and a justification for its choice. In the study summaries available to RAC no justification for the choice of the respective vehicle was provided for any of the three studies. However, all three chosen vehicles (paraffin oil, petrolatum, and Alembicol-D [triglycerides from coconut oil]) are lipophilic mixtures of several organic compounds. While paraffin oil and petrolatum contain long-chain hydrocarbons, Alembicol-D consists of triglycerides. It seems unlikely that vehicle choice could have influenced the outcome of the studies.
e) Nevertheless, it should be noted that slight irritative effects were reported in control animals after topical induction with the vehicle Alembicol-D. No such findings were reported for the other two vehicles but summaries of the study results were less detailed.
f) The two negative studies included concurrent positive controls, this was not the case for the third (positive) study (sensitivity of the test system was tested periodically using formalin as the test substance).
g) According to the CLP Regulation, substances should be placed in Cat. 1A for skin sensitisation when ≥ 60% of animals are responding at > 0.1% to ≤ 1% intradermal induction dose. While two studies (one negative, one positive) used 1% cymoxanil as intradermal induction concentration, one of the studies used 3% cymoxanil and still yielded negative results.

Overall, RAC considers all of the presented studies to be of similar reliability. Taking into account all of the above and based on the uncertainties surrounding the discrepancy in the results of the three studies, RAC does not consider any amendment to their previous assessment to be warranted. Thus, in contrast to the DS, RAC proposes to retain the existing classification of cymoxanil as **Skin Sens. 1, H317 without sub-categorisation**.

RAC concurs with the DS that no concern regarding a potential extreme sensitisation potency for cymoxanil could be identified and no SCL needs to be set.

These recommendations are in line with RAC's previous evaluation (RAC, 2012) of Cymoxanil.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS highlighted five studies as relevant for classification (two 90 day studies in dogs, two 1-year studies in dogs, one combined chronic toxicity/ carcinogenicity study in rats). The target tissues of orally administered cymoxanil were testes, epididymis, blood, thymus and eyes.

Adverse effects on the testes and epididymis justify classification of cymoxanil as toxic to male fertility (RAC Opinion, 14 September 2012) based on the findings in the repeated dose toxicity studies in rats, mice and dogs. Therefore, further discussion on these effects can be found in the section on reproductive toxicity.

According to the CLP Guidance (2017) a reduction in haemoglobin ≥ 20% is considered as an adverse effect on haematology and should be considered in the classification for STOT RE. Statistically significant reductions in haemoglobin in males (24.4%) at 10.56 mg/kg bw/d and females (22.2%) at 10.51 mg/kg bw/d were reported in a 90-day study in dogs (Anonymous,

1993). A similar but weaker effect was repeated in a second 90-day study in dogs (Anonymous, 1999): a dose-related and statistically significant reduction (13.9%) in haemoglobin in females was reported at 15.5 mg/kg bw/d. In addition, at the highest dose (5.7 mg/kg bw/d) a statistically significant reduction in haemoglobin in males was reported at week 2, 12 and 25 (11.0%, 18.8% and 10.8%, respectively) in a 1-year dog dietary study (Anonymous, 1994).

A dose-dependent increase of thymus atrophy from 9.7 mg/kg bw/d was reported in a 90-day study in male and female dogs (Anonymous, 1999). Male thymus atrophy was repeated at 5.6 mg/kg bw/d in a 1-year dog study (Anonymous, 2003). Some findings indicate that changes in thymus might be a substance specific effect rather than a result of generalised high dose stress response.

Similar effects on eyes were observed in two 1-year dog dietary studies (Anonymous, 1994 and Anonymous, 2003): slight bilateral cataract in one male out of five at a dose of 5.7 mg/kg bw/d and slight bilateral lenticular degeneration were recorded in a single male (out of four) at 5.6 mg/kg bw/d, respectively. In addition, slight unilateral cataract in one female (out of five) was recorded at 3.1 mg/kg bw/d in one 1-year dog study (Anonymous, 1994). Given the small number of dogs, the young age of animals, and similar dose levels in two studies, it is considered that three incidents of eye damage were not incidental and might represent a treatment-related effect. Additionally, from 30.3 mg/kg bw/d and above, both males and females showed statistically significant retinal atrophy in a two-year study in rats (Anonymous, 1994a). It is noteworthy that the 1-year interim sacrifice revealed statistically significant increases in the incidence of retinal atrophy in males from the 32.8 mg/kg bw/d dose group.

The effects on haematology and the thymus atrophy reported in dogs were at doses at the boundary between the guidance values for a classification in STOT RE Cat. 1 and Cat. 2. These effects were not reported in all short-term dog studies available for evaluation and/or in other repeated exposure studies in other species. Therefore, it is considered that a classification of cymoxanil for these effects as STOT RE 2 is more appropriate.

The effects on eyes (bilateral cataract/bilateral lenticular degeneration) reported in two 1-year dog studies are also relevant to a classification of cymoxanil in STOT RE 2. These results on eyes are supported by the combined chronic toxicity/carcinogenicity (including at the 1-year interim sacrifice) study on rats.

Conclusion on classification

The DS stated that the effects reported on blood parameters, thymus and eyes in the dogs following 90-day and 1-year exposure to cymoxanil are relevant for classification for **STOT RE 2, H373 (blood, thymus, eyes)**.

Comments received during consultation

One MSCA commented on this endpoint. They considered classification as STOT RE 1, H372 (blood, thymus) appropriate according to the effects observed in 90-day studies in dog below the cut off values (10 mg/kg bw/d).

- In the first 90-day dog study (1993) at 5.13 mg/kg bw/d in males, effects on haematology were observed: mild anaemia with a decrease of red blood cells (RBC, 15.9%), haemoglobin concentration (Hb, 16.7%) and haematocrit (Ht, 14.9%)
- In the second 90-day dog study (1999) thymus toxicity in males was observed at 9.7 mg/kg bw/d: ↓ absolute (> 55%) and relative (> 45%) thymus weight and histological alterations (lymphoid atrophy) with increasing severity

- In addition, in the second 90-day dog study (1999) in females, effects on haematology as well as liver and thymus toxicity were observed at 9.9 mg/kg bw/d:
    - Haematology effects: mild anaemia with a decrease of RBC (13.2%) and haemoglobin concentration (10.1%)
    - Liver toxicity: ↑ relative liver weight (28.6%) with alterations of clinical chemistry [↑ gamma-glutamyl transferase (GGT, 89%), ↑ total bilirubin (17%)]
    - Thymus toxicity: ↓ absolute (> 56%) and relative (> 50%) thymus weight and histological alterations (lymphoid atrophy)

The DS argued that the effects on haematology and thymus reported in dogs were at doses at the boundary between the guidance values for a classification in STOT RE 1 and 2. These effects were not reported in all short-term dog studies available for evaluation and/or in others repeated exposure studies on other species. Therefore, it is considered that the effects reported are in accordance with a classification of cymoxanil in STOT RE 2, H373.

Assessment and comparison with the classification criteria

To evaluate the specific target organ toxicity of cymoxanil after repeated exposure, 26 studies were assessed for relevance for classification. In the table below, the relevant studies for classification and their results are listed. Findings from other studies are not sufficient for classification and are regarded as supportive data.

***Table**: Summary of animal studies on repeated dose toxicity relevant for classification STOT RE (modified from the CLH report table 26). * p < 0.05, ** p < 0.01, effects relevant for classification are in **bold** text*

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance (Batch No; purity w/w), route of exposure, dose levels, duration of exposure**	**Results relevant for STOT RE classification** **Cat. 1: > 10 mg/kg bw/d** **Cat. 2: between 10 and 100 mg/kg bw/d** (based on the 90d oral toxicity study in rats)	**Reference**
90 days dog oral, dietary OECD TG 409 GLP Beagle dog 4/sex/dose	Cymoxanil DPX-T3217-113, 97.8% 0, 100, 200, 250/500 ppm equal to 0, 3.13, 5.13, 10.56 mg/kg bw/d (males) 0, 3, 5.27, 10.51 mg/kg bw/d (females)	<u>Effects in males</u> **At 5.13 mg/kg bw/d:** -alterations of haematological parameters [↓ RBC (15.9%**), ↓ Hb (16.7%**), ↓ Ht (14.9%)] **At 10.56 mg/kg bw/d:** -↓ body weight (35.1%*), loss overall body weight gain (g), ↓ food consumption during eleven weeks (34.5 – 68.9%*) -**↓ RBC (23.0%**), ↓ Hb (24.4%**), ↓ Ht (23.4%)** <u>Effects in females</u> **At 5.27 mg/kg bw/d:** -↓ RBC (11.5%), ↓ Hb (12.8%), ↓ Ht (9.1%) -loss in overall body weight gain (g) at termination, ↓ food consumption (g/animal/day) during 11 weeks (31.8 – 46.2%**)	Anonymous, 1993

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance (Batch No; purity w/w), route of exposure, dose levels, duration of exposure**	**Results relevant for STOT RE classification** **Cat. 1: > 10 mg/kg bw/d** **Cat. 2: between 10 and 100 mg/kg bw/d** (based on the 90d oral toxicity study in rats)	**Reference**
		At 10.51 mg/kg bw/d: -↓ body weight from fourth week to termination (21.9 - 41.9%), loss of overall body weight gain (g), ↓ food consumption throughout all the dosing period (13 weeks, 40.6 - 74.0%) -↓ **RBC (25.6%*), ↓ Hb (22.2%*),** ↑ MCV (8.7%*)	
90 days dog oral, dietary OECD TG 409 GLP Beagle dog 4/sex/dose	Cymoxanil 498VF973, 98.8% 0, 200, 400, 800 ppm equal to 0, 4.9, 9.7 and 14.2 mg/kg bw/d (males) 0, 5.2, 9.9 and 15.5 mg/kg bw/d (females)	Effects in males **At 9.7 mg/kg bw/d:** -↓ **absolute (> 55%) and relative (> 45%) thymus weight; histological alterations in thymus (2/4 versus 0 in controls) with increasing severity** **At 14.2 mg/kg bw/d:** -clinical signs ('weakness'), ↓ body weight (32.4%), loss body weight gain*, ↓ food consumption up to 60.0%* -↓ **absolute (> 52%) and relative (> 30%) thymus weight; histological alterations in thymus (3/4 versus 0 in controls)** Effects in females **At 5.2 mg/kg bw/d:** -↓ RBC (9.4%*), ↑ MCV (4.7%*) **At 9.9 mg/kg bw/d:** -↓ body weight (11.2%) -↓ RBC (13.2%*), ↓ Hb (10.1%) -↓ **absolute (> 56%*) and relative (> 50%*) thymus weight; histological alterations in thymus (2/4 versus 0 in controls)** **At 15.5 mg/kg bw/d:** -clinical signs ('weakness'), ↓ body weight (30.8%), loss body weight gain*, ↓ food consumption during five weeks (37.8 – 56.1%*) -↓ RBC (14.7%*), ↓ Hb (13.9%*), ↓ Ht (11.1%*), ↑ MCV (3%) -↑ **GGT (111%*), ↑ total bilirubin (33%*)** -↓ **absolute (> 66%*) and relative (> 55%*) thymus weight; histological alterations in thymus (2/4 versus 0 in controls)**	Anonymous, 1999

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance (Batch No; purity w/w), route of exposure, dose levels, duration of exposure**	**Results relevant for STOT RE classification** **Cat. 1: > 10 mg/kg bw/d** **Cat. 2: between 10 and 100 mg/kg bw/d** (based on the 90d oral toxicity study in rats)	**Reference**
1 year dog oral, dietary OECD TG 452 (1981) GLP Beagle dog 5/sex/dose	Cymoxanil DPX-T3217-113, 97.8% males: 0, 50, 100, 200 ppm females: 0, 25, 50, 100 ppm equal to 0, 1.8, 3.0, 5.7 mg/kg bw/d (males) 0, 0.7, 1.6, 3.1 mg/kg bw/d (females)	<u>Effects in males</u> **At 5.7 mg/kg bw/d** (LOAEL): - body weight gain loss ** during the weeks 1 and 19 - ↓ mean food consumption (g/animal/day) (> 33%*) during the weeks 1 - ↑ MCV (4.2%**) at termination, ↓ RBC (18.3%*/10.2%*) at week 12/25, ↓ Hb (11.0%*/18.8%**/10.8%*) at week 2/12/25 - **bilateral cataract (slight, grade 1) in one male versus 0 in controls** <u>Effects in females</u> **At 3.1 mg/kg bw/d:** - **unilateral cataract (slight, grade 1) in one female versus 0 in controls** - stat. sign. body weight loss during week 1 - ↓ mean food consumption (g/animal/day) (> 33%*) during the week 1	Anonymous, 1994
1 year dog oral, dietary OECD TG 452 (1981) GLP Beagle dog 4/sex/dose	Cymoxanil 89800028, 98.8% (first 4 weeks) 19800042, 99.2% (for remainder of study) males: 0, 50, 100, 200 ppm females: 0, 25, 50, 100 ppm equal to 0, 1.3, 2.8, 5.6 mg/kg bw/d (males) 0, 0.8, 1.4, 2.9 mg/kg bw/d (females)	<u>Effects in males</u> **At 1.3 mg/kg bw/d:** - ↓ absolute thymus weight (23.2%) - ↓ relative thymus weight (24.2%) - microscopic thymic lymphoid atrophy (2/4 versus 0 in controls) **At 2.8 mg/kg bw/d:** - ↓ absolute thymus weight (38.5%) - ↓ relative thymus weight (35.4%) - Microscopic thymic lymphoid atrophy (3/4 versus 0 in controls) **At 5.6 mg/kg bw/d:** - ↓ **absolute thymus weight (52.7%*)** - ↓ **relative thymus weight (44.4%)** - **microscopic thymic lymphoid atrophy (3/4 versus 0 in controls)** - **lenticular degeneration in both eyes (1/4 versus 0 in controls)** <u>Effects in females</u> **At 0.8 mg/kg bw/d:** - ↓ absolute thymus weight (16.7%) - ↓ relative thymus weight (6.7%)	Anonymous, 2003

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance (Batch No; purity w/w), route of exposure, dose levels, duration of exposure	Results relevant for STOT RE classification **Cat. 1: > 10 mg/kg bw/d** **Cat. 2: between 10 and 100 mg/kg bw/d** (based on the 90d oral toxicity study in rats)	Reference
		At 1.4 mg/kg bw/d: -↓ absolute thymus weight (23.5%) -↓ relative thymus weight (18.8%) **At 2.9 mg/kg bw/d (NOAEL):** -↓ absolute thymus weight (29.6%) -↓ relative thymus weight (23.8%)	

MCV: mean corpuscular volume

According to the existing RAC opinion from 2012, based on the adverse effects on testes and epididymis, reported in repeated dose toxicity studies, a classification for fertility is more appropriate that a classification for STOT RE. This is in line with the CLP criteria for reproductive toxicity, where it is indicated that a classification for fertility is justified based on the adverse effects on testes and epididymis in repeated dose toxicity studies. For further information and discussion on these effects, please refer to the section on reproductive toxicity.

Blood (haematology)

One 90-day study in dogs (Anonymous, 1993) showed a significant dose dependent reduction (> 10%) in the number of red blood cells (15.9% and 23.0%), haemoglobin (16.7% and 24.4%) and haematocrit (14.9% and 23.4%) in mid and high dose males (5.13 and 10.56 mg/kg bw/d). The findings in males at 5.13 mg/kg bw/d were not related to the presence of general toxicity. Findings in males at 10.56 mg/kg bw/d were observed in the presence of general toxicity. The number of red blood cells (25.6%) and haemoglobin (22.2%) were statistically significantly reduced in females of the high dose group (10.51 mg/kg bw/d). In the same dose group, the mean corpuscular volume (MCV) was increased (8.7%). In females of the mid dose group the number of red blood cells (11.5%), haemoglobin (12.8%) and haematocrit (9.1%) were not statistically significantly reduced. During week 10 of the study one high dose female was euthanized in extremis, and at necropsy changes in blood parameters were present. Macroscopic examination revealed dark red contents and reddened mucosa throughout the gastrointestinal tract. In males at scheduled necropsy, no such changes were reported.

