Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Cymoxanil

EC Number: 261-043-0

CAS Number: 57966-95-7

ECHA/RAC/CLH-O-0000002970-73-01/F

Adopted

14 September 2012

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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 the Regulation (EC) No 1272/2008 (CLP Regulation), the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling of

Substance Name: **Cymoxanil**

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

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

The proposal was submitted by **Austria** and received by RAC on **14 June 2011.**

The proposed harmonised classification

	CLP Regulation (EC) No 1272/2008	**Directive 67/548/EEC**
Current entry in Annex VI of CLP Regulation (EC) No 1272/2008	Acute Tox 4*, H302 Skin Sens. 1, H317 Aquatic Acute 1, H400 Aquatic Chronic 1, H410	Xn, R22, R43 N R50/53
Proposal by dossier submitter for consideration by RAC	Acute Tox 4, H302 Skin Sens. 1A, H317 STOT RE Cat 2, H373 Repr. Cat 2, H361d Aquatic Acute 1, H400 Aquatic Chronic 2, H411 M=1	Xn, R22 Xn, R48/22 Xn, R63 R43 N R50/53
Resulting harmonised classification (future entry in Annex VI of CLP Regulation) as proposed by dossier submitter	Acute Tox 4, H302 Skin Sens. 1A, H317 STOT RE Cat 2, H373 Repr. Cat 2, H361d Aquatic Acute 1, H400 Aquatic Chronic 2, H411 M=1	Xn, R22 Xn, R48/22 Xn, R63 R43 N R50/53

PROCESS FOR ADOPTION OF THE OPINION

Austria 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/web/guest/harmonised-classification-and-labelling-consultation*** Parties concerned and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **29 July 2011**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Christine Bjørge**
Co-rapporteur, appointed by RAC: **José Luis Tadeo**

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

The RAC opinion on the proposed harmonised classification and labelling has been reached on **14 September 2012,** in accordance with Article 37 (4) of the CLP Regulation, giving parties concerned the opportunity to comment. Comments received are compiled in Annex 2.

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

OPINION OF RAC

The RAC adopted the opinion that **cymoxanil** should be classified and labelled as follows:

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

Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
				Hazard Class and Category Code(s)	Hazard state-ment Code(s)	Pictogram, Signal Word Code(s)	Hazard state ment Code(s)	Suppl. Hazard statement Code(s)		
616-035-00-5	Cymoxanil (ISO): 2-cyano-*N*-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide	261-043-0	57966-95-7	Repr. 2 Acute Tox. 4 Skin Sens. 1 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H361fd H302 H317 H373 (Blood, thymus) H400 H410	GHS07 GHS08 GHS09 Wng	H361fd H302 H317 H373(Blood, thymus) H410		M(acute) = 1 M(chronic) = 1	

Classification and labelling in accordance with the criteria of Directive 67/548/EEC

Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits	Notes
616-035-00-5	Cymoxanil (ISO): 2-cyano-*N*-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide	261-043-0	57966-95-7	Xn; R22-48/22-62-63 R43 N; R50/53	Xn, N R : 22-48/22-43-62-63-50/53 S : (2-)36/37-46-60-61	N; R50/53: C ≥ 25 % N: R51/53 : 2.5% ≤ C < 25% R52/53 : 0.25% ≤ C < 2.5%	

SCIENTIFIC GROUNDS FOR THE OPINION

The opinion relates only to those hazard classes that have been reviewed in the proposal for harmonised classification and labelling, as submitted by Austria. This includes mutagenicity, carcinogenicity, toxicity to reproduction and respiratory sensitisation amongst others.