In the **second 90-day study in dogs** (Anonymous, 1999) a statistically significant dose dependent reduction (> 10%) in the number of red blood cells (13.2% and 14.7%) was observed in females of the mid and high dose groups (9.9 and 15.5 mg/kg bw/d). These values were within the range of the historical control data (HCD) submitted (No of females 105; mean 1-SD range: 5.88 – 7.18 x 10^6/µL) but it should be noted that the HCD for haematology parameters provided was of limited relevance due to the method of collection. In males of the high dose group the number of red blood cells (10.4%) was not statistically significantly or dose dependently reduced and the value was within the HCD submitted (No of males 98; mean 1-SD range-L: 5.55 – 6.59 x 10^6/µL). A dose related reduction in haemoglobin (> 10%) was reported for females of the mid and high dose groups (10.1% and 13.9% at 9.9 and 15.5 mg/kg bw/d, respectively), which reached statistical significance at the high dose. The latter Hb value (136 g/L) was lower than the HCD range (No of females 105; mean 1-SD range-L: 137.54 – 160.66 g/L). The MCV was statistically significantly increased in females of the low and mid dose groups (4.7% and 3%, respectively) but not in the high dose group (3%). Regarding haematology, females were more severely affected than males in this study.

In a **one-year study in dogs** (Anonymous, 1994) a statistically significant reduction (> 10%) in the number of red blood cells in males of the high dose group (5.7 mg/kg bw/d) was recorded at week 12 and 25 (18.3% and 10.2%, respectively). However, these finding were not always dose dependent. Additionally, in the high dose group a statistically significantly reduction (> 10%) in haemoglobin was reported in males at week 2, 12 and 25 (11.0%, 18.8% and 10.8%, respectively). This effect was also not dose dependent. At termination MCV was statistically significantly increased (4.2%) in males of the high dose group. This reduction was concomitant with slight reduction (3%) of mean corpuscular haemoglobin concentration when compared to control. No relevant changes in haematology parameters were reported for female dogs in any of the dose groups.

It should be noted that the results of the analytical determination of cymoxanil in the diet of 100 ppm group of animals represent sources of uncertainties, as the concentrations of the substance were 56.1 – 65.4% of expected values for about one third of the study duration.

The **two-year study in rats** (Anonymous, 1994a) revealed a statistically significant increase (5.8%) in MCV at the 3-month and 6-month time points in males of the high dose group (123 mg/kg bw/d). These alterations were not accompanied by changes in other relevant haematological parameters.

In one **carcinogenicity study in mice** (Anonymous, 1994b) a statistically significant increase (6.4% and 10.6%, respectively) in MCV was seen in males of the two highest dose groups (216 and 446 mg/kg bw/d) after 18 months of treatment. A similar statistically significant increase in MCV (6.1%) in males was seen after 3 months of treatment. This increase was accompanied by a statistically significantly decreased number of red blood cells (22.4%). No significant changes in haematology were found in females.

Thymus atrophy

In one **90-day dog study** (Anonymous, 1999) a dose-dependent statistically significantly reduction in absolute (> 56%) and relative (> 50%) thymus weight at ≥ 9.9 mg/kg bw/d dose was observed in females. Additionally, there was histological evidence of lymphoid atrophy in the thymus. In the 9.9 mg/kg bw/d dose group two, out of four females were affected (minimal and moderate), whereas in the 15.5 mg/kg bw/d dose group 4 out of four females showed these effects (two minimal, two moderate). These findings indicate a dose-response relationship. A dose dependent increase in lymphoid atrophy of male thymus with increasing severity was reported for the 9.7 mg/kg bw/d dose group (2/4, minimal and moderate) and for the 14.2 mg/kg bw/d (3/4, 2 moderate and 1 severe). A reduction in absolute and relative thymus weights in males was non-statistically significant and not clearly dose-dependent. No effects on the thymus were reported in the control and low dose groups.

It has to be noted, that the thymus is sensitive to stress and toxic insult. A decrease in thymus weight and/or thymus atrophy cannot be used as a stand-alone parameter for classification. However, a dose-response relationship and changes in other lymphoid tissues might be supportive evidence. It can be difficult to distinguish between substance related effects and changes which are a result of general stress. Substance-related effects are considered to appear in a dose-dependent manner with decreased thymus weight and atrophy starting at non-toxic dose levels. Thymus atrophy as a response to stress is usually limited to high doses.

In this case, there was a clear dose-dependent and statistically significant reduction in relative thymus weight in females (42%, 51.9% and 55.6% for 5.2, 9.9 and 15.5 mg/kg bw/d). However, there was no significant loss of body weight gain as well as lymphoid atrophy in the thymus in females at 5.2 mg/kg bw/d. Changes were found in other lymphoid tissues in females of the high dose group (e.g. bone marrow, lymph nodes) but these tissues were not examined in females of other dose groups. In one emaciated female the following histopathological findings of lymphoid

tissues were noted: lymphoid atrophy in the thymus (moderate), lymphoid atrophy in the mesenteric lymph nodes (mild), atrophy in the bone marrow (severe) and in the sternum marrow (severe). There was no such finding in one emaciated male with lymphoid atrophy in the thymus (severe) at high dose. Neither of the control groups also revealed such histopathological findings in the lymphoid tissues.

In a **one-year dog study** (Anonymous, 2003) a statistically significant decrease in absolute thymus weight was observed in high dose males (5.6 mg/kg bw/d). Additionally, a dose-dependent but not statistically significant decrease in mean absolute and relative thymus weights in males of all three treated groups were recorded (23.2-57.7% and 24.2-44.4% for 1.3, 2.8 and 5.6 mg/kg bw/d). In the macroscopic examination, the thymus size was found to be reduced in two males of the high dose group and in one male of the mid dose group. Microscopic examination revealed thymic lymphoid atrophy – involution in males of all dose groups (1.3, 2.8 and 5.6 mg/kg bw/d) but not in the control group (0/4; 2/4; 3/4; 3/4, respectively). In females the absolute and relative thymus weights of all three treated groups (0.8, 1.4 and 2.9 mg/kg bw/d) were found to be dose-dependently decreased (16.7, 23.5, 29.6% and 6.7, 18.8, 23.8%, respectively). These findings did not reach statistical significance. A microscopic examination revealed that thymic lymphoid atrophy – involution was found in all treated groups of females and also in females of the control group (4/4, 2/4, 2/4 and 3/4 for 0, 0.8, 1.4 and 2.9 mg/kg bw/d, respectively). These microscopic findings are considered to be normal age-associated thymic involution.

The dose-response relationship in absolute and relative thymus weights of males and the microscopic observations indicate that the effects seen in high dose males are not due to stress but might be a substance specific effect.

In a **90-day dog study** (Anonymous, 1993) and a **one-year dog study** (Anonymous, 1994) the thymus weights were not investigated. Macroscopic and histologic examination did not show any effects in thymus.

Eyes

In a **one-year dog study** (Anonymous, 1994), bilateral cataract in one male out of five (slight, grade 1) and unilateral cataract in one female out of five (slight, grade 1) were recorded at high dose level (5.7 mg/kg bw/d and 3.1 mg/kg bw/d, respectively). These findings might be treatment-related, since they are uncommon in young dogs.

Another **one-year dog study** (Anonymous, 2003) showed lenticular degeneration of a slight degree in both eyes of one out of four high dose males (5.6 mg/kg bw/d). This finding might be treatment-related, since this effect occurred in untreated beagle dogs at a very low incidence. No information was included on whether the retina was included in histopathological examinations of the eyes.

In a **two-year rat study** (Anonymous, 1994a) the histological evaluation revealed retinal atrophy incidence in males (10/45, 18/46, 19/46, 35/46 and 52/54 at 0, 1.98, 4.08, 30.3 and 90.1 mg/kg bw/d, respectively) and females (33/55, 34/54, 28/48, 47/52 and 54/55 at 0, 2.71, 5.36, 38.4 and 126.0 mg/kg bw/d, respectively). The incidence and severity of these findings behaved in a dose dependent manner and reached statistical significance at the two highest dose levels (males: 30.3 and 90.1 mg/kg bw/d; females: 38.4 and 126.0 mg/kg bw/d). Either bilateral or unilateral atrophy ranged in severity from single discrete foci to complete loss of photoceptor and outer nuclear layers. As this lesion is typical of age-related photoceptor cell atrophy described for albino rats, it was observed in all groups at termination. However, it should be noted, that retinal atrophy was exacerbated by the test compound. At the one-year interim sacrifice significant increase in the incidences of retinal atrophy were observed in males of the two highest dose groups (1/9, 1/10, 1/10, 4/10 and 9/10 at 0, 2.25, 4.58, 32.8 and 97.4 mg/kg bw/d,

respectively) and in females of the highest dose group (3/10, 3/10, 5/10, 3/10 and 9/10 at 0, 3.07, 6.09, 43.5, 134 mg/kg bw/d, respectively). As this lesion is unusual in one year control rats, a treatment related effect cannot be excluded.

One **28-day mice study** (RAR B.6.3.1.2., 1999a) recorded a bilateral cataract in a single male at 624.4 mg/kg bw/d during gross necropsy. As this study was performed as range finding study it is considered supportive only.

Conclusion on classification

No human data on the specific target organ toxicity after repeated exposure are available.

Several animal studies did not record any toxicologically relevant findings for effects of cymoxanil on the liver or kidney. There is also no evidence for neurotoxicity and immunotoxicity.

Target organs for repeated exposure with cymoxanil were testes, epididymis, blood, thymus and eye. In line with the DS and the RAC opinion from 2012 a classification for fertility is justified based on adverse effects on testes and epididymis in the repeated dose toxicity studies in rats, mice and dogs. For further discussion please refer to the reproductive toxicity section.

***Table**: Extrapolation of equivalent effective dose for relevant toxicity studies (modified from CLH report)*

Study reference	Effective dose (mg/kg bw/d) and affected organ	Duration of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Guidance values for oral repeated exposure studies (mg/kg bw/d)
90-day dog study (Anonymous, 1993)	10.51 Blood	90 days	10.51	STOT RE 1: C ≤ 10 STOT RE 2: 10 < C ≤ 100
90-day dog study (Anonymous, 1999)	9.7 Thymus	90 days	9.7	
1-year dog study (Anonymous, 1994)	5.7 Eyes	1 year	22.8	
1-year dog study (Anonymous, 2003)	5.6 Thymus/Eyes	1 year	22.4	

The table above summarises the most relevant animal studies for STOT RE classification. Statistically significant reductions in haemoglobin in males (24.4%) at 10.56 mg/kg bw/d and females (22.2%) at 10.51 mg/kg bw/d were recorded in a 90-day dog study (Anonymous, 1993). Similar but weaker effects were reported in another 90-day dog study (Anonymous, 1999). Such effects were also reported to some extent in rats and mice.

According to the CLP Guidance (2017), a reduction in haemoglobin at ≥ 20% is considered a significant adverse effect in haematology. The increase of the MCV, as the most significant haematological parameter for assessing anaemia, of about 5% might indicate morphological abnormality of erythrocytes and should be considered as a toxicologically significant change, according to WHO Guidance document (WHO, Guidance document for WHO monographers and reviewers, 2015). Thus, haematological changes observed in dogs at or above but not below 10.5 mg/kg bw/d (supported by findings in other species) warrant classification as STOT RE 2.

One study in dogs (2003) showed a dose-dependent increase in male and female thymus atrophy from 9.7 mg/kg bw/d in females. Male thymus atrophy was recorded in a one-year dog study (Anonymous, 2003) at 5.6 mg/kg bw/d. Some findings indicate that changes in the thymus might be a substance related effect and not a high-dose stress response. These effects were borderline

between guidance values for classification as STOT RE 1 and STOT RE 2. Moreover, in the 1-year dog study effects of similar severity were observed at considerably higher doses indicating a shallow dose-response curve. Although thymus effects were also observed at doses below the guidance value for category 1, RAC considers these not sufficiently adverse for classification. In the RAC opinion from 2012, it was considered that the effects reported are in accordance with a classification as STOT RE 2, as the effects were not reported in all available studies. This is in line with the evaluation by the DS. Therefore, RAC supports classification as STOT RE 2 for the target organs blood and thymus.

With respect to eyes as target organ, two one-year dog studies (Anonymous, 1994 and Anonymous, 2003) reported slight bilateral cataract in one out of five males at 5.7 mg/kg bw/d and bilateral lenticular degeneration of a slight degree in one male out of four at 5.6 mg/kg bw/d. Additionally, a slight unilateral cataract was reported in one female out of five at 5.6 mg/kg bw/d in the one-year dog study (Anonymous, 1994). These three incidents of eye damage in dogs of young age are not considered to be incidental and might represent a substance-related effect. These effects are supported by a statistically significant retinal atrophy in males and females in a two-year study in rats (Anonymous, 1994a). It is noted that the 1-year interim sacrifice revealed statistically significant increases in the incidence of retinal atrophy in males at 32.8 mg/kg bw/d. Although effects in dogs and rodents are different, classification is based on target organ toxicity and is not restricted to a specific effect. Therefore, RAC considers eye effects in rodents to be supportive of eyes as the target organs. Effects observed in dogs are in line with classification as STOT RE 2.

In conclusion, RAC supports the dossier submitter's proposal to classify cymoxanil as **STOT RE 2 H373 (blood system, thymus, eye).**

This recommendation is broadly in line with RAC's previous evaluation (RAC, 2012) of Cymoxanil. However, 'blood' has been amended to 'blood system' in line with the nomenclature used in recent RAC opinions and 'eye' has been added as a target organ.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

In vitro

Six Ames tests performed on strains of *Salmonella typhimurium* and *Escherichia coli* with and without metabolic activation were negative. Another assay was considered not acceptable due to lack of sensitivity to detect cross-linking agents, and too low dose levels tested.

Studies on mammalian cell gene mutation *in vitro* (one HPRT tests on Chinese hamster ovaries and one TK locus assay on mouse lymphoma cells) did not show any mutagenic potential caused by cymoxanil. A third assay was considered not acceptable because the highest exposure concentration chosen was too low.

Two of three *in vitro* studies on chromosomal aberrations showed positive results indicating structural chromosomal damage in human lymphocytes and Chinese hamster ovary cells induced by cymoxanil.

One *in vitro* UDS assay using rat hepatocytes indicated that cymoxanil induced unscheduled DNA synthesis. It should be noted that the OECD TG 482 has been deleted on 2nd April 2014 as this test was not considered sensitive enough and this assay is now non-preferred. The results of an *in vivo* UDS assay in rat hepatocytes and spermatocytes did not confirm this finding.

In vivo

Four *in vivo* studies provided (three micronucleus tests in mice and one_bone marrow chromosomal aberration test in rats) did not show any potential of cymoxanil to produce chromosomal damage.

The results of an *in vivo* UDS assay (RAR B.6.4.2.3., 1994) in rat hepatocytes and spermatocytes were negative: no statistically significant increases or increasing dose-related trends in UDS response were observed at any dose of cymoxanil technical at any harvest time.

Conclusion on classification

The DS summarised that, considering available data from Ames and mammalian gene mutation tests, the compound is unlikely to be of gene mutation concern. Based on negative micronucleus tests *in vivo* where evidence of bone marrow exposure was demonstrated, cymoxanil is unlikely to be genotoxic *in vivo*. The DS concluded that **no classification** of cymoxanil for mutagenicity is warranted.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

A number of studies conducted *in vitro* and *in vivo* address the genotoxicity of cymoxanil. Twelve Studies have been previously evaluated in the Cymoxanil DAR (2007) including eight *in vitro* studies (three Ames tests, two Chinese hamster ovary (CHO) cells gene mutation assays, one UDS assay and two chromosome aberration tests) and four *in vivo* studies (one chromosome aberration test in rat bone marrow, two micronucleus tests and one UDS assay). In addition to these studies, a wide range of new OECD compliant *in vitro* and *in vivo* studies are available (*in vitro*: five Ames tests, one mouse lymphoma gene mutation test, one Chinese hamster ovary cells chromosome aberrations assay; *in vivo*: one micronucleus test).

In vitro

All available studies on genotoxicity/ germ cell mutagenicity *in* vitro are listed in the table below.

***Table**: Summary table of genotoxicity/ germ cell mutagenicity tests in vitro (modified from CLH Report table 29)*

Method, guideline, deviations if any	**Test substance (Batch No; purity)**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations /Results**	**Reference**
Bacterial Reverse Mutation Test (Ames test)				
OECD TG 471 and 472 (1983) GLP Acceptable	Cymoxanil DPX-T3217-113 97.8%	Organism/ Strain(s): *S. typhimurium* strains TA98, TA100, TA1535, TA1537 Concentrations tested: 0, 31.3, 62.5, 125, 250, 500, 1000 and 2000 µg/plate (-/+ S9) *E. coli* WP2 uvrA Concentrations tested: 0, 313, 625, 1250, 2500 and 5000 µg/plate (-/+ S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity: TA100, TA98 at 2000 µg/plate (-S9); TA1535, TA1537 at ≥ 1000 µg/plate (-S9); TA100, TA1535, TA98 at 2000 µg/plate (+S9); TA1537 at 1000 µg/plate (+S9)	Anonymous, 1994
OECD TG 471 (1983) GLP Not acceptable Lack of sensitivity to detect cross-linking agents & too low dose levels tested	Cymoxanil 0972 98.8%	Organism/ Strain(s): *S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 Concentrations tested: 0, 50, 85, 140, 235 and 400 µg/plate (-/+S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity (preliminary dose range finding): TA100 at ≥ 800 µg/plate (+/-S9)	Anonymous, 1997
OECD TG 471 (1997) GLP Acceptable	Cymoxanil DPX-T3217-266/ LS1207012 99.7%	Organism/ Strain(s): *S. typhimurium* strains TA98, TA100, TA1535, TA1537 *E. coli* WP2 uvrA Concentrations tested (range): I and II Experiment: 333 - 5000 µg/plate (-/+S9) II Experiment: TA98 - 66.7, 100, 333, 667, 1000 µg/plate (-S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity (toxicity-mutation test): TA98, TA1535, TA1537 (-/+ S9), TA100 (+ S9) at ≥ 3333 µg/plate	Anonymous, 2013
OECD TG 471 (1997) GLP Acceptable	Cymoxanil 080906 100.85%	Organism/ Strain(s): *S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537 Concentrations tested (range): 12 - 1250 µg/plate (-/+ S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity (preliminary dose range finding): TA100 (-/+S9) at 2500 µg/plate	Anonymous, 2008a

Method, guideline, deviations if any	Test substance (Batch No; purity)	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
OECD TG 471 (1997) GLP Acceptable	Cymoxanil 143/092013 98.3%	Organism/ Strain(s): *S. typhimurium* strains TA98, TA100, TA1535, TA1537 *E. coli* WP2 uvrA Concentrations tested (range): 5 - 5000 µg/plate (-/+ S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity: all *S. typhimurium* strains Plate Incorporation Method: at 5000 µg/plate (-/+ S9) Pre-Incubation Method: at 1581 and 5000 µg/plate (-/+ S9)	Anonymous, 2015
OECD TG 471 (1997) GLP Acceptable	Cymoxanil Batch No not available 99% (CoA not available)	Organism/ Strain(s): *S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537 Concentrations tested (range): 5 - 5000 µg/plate (-/+ S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity (preliminary toxicity test): TA100 at ≥ 1500 µg/plate (-/+ S9)	Anonymous, 2006
OECD TG 471 (1997) GLP Acceptable	Cymoxanil U028/09-B 99.09%	Organism/ Strain(s): *S. typhimurium* strains TA98, TA100, TA1535, TA1537 *E. coli* WP2 uvrA (pKM101) Concentrations tested (range): 5 - 5000 µg/plate (-/+ S9) Dissolved in DMSO	Negative (+/- S9 mix) Cytotoxicity: all *S. typhimurium* strains at 5000 µg/plate (-/+ S9)	Anonymous, 2009
Mammalian Cell Gene Mutation Test				
OECD TG 476 (1984) GLP Supportive only The low number of cells plated for mutagenicity leading to a low number of spontaneous mutant frequency	Cymoxanil DPX-T3217-113 (Blend of lots 80317145 and 80321154) 97.8% (CoA not available)	HPRT-locus in Chinese hamster ovary (CHO K1-BH4) cells 0.005, 0.01, 0.05, 0.1, 0.25, 0.50, 0.75, 1.25 and 1.5 mg/mL Dissolved in DMSO	Negative (+/- S9 mix)	Anonymous, 1993

Method, guideline, deviations if any	Test substance (Batch No; purity)	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
OECD TG 476 (1984) GLP Not acceptable The highest exposure concentration chosen was too low	Cymoxanil 0972 98.6%	HPRT-locus in Chinese hamster ovary (CHO K1) cells 0, 100, 160, 250 and 400 µg/mL dissolved in DMSO	Negative (+/- S9 mix)	Anonymous, 1998
OECD TG 476 (1997) GLP Acceptable	Cymoxanil 2008112201 98.4%	TK-locus in Mouse lymphoma cells L5178Y 62.5- 1000 µg/mL (-/+S9) 4h exposure; 12.5- 125 µg/mL (-S9) 24 h exposure, 200 – 1000 µg/mL (+S9) 4 h exposure Dissolved in DMSO	Negative (+/- S9 mix)	Anonymous, 2009
Mammalian Chromosome Aberrations Test				
OECD TG 473 (1983) GLP Supportive only Too low number of total cells analysed per dose and no long term treatment (-S9) available	Cymoxanil DPX-T3217-113 (Blend of lots 80317145 and 80321154) 97.8% (CoA not available)	Human lymphocytes 0, 0.1, 0.5, 0.75, 0.85, 1.0, 1.25, 1.5 mg/mL Dissolved in DMSO	**Clastogenic** (+/-S9 mix) 3-4 h exposure	Anonymous, 1993
US EPA OPPTS 870.5375 (1998), equivalent to OECD TG 473 (1997) GLP Acceptable	Cymoxanil 498VF973 98.8%	Chinese hamster ovary (CHO K1) cells 0, 16, 19, 36, 38, 76 and 81 µg/mL Dissolved in DMSO	Negative (+/- S9 mix) 3 and 19-21 hours exposure	Anonymous, 2000
OECD TG 473 (1997) GLP Acceptable	Cymoxanil PUS-056200-07/05 > 97.5% (CoA not available)	Chinese hamster ovary (CHO K1) cells 0, 5 , 12.5, 25 and 50 µg/mL Dissolved in McCoys 5A culture medium	**Clastogenic** (-S9) 20 hours exposure	Anonymous, 2005

Method, guideline, deviations if any	Test substance (Batch No; purity)	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Unscheduled DNA Synthesis Assay				
OECD TG 482 (1986), now obsolete (has been deleted on 2014) GLP Acceptable	Cymoxanil DPX-T3217-113 (Blend of lots 80317145 and 80321154) 97.8%	Primary Sprague-Dawley rat hepatocytes 0 (solvent control), 5, 10, 50, 100, 250, 500 µg/mL Dissolved in DMSO	**Positive:** UDS induced ($p < 0.05$) I trial: at 5, 10, 50,100, 250 and 500 µg/mL II trial: at 5, 10, 100 and 250 µg/mL Cytotoxicity: at ≥ 500 µg/mL (II trial) determined by an elevation of LDH activity	Anonymous, 1993

Six out of seven Ames tests performed on strains of *S. typhimurium* and *E. coli* with and without metabolic activation were negative. One Ames test (Anonymous, 1997) has a lack of sensitivity to detect cross-linking agents and too low dose levels. Therefore, it is considered not acceptable.

Both studies on mammalian germ cell gene mutation in vitro (HPRT test on Chinese hamster ovaries and TK locus assay on mouse lymphoma cells) did not show any mutagenic potential caused by cymoxanil. The HPRT test on Chinese hamster ovaries (Anonymous, 1993) is considered as supportive only due to the low number of cells plated for mutagenicity. A third study (Anonymous, 1998) is considered to be not acceptable because the highest exposure concentration was chosen too low.

The chromosomal aberration test in human lymphocytes and one chromosomal aberration test in Chinese hamster ovary cells showed positive results indicating structural chromosomal damage induced by cymoxanil. In the chromosome aberration test in human lymphocytes (Anonymous, 1993), the number of total cells analysed per dose was too low and no long-term treatment without metabolic activation was available. This study is regarded as supporting evidence only. One additional chromosome aberration test in Chinese hamster ovary cells (Anonymous, 2000) did not find any evidence of clastogenic activity of cymoxanil.

The *in vitro* UDS assay using rat hepatocytes was positive and indicated that cymoxanil induced unscheduled DNA synthesis. It is noted that the OECD TG 482 was not considered sensitive enough and has been deleted on 2nd April 2014. Therefore, it is now not a preferred test.

In vivo

Three studies evaluated cymoxanil for induction of micronuclei to mouse bone marrow polychromatic erythrocytes (PECs). None of the treated groups showed statistically significant increase in the frequency of micronucleated PCEs. Evidence of bone marrow exposure were obtained from different signs, such as reduction in proportion of immature erythrocytes among total erythrocytes and systemic toxicity observed in all bone marrow studies at high doses. Furthermore, the radiolabelled cymoxanil was detected in the bone and systematically in the blood/plasma in toxicokinetic studies. Cymoxanil showed no genotoxic potential in the mammalian erythrocyte micronucleus tests.

The bone marrow chromosomal aberration test in rats also revealed a negative outcome as no increase in the frequency of chromosomal aberration at any of the cymoxanil dosages were observed. Due to a too low number of metaphases analysed for each animal/group, this study is considered supplementary only.

One *in vivo* UDS assay in rat hepatocytes and spermatocytes showed negative results.

Study results are summarized in the table below.

***Table**: Summary table of genotoxicity/mutagenicity tests in mammalian somatic or germ cells in vivo (modified from CLH report table 30)*

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study (as applicable)**	**Observations/Results**	**Reference**
Micronucleus Test				
OECD TG 474 (1983) GLP Acceptable	Cymoxanil DPX-T3217-113 (Blend of lots 80317145 and 80321154) 97.8% (CoA not available)	Crl:CD-1 mice 0, 125, 225, 350/450 mg/kg bw suspended in sterile water Single oral exposure Time points of bone marrow samples: 24, 48 or 72h	Negative Cytotoxicity: depression of PCEs/1000 erythrocytes at 48 h at 350 mg/kg (♀); PCE/NCE ratio not affected	Anonymous, 1993
OECD TG 474 (1997) GLP Acceptable	Cymoxanil 0972 98.6%	Albino Swiss mice, HsdOla: MF1 strain 0, 50, 250, 500 mg/kg bw suspended in 0.5% aqueous carboxymethyl cellulose Twice oral exposure (24 hour intervals) Time point of bone marrow samples: 24h	Negative Cytotoxicity: reduced PCE/RBC ratio at 50, 250, 500 mg/kg bw (except the low dose, ♀)	Anonymous, 1999
OECD TG 474 (1997) GLP Acceptable	Cymoxanil 080906 100.85%	Albino Swiss mice 0, 125, 250, 500 mg/kg bw suspended in corn oil Twice oral exposure (24 hour intervals) Time point of bone marrow samples: 24h	Negative Cytotoxicity: P/E ratio not affected	Anonymous, 2008b

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Bone Marrow Cytogenetic Test – Chromosomal Analysis				
Not stated Original guideline OECD TG 475 only adopted in 1984. The study complies with it. Quality assurance statement Supportive only Too low number of metaphases analysed for each animal and/or group	Cymoxanil Batch No not available 98% assumed (CoA not available)	Sprague-Dawley rats (bone marrow cells) 0, 50, 100, 500 mg/kg bw suspended in corn oil Single oral exposure Time points of bone marrow samples: 6, 12, 24 or 48h after dosing	Negative No cytotoxicity: no changes in the mitotic indices	Anonymous, 1982
Unscheduled DNA synthesis test				
Draft OECD GLP 486 (1997) and reference Butterworth *et al*., 1987 GLP Supportive only Dose selection criteria are not fulfilled	Cymoxanil DPX-T3217-113 (Blend of lots 80317145 and 80321154) 97.8%	Sprague-Dawley rats (hepatocytes and spermatocytes) 0, 500, 1000 mg/kg bw suspended in 0.5% methyl cellulose Single oral exposure Time points of samples: 2 and 16h after dosing	Negative No cytotoxicity: hepatocytes /spermatocytes viability > 90%	Anonymous, 1994

Conclusion on classification

There are no human epidemiological data available for cymoxanil. The animal studies did not show any indication that cymoxanil could induce heritable mutations in the germ cells of humans. The criteria for classification for mutagenicity were not met. Therefore, RAC supports the DS proposal that **no classification** for mutagenicity is warranted.

This recommendation is in line with RAC's previous evaluation (RAC, 2012) of Cymoxanil.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The carcinogenicity of cymoxanil has been investigated in rats and mice (two studies each).

Cymoxanil did not reveal any oncogenic potential in any of the four studies, up to and including the highest dose levels tested. The DS proposed **no classification** of cymoxanil for carcinogenic effects.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

The conclusion of the RAC Opinion for cymoxanil (2012) was that "*no oncogenic effects were observed in studies conducted with cymoxanil, either in rat or in mouse carcinogenicity studies*". No new information has been submitted for the renewal of approval of cymoxanil regarding carcinogenicity since then with the exception of a QSAR evaluation of the metabolite of cymoxanil, which has yielded no structural alerts for carcinogenicity. Since the 2012 opinion was very condensed, the studies are presented here in a somewhat more comprehensive form.

The long-term toxicity and carcinogenicity have been investigated in rats and mice (two studies each): 2-year combined chronic toxicity/carcinogenicity study in rats (RAR B.6.5.1.1., 1994a and RAR B.6.5.1.2., 2003); carcinogenicity study in mice (RAR B.6.5.2.1., 1994b and RAR B.6.5.2.2., 2002).

In the **first two-year study in rats** (RAR B.6.5.1.1., 1994a) the survival rate was decreased in all groups, including the control group without any dose dependency (table below). Therefore, this effect is considered not treatment related.

***Table**: Overall survival (%) after 23 months of exposure to cymoxanil*

	Sex	**Concentration in diet (ppm)**				
		0	**50**	**100**	**700**	**2000**
% Survival	Males	26	29	24	45	34
	Females	21	34	34	27	40

Relevant histopathological findings of this study are summarized in the table below.