Carcinogenicity

Summary of the Dossier submitter's proposal

The long term toxicity and carcinogenicity of Cymoxanil has been investigated in rats and mice (two studies each):

In the first 2-year combined chronic toxicity/carcinogenicity study in rats (*Cox, 1994a*), treatment related effects were reported as increased incidences of clinical findings (increased hyperactivity and aggressiveness), reductions in body weight and weight gain and adverse macroscopic/ histopathological changes in various organs (degenerative and or inflammatory effects of the retina, nerves, lung, liver, pancreas, testes): The NOAEL was established at 100 ppm (equivalent to 4.1 mg/kg bw for males and 5.4 mg/kg bw for females). Histological findings with respect to testes (elongate spermatid degeneration, multinucleated spermatids) were found at $\geq$ 700 ppm supporting the conclusions drawn from the results of the available short term toxicity studies.
Based on the treatment related findings of the second chronic toxicity/carcinogenicity study on rats (*Malleshappa, 2003*) the NOAEL for males was set at 100 ppm (equivalent to 4.7 mg/kg bw) based on reduced body weight and body weight gain as well as histological findings in different organs (rectum, lung, testes). In females, treatment related effects had been observed at 1200 ppm (changes in haematological and clinical parameters, histological findings in colon and lung); therefore the NOAEL for females can be set at 500 ppm (equivalent to 31.6 mg/kg bw).
In the first carcinogenicity study in mice (*Cox, 1994b*), the NOAEL was set at 30 ppm (equivalent to 4.19 mg/kg bw for males and 5.83 mg/kg bw for females) based on clinical symptoms, reduction of body weight gain, organ weight changes and histological findings in some organs (centrilobular hepatocellular hypertrophy, testicular atrophy, epididymal oligospermia and focal sperm cyst/cystic dilatation).
The results of the second carcinogenicity study on mice (*Krishnappa, 2002*) indicated treatment related findings with respect to reduced food consumption, changes in the differential leukocyte count and also macroscopic findings (haemorrhagic mesenteric lymph nodes in males) as well as histological alterations (follicular cysts in ovaries) in the highest dose group. The NOAEL can be set at 600 ppm (equivalent to 91.4 mg/kg bw for males and 91.9 mg/kg bw for females).
In all four studies, cymoxanil did not reveal any oncogenic potential up to and including the highest dose levels tested. No classification for carcinogenicity was proposed by the dossier submitter.
Human information
Based on the documentation submitted, no health disturbances caused by cymoxanil have been observed in personnel involved in product manufacturing and from the formulation plants. No cases of acute cymoxanil intoxication have been reported and also no specific clinical signs are expected from acute and/or accidental exposure to humans. This human information is relevant for all hazard classes assessed for cymoxanil.

Information received during public consultation

No new information was received during the public consultation. The proposal by the Dossier Submitter for no classification for carcinogenicity was supported by several MSCA. The Dossier Submitter included some more information on the observed effects on liver- and uterus malignancies, which is presented below.

Liver adenocarcinomas were found, however they were not primary liver tumours but appeared to have metastasized from uterus adenocarcinomas which did not show any clear relationship to treatment with cymoxanil. The primary liver tumour (hepatocellular carcinoma) was observed only in a single high dose terminally sacrificed female.

The incidences of the hepatocellular carcinoma and the uterine adenocarcinoma in the females are presented in the table below.

	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 (B)	-	-	-	-	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

If a weight of the evidence approach is used, with other factors such as:

- absence of increased liver weight,
- absence of preneoplastic changes such as hyperplasia, foci, or adenoma,
- lack of histological evidence of liver cell cytotoxicity,
- no increases in serum liver enzyme levels indicative of liver cell toxicity,
- lack of statistical significance,
- absence of the adenocarcinomas in either males within the study or in a second study conducted in another rat strain,

it can be concluded that the very slight increase in female rats is not test substance related.

RAC assessment and comparison with the criteria

No oncogenic effects were observed in studies conducted with cymoxanil, either in rat or in mouse carcinogenicity studies (according to both DSD and CLP).

RAC conclusions

RAC agrees with the Dossier Submitter that the available information does not support a classification of cymoxanil for carcinogenicity.

Mutagenicity

Summary of the Dossier submitter's proposal

Cymoxanil was tested in a sufficient range of *in vitro* and *in vivo* mutagenicity assays measuring different mutagenic endpoints like gene mutation in bacterial and mammalian cells *in vitro* and chromosomal mutations and unscheduled DNA synthesis *in vitro* as well as *in vivo*.