***Table**: Incidence of relevant histopathological findings after 23 months treatment with cymoxanil. * p < 0.05 Cochran-Armitage trend test or Fisher's exact test*

Organ/tissue	**Concentration in diet (ppm)**									
	Males					**Females**				
	0	**50**	**100**	**700**	**2000**	**0**	**50**	**100**	**700**	**2000**
Lung										
haemorrhage	0/63	1/62	2/62	0/56	**6/61***	3/62	15/62	19/62	18/62	10/62
histiocytosis	14/63	16/62	19/62	15/56	19/61	15/62	2	2	24/62	**39/62***
inflammation (alveolar)	3	2	2	6/56	7/61	2	20/62	27/62	4/62	
inflammation (granulomatous)	4/63	3/62	3/62	4/56	**11/61***	7/62	2	2		**16/62***
fibrosis/inflammation	6/63	3/62	3/62	1/56		1/62	5/62	7/62	6/62	
polyarteritis	4/63	3/62	1/62		3/61	0/62	6/62	9/62	1/62	**15/62***
metaplasia (alveolar walls)	-	-	-	-	-	1/62	0/62	0/62	**2/62***	
type II cell hyperplasia	-	-	-	-	-	0/62	0/62	0/62	0/62	**5/62***
	-	-	-	-	-	0/62	0/62	3/62	3/62	**7/62***
							2/62	3/62		**4/62***
										9/62*

Testes elongate spermatid degeneration multinucleated spermatids	7/63 1/63	5/62 5/62	4/62 1/62	**17/56** * 3/56	**29/62** * **8/62***	-	-	-	-	-
Retina atrophy	10/45	18/46	19/46	**35/46** *	**52/54** *	33/55	34/54	28/48	**47/52** *	**54/55** *
Liver inflammation, (portal) inflammation/ necrosis/fibrosis/ haemorrhage	4/63 11/63	0/62 7/62	1/62 11/62	2/56 9/56	4/62 14/62	0/62 9/62	0/62 3/62	1/62 7/62	0/61 **14/61** *	4/62 **15/62** *
Pancreas focal basophilic alteration inflammation inflammation/ fibrosis/pigment polyarteritis	2/62 - 10/62 7/62	1/51 - 10/51 3/51	2/50 - 12/50 2/50	0/56 - 8/56 2/56	4/62 - 10/62 11/62	3/61 0/61 12/61 2/61	4/62 0/62 7/62 0/62	6/62 0/62 9/62 2/62	**13/61** * 1/61 16/61 0/61	**7/62*** * **8/62*** **25/62** * **11/62** *
Sciatic nerve axon/myelin degeneration	17/63	7/50	10/48	10/32	20/62	10/61	9/62	14/62	**22/61** *	**28/61** *

There was no significant increase in the incidence in specific neoplasms, nor in the number of rats bearing neoplasms, in either sex. Therefore, the NOAEL for carcinogenicity was ≥ 2000 ppm (90.1 and 126 mg/kg bw/d for males and females, respectively), the highest dose tested.

In the **second two-year study in rats** (RAR B.6.5.1.2., 2003) no dose related trends in mortality for male or female rats were observed. The survival rate was above 60% in all dose-groups (table below).

***Table**: Overall survival (%) after 24 months of exposure to cymoxanil*

	Sex	**Concentration in diet (ppm)**			
		0	**100**	**500**	**12000**
% Survival	Males	82	64	70	60
	Females	76	86	78	72

Concerning the number of rats with benign and/or malignant neoplasms and rats with metastatic/infiltrative neoplasms, the only statistically significant increase was observed for malignant neoplasms in males of the mid dose group found dead or moribund sacrificed. However, this finding was not considered relevant since the incidence in the high dose group males was of no statistical significance and no dose-relationship is evident. No HCD were available.

The incidences of specific neoplastic findings in liver and uterus of females are presented in the table below.

***Table**: Incidences of neoplastic findings in liver and uterus of female rats in the 2003 rat carcinogenicity study. B – Benign, M – Malignant, MM - Metastatic*

	Dead and moribund				**Terminal sacrifice**				**Combined fates**			
	C	**L**	**M**	**H**	**C**	**L**	**M**	**H**	**C**	**L**	**M**	**H**
No. of rats examined	12	7	11	15	38	43	39	35	50	50	50	50
Liver												
Adenocarcinoma-metastatic (MM)	1	1	2	5	-	-	-	-	1	1	2	5
Hepatocellular carcinoma (M)	0	0	0	0	0	0	0	1	0	0	0	1
Hepatocellular adenoma	-	-	-	-	0	1	0	1	0	1	0	1
No. of rats examined	12	7	11	15	38	17	15	35	50	24	26	50
Uterus												
Adenocarcinoma (M)	4	2	7	10	6	5	5	2	10	7	12	12
Adenoma (B)	0	0	0	1	1	6	1	3	1	6	1	4
Polyp(s) (B)	3	0	0	0	7	4	9	8	10	4	9	8
Leiomyosarcoma (M)	-	-	-	-	0	0	1	0	0	0	1	0
Squamous cell carcinoma (M)	-	-	-	-	1	1	1	1	1	1	1	1

In high dose dead and moribund females, a higher incidence of metastatic adenocarcinoma of liver and stomach was observed. It is noteworthy, that these were neither primary liver tumours nor primary stomach tumours. In dead and moribund females of the mid and high dose, a non-statistically significant increase in the incidence of adenocarcinoma of the uterus was observed. In these animals, the uterine adenocarcinomas were metastatic to several organs including the liver and stomach. A primary liver tumour (hepatocellular carcinoma) was observed in one high dose female.

For combined subgroups (i.e. animals found dead and moribund plus animals sacrificed at study termination), the following incidences of neoplasms appeared to be dose related: liver adenocarcinoma in females, stomach adenocarcinoma in females, uterus adenocarcinoma, and adenoma. However, the difference compared to the control group was not statistically significant for any dose group.

In a weight of evidence approach considering factors such as absence of increased liver weight, absence of preneoplastic changes, lack of histological evidence of liver cell cytotoxicity, no increases in serum liver enzyme levels indicative of liver cell toxicity, lack of statistical significance, and absence of adenocarcinomas in either males within the study or in a second study conducted in another rat strain, it can be concluded that the slight increase of neoplasms in female rats is not test substance related.

Since no oncogenic effects were observed in this study the NOAEL for carcinogenicity was ≥ 1200 ppm (equal to 58.8 and 75.8 mg/kg bw/d for males and females, respectively), the highest dose tested.

The available data from two rat studies do not support a classification of cymoxanil for carcinogenicity.

In the **first carcinogenicity study in mice** (RAR B.6.5.2.1., 1994b), the survival rate was statistically significantly decreased in high dose (582 mg/kg bw/d) females (survival 57%) at the end of the study after 18 months. Male rats, however, did not show increased mortality in any dose group (table below).

***Table**: Overall survival for male and female mice after 18 months of dosing (% survival) * p < 0.051 Cochran-Armitage trend test*

	Sex	Concentration in diet (ppm)				
		0	30	300	1500	3000
% survival	Males	67	70	78	65	73
	Females	69	76	78	74	57*

There was no significant increase in the incidence of the total number of mice bearing neoplasms, or the total number of specific neoplasms over the whole study period in either sex.

Since no oncogenic effects were observed in the study including the highest dose group, the NOAEL for carcinogenicity was ≥ 3000 ppm (equal to 446 mg/kg bw/d), the highest dose tested for males.

In the **second carcinogenicity study in mice** (RAR B.6.5.2.2., 2002) no dose related trends in mortality in males or females were observed (table below).

***Table**: Overall survival for male and female mice after 18 months of dosing (% survival)*

	Sex	Concentration in diet (ppm)				
		0	60	120	600	1200
% survival	Males	64	56	64	76	52
	Females	74	64	76	60	70

Includes mice found dead or found moribund and sacrificed

There was no significant increase in the incidence of the total number of mice bearing neoplasms, or the total number of specific neoplasms over the whole study period in either sex.

Since no oncogenic effects were observed in the study including the highest dose group, the NOAEL for carcinogenicity was ≥ 3000 ppm (equal to 446 and 582 mg/kg bw/d for males and females, respectively), the highest dose tested.

In the **second carcinogenicity study in mice** (RAR B.6.5.2.2., 2002) no dose related trends in mortality in males and females were observed (table below).

***Table**: Overall survival for male and female mice after 18 months of dosing (% survival)*

	Sex	Concentration in diet (ppm)				
		0	60	120	600	1200
% survival	Males	64	56	64	76	52
	Females	74	64	76	60	70

No significant increase in neoplasms could be identified compared to control groups. The number and types of neoplasms noted in mice of all dose groups were similar in both treated and control animals and were within the range of historical controls (see the table below). However, HCD provided did not meet the criteria as set out in Commission Regulation (EU) No 283/2013.

***Table**: Carcinogenic study in mice, neoplastic findings (number of animals affected).*

Parameter	Concentration in diet (ppm)									
	Males					Females				
	0	60	120	600	1200	0	60	120	600	1200
Malignant lymphoma										
Animals found dead or sacrificed moribund	4/18 22%	3/22 14%	5/18 28%	0/12 0%	6/64 25%	2/13 15%	9/19 47%	6/12 50%	6/20 30%	3/15 20%
Animals terminally sacrificed	3/32 9%	9/28 32%	4/32 13%	8/38 21%	4/26 15%	7/37 19%	8/31 26%	11/38 29%	6/30 20%	9/35 26%
Total	7/50 14%	12/50 24%	9/50 18%	8/50 16%	10/50 20%	9/50 18%	17/50 34%	17/50 34%	12/50 24%	12/50 24%
Total mice with any tumour all animals (50 animals per group)										
Total mice with benign tumours	7	4	5	5	6	11	8	9	7	10
Total mice with malignant tumours	8	12	14	10	11	16	19	18	16	15
Total benign and malignant tumours	15	13	18	14	15	21	21	23	22	23

No oncogenic effects were observed in this study. The NOAEL for carcinogenicity was ≥ 1200 ppm (equal to 178.3 and 179.8 mg/kg bw/d for males and females, respectively), the highest dose tested.

Conclusion on classification

Studies performed with cymoxanil in rats and mice did not provide sufficient evidence of carcinogenicity based on overall weight and strength of evidence. However, it should be noted that the maximum tolerated dose was not reached for either sex in the second carcinogenicity study in mice and for females in the second combined chronic toxicity/carcinogenicity study in rats.

No new information has been submitted for the renewal of approval of cymoxanil regarding carcinogenicity. After a second thorough evaluation of the data no change of the harmonised classification of cymoxanil is proposed.

RAC concurs with the DS that **no classification** for cymoxanil for carcinogenicity is warranted.

This recommendation is in line with RAC's previous evaluation (RAC, 2012) of Cymoxanil.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

For the evaluation of reproductive toxicity, two multigenerational studies in rats were reviewed. The DS noted that some endocrine activity parameters required by the updated OECD TG 416 (2001) were not investigated in these studies, which were compliant with OECD TG 416 from 1983. An additional, reduced-size one generation study for range finding in rats that was submitted for renewal was considered as supportive only and had no measurements of endocrine activity parameters either. Furthermore, several repeated dose studies in rats, mice and dogs

contained information on fertility and sexual function and were also evaluated. It should be noted that the majority of these repeated dose studies have limitations due to missing macroscopic/histopathological investigations.

Generation studies

In the **first two-generation study in rats** (RAR B.6.6.1.1., 2001), the F1 generation showed statistically significant reductions of mean number of corpora lutea, mean number of implantations, mean litter size and live born pups as well as a statistically significantly increased post-implantation loss at top dose.

In the **second two-generation study in rats** (RAR B.6.6.1.2., 1993), the mean relative testes weight of F0 parental males was statistically significantly increased at the mid and high doses, whereas the absolute weight of the testes in F1 males was reduced at high dose only. Nevertheless, no histopathological correlates were found. Thus, these effects on testes were not considered adverse.

In the **additional reduced-size one generation study in rats** (RAR B.6.6.1.3., 1998), the parental animals had a reduced food consumption during all phases at the highest dose tested (females: 240.8 mg/kg bw/d; males 226.2 mg/kg bw/d). Furthermore, there was a statistically significant reduction of body weight gain in females (during gestation) at mid and high dose and in males at high dose only. Concerning reproductive toxicity, statistically significant reductions of female fertility index, mean number of corpora lutea, mean number of implantations and mean litter size as well as an increase in pre-implantation loss and post-implantation loss were identified at the high dose. Additionally, 5 males showed bilateral small and flaccid testes at the top dose. The bodyweight of the offspring was statistically significantly reduced from the mid dose (females: 136.1 mg/kg bw/d; males 114.3 mg/kg bw/d). At the top dose, a statistically significantly lower mean live litter size including lower live birth index was reported.

Repeated dose studies

Rats

In the **28-day dietary study in rats** (RAR B.6.3.1.1., 1999a), a statistically significant reduction of testes weight as well as increase of epididymis weight were observed in the animals of the two highest dose levels. The changes in organ weight were considered to be linked to the reduction in body weight and body weight gain, that occurred in these dose levels. However, no histopathology has been performed in this study.

In the **first 90 -day dietary rat study** (RAR B.6.3.2.1. Study 1, 1993), male animals of the top dose had a statistically significantly reduced body weight, body weight gain and overall food conversion efficiency. With respect to fertility and sexual function, there were dose-related increases in mean relative testis weights at the mid and two high dose groups. Histological changes like bilateral elongated spermatids, multinucleated spermatids, cell debris, and hypospermia were also observed.

Neither changes in the weight of testes nor macroscopic/histopathological effects/changes in the testes were observed in the **second 90-day dietary rat study** (RAR B.6.3.2.1. Study 2, 1999b) up to the highest dose tested (174.3 mg/kg bw/d).

In the **first 2-year dietary rat study** (RAR B.6.5.1.1., 1994a), a statistically significant elongated spermatid degeneration and an increase in relative testes weight accompanied by a statistically significant increase of multinucleate spermatids were observed.

In the **second 2-year dietary rat study** (RAR B.6.5.1.2., 2003), the incidence of mild-to-moderate seminiferous tubule atrophy in the testis was statistically significantly increased.

Mice

No effects on testes caused by cymoxanil were evident in a **28-day dietary study** (RAR B.6.3.1.2., 1999a) or a **90-day dietary study** (RAR B.6.3.2.2., 1999b) **in mice** up to a dose of 624.4 mg/kg bw/d. No histology had been performed in the shorter study.

In the **first 18-month dietary mice study** (RAR B.6.5.2.1., 1994b), tubular dilatation, aggregate lymphoid and sperm cysts/cystic dilation of the epididymis were statistically significantly increased in a dose-dependent manner from the mid-dose. A statistically significantly increased unilateral and bilateral oligospermia and sperm granuloma in the epididymis were reported from the second highest dose. The animals of the top dose showed a statistically significant increase in the incidences of small and "soft" testes, tubular atrophy of testes and reduction in the absolute testes weight.

No effects on testes/epididymis caused by cymoxanil were evident in the **second 18-month dietary mice study** (RAR B.6.5.2.2., 2002), up to the highest dose tested (178.3 mg/kg bw/d). However, the weights of the testes were not recorded in this study.

Dogs

In the **first 90-day dog study** (RAR B.6.3.2.3. Study 1, 1993), small testes in one male, aspermatogenesis in the testes of two animals as well as a statistically significant decrease of relative and absolute epididymis weight were observed in the highest dose group (10.56 mg/kg bw/d). However, no histopathological findings were reported in any of the groups tested.

In the **second 90-day dog study** (RAR B.6.3.2.3. Study 2, 1999), the males of mid and high dose showed a statistically significant dose-dependent decrease in absolute testes weight. However, no histopathological changes were observed in these groups.

No effects on testes/epididymis caused by cymoxanil technical were evident in the **first 1-year dog dietary study** (RAR B.6.3.3.1., 1994), up to the highest dose tested (5.7 mg/kg bw /d).

In the **second 1-year dog study** (RAR B.6.3.3.2., 2003), histological changes in the testes (minimal/slight bilateral atrophy) with an apparent trend in the incidence and severity were determined. The epididymis of one animal showed bilateral seminiferous cell debris and unilateral atrophy with aspermia at the top dose. Furthermore, the size of the testes and epididymis were reduced in one male, and a thickened epididymis was reported in another male at this dose.

Conclusion on classification

Based on some adverse effects on testes and epididymis in rats, mice, and dogs that were not consistently reported in the repeated dose toxicity and multi-generational studies, the DS proposed a classification of cymoxanil with **Repr. 2, H361f** according to Regulation (EC) No 1272/2008.

Development

For the renewal, two 2-generation reproduction toxicity studies in rats, one developmental neurotoxicity rat study, two developmental toxicity studies in rats and four developmental toxicity studies in rabbits were re-evaluated. Additionally, one reduced-size one-generation study in rats was reviewed. It must be pointed out, that the developmental toxicity studies were not compliant with the updated OECD TG 414 (2018) due to the following identified deviations: dosing period covered solely the period of major organogenesis, no evaluation of endocrine sensitive end-points, limited information on the HCD, and some groups had fewer than 16 animals with implantation sites at necropsy. Furthermore, the validity of one rabbit developmental toxicity study was limited for the assessment of developmental effects, based on the insufficient number of females with implantations and no maternal toxicity reported at any dose levels tested.

Generation studies

The two 2-generation reproduction toxicity studies in rats, and one reduced-size one-generation study in rats have been described in the fertility section. Developmental effects comprised reduced offspring body weight, viability index, and percentage of live pups born. Additionally, there was a statistically significant increase in clinical observations in one of the studies (e.g. gasping, no milk spot, subcutaneous haemorrhage, and weakness) in all generations at the high dose.

Developmental studies

It should be noted that the HCD provided for the following studies have clear limitations: no detailed information (e.g. identification of species and strain, name of the supplier, and laboratory, dates when the studies were performed), and differences in the covered period (shorter/longer than the requested five-year-period).

Rats

The females of the **first developmental rat study** (RAR B.6.6.2.1., 1993) showed maternal toxicity, evidenced by statistically significant reductions of mean body weight, body weight gain, and food consumption from the mid-dose. In addition, foetotoxicity was evident as increased incidences of skeletal variations (partially ossified skull, partially ossified/unossified sternebra, partially ossified vertebra, wavy ribs, unossified hyoid and partially ossified pelvis) as well as malformations (external, visceral or skeletal) from this dose level. Some malformations as hemi vertebra occurred only in the higher dose groups accompanied by maternal toxicity.

In the **second developmental rat study** (RAR B.6.6.2.2., 1998), maternal toxicity was evident from the reduced mean body weight gain and food consumption at the highest dose tested. With respect to foetotoxicity, statistically significantly increased minor anomalies (dumb-bell shaped thoracic vertebra 6/13) occurred already from the low dose and were above the HCD range. From the mid-dose, incidences of several skeletal variations as delayed ossification (cervical vertebra and supraoccipital), and some related minor anomalies (hypoplasia of sternebra no. 1/2, and rudimentary 14^{th} rib) were statistically significantly increased above the range of the HCD. Further statistically significantly increased incidences of delayed ossification (sternum, phalanges) and minor anomalies (vertebra) were reported at the high dose, and were also above the HCD range.

In the **developmental neurotoxicity study in rats** (RAR B.6.7.1.2., 2001), no developmental neurotoxic effects were evident up to the highest dose level tested (100 mg/kg bw/d). Maternal toxicity was characterised by reduced body weight gain and food consumption at high dose. With respect to the developmental toxicity, the offspring showed statistically significant reductions in viability index, lactation index, number of live pups/litter and live litter size as well as increases in pup mortality and incidences of clinical observations (cold to the touch, not nursing and not nesting) at 100 mg/kg bw/d.

Rabbits

Based on the limited validity in assessing developmental effects, the **first developmental rabbit study** (RAR B.6.6.2.3., 1980) was considered supportive only. No test compound related effects as maternal toxicity, gross pathological changes, changes in pregnancy parameters and foetal parameters were reported at all dose levels tested (highest dose: 16 mg/kg bw/d).

In the **second developmental rabbit study** (RAR B.6.6.2.4., 1981), statistically significantly increased incidences of anorexia and reduced faecal output were observed from 16 mg/kg bw/d. A statistically significantly reduced maternal body weight gain occurred at the high dose only (32 mg/kg bw/d). Concerning developmental toxicity, incidences of skeletal malformations (vertebral and/or rib alterations, including hemi vertebra, absent or fused vertebrae, misaligned vertebral

centra/arches, fused/absent ribs, and various degrees of resulting scoliosis) were not statistically significantly increased at the high dose, but were above the range of the HCD.

In the **third developmental rabbit study** (RAR B.6.6.2.5., 1982), no maternal toxicity was reported up to the highest dose (32 mg/kg bw/d). Regarding foetal findings, visceral malformations (cleft palate and hydrocephaly) were found in two foetuses each of the highest dose group (32 mg/kg bw/d). The incidences of hydrocephaly were not statistically significantly increased, but were above the range of HCD. The foetuses with cleft palate were from dams that showed anorexia. The incidence of this effect was statistically significantly increased and above the HCD.

In the **fourth developmental rabbit study** (RAR B.6.6.2.6., 1999), maternal toxicity was characterised by statistically significantly reduced body weight gain at the top dose (25 mg/kg bw/d) and statistically significantly reduced food consumption from the mid dose (15 mg/kg bw/d). The foetuses showed statistically significantly increased incidences of dilated heart ventricles at the highest dose, which were above the range of HCD. Furthermore, visceral variants (slight renal pelvis dilation), skeletal alterations (incomplete/poor ossification of fore limb - middle phalange: 1/5) and skeletal minor anomalies (accessory floating rib no. 13) were statistically significantly increased at the high dose.

Conclusion on classification

The DS summarised that the available data on developmental toxicity reported in rats and rabbits did not show a clear and consistent pattern regarding developmental toxicity following exposure to cymoxanil. However, effects were reported in five developmental toxicity studies as well as post-implantation loss in a 2-generation study. Thus, the DS proposed classification according to CLP as **Repr. 2, H361d**.

Effects on or via lactation

The DS described some effects on pup body weight and litter survival from the generational studies as well as some pups found without milk spot or milk in the stomach in the developmental neurotoxicity study. In the latter a reduced lactation index was also reported in the high dose group. In none of the four studies clear effects on body weight were reported for F0 and/or F1 females during the lactation period. However, no information on the quality or quantity of milk produced by the dams and no analytical data on potential transfer to rat milk were available. Moreover, ADME studies showed that cymoxanil is rapidly eliminated mainly via the urine, faeces, and bile. Physico-chemical properties indicate a low potential for bioaccumulation and a limited lipophilicity. Therefore, the DS argued that it was not possible to link effects seen in pups to a transfer of cymoxanil to the milk or an effect of cymoxanil on lactation. Thus, they concluded that **no classification for effects on or via lactation** is warranted.

Comments received during consultation

One Member State Competent Authority (MSCA) commented on this hazard class and stated that multiple malformations were seen in development studies:

- In the first rat study (RAR B.6.6.2.1., 1993), an increased incidence in malformations was observed; these findings showed a low incidence but were above the HCD range and showed a dose-response relationship.
- In the second rat study (RAR B.6.6.2.2., 1998), one foetus was found with a cleft palate at 120 mg/kg bw/d. Increased incidences of variants and minor anomalies were shown to be statistically significantly increased and above the HCD.

- In one rabbit study (RAR B.6.6.2.4., 1981), increased incidences of skeletal malformations associated with scoliosis, such as "vertebra and/or rib alterations" and "vertebral and other changes between upper cervical and mid-thoracic regions /or rib alterations" were observed above the HCD range at 32 mg/kg bw/d.
- In a further rabbit study (RAR B.6.6.2.5., 1982) statistically significantly increased incidences (above the range of HCD) of major malformations [(hydrocephaly (2 foetuses), cleft palates (2 foetuses)] occurred at 32 mg/kg bw/d.
- Finally, incidence of dilated heart ventricles in a third study in rabbits (RAR B.6.6.2.6., 1999) increased from 15 mg/kg bw/d, reaching statistical significance only at 25 mg/kg bw/d. However, they acknowledged that even the control value for this malformation was above the range of HCD in this study. In addition, the incidences of visceral and skeletal variants as well as skeletal anomalies were shown to be relevant.

The MSCA concluded that some major malformations were found at low dose levels and above the range of HCD range for both tested species cannot be attributed to maternal toxicity or to a mechanism of action not relevant for humans. Thus, they proposed a classification as Repr. 1B, H360Df.

The DS argued that the effects were inconsistently observed. Furthermore, the DS noted that the HCD submitted for all studies under evaluation should be used with caution due to their low reliability (differences in the covered period, no correct summary data, missing information). In addition, in the third rabbit study (RAR B.6.6.2.6., 1999), the incidence of dilated heart ventricles was statistically significantly increased in the high dose animals only. Moreover, there was high percentage of this finding (15.2%) outside the HCD range in the control group.

Assessment and comparison with the classification criteria

Fertility

No human data on adverse effects of cymoxanil on sexual function and fertility are available.

Studies available for evaluation of this endpoint were already considered in the 2012 RAC opinion on cymoxanil except for a reduced-size one-generation study in rats that was submitted in the renewal process. In their last opinion, RAC concluded that there was some evidence of adverse effects on sexual function and fertility from the two, two-generation studies as well as several repeated dose toxicity studies (reduced number of corpora lutea and mean number of implantation sites in one of the two-generation studies, and effects on testes and epididymis in several but not all repeated dose toxicity studies). Thus, taking into account both positive and negative results, RAC concurred with the DS at that time (Austria) and proposed classification as Repr. 2, H361f.

According to CLP Guidance (section 3.7.2.3.1), regarding effects on fertility, "*in case of contradictions between the standard repeat dose studies and reproductive studies, the result from the latter should be considered more relevant*", and "*appropriate classification will always depend on an integrated assessment of all available data and their interrelationship using a weight of evidence approach*". Therefore, all available data regarding effects on sexual function and fertility were compiled in the table below to assist re-evaluation.

Table: *Effects of cymoxanil on sexual function and fertility reported in generational studies and repeat dose toxicity studies in rats, mice, and dogs, including studies with no effects, and limitations in reporting. Doses are rounded to whole numbers. Reductions in body weights are given as compared to controls unless otherwise stated.*

Study	Effects on male fertility	Effects on female fertility	Limitations/ Remarks
2-generation study (2001) in rats (HsdCpb:WU)	*No effects up to 94 mg/kg bw/d*	Decreased number of corpora lutea, mean number of implantations, mean litter size, percentage of live pups born + increased post-implantation loss in high dose F1 (116 mg/kg bw/d); in these dams body weight (bw) and food consumption (fc) was reduced stat sign. weeks 0-14 premating/ days 0-20 gestation (uncorrected)/ days 1-21 lactation: week 14 bw -8%, fc -9% GD20 bw -12%, fc -8% LD0 bw -12%, fc -26%	Number of corpora lutea and number of implantations were reduced compared to F1 controls but not compared to F0 controls; reproductive organs were not weighed but subjected to gross and microscopic pathology; no examination of sperm parameters, oestrus cyclicity, and sexual maturation; no corrected body weights available
2-generation study (1993) in rats (Crl:CD BR)	*No biologically relevant effect up to 98 mg/kg bw/d*	*No effects up to 103 mg/kg bw/d*	Reproductive organs (other than testes) were not weighed but subjected to gross and microscopic pathology; no examination of sperm parameters, oestrus cyclicity, and sexual maturation
reduced-size one-generation study (1998) in rats (HsdCpb:WU)	Bilateral and small flaccid testes in 5/15 at high dose (226 mg/kg bw/d); in high dose males body weight was reduced compared to controls stat sign. weeks 1-15: -10% week 1 -15% week 3 -11% week 10 -12% week 15	Decreased fertility index (-53%), mean number of corpora lutea, mean number of implantations, mean litter size; increased pre- and post-implantation loss (by 12.9% and 30.5%) in high dose group (241 mg/kg bw/d); in high dose females body weight was reduced stat. sign. weeks 1-3: -11% week 1 -8% week 3 -5% week 10	Only 15 animals/sex/dose; lower mean litter size presumably due to reductions in other parameters; high proportion of post-implantation loss compared to control due to low number of implantations and pregnant females (7/15); stat. sign. reduced body weight gain in high dose females during gestation (by 43%), body weight gain in high dose males reduced by 18% no corrected body weights available
28-d dietary study (1999a) in rats (HsdCpb:WU)	Decreased absolute testis weights (by 15% and 30%); increased relative epididymis weights (by 27% and 43%) in two highest dose groups (260 and 400 mg/kg bw/d);	Decreased absolute weight of ovaries in high dose group (416 mg/kg bw/d); in high dose females body weight was reduced week 1-4 (stat sign week 4): -26% week 1	Weight of prostate and seminal vesicles not recorded; no histopathology; reduction in body weight gains (by 45% and 66%) and mean food

Study	Effects on male fertility	Effects on female fertility	Limitations/ Remarks
	body weight reduced stat. sign, weeks 1-4 in two high doses: -17%/-26% week 1 -28%/-41% week 4	-28% week 4	consumption (by 13% and 20%) in males of the two highest dose groups, and females (bw by 54%, fc by 21%) of high dose group
28-d dietary study (1999a) in mice (Swiss albino)	*No effects up to 303 mg/kg bw/d (mid dose group)*	Decreased absolute weight of ovaries in low and mid dose group (179 and 330 mg/kg bw/d)	Weight of prostate, epididymis, seminal vesicles, and uterus not recorded; no histopathology; no weight gain in second highest dose groups and weight reduction in the highest dose groups of both males and females throughout study period
90-d dietary study (1993) in rats (Crl:CD BR)	increased relative testis weights, dose-related increase in bilateral elongated spermatid degeneration from 48 mg/kg bw/d; cell debris and bilateral hypospermia in epididymis in high dose group (224 mg/kg bw/d); body weights were reduced D97 in two high doses (stat. sign. in highest dose): -5%/-15%	*no toxicologically relevant macroscopic or histopathological changes in ovaries, uterus, cervix, and vagina up to 333 mg/kg bw/d*	weight of prostate, epididymis, and seminal vesicles not recorded; sperm morphology not examined; stat. sign. reduced body weight gain (by 22%) in high dose group males no effect on food consumption
90-d dietary study (1999b) in rats (HsdCpb:WU Wistar)	*No changes in testis weight and no macroscopic or histopathologic changes in testis up to 174 mg/kg bw/d;* small sized and flabby testes with atrophy/calcification of seminiferous tubules in 1/10 males of recovery group; body weight was reduced stat. sign. weeks 1-17 in recovery group: -12% week 1 -12% week 17	*No changes in weight of ovaries and no macroscopic or histopathologic changes in ovaries and uterus up to 188 mg/kg bw/d*	Weight of prostate, epididymis, and seminal vesicles not recorded, and no histopathology of these organs was performed; food consumption stat. sign. reduced in recovery group weeks 1-13 (by 27% week 1, by 8% week 13)
90-d dietary study (1999b) in mice (Swiss albino)	*No changes in testis weight and no macroscopic changes in testis up to 267 mg/kg bw/d;* decreased spermatogenesis in 1/10 males of high dose group°; body weight was reduced weeks 1-13 (stat. sign. week 2) in high dose:	*No changes in weight of ovaries and no macroscopic or histopathologic changes in ovaries and uterus up to 303 mg/kg bw/d*	Weight of prostate, epididymis, and seminal vesicles not recorded and no histopathology of these organs was performed; °the DS reported this as an effect in the recovery group but in table 6.3.2.2-5 provided with the study summary it is attributed to the high dose group of the main study;