Studies on gene mutation *in vitro* (bacterial tests, HPRT test on Chinese hamster ovaries) did not show any mutagenic potential caused by cymoxanil.
With respect to chromosomal aberrations, one of two *in vitro* studies showed positive results indicating chromosomal damage in human lymphocytes induced by the test substance. However, the results of a second study submitted on chromosomal aberrations on Chinese hamster ovary cells did not confirm the potential of cymoxanil with respect to possible genotoxicity. Furthermore, the results of 3 *in vivo* studies provided (2 micronucleous tests on mice, one *in vivo* chromosomal aberration assay in rats – bone marrow) did not show any potential of the test substance to produce chromosomal damage.
One *in vitro* UDS assay in primary rat hepatocytes indicated that under the test conditions used cymoxanil induces unscheduled DNA synthesis; again, the results of an *in vivo* study on unscheduled DNA synthesis (hepatocytes and spermatocytes) could not confirm the possible influence of cymoxanil to unscheduled DNA synthesis: the net nuclear grains observed in both hepatocytes and spermatocytes of treated animals were not statistically increased when compared to the negative (solvent) control.
Based on the results of all studies provided, the weight of evidence leads the dossier submitter to propose no classification for cymoxanil.

Information received during public consultation

No new information regarding the mutagenic potential of cymoxanil was received during public consultation. Several MSCA agree with the dossier submitter with no classification for germ cell mutagenicity.

RAC assessment and comparison with the criteria

Based on the results of all studies provided, the weight of evidence suggests (according to both DSD and CLP) no genotoxic potential of cymoxanil.

RAC conclusions

RAC agrees with the Dossier Submitter that the available information does not support a classification of cymoxanil for mutagenicity.

Reproductive Toxicity

Effects on sexual function and fertility

Summary of the Dossier submitter's proposal

With respect to reproductive toxicity, two multigeneration studies in rats have been submitted:

Based on the results of the first two-generation study (*Kreckmann,* 1993), the reproductive parameters investigated did not indicate a possible reproductive influence caused by cymoxanil up to 1500 ppm (97.9 – 103.0 mg/kg bw/day) through the diet. For parental animals, reduced body weight of females (F_1

generation during gestation/lactation), reduced body weight gain as well as reduced food consumption of males (F_0 generation) and increased relative testes weight (adults of the F_0 generation) were shown to be of statistical significance at the mid dose group (32.1 – 34.7 mg/kg bw/day) and above. Litter data: 0 – 4 day viability was statistically significantly reduced for the F_1 pups (this finding was not evident at both F_2-generations). Concerning pup weight, statistically significant reductions were evident at 1500 ppm (all generations) and also at the mid dose level of 500 ppm for the F_{2b}-generation.
Based on these findings, the NOAEL for both parental and offspring effects is to be set at 100 ppm equivalent to 6.5 mg/kg bw/day (males) and 6.65 mg/kg bw/day (females); the reproductive NOAEL is 1500 ppm (equivalent to 97.9 mg/kg bw/day – males – and 103 mg/kg bw/day – females).
In the second two-generation study (*Ganiger*, 2001), parental toxicity was evident by reduced body weights of the males (F_1 generation) and of females (F_0 and F_1 generation during premating) as well as reduced food consumption (F_0 females during premating and gestation) in the mid and high dose groups. With respect to reproductive parameters, there was a statistically significant decrease in the percentage of live pups born, together with a reduced mean number of corpora lutea, mean number of implantations and an increased percentage of post-implantation loss in the high dosed F_1 generation. Concerning pup development, statistically significant decreased body weights were observed for F_1 and F_2 pups (males, females and combined sex) at the mid dose level of 450 ppm and above.
Based on findings in the second study, the NOAEL for both parental and offspring effects can be set at 150 ppm equivalent to 10.5 mg/kg bw/day (males) and 14.9 mg/kg bw/day (females); the reproductive NOAEL is 450 ppm (equivalent to 31.6 mg/kg bw/day in males and 42.8 mg/kg bw/day in females).
No classification for effects on sexual function and fertility was proposed. However, the dossier submitter acknowledged that the effects seen on testes in repeated dose studies could lead to such a classification and suggested that the RAC draws conclusions on this aspect.
The dossier submitter reported the following results regarding effects on testes and epididymis in rats, mice and dogs in repeated dose toxicity studies:
Rats:

- In the 28 days dietary study in rats (*Ramesh, 1999a*), animals of the two highest dose levels(260 mg/kg bw/day and 400.3 mg/kg bw/day) in rats showed changes in testes and epididymis weight, which might be linked to the reduction in body weight and body weight gain that occurred at the two highest dose groups. However, no histology has been performed in this study.
- In a 90 days dietary rat study (*Malek, 1992*), at 47.6 mg/kg bw/day, bilateral elongate spermatid degeneration in testes was already observed. At 102 mg/kg bw/day and above increase of testes weight of animals had been accompanied by histological changes in testes and epididymis (multinucleated spermatids, cell debris, hypospermia).
- In a second 90 days dietary rat study (*Ramesh, 1999b*), the macroscopic examination provided no information on damage to organs and tissues caused by the test substance; with respect to histopathology, no test substance related changes in testes and epididymis have been shown up to the highest dose tested (174.3 mg/kg bw/day).
- In a first 2 years dietary rat study (*Cox, 1994a*), histological findings with respect to testes (statistically significant elongate spermatid degeneration) were observed at 30.3 mg/kg bw/day, whereas the relative testes weight was increased and statistically significant increase of multinucleated spermatids observed at 90.1 mg/kg bw/day. Additionally it should be noted that at 700 ppm (30.3 mg/kg bw/day males and 38.4 mg/kg bw/day females) and above, both males and females showed statistically significant retina degeneration.

- In a second 2 years dietary rat study (*Malleshappa, 2003*), histological findings with respect to testes (atrophy of seminiferous tubules) were observed at 58.8 mg/kg bw/day.

Mice:

- In the 28 days dietary study in mice (*Krishnappa, 1999a*), no effects on testes/epididymis caused by cymoxanil technical were evident. However, no histology was performed in this study.
- In the 90 days dietary mice study (*Krishnappa, 1999b*), the only histopathological findings were vacuolar changes of liver cells; no effects on testes/epididymis were evident up to the highest dose tested 256.6 mg/kg bw/day.
- In the first 18 months dietary mice study (*Cox, 1994b*), at 3000 ppm (446 mg/kg bw/day) testis weight was statistically significantly lower (small and soft testes were observed) and tubular atrophy was statistically increased. However, already at 300 ppm (42 mg/kg bw/day) tubular dilation, aggregate lymphoid and sperm cysts/cystic dilation of epididymis were statistically significantly increased. At 1500 ppm (216 mg/kg bw/day) and above, additionally, statistically significantly increased unilateral and bilateral oligospermia and sperm granuloma in epididymis were observed.
- In the second 18 months dietary mice study (*Krishnappa, 2002*), no effects on testes/epididymis caused by cymoxanil were evident up to the highest dose tested (178.3 mg/kg bw/day).

Dogs:

- In the first 90 days dog study (*Tompkins, 1993*), "small" testes, reduced epididymis weight as well as aspermatogenesis were reported at a dose level of 500 ppm(10.56 mg/kg bw/day).
- In the second 90 days dog study (*Venugopala, 1999*), no effects on testes/epididymis caused by cymoxanil technical were evident up to the highest dose tested (14.2 mg/kg bw/day).
- In the first 1 year dog dietary study (*Tompkins, 1994)* the highest dose administered (200 ppm; 5.7 mg/kg bw/day) was much lower than the "effect dose" in the 90 days study. In this study, no effects on testes/epididymis caused by cymoxanil technical were evident.
- In the second 1 year dog study (*Teunissen, 2003*), pathological examination exhibited atrophy of testes in 2 out of 4 dogs at 2.8 mg/kg bw/day and above (3 from 4 animals at 5.6 mg/kg bw/day). Additionally, at 200 ppm (5.6 mg/kg bw/day), reduced size of testis as well as reduced size of epididymis and thickened epididymis were observed in one of 4 animals. The histological findings comprised atrophic changes of testes and epididymis (seminiferous cell debris) in 1 of 4 dogs.

The dossier submitter proposed, based on the adverse effects on testes and epididymis in several repeated dose toxicity studies in rats, mice and dogs a classification of cymoxanil with STOT RE 2 (CLP) and Xn; R48/22 (DSD).

Information received during public consultation

No new information was received during public consultation. Several comments received from MSCAs considered that the adverse effects on testes and epididymis reported in rats, mice and dogs in repeated dose toxicity studies should be discussed in relation to a classification of cymoxanil for fertility in Repr Cat. 2; H361f under CLP (DSD Repr. Cat 3; R62).

RAC assessment and comparison with the criteria

In the 2-generation study by Kreckmann (1993) no adverse effects on fertility parameters were reported. However, in the 2-generation study by Ganiger (2001) minor effects on fertility parameters were reported in the F1 generation. These

included a statistically significant (*) reduction in the percentage of live pups born (90.1, 91.0, 88.8, 81.0* at 0, 14, 45 and 116 mg/kg bw/day) together with a reduced number of corpora lutea (14.3, 14.1, 13.8, 12.2* at 0, 14, 45 and 116 mg/kg bw/day, historical control data (HCD) 12.6-13.3), reduced mean number of implantations (11.7, 12.0, 12.0, 10.1* at 0, 14, 45 and 116 mg/kg bw/day, HCD 11.3-12.1) and an increase in the percentage of post-implantation loss (9.9, 9.0, 11.2, 19.0* at 0, 14, 45 and 116 mg/kg bw/day, HCD 8.2-13.5). In F1 and F2 pups a statistically significant decreased body weight were reported from the mid dose (45.0 mg/kg bw/day) and above. As regards maternal toxicity no reduction in body weight gain was reported in the pre-mating period of the F1. During gestation in F1 there was a 20% reduction in body weight gain in the high dose with an 8% reduction in food intake. It is considered that the effects reported in F1 are not related to maternal toxicity.

Several repeated dose toxicity studies in rats, mice and dogs have been performed with cymoxanil. In these studies statistically significant adverse effects on testes and epididymis were reported in all three species. These effects included bilateral elongate spermatid degeneration, atrophy of the seminiferous tubules and histological changes in testes and epididymis starting around 50 mg/kg bw/day in rats. However, in repeated dose toxicity studies in rats, mice and dogs, studies were also reported that induced minor or no effects on male reproductive organs. The difference in the results in the rat and mouse studies could have been due to difference in the rat or mouse strains used in the various studies. In a weight of evidence analysis, the effects on male reproductive organs observed in rats are considered most relevant for classification. These included in a 90 day study a dose related increase in bilateral spermatid degeneration from 47.6 mg/kg bw/day and an increase in bilateral hypospermia in epididymis at 224 mg/kg bw/day. In a 2-year study in rats a dose related increase in elongated spermatid degeneration from 30 mg/kg bw/day were reported as well as an increase in multinucleated spermatids at 90 mg/kg bw/day. The effects on male reproductive organs in mice were reported at higher doses than effects reported in rats and the effects on male reproductive organs in dogs may have been related to marked body weight loss resulting in delayed puberty. The absence of effects on male fertility parameters in the 2-generation studies are not considered contradictory to or inconsistent with the testis and epididymis toxicity reported in several animal species in repeated dose toxicity studies. It is well known that as to male reproduction in animal species the most sensitive endpoint is histopathology of the testis which has a higher sensitivity compared to fertility parameters (Magelsdorf et al., 2003). This is related to the fact that rats have a high sperm reserve; they are still fertile after a reduction in sperm counts up to around 90%. In contrast, human fertility may already be affected by a small reduction in sperm count. Therefore, the toxicity on testes and epididymis are considered more relevant for humans than the minor effects on fertility parameters reported in the two 2-generation studies in rats regarding male reproductive organ toxicity.
The effects on testis and epididymis are in accordance with the CLP and DSD classification criteria for effects on sexual function and fertility. According to the CLP criteria (CLP section 3.7.1.3) adverse effects on sexual function and fertility includes: *"any effect of a substance that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the male or female reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modification of other functions that are dependent on the integrity of the reproductive systems".*
In Annex I section 3.7.2.5.3 of the CLP Regulation, it is further described that *"Adverse effects or changes, seen in short- or long-term repeated dose toxicity studies, which are judged likely to impair reproductive function and which occur in the absence of significant generalised toxicity, may be used as a basis for*

classification, e.g. histopathological changes in the gonads". In the related CLP Guidance the use of data from repeated dose toxicity studies is further discussed in Section 3.7.2.3.1 under the heading fertility effects as follows: "*Toxicological effects, including marked effects, observed in a standard repeated dose study could be considered valid for the pre-mating phase for adult females and the pre- and post-mating phase for adult males. However, in case of contradictions between the standard repeat dose studies and reproductive studies, the result from the latter should be considered more relevant."*
Furthermore, in CLP Annex I section 3.7.2.3.1 it is described that both positive and negative results are assembled together into a weight of evidence determination. A single positive study performed according to good scientific principles and with statistically or biologically significant positive results may justify classification.
According to the DSD criteria: Effects on male or female fertility include adverse effects on libido, sexual behaviour and aspects of spermatogenesis or oogenesis, or on hormonal activity or physiological response which would interfere with the capacity to fertilise, fertilisation itself or the development of the fertilised ovum up to and including implantation.
In one of the 2-generation reproductive toxicity studies minor effects on fertility parameters were reported in the F1 generation in the high dose group (116.0 mg/kg bw/day) including reduction in number of live pups born together with a reduced number of corpora lutea, mean number of implantations and an increase in post-implantation loss in the absence of clear maternal toxicity. In the repeated dose toxicity studies in rats clear evidence of toxic effects in testes and epididymis were reported starting around 50 mg/kg bw/day. The effects in rats on male reproductive organs were supported by some evidence of effects on male reproductive organs in mice and dog in repeated dose toxicity studies.

RAC conclusions

RAC agrees with the comments received from MSCA during public consultation that the adverse effects on testes and epididymis in repeated dose toxicity studies in rats, mice and dogs favour a classification of cymoxanil for effects on sexual function and fertility. There was clear evidence of adverse effects on male reproductive organs in a 90 day- and 2-year repeated dose toxicity study in rats. The effects observed in rats are not considered to be a secondary consequence of other toxic effects. In the 90 days study in dogs the effects on epididymis and testes may be related to decreased body weight which could have delayed the puberty. In repeated dose toxicity studies in rats, mice and dogs, studies were also reported that induced no effects on male reproductive organs. In one of the 2-generation reproductive toxicity studies minor effects on fertility parameters were reported in the F1 generation. These included a statistically significant reduction in number of live pups born together with a reduced number of corpora lutea and mean number of implantations in the absence of clear maternal toxicity.

As no evidence from humans are available a classification in Repr. 1A (CLP) and Repr. Cat. 1; R60 (DSD) is not considered appropriate.
Since the effects on male reproductive organs were not consistent in all repeated dose toxicity studies in rats, dogs and mice and since no effects on male reproductive organs were reported in the two 2-generation studies RAC is of the opinion that the evidence is not sufficient to classify cymoxanil in Repr. 1B H360F (CLP) and Repr. Cat. 2; R60 (DSD).
In a weight of evidence analysis both positive and negative results are assembled together and a single positive study performed according to good scientific principles and with statistically or biologically significant positive results may justify classification. Clear effects were reported in repeated dose toxicity studies in rats, mice and dogs, although in dogs there might have been an influence of general toxicity. However, in repeated dose toxicity studies in rats, mice and dogs, studies were also reported that induced minor or no effects on male reproductive organs.

Since there is some evidence from animal studies of an adverse effect on sexual function and fertility, RAC concludes that cymoxanil should be classified according to the CLP as Repr. 2, H361f (DSD Repr. Cat. 3; R62).

Effects on development

Summary of the Dossier submitter's proposal

Developmental toxicity of cymoxanil was investigated in rats (2 studies) and rabbits (4 studies):

In the first study in *rats* (*Murray*, 1993) statistically significant reductions of mean maternal body weights, reduced body weight gain and reduced food consumption indicate dose related maternal toxicity at 25 mg/kg bw and above. Concerning foetotoxicity, increased incidences of treatment related variations (partially ossified and unossified sternebra, wavy ribs and partially ossified pelvis) could be observed at maternal toxic dose levels. Concerning malformations, incidences of hemi vertebra, exencephalic head and fused ribs were shown to be above the range of historical control at dose levels of clear maternal toxicity (150 mg/kg bw/day). Although incidences of these malformations observed were low, it cannot be excluded that these findings are treatment-related.
In the second developmental rat study (*Veena*, 1998), no maternal toxicity was reported up to 120 mg/kg bw/day (highest dose level tested) based on the findings with respect to body weight, body weight gain, food consumption and reproductive parameters. Concerning foetal findings incidences for minor anomalies (dumb-bell shaped thoracic vertebra 6/13) were shown to be statistically significantly increased and above the historical control data even at the lowest dose tested (i.e. 30 mg/kg bw/day). These alterations are demonstrating an impact of the test material to the development of foetuses. In the first developmental rabbit study (*Cozens et al*., 1980), no treatment related effects regarding maternal toxicity, litter data and foetal parameters were observed at any dose level tested up to 16 mg/kg bw/day. However, the small number of litters available limited the validity of the assessment of developmental effects. Therefore, the study was regarded as providing supplementary information only.
In the second developmental rabbit study (*Palmer et al*., 1981), maternal toxicity (body weight gain and clinical observations) was evident in the mid (16 mg/kg bw/d) and high dose females (32 mg/kg bw/d). Concerning foetal findings, increased incidences of skeletal malformations (scoliosis and the presence of cervical ribs including "borderline cases between malformations and variants") have been observed in all dose groups from 8 mg/kg bw/day, but revealed no statistical significance. Even after re-evaluation and re-categorisation of these findings ("vertebra and/or rib alterations" associated with scoliosis) increased incidences (but without statistical significance) could be observed. For the high dose group, the number of foetuses with these malformations was above the historical control data submitted.
In the third study in rabbits (*Feussner et al*., 1982), no maternal toxicity occurred, even at the highest dose (32 mg/kg bw/d). Concerning foetal findings, hydrocephaly was found in two foetuses of the highest dose group (32 mg/kg bw/day); the increased number of foetuses affected was without statistical significance but clearly above the range of historical control data. In addition, incidences of foetuses with cleft palates were found in the highest dose tested, the increased number of foetuses affected showed statistical significance and was above the range of historical control. These malformations occurring in the highest dose group were found in two foetuses from dams that showed anorexia.
In the fourth developmental study in rabbits (*Ponnana*, 1999), maternal toxicity was evident for high dose females at 25 mg/kg bw/day (reduced body weight gain and reduced food consumption). Concerning foetal findings, the incidence of dilation of heart ventricles was statistically significantly increased in the high dose animals and

was also above historical control data. As dilation of heart ventricles must be classified as a structural change that could impair foetal survival, development or function, this alteration should be indicated as a major malformation rather than an anomaly. In addition, the incidences of visceral variants (slight renal pelvis dilation) and skeletal variants (incomplete/poor ossification of fore limb) as well as skeletal anomalies (accessory floating rib no. 13) were also shown to be relevant at maternal toxic dose levels. Based on the study result the dossier submitter proposed a CLP classification of Repr. 2, H361d (DSD: Repr. Cat. 3; R63).

Information received during public consultation

No new information was received during public consultation. Several MSCAs supported the classification of cymoxanil in CLP Repr. 2; H361d (DSD, Repr. Cat. 3; R63). One MSCA indicated that the foetal effects should be further discussed. A MSCA indicated that more information from the Palmer, 1981 study and Feussner, 1982 study on vertebra and/or rib alterations should be included since this would facilitate the interpretation of the data in terms of dose-response relationship and historical controls for each malformation, however, no further evaluation of the data was found by the dossier submitter. One MSCA asked for a clarification regarding the reproductive parameters in dams from the study by Veena, 1998 especially to include the effects as mean percentages of post-implantation loss per dose group. This clarification has been included by the dossier submitter in the revised CLH report. The clarification in table 129 in the CLH report that was presented for public consultation is revised as follows (is table 131 in the revised CLH report, provided as an appendix to the RCOM):

Table 131: Teratogenicity study in rats: Reproductive parameter of dams

Parameter	Dose group levels [mg/kg bw/day]			
	0	**30**	**60**	**120**
Number of late resorptions per dose group	0	1	2	41[1)]
Post-implantation loss in total (number of early and late resoprtions per dose group) - post implantation loss(%) per dose group	17 5.6%	13 4.6%	12 5.2%	59[1)] 20.4%
Number of dams (and %) with any resoptions	9 (36.0%)	10 (43.5%)	8 (40.0%)	15 (60.0%)[1)]

1) Statistically not significant altered (Mann Whitney test/Contingency test; level of significance: $p \leq 0.05$) but marked higher than the other dose groups.

Industry questioned if the studies on developmental toxicity should lead to a classification for cymoxanil in CLP Repr. 2; H361d. Their conclusion was that on the basis of the available data, the classification of cymoxanil for developmental toxicity with H361d (CLP) or R63 (DSD) was not scientifically justified. Further details can be found in the RCOM.

RAC assessment and comparison with the criteria

Taking into account the results of the developmental studies available as well as the 2-generation study, there is reasonable evidence that cymoxanil can impair foetal development producing also malformations (demonstrated in two developmental toxicity studies in rats and in three out of four studies in rabbits).

- In the 2-generation study by Ganiger (2001), effects on development were reported. These included a statistically significant increase in post-implantation loss in the high dose F1 generation (132.4 mg/kg bw/day). In F1 and F2 pups, a statistically significant decrease in body weight was

reported from the mid dose (45.0 mg/kg bw/day) and above. During gestation in F1 there was a 20% reduction in body weight gain in the high dose with an 8% reduction in food intake.

- In the first rat developmental toxicity study (*Murray, 1993*) increased incidences of malformations (hemi vertebra, exencephalic head and fused ribs were reported at 150 mg/kg bw/day; findings above the range of historical control values). These effects were observed in the presence of maternal toxicity evident as statistically significant reduced maternal body weight gain*.
- Also in the second rat developmental toxicity study (*Veena; 1998*), increased incidences of variants from 30 mg/kg bw/day (some of variants were above the historical control values) and minor anomalies at non-maternally toxic dose levels indicate the potential of cymoxanil to disturb the development of foetuses. Increases in post-implantation loss were reported at 120 mg/kg bw/day.
- In one rabbit developmental toxicity study (*Palmer et al., 1981*), there was a clear dose dependent increase of "vertebra and/or rib alterations" from 8 mg/kg bw/day, sometimes associated with scoliosis, without statistical significance but above the historical control data. These effects were observed in the presence of maternal toxicity evident as statistically significant reduced maternal body weight gain*.
- In a further rabbit study *(Feussner et al., 1982*) increased incidences of malformations (hydrocephaly, cleft palates) occurred at the highest dose tested (32 mg/kg bw/day). Incidences were statistically significantly increased and above historical background of these findings*.
- Finally the incidence of dilation of heart ventricles of a third developmental toxicity study in rabbits (*Ponnana, 1999*) was statistically significant increased in the high dose animals (25 mg/kg bw/day) and were above the historical control data*

In section 3.7.2.4.2 of Annex I to Regulation (EC) No 1272/2008 it is clearly stated that *"developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies".*

According to the CLP criteria a classification of a substance in **Category 1B** is largely based on data from animal studies. Such data shall 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.

According to the CLP criteria, substances are classified in **Category 2** for reproductive toxicity when there is **some evidence** from human or experimental animals, possible supplemented with other information, of an adverse effect on development, and where the evidence is not sufficiently convincing to place the substance in Category 1.

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, marked effects were reported in five developmental toxicity studies as well as post-implantation loss in a 2-generation study. All studies were performed according to relevant test guidelines. As no evidence from humans is available, classification in CLP Repr. 1A (DSD, Repr. Cat. 1; R61) is not considered appropriate. Since the developmental toxicity reported in

* "Severe malformations in the foetus, even at marked maternal toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma) should not be dismissed for classification (ECBI/30/4 "Expert discussion on classification of substances toxic to reproduction").

rats and mice was not consistently observed in the studies, a classification according to CLP in Repr.1B H360D (DSD, Repr. Cat. 2; R62) is not considered appropriate. Based on the evaluated data, a classification according to CLP in Repr. Cat 2 H361d (DSD, Repr. Cat 3; R63) is warranted.

RAC conclusions

RAC agrees to the proposed classification presented