Study	Effects on male fertility	Effects on female fertility	Limitations/ Remarks
	-7% week 1 -6% week 2 -7.5% week 13		stat. sign. reduction in body weight gain in high dose males (by 21%) and recovery group males (by 15%) food consumption reduced stat. sign. weeks 1, 3, 4 in high dose, and weeks 1, 2, 8 in recovery males
90-d study (1993) in dogs	Decrease of relative (24.1%) and absolute (48.4%) epididymis weight, decreased relative and absolute testis weight at high dose (11 mg/kg bw/d); small testes with aspermatogenesis and minimal or no spermatid formation in 2/4 males of high dose (11 mg/kg bw/d); in high dose males body weight was reduced weeks 1-13 (stat sign week 13): Week 1: -1% vs ctrl/ -2.8% vs week 0 Week 13: -31.6% vs ctrl/ -20% vs week 0	Increased absolute and relative weight of ovaries in low dose (3 mg/kg bw/d); in low dose females, body weight was reduced weeks 1-13: Week 1: -1% (-6%) Week 13: -4.7% (-12.5%) One ctrl female had particularly high bw and was excluded for comparison in study summary (when using mean for all ctrl females)	No histopathological findings in ovaries and uterus; body weight loss in males of high dose and females of mid and high dose groups over the whole study period; reduced body weight gain in females of low dose group (by 51%); food consumption reduced in high dose males weeks 1-13 (stat. sign. weeks 1-8; no data for weeks 9-12) by 51 to 69%, and in low dose females (stat. sign. weeks 1, 8) by 16 to 28%
90-d dietary study (1999) in dogs	Decreased absolute testes weight in mid and high dose groups (10 and 15 mg/kg bw/d); reduced body weight weeks 2-13 in mid and high dose: Week 2: -7.3%/ -12% vs ctrl -6%/ -9.8% vs week 0 Week 13: -16%/ -31% vs ctrl -12%/ -28% vs week 0	Decreased absolute and relative uterus weight and weight of ovaries in high dose; reduced body weights weeks 1-13 in high dose: Week 1: -6.7% vs ctrl -6.7% vs week 0 Week 13: -30.8% vs ctrl -29% vs week 0	No histopathological correlates; body weight loss in males and females of mid and high dose groups and no weight gain in males and females of low dose groups over the whole study period (however, only slight weight gain in control females: 0.2 kg); reduced food consumption in males and females of all dose groups (stat. sign. for males high dose weeks 1-13, mid dose weeks 1-8, low dose weeks 4, 5, and females high dose weeks 1-4, 8)
1-year study (1994) in dogs	Non-significant decrease in absolute and relative testis weight in high dose (6 mg/kg bw/d); reduced body weights weeks 1-13 in high dose: -8.6% week 1 -15% week 8 -10% week 13	*No histopathological findings in vagina (other organs not included in study summary available to RAC) up to 6 mg/kg bw/d*	No histopathological correlates; weights of ovaries and uterus not recorded; no histopathological examination of seminal vesicles and cervix; stat sign. reduced food consumption in high dose males weeks 1, 2

Study	Effects on male fertility	Effects on female fertility	Limitations/ Remarks
	Increased body weights weeks 26, 39, 52 (other weeks not available): +3% week 26 +9% week 39 +9.6% week 52		
1-year study (2003) in dogs	*No effects on testis and epididymis weights up to 6 mg/kg bw/d;* unilaterally reduced testis and epididymis size in 1/4 and thickened epididymis in another male of high dose group (6 mg/kg bw/d); testis atrophy in 2/4 and 3/4 males of mid (3 mg/kg bw/d) and high dose groups; bilateral seminiferous cell debris in 1/4 and unilateral epididymis atrophy with aspermia in another male of high dose group; reduced body weights weeks 2-53 in mid and high dose: Week 2: -3.3% / -6.6% vs ctrl -2% / -4.8% vs week 0 Week 53: -5.3% / -14.4% vs ctrl -2% / -11% vs week 0	*No toxicologically relevant changes in weights and histopathology of ovaries and uterus up to 3 mg/kg bw/d*	Histopathological examination of epididymis not specified; stat. sign. but not dose-dependently reduced body weight gain in females of all treated groups (by 11-18%), male controls did not gain weight from acclimatisation period to end of study, weight loss in males of mid and high dose groups up to week 39, some weight gain in weeks 39-53 (but animals did not regain their initial weight); reduced food consumption in mid and high dose males weeks 1-6 (stat. sign. for high dose) by 5 to 42%
18-months dietary study (1994b) in mice (Crl:CD-1 BR)[#]	Reduced absolute testis weight, increased incidence of small testis and tubular atrophy in high dose (446 mg/kg bw/d); dose-related increase of tubular dilatation, aggregate lymphoid and sperm cysts/cystic dilation of epididymis from 42 mg/kg bw/d (mid dose); increased incidence unilateral and bilateral oligospermia and sperm granuloma in epididymis from 216 mg/kg bw/d (second highest dose); body weight reduced after 18 months (no other data available; stat. sign. for 2 high doses): -1.2%, -3.4%, -7.4%, -11%	*No changes in weights and histopathology of reproductive organs up to 582 mg/kg bw/d*	Stat. sign. reduction in body weight gain for both males and females of the two highest dose groups (by 15-48%); no effect on mean food consumption;

Study	Effects on male fertility	Effects on female fertility	Limitations/ Remarks
18-months dietary study (2002) in mice (Swiss albino)#	*No effects on testes and epididymis up to 178 mg/kg bw/d*	Increased incidence of follicular cysts in ovaries in 4/50 females of high dose group; significantly decreased body weight in high dose females weeks 2 and 25 (no values were available to RAC)	No histopathology of cervix and vagina
2-year dietary study (1994a) in rats (Sprague Dawley)#	Increased relative testis weight at high dose (90 mg/kg bw/d); elongate spermatid degeneration from second highest dose (30 mg/kg bw/d) at interim sacrifice and termination; increased incidence of multinucleated spermatid in epididymis in high dose (97 mg/kg bw/d) at interim sacrifice; study summary provides body weight data after 23 month only: stat. sign. reduction in 2 high doses: -15% /-28%	*No adverse effects on histopathology of ovaries, uterus, vagina, and mammary glands and no effect on weight of ovaries up to 134 mg/kg bw/d*	Weights of epididymis and ovaries not recorded; histopathological examination of cervix not specified; stat. sign. reduction of body weight gain for males of two highest dose (by 22 and 36%) and females of high dose groups (by 26%); poor survival in all groups including controls (45-21%) at 23 months, study terminated early
2-year dietary study (2003) in rats (HsdCpb:WU)#	Mild to moderate atrophy of seminiferous tubules in 12/50 males of high dose group (59 mg/kg bw/d); increased combined incidence of epididymal oligospermia with aspermia (a low sperm count and the complete lack of semen) at high dose only at terminal sacrifice; stat. sign. reduction in body weight (by 6 and 13%) for mid and high dose males	*No effects on organ weights or histopathology up to 67 mg/kg bw/d*	Organ weights recorded only for 10 animals per group; histopathological examination of testis and epididymis only for controls and high dose; no histopathological examination of vagina; cervix not specified; stat. sign. reduced body weight gain (by 7 and 15%) for males of mid and high dose; no effect on food consumption

neoplastic effects discussed in section on carcinogenicity

Overall, effects on male and female fertility parameters and reproductive organs were observed in several studies in rats, mice, and dogs with oral exposure to cymoxanil. However, these effects were accompanied by reductions in body weights and body weight gain as compared to controls or sometimes body weight loss in the affected groups, occurred at single incidences only, or were without histopathological correlates.

Conclusion on classification

Thus, RAC concurs with the DS that **Repr. 2, H361f** is the most appropriate classification based on a weight of evidence approach evaluating effects on male and female fertility observed in generational and repeat dose toxicity studies presented in the CLH dossier.

Development

No new developmental toxicity studies have been submitted since the first assessment of this endpoint by RAC in 2012. However, the developmental neurotoxicity study was not mentioned in the reproductive toxicity section of the 2012 opinion (but rather in the section on other effects) and its results were seemingly not considered for this endpoint. In their evaluation at that time, RAC concluded the "*the available data on developmental toxicity reported in rats and rabbits did not show a clear and consistent pattern regarding developmental toxicity following exposure to cymoxanil*" but that effects observed in two rat developmental toxicity studies, three out of four rabbit developmental toxicity studies, and one of the two 2-generation studies in rats warranted classification as Repr. 2, H361d (as was proposed by the previous DS Austria).

Effects relevant for classification regarding developmental toxicity are compiled in the table below. It should be noted that none of the studies, except the developmental neurotoxicity study, was compliant with the respective current guidelines (shorter exposure periods).

***Table**: Effects observed in reproductive toxicity studies relevant for classification for developmental toxicity (modified from table 36 of the CLH report).*

Study	Effects on offspring	Effects on maternal animals	Limitations/ Remarks
2-generation study (2001) in rats (HsdCpb:WU) males (F0): 0,10.5, 31.6, 94 mg/kg bw/d females (F0): 0, 14.9, 42.8, 116.3 mg/kg bw/d via diet	F1: decreased survival indices at PND 14 (94.6% vs. 100% in ctrl.) and 21 (92.4% vs. 100% in ctrl) in high dose; F2: increased post-implantation loss (19% vs. 9% in ctrl.), decreased percentage of live pups born (81% vs. 90.1 in ctrl.) and number of pups alive on PND 4 (229 vs. 267 in ctrl.) in high dose	Decreased number of corpora lutea, mean number of implantations, mean litter size in high dose F1; F1: body weight reduced stat sign. weeks 0-14 premating (uncorrected)/ days 0-20 gestation/ days 1-21 lactation: GD20 -12% LD1 -12% LD21 -12%	Number of corpora lutea and number of implantations were reduced compared to F1 controls but not compared to F0 controls; no corrected body weights available; decreased body weight gain in both F0 and F1 high dose dams during lactation (by 78% and 20%, respectively); F0 mid and high dose dams: stat. sign. reduced food consumption during premating (-7% and -9%), during gestation (-9% and -11%), and high dose during lactation (-33%); F1 high dose dams: stat. sign. reduced food consumption during premating (-9%), gestation (-8%), and lactation (-26%)
2-generation study (1993) in rats (Crl:CD BR) males (F0):	F1: decreased viability PND 1-4 (85.3% vs. 100% in ctrl)) and lactation indices (84% vs. 100% in ctrl.), consistently lower body	F0 high dose: body weight reduced stat. sign. d0-70 premating/ days 0-21 gestation (uncorrected)/ days 0-14 lactation	No corrected body weights available; decreased body weight gain in F0 and F1 high dose dams during premating (by 23% and

Study	Effects on offspring	Effects on maternal animals	Limitations/ Remarks
0, 6.5, 32.1, 97.9 mg/kg bw/d females (F0): 0, 6.65, 34.7, 103 mg/kg bw/d via diet	weights during lactation (by 32% on PND 21) in high dose F2A: consistently lower body weights during lactation (by 40% on PND 21) in high dose F2B: consistently lower body weights during lactation (by 37% on PND 21)	PMD70 -11%, GD0 -9%, GD21 -12%, LD0 -9%. LD14 -8% F1 high dose: body weight reduced stat. sign. d7-105 premating/ days 0-21 gestation (uncorrected)/ days 0-21 lactation PMD105 -17%, GD0 -13/16%, GD21 -12/15%, LD0 -15/17%, LD21 -14/10% F1 mid dose: body weights stat. sign reduced days 0-7 premating/ day 0-7 2nd gestation (uncorrected)/ days 0-7 2nd lactation	14%), and in F0 high dose dams during gestation (by 12%); no effects on food consumption for F0 dams; F1 dams: stat. sign. reduced food consumption during premating (-7%) and 1st gestation (-10%); no food consumption data for dams during lactation available
Reduced-size one-generation study (1998) in rats (HsdCpb:WU) males: 0, 57.7, 114.3, 226.2 mg/kg bw/d females: 0, 75.1, 136.1, 240.8 mg/kg bw/d via diet	Increased pre- and post-implantation loss (by 12.9% and 30.5%) in high dose group	Decreased fertility index (-53%), mean number of corpora lutea, mean number of implantations, mean litter size in high dose; Body weights reduced week 1-10 (stat sign. 1-3) in high dose: -11% week 1 -8% week 3 -5% week 10	Only 15 animals/sex/dose; only 7 females pregnant, only 6 delivering live litters in high dose; lower mean litter size presumably due to reductions in other parameters; high proportion of post-implantation loss compared to control due to low number of implantations and pregnant females; no corrected weights available; significantly reduced body weight gain in high dose females during gestation (by 43%); no food consumption data available
Developmental neurotoxicity study (2001) in rats (CD (SD)IGS VA Plus)	Decreased viability index (85.6% vs. 98.7% in ctrl.) and lactation index (95.4% vs. 100% in ctrl.) at high dose; single incidences of pups that were cold to touch	Body weights reduced GD6-21 (stat. sign. from GD7; uncorrected)/ LD1-19 (stat sign. LD2-7) in high dose: GD7 -3.7%	*No neurotoxic effects;* no corrected body weight available; stat. sign. reduced food consumption and body weight gain in high dose dams (by 9% and 15%,

Study	Effects on offspring	Effects on maternal animals	Limitations/ Remarks
0, 5, 50, 100 mg/kg bw/d gavage	(4), not nursing (2), not nesting (2), dehydrated (1), and emaciated (1) in high dose	GD21 -5.7% LD1 -4.3% LD19 -1% LD22 +2%	respectively) during gestation and first days of lactation
Developmental toxicity study (1993) in rats (Crl:CD BR) 0, 10, 25, 75, 150 mg/kg bw/d gavage	*Control:* *partially ossified vertebra [12.8%], partially ossified skull [2.7%], partially ossified sternebra [4%]* 25 mg/kg: incidence of skeletal variations and delayed ossification (partially ossified vertebra [33%] and partially ossified skull [6.7%]) ↑* 75 mg/kg: incidence of skeletal variations and delayed ossification (partially ossified vertebra [44.9%], partially ossified skull [9.7%] and partially ossified sternebra [5.4%]) ↑* skeletal malformations: number of foetuses with hemivertebra (2 from 2 litters) ↑* 150 mg/kg: incidence of skeletal variations and delayed ossification (partially ossified sternebra [21.6%], unossified sternebra [2.1%], wavy ribs and partially ossified pelvis [3.1%]) ↑* skeletal malformations: number of foetuses with hemivertebra and fused ribs (3 from 2 litters) ↑* number of foetuses with exencephalic head (1) ↑*	Initial body weight loss, reduced (uncorrected) body weights (by 5% and 10% on GD21) throughout gestation in two highest dose groups	Dosing: GD 7-16; one litter in the mid dose group (25 mg/kg bw/d) showed various malformations and variations that were not present in other dose groups; foetus with exencephaly was overall small and showed several malformations of different organs; no corrected body weights available; reduced body weight gain GD 1-11 and GD 17-22 (by 9-15%) in two highest dose groups; reduced food consumption in dams GD 7-9 from 25 mg/kg bw/d by 12, 30, 58%; consistently reduced food consumption until GD17 in two highest dose groups compared to controls (but increased over time)
Developmental toxicity study (1998) in rats (Wistar) 0, 30, 60, 120 mg/kg bw/d	30 mg/kg: delayed or incomplete ossification (e.g. sternum, scull) ↑* incidence of minor skeletal anomalies (dumb-	(Uncorrected) body weights reduced GD15-20 in mid and high dose (stat sign. GD 15 high dose), [data for GD0, 6, 15, 20 only]:	Dosing GD 6-15; no corrected body weights available; reduced body weight gain on GD 6-15 in mid and high dose groups (by 25

Study	Effects on offspring	Effects on maternal animals	Limitations/ Remarks
gavage	bell shaped thoracic vertebra 6/13) ↑* 60 mg/kg: delayed or incomplete ossification (e.g. vertebra, supraoccipital) ↑* incidence of minor skeletal anomalies (dumb-bell shaped thoracic vertebra 6/13, hypoplasia of sternum: sternebra no. 1/2 and rudimentary 14th rib) ↑* 120 mg/kg: delayed or incomplete ossification (e.g. sternum, vertebra, phalanges, supraoccipital) ↑* incidence of minor skeletal anomalies (dumb-bell shaped thoracic vertebra 6/13, hypoplasia of sternum: sternebra no. 1/2, rudimentary 14th rib and vertebra) ↑*	GD15 -3.5%/ -7% GD20 -4.3%/ -6.9%	and 50%) and GD 0-20 in high dose group (by 20%); reduced food consumption on GD 6-16 in mid and high dose groups (by 9 and 25%) and GD 0-20 in high dose group (by 13%)
Developmental toxicity study (1980) in rabbits (NZW) 0, 4, 8, 16 mg/kg bw/d gavage	*No adverse foetal findings*	*No maternal toxicity*	Dosing GD 6-18; low number of females with implantations
Developmental toxicity study (1981) in rabbits (NZW) 0, 8, 16, 32 mg/kg bw/d	32 mg/kg: incidence of vertebra and rib alterations [8 in 2 litters] ↑ (including hemivertebra, absent or fused vertebrae, misaligned vertebral centra/arches, fused/absent ribs, and various degrees of resulting scoliosis)	Although body weight gain was increased by 74% compared to controls on GD 19-23, (uncorrected) body weight of dams remained lower throughout gestation (by 6% on GD 29) in high dose group; anorexia and reduced faecal output in 5/15 and 10/15 does of mid and high dose groups	Dosing GD 6-18; only 13-15 pregnant does per group; maternal toxicity findings inconsistent with 1982 study (see below); 6/8 foetuses with vertebra and rib alterations were in one litter (all foetuses of that litter); no corrected body weights available; reduced body weight gain in mid and high dose groups on GD 6-10 (by 11 and 85%) and on GD 6-19 in high dose group (by 39%)

Study	Effects on offspring	Effects on maternal animals	Limitations/ Remarks
Developmental toxicity study (1982) in rabbits (NZW) 0, 1, 4, 8, 32 mg/kg bw/d gavage	32 mg/kg: incidence of vertebra and rib alterations [2 foetuses of 2 litters] ↑* Visceral malformations: hydrocephaly (2/117 [1.7%] foetuses) cleft palate (2/117 [1.7%] foetuses) *	*No maternal toxicity*	Dosing only GD 6-18; maternal toxicity findings inconsistent with 1981 study; no visceral malformations observed in 1981 study at same dose (see above); hydrocephaly occurred also in 1 foetus of the control group; hydrocephaly and cleft palate in high dose occurred in the same 2 foetuses
Developmental toxicity study (1999) in rabbits (NZW) 0, 5, 15, 25 mg/kg bw/d gavage	25 mg/kg: incidence of skeletal variants [incomplete/poor ossification of fore limb (middle phalange: 1/5)] ↑* incidence of visceral variants (slight renal pelvis dilation) [7.8%]↑* incidence of skeletal minor anomaly (accessory floating 13th rib) ↑* Visceral malformations: incidence of dilation of heart ventricles [31%] ↑*	Minimal body weight loss (30 g) and stat. sign. reduced food consumption on GD 6-18 in high dose group (-17%); body weight values (uncorrected) were only available for GD 0, 6, 18, 29, reduced body weight GD18, 29: GD18 -2.8% vs ctrl - 1% vs GD6 GD29 -2% vs ctrl	Dosing GD 6-18; no corrected body weights available; dilation of heart ventricles considered of doubtful biological significance by study authors (likely dependent on phase of heart contraction in which foetus was killed by sodium thiopentone injection); incidence of dilated heart ventricles was considerably above HCD range in all groups including controls (15, 13, 18% in control, low, and mid dose, respectively; HCD: 0-8.6%);

* statistically significant

Conclusion on classification

According to the CLP criteria, a classification of a substance in Category 1B is based on data that provide *clear* evidence of an adverse effect on development in the absence of other toxic effect, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects.

Substances are classified in Category 2 for reproductive toxicity when there is *some* evidence from human or experimental animals of an adverse effect on development, and where the evidence is not sufficiently convincing to place the substance in Category 1.

No human data are available on reproductive toxicity of cymoxanil. There was some indication from animal studies that cymoxanil has the potential to induce adverse effects on the

development of the offspring in rats and rabbits. Although some of the effects in the developmental toxicity studies were outside the range of the provided historical control data, these data did not meet the requirements set out in Commission Regulation (EU) No. 283/2013 in terms of length of the period covered, information on the used strain and/or age of animals, and provided descriptive statistics such as ranges, means and/or standard deviations.

Malformations observed were namely:

- An exencephalic head in one foetus, and hemivertebra and fused ribs in 3 foetuses of 2 litters of the highest dose group were observed in the first rat developmental toxicity study. Dams of the high dose lost weight until GD9 and did not regain weight until GD15. Hemivertebra and fused ribs were also observed in 2 foetuses of 2 litters in the second highest dose.
- Hemivertebra and absent or fused vertebrae were reported in all six foetuses of one litter and two foetuses of another litter in the highest dose group (32 mg/kg bw/d) of the second rabbit developmental toxicity study. In this dose group, maternal toxicity occurred as anorexia and reduced faecal output in 10/15 dams, a reduced body weight gain (-85% GD6-10 and -39% GD6-19 as compared to controls), and reduced body weights throughout gestation as compared to controls (-6% at GD29).
- Hydrocephaly and cleft palate were observed in 2 foetuses of 117 in the highest dose group in the third rabbit developmental toxicity study. One foetus with hydrocephaly was also found in the control group in this study. In contrast to the second rabbit study, in this study no maternal toxicity was reported although the highest dose group was also 32 mg/kg bw/d.
- A statistically significantly increased incidence of dilated heart ventricles was reported in the fourth rabbit developmental toxicity study in the highest dose group (25 mg/kg bw/d) which was not observed in any other study. Dilated heart ventricles were also observed at high incidences in all other dose groups of this study, including controls.

Overall, malformations were not consistently observed throughout the studies (even at similar dose levels in the same species) and occurred at low or single incidences in litters/foetuses affected by several effects. The effects observed were not considered to be related to maternal toxicity (weight loss during gestation or reduced body weight gain).

Therefore, RAC concurs with the DS that classification as **Repr. 2, H361d** is appropriate.

Effects on or via lactation

Some effects on pup survival and body weight as well as incidences of pups without milk in the stomach or without milk spot were noted in the generational and developmental neurotoxicity studies. However, no clear link could be established to cymoxanil transfer to the milk or adverse effects of the substance on lactation performance of the dams. No information is available on the quality and quantity of milk, and physico-chemical properties of the substance do not necessarily indicate a potential to accumulate in the milk. Thus, RAC concurs with the DS that **no classification** for effects on or via lactation is warranted.

These recommendations on sexual function and fertility, development and lactation are in line with RAC's previous evaluation (RAC, 2012) of Cymoxanil.

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

According to CLP guidance "a classification is based on reliable and good quality human evidence or if the substance is a hydrocarbon and has a kinematic viscosity of 20.5 mm^2/s or less, measured at 40 °C". No animal or human data are available on a potential aspiration hazard for cymoxanil and the substance is not a hydrocarbon.

Therefore, the DS concluded that **no classification** was warranted for Aspiration Hazard.

Comments received during consultation

No comments were received.

Assessment and comparison with the classification criteria

Since no data on this endpoint are available and cymoxanil is not a hydrocarbon (thus, does not fulfil classification criteria), RAC concurs with the DS that **no classification** of cymoxanil for Aspiration Hazard is warranted.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Cymoxanil has gone through the following regulatory processes:

(i) an initial risk assessment provided by the Rapporteur Member State Austria (Draft Assessment Report (DAR) published in 2007)

(ii) CLH process

A harmonised classification and labelling for cymoxanil has been adopted by the ECHA Committee for Risk Assessment (RAC) on 14 September 2012 (ECHA/RAC/CLH-O-0000002970-73-01/F). The substance was classified as Aquatic Acute 1, H400, M=1 based on *Anabaena flos-aquae* with E_rC_{50} = 0.254 mg/L and Aquatic Chronic 1, H410, M=1 based on *Oncorhynchus mykiss* with 90d NOEC = 0.044mg/L, non-rapid degradable and low bioaccumulation potential.

(iii) a renewal of the approval of the active substance (Renewal Assessment Report (RAR) prepared according to the Commission Regulation (EC) No 1107/2009 by Lithuania and Finland in February 2020)

(iv) current CLH process

The substance is currently listed in Annex VI of Regulation (EC) No 1272/2008 with a classification for environment hazard as Aquatic Acute 1, H400, M=1 and Aquatic Chronic 1, H410, M=1 based on the data indicated above (see point (ii)). Based on re-evaluation of all relevant old and new acute and chronic aquatic toxicity studies that were provided for the renewal of the approval of the cymoxanil same classification of the substance was proposed by the RMS/DS. The DS proposed to classify the substance as Aquatic Acute 1, H400, M=1 based on 48h EC_{50}

value of 0.8 mg/L for *Daphnia magna* for metabolite IN-KQ960 and Aquatic Chronic 1, H410, M=1 based on lack of rapid degradation and a 90d NOEC value of 0.044 mg/L for the *Oncorhynchus mykiss* for cymoxanil.

Degradation

Hydrolysis of cymoxanil was investigated in sterile buffer solutions at pH 4, 5, 7 and 9 in two independent studies, which gave consistent results. Cymoxanil undergoes extensive hydrolysis strongly depending on the pH of the solution, leading to the formation of numerous metabolites. Cymoxanil is considered stable at a pH of 4, half-life times at pH 5, 7 and 9 were 144, 1.1 and 0.02 days at 25°C and at 20°C half-life times at pH 7 and 9 were determined to be 2.1 and 0.04 days. At pH 5 only minor metabolites were observed, no major metabolites were found. The major metabolites formed under sterile conditions at pH 7 and 9 were: IN-U3204 (60.8% AR), IN-JX915 (11.0% AR), IN-W3595 (41.5% AR), IN-KP533 (57.4% AR), IN-R3273 (10.2% AR), IN-KQ960 (14.1% AR). The metabolites IN-W3595, IN-KQ960, IN-R3273, IN-KP533 were considered stable under the conditions of sterile hydrolysis at both pHs. Metabolite IN-U3204 is highly unstable in aqueous solutions, rapidly degrading into IN-KP533, IN-T4226 and IN-KQ960. IN-T4226 is a further transient hydrolysis metabolite rapidly degrading into IN-KP533. Metabolites IN-KQ960 and IN-KP533 have to be considered stable under the conditions of sterile hydrolysis. Metabolite IN-JX915 rapidly degrades into IN-R3273, which in turn slowly degrades into IN-T4226. The parent cleavage product IN-W3595 is considered rather stable under the conditions of hydrolysis in sterile buffer solutions. Ethyl urea, which is likely to be formed together with IN-W3595, was never quantified in environmental fate studies, since the labelling of the parent (cyanoacetamide position) does not allow to follow the fate of this cleavage product. Nevertheless, ethyl urea has to be considered a major degradation product of the hydrolysis of cymoxanil in sterile buffer solutions at neutral and alkaline pH, too. Hydrolysis half-life of the transient metabolites IN-U3204, IN-JX915 and IN-T4226 at pH 7 and pH 9 were estimated to be 2.5 and 0.5 days, 0.7 and 1.7 days, and 7.2 and 2.0 days, respectively. The metabolites IN-W3595, IN-KQ960, IN-R3273 and IN-KP533 have to be considered rather stable under the conditions of sterile hydrolysis at each pH, their amounts remained almost stable once the hydrolysis process has finished (which occurred by approx. 15 DAT at pH 7 and by 7 DAT at pH 9).

In the Oxon study (Slangen and Willams, 2003) several degradation products, not exceeding 10% of AR individually, remained unidentified.

Another hydrolysis study (Anand, 2007) showed that cymoxanil is stable at pH 5 and very quickly hydrolyses under basic conditions with half-life values of 0.02 days at pH 7 and 1.4 days at pH 9. Under acidic conditions, cymoxanil slowly hydrolyses with half- life value of 148 days at pH 4 at 25°C. At pH 7 and 25°C, degradation of IN-U3204 was observed up to 52.7% of AR after 2 days, IN-W3595 up to 16.2% of AR after 15 days, IN-KP533 up to 57.0% of AR after 30 days. At pH 9 and 25°C, degradation of IN-W3595 was observed up to 39.0% of AR after 3 days, IN-KP533 up to 31.6% of AR up to 10 days, IN-U3204 up to 60.8% of AR after 0.17 days and IN-KQ960 up to 13.5% of AR after 7 days.

The photolytic degradation of cymoxanil in water has been investigated under sterile conditions in acetate buffer solutions at pH 5 for up to 30 days. Cymoxanil degraded rapidly under photolytic conditions at pH 5. Two major degradants were seen at > 10% IN-JX915 and IN-R3273, plus a further two metabolites IN-KP533 and IN-T4226 were seen at 7.9 and 6.7% AR respectively, several additional minor components were also seen.

The DT_{50} for cymoxanil under continuous irradiation was 1.8 days. Under the impact of irradiation, degradation of cymoxanil owing to photolysis is strongly driven by formation of metabolite IN-JX915 (52.6% of AR), which rapidly further degrades to IN-R3273 (35.4% of AR). No other major metabolites were observed. This pathway is clearly the major degradation route of cymoxanil in

acidic solutions exposed to irradiation. The alternative hydrolysis processes (cyclisation to IN-U3204 and cleavage of the parent to form IN-W3595) were almost negligible at the investigated pH value. In the dark control samples almost no degradation of cymoxanil was observed.

Net photolysis half-life time of cymoxanil in sterile buffer solution at pH 5 was calculated to be 1.7 and 3.0 days. The experimental net photolysis of cymoxanil ranged between 4.3 and 12.1 days under environmental conditions (midsummer day, approx. 40 °N). As demonstrated in one additional experiment, conducted in non-sterile pond water at pH 7.0, the impact of irradiation on the overall dissipation of cymoxanil in aquatic ecosystems loses its significance at neutral and alkaline conditions owing to the extensive abiotic hydrolysis of cymoxanil at higher pH values. Quantum yield of cymoxanil was calculated in two studies to range between 0.0052 and 0.00058.

The DT_{50} of IN-JX915, owing to the influence of photolysis and hydrolysis, was calculated to be approx. 6.6 days at the investigated pH of 5.0. However, owing to the highly transient character of IN-JX915 during hydrolysis under neutral and alkaline conditions (hydrolysis DT_{50} < 2 days) it is expected that levels of photolytically formed IN-JX915 will be significantly lower in aquatic systems under environmental conditions (without considering biotic degradation).

Degradation half-life of IN-R3273 at pH 5.0, owing to the influence of photolysis and hydrolysis, was calculated to be 32.7 days, no reliable half-life time could be calculated for IN-R3273. Further minor photolysis products (< 10% of AR) were IN-T4226 and IN-KP533 which derive from the degradation of IN-JX915 and IN-R3273.

There are three ready biodegradation studies available on cymoxanil. In the first study (Luit, 2001) the biodegradation of the cymoxanil was determined with modified Sturm test (OECD TG 301B) over 10 days at 10 mg TOC/L and 21 – 23.5°C. No significant degradation (< 10%) of cymoxanil technical was observed under test conditions. Also the second study (Desmares-Koopmans, 2008) which was performed with modified Sturm test (OECD TG 301B) but over 28 days at 12 mg TOC/L and 21.3 – 22.6°C in darkness indicated no significant degradation (< 20%) of cymoxanil. Third study (Feil, 2009) carried out using Manometric respirometry test (OECD TG 301F) show no significant degradation (23% based on $ThOD_{NO3}$) of cymoxanil over 28 days at 21 – 22°C in darkness. Based on these studies, the DS concluded that cymoxanil is not readily biodegradable.

In an aerobic mineralisation study (Irmer, 2019) after 61 days the mineralisation of cymoxanil to CO_2 was observed in amounts up to 24.1% AR and 29.0% AR for the low (9.4 µg/L) and high (96.0 µg/L) test concentration, respectively. Degradation was very rapid. The DT_{50} values for cymoxanil were 2.52 hours (9.4 µg/L) and 2.42 hours (96.0 µg/L). The DT_{90} values were 10.8 hours (9.4 µg/L) and 99.1 hours (96.0 µg/L). Calculated DT_{50} for cymoxanil in surface water were 0.09-0.15 days in non sterile system. Six major metabolites were detected: IN-KP533 (25.8% AR), IN-W3595 (30.3% AR), IN-U3204 (34.3% AR), IN-R3274 (15.0% AR), IN-KQ960 (21.5% AR) and IN-R3273 (7.3% AR). Two major metabolites (M125 and M103) could not be identified. The metabolite M125 was very transient and its instability did not allow identification. M103 was sufficiently stable but it is very high polarity indicated a very small molecular structure. Due to this, it was not possible to identify M103 but it is assumed that it consists of oxalic acid.

Aerobic water/sediment studies were conducted in five aerobic/water systems (pH of water phase 6.7 to 8.9). Cymoxanil dissipated from the water phase to the sediment very rapidly.

Seven metabolites (> 5% AR) were observed in water phase: IN-W3595, IN-KP533, IN-U3204, IN-KQ960, AS999 (M5), IN-JX915 and IN-T4226. The maximum level of cymoxanil in the sediment phase never reached above 5% in any studies. No metabolite was observed < 5% AR in the sediment phases of all test systems investigated. Degradation of cymoxanil in the whole system was fast with DT_{50} values in a range of 0.056-1.6 days following SFO kinetics with a geometric mean of 0.268. Since transfer of cymoxanil into sediment layer was negligible, dissipation in the water layer is almost consistent to degradation in the entire system. Based on

the entire system, the following metabolites are considered major (> 10% of AR): IN-U3204 (maximum occurrence 25.4% of AR by 1 DAT), IN-W3595 (24.6% of AR by 4 DAT), IN-KQ960 (14.25% of AR by 10 DAT), IN-JX915 (11.5% of AR by 2 DAT), IN-KP533 (21.6% of AR by 8 DAT), IN-T4226 (12.0% of AR by 3 DAT), M5/ASS999 (29.2% of AR by 3 DAT). None of the observed metabolites in the water/sediment studies was persistent. In the Table 66 of the CLH report the whole system DT50s are presented for cymoxanil and its metabolites. Whole system DT50s for major metabolites were between 0.794 and 160 days.

Based on the available data, the DS concluded that cymoxanil is considered as not rapidly degradable for classification purposes.

Bioaccumulation

No experimental data on bioaccumulation of cymoxanil in fish has been performed as the measured octanol-water partition coefficient (log K_{OW}) is < 3 (0.67 - 0.59 at 20 °C). Based on the data presented DS concluded that cymoxanil has a low potential for bioaccumulation as log K_{OW} of cymoxanil is below the cut-off value of 4 given in the CLP Regulation.

Aquatic Toxicity

For cymoxanil, reliable aquatic toxicity data are available for two tropic levels, fish and aquatic invertebrates. In addition to aquatic toxicity studies using cymoxanil, reliable aquatic toxicity studies on fish, invertebrates and algae using different metabolites are presented in the CLP report. The summary of the relevant information on aquatic toxicity for cymoxanil and its metabolites are provided by the DS in Tables 75 - 80 of the CLP report.

Acute toxicity

Three valid acute toxicity studies with three different fish species (*Oncorhynchus mykiss, Lepomis macrochirus* and *Cyprinodon variegatus*) using cymoxanil and seventeen studies with one fish species (*Oncorhynchus mykiss*) using all relevant metabolites for the surface water (IN-W3595, IN-KP533, IN-U3204, IN-R3273, IN-KQ960, IN-T4226, M5 and IN-R3273) with exception IN-JX915 were available. The lowest acute endpoint for cymoxanil for fish is mean measured 96h LC_{50} value of 29 mg/L for *Lepomis macrochirus*. Based on the available data (summarised in the Table 75 of CLH report) DS concluded that metabolites are less toxic to fish than the parent compound with the exception of IN-KQ960 metabolite with mean measured 96h LC_{50} value of 23.923 mg/L for *Oncorhynchus mykiss*. As the lowest acute endpoints for cymoxanil and metabolites are higher than 1 mg/L, cymoxanil or its metabolites are not considered hazardous for fish on acute toxicity.

Three valid acute toxicity studies with three different invertebrate species (*Daphnia magna*, *Mysidopsis bahia* and *Crassostrea virginica*) using cymoxanil and fourteen studies with invertebrate *Daphnia magna* using relevant water metabolites (IN-W3595, IN-U3204, IN-R3273, IN-KQ960, IN-T4226, M5) were provided. The lowest acute endpoint for invertebrates is mean measured 48h EC_{50} value of 27 mg/L for *Daphnia magna* using cymoxanil. Based on the available data (summarised in the Table 76 of CLH report) DS concluded that metabolites are less toxic to daphnia than the parent compound cymoxanil with exception of IN-KQ960 (48h LC_{50} = 0.8 mg/L) which showed higher toxicity to *Daphnia magna* in comparison with cymoxanil. The lowest derived LC_{50} value for cymoxanil is higher than 1 mg/L, however its metabolite IN-KQ960 can be considered acutely toxic to aquatic invertebrates.

In the CLH report, aquatic toxicity studies with algae and aquatic plants are available for cymoxanil but are considered not valid by the DS as the concentrations of cymoxanil could not be sufficiently maintained and also not all validity criteria according the OECD TG 201 guideline were met for some algae studies. The toxicity studies on algae with relevant metabolites (IN-

W3595, IN-U3204, IN-R3274, IN-KQ960, IN-T4226, IN-JX915, M5 and IN-R3273) for the surface water are provided. The lowest acute endpoint for algae is the result from the study using fresh water green algae *Raphidocelis subcapitata* with geometric mean measured 72h E_rC_{50} value of 0.931 mg/L using metabolite IN-JX915.

DS based the classification proposal on studies conducted with cymoxanil and metabolites as metabolite IN-KQ960 showed higher acute toxicity to fish and aquatic invertebrates than the cymoxanil. Furthermore metabolite IN-JX915 shows similar toxicity to algae as metabolite IN-KQ960 to aquatic invertebrates. From the available aquatic toxicity data, invertebrates are the most acutely sensitive trophic group, therefore the acute aquatic classification proposed by the DS was based on *Daphnia magna* toxicity study (48h EC_{50} of 0.8 mg/L) with metabolite IN-KQ960. The lowest acute toxicity value of 0.8 mg/L is lower than classification threshold value of 1 mg/L, therefore the substance should be classified as Aquatic Acute 1, H400 with M-factor of 1 ($0.1 < L(E)C_{50} \leq 1$ mg/L).

Chronic toxicity

Two chronic toxicity studies with two different fish species (*Oncorhynchus mykiss* and *Cyprinodon variegatus*) were available. The lowest chronic endpoint for cymoxanil for fish is mean measured 90d NOEC value of 0.044 mg/L for *Oncorhynchus mykiss.*

Two valid chronic toxicity studies using cymoxanil and three studies using metabolite IN-KQ960 were provided for *Daphnia magna*. The lowest chronic endpoint for invertebrates is 21d EC_{10} value of 0.0619 mg/L based on time-weighted average for *Daphnia magna* using cymoxanil. Based on the available data (summarised in the Table 79 of CLH report) DS concluded that metabolite IN-KQ960 is less toxic to *Daphnia magna* than the cymoxanil.

In the CLH report, aquatic toxicity studies with algae and aquatic plant are available for cymoxanil but are considered not valid by the DS as the concentrations of cymoxanil could not be sufficiently maintained and also not all validity criteria according the OECD TG 201 guideline were met for some algae studies. Toxicity studies using relevant metabolites for the surface water (IN-W3595, IN-U3204, IN-R3274, IN-KQ960, IN-T4226, IN-JX915, M5 and IN-R3273) were provided for algae. The lowest endpoint for algae was derived for metabolite IN-JX915 from the study on *Raphidocelis subcapitata* with geometric mean measured 72h NOEC value of 0.06 mg/L and geometric mean measured 72h E_rC_{10} value of 0.268 mg/L.

DS based the classification proposal on aquatic toxicity studies conducted with cymoxanil although there are chronic toxicity studies available for metabolite IN-KQ960 for daphnia and toxicity studies for all relevant aquatic metabolites for algae. The lowest and the most reliable endpoint values for classification purpose were obtained from the studies with the parent substance cymoxanil. The results of long-term aquatic toxicity studies indicate that fish are the most sensitive taxon therefore chronic aquatic classification proposed by DS was based on the fish *Oncorhynchus mykiss* toxicity study (90d NOEC = 0.044 mg/L). The DS proposed Aquatic Chronic 1, H410 with an M-factor = 1 ($0.01 < NOEC \leq 0.1$ mg/L) along with the understanding that the substance is not rapidly degradable.

Comments received during consultation

Two MSCAs and one National Authority provided comments. Both MSCA agreed with the proposed classification for environmental hazards by DS.

One MSCA pointed out that proposed classification is based only on reliable data for fish and invertebrates, while the not acceptable algae studies (e.g. Hughes 1996) suggested that algae

could be the most sensitive taxon to cymoxanil. Therefore, the new study on algae with cymoxanil, which was not submitted until finalisation of the draft RAR, could be vital for the final classification of the substance and defining study for setting M-factors. MSCA indicated that both M-factors should be updated as soon as the new study results are available. DS confirmed that new algae study has been submitted and agreed with commenting MSCA regarding M-factors.

National Authority asked for clarification regarding the proposed current environmental classification as it is the same as harmonised classification for cymoxanil that was agreed by RAC in 2011. DS clarified that current classification was proposed based on re-evaluation of all relevant aquatic toxicity old and new studies provided for the renewal of the approval of cymoxanil. National Authority agreed that the substance is not rapidly degradable due to the toxicity of the degradation products.

In the view of the National Authority it would be useful to consider this to support the conclusion on the degradation potential of the parent substance and the relevance of new degradant ecotoxicity data. DS explained that cymoxanil is considered as not readily biodegradable based on three ready biodegradation studies. However, cymoxanil degrades very rapidly in water under neutral and alkaline conditions. The degradation is mainly driven by hydrolysis. Due to the rapid conversion of cymoxanil to metabolites and higher toxicity of some metabolites than the parent, toxicity data of metabolites are considered in the classification of the substance.

National Authority also commented that current proposal considers available algal toxicity data for cymoxanil not reliable, however the validity criteria were met in the algal study by Boeri (1999) which was considered by DS not acceptable because test concentrations were not maintained and below the LOQ after 72 hours. The DS referenced an EFSA technical report (2015) to support this view that the use of half the LOD or LOQ is not acceptable to calculate geometric mean measured concentrations where intermediate measured concentrations are not available. National Authority referred to ECHA's Guidance on Application of CLP Criteria (2017) section I.4.1 where is indicated that the use of half the LOQ or LOD is suitable for hazard classification. Therefore, reliable chronic and acute toxicity endpoints should be derived for the 72h endpoint from this study. These endpoints would be within the same concentration range as the key endpoints used for the proposed classification and would therefore support this proposal. DS agreed with National Authority in regard to CLP guidance and use of half the LOQ or LOD and added that new algae study will be submitted.

RAC notes that a new algae study (see BD) generated during the procedure for renewal of the approval of cymoxanil did not affect the acute and chronic classification of the cymoxanil as the values are in the same range as the key values used for classification by DS.

Assessment and comparison with the classification criteria

Degradation

The substance does not undergo rapid abiotic degradation at pH 4 (DT_{50} = 148 days at 25°C) and pH 5 (DT_{50} = 144 days at 25°C) but undergoes extensive hydrolysis with increasing alkalinity (DT_{50} are 2.1 days (pH 7) and 0.04 days (pH 9) at 20°C and 1.1 days (pH 7) and 1.4 days (pH 9) at 25°C). Several major hydrolysis metabolites were formed. Data on hydrolysis might be considered for classification purposes only when the longest half-life determined within the pH range 4 - 9 is less than 16 days (corresponding to a degradation of > 70% within 28 days). Accordingly, cymoxanil is hydrolytically stable.

In the ready biodegradability tests (OECD TG 301B and OECD TG 301F) no significant degradation was observed, indicating that cymoxanil is not readily biodegradable.

Limited mineralisation after 61 days was observed for cymoxanil in an aerobic mineralisation study. Degradation of cymoxanil was very rapid with DT_{50} values of 2.52 hours (9.4 µg/L) and 2.42 hours (96.0 µg/L). Eight major metabolites were formed (IN-KP533, IN-W3595, IN-U3204, IN-R3274, IN-KQ960, IN-R3273, M125 and M103). Calculated DT_{50} for cymoxanil in surface water were 0.09-0.15 days in non sterile system.

In an aerobic water/sediment simulation studies, whole system DT_{50} for the cymoxanil was between 0.056 and 1.6 days following SFO kinetics with a geometric mean of 0.268. Seven major metabolites were observed (IN-W3595, IN-KP533, IN-U3204, IN-KQ960, AS999 (M5), IN-JX915 and IN-T4226). Whole system DT50s for major metabolites were between 0.794 and 160 days. Classification information for metabolites is lacking. Based on the acute toxicity data available in CLH report the metabolite IN-KQ960 is more toxic to fish and invertebrates than cymoxanil. In line with CLP guidance (version 5, June 2017, section II.3.4.) data on primary degradation may be used for demonstrating rapid degradability only when it can be satisfactory demonstrated that the degradation products formed do not fulfil the criteria for classification as hazardous to the aquatic environment.

Overall, the substance does not pass the ready biodegradability tests, the available abiotic and biotic degradation information does not indicate that cymoxanil is ultimately degraded in the aquatic environment with a half-life of < 16 days (corresponding to a degradation of > 70% within 28 days) or that it is transformed to non-classifiable products. Consequently, RAC considers the substance to be not rapidly degradable for the purpose of environmental classification.

Bioaccumulation

RAC agrees with the DS conclusion to consider cymoxanil having low potential for bioaccumulation based on the estimated log K_{OW} values of 0.67 - 0.59 which is below the decisive CLP Regulation threshold of 4.

Aquatic toxicity

In the CLH report, no reliable toxicity data were reported for algae and aquatic plants. During the opinion development process, additional toxicity study with algae was provided by the DS. EC_{50}, NOEC and EC_{10} values based on geometric mean measured concentrations were reported for toxicity study carried out on green algae *Raphidocelis subcapitata*. The algae study meets all validity criteria according to the current version of OECD TG 201 and is considered acceptable by the RMS/DS. RAC is of the opinion that it is appropriate to consider this data relevant for classification of the substance. According to current CLP Guidance (Version 5.0, July 2017), the endpoint based on growth rate reduction is preferred for algae. Therefore the 72h E_rC_{50} of 0.69 mg/L, 72h NOE_rC of 0.0091 mg/L and 72h E_rC_{10} of 0.051 mg/L were selected to be used for classification by RAC. In addition, in line with the current CLP Guidance (Version 5.0, July 2017), the preference is given to EC_{10} value over the NOEC value, therefore RAC considers that the E_rC_{10} should take precedence over NOE_rC. According to recent scientific developments, E_rC_{10} values are preferred as these are statistically derived from the entire dataset, and less dependent on test design considerations than the NOEC.

Acute toxicity

RAC is of the opinion that in case of cymoxanil, reliable acute toxicity data are available for all three trophic levels. Also studies with different metabolites were available for all three trophic levels. The toxicity studies with different degradation products generally derive effect values higher (namely, much lower toxicity) than for cymoxanil with exception of IN-KQ960 which was more acutely toxic to fish and daphnia than parent compound. Based on all available data algae are the most acutely sensitive group, and the lowest result is a 72h E_rC_{50} value of 0.69 mg/L for

Raphidocelis subcapitata using cymoxanil. Cymoxanil shows similar toxicity to algae (0.69 mg/L) as metabolite IN-JX915 (0.931 mg/L). The lowest acute toxicity value of 0.69 mg/L is below the classification threshold value of 1 mg/L, RAC concludes that a classification as Aquatic Acute 1, H400, is justified. As $0.1 < E_rC_{50} \leq 1$ mg/L, the acute M-factor is 1.

Chronic toxicity

Reliable long-term aquatic toxicity data on cymoxanil are available for all three trophic levels while studies with different metabolites are available only for invertebrates and algae. The toxicity studies with different degradation products derive effect values higher (namely, much lower toxicity) than for cymoxanil. The lowest chronic effect value corresponds to a test with fish *O. mykiss* with mean measured 90 d NOEC value of 0.044 mg/L for cymoxanil. As the value is below the classification threshold value of 1 mg/L and the substance is considered not rapidly biodegradable, RAC concludes that a classification as Aquatic Chronic 1, H400, is justified. As $0.01 < NOEC \leq 0.1$ mg/L, the chronic M-factor is 1.

In summary, on the basis of the available data, RAC considers that cymoxanil should be classified according to CLP as:

Aquatic Acute 1, H400, M-factor = 1 and

Aquatic Chronic 1, H410, M-factor = 1

This is consistent with the conclusion of the Dossier Submitter. These recommendations are in line with RAC's previous evaluation (RAC, 2012) of Cymoxanil.

ANNEXES:

Annex 1 The 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 RAC (excluding confidential information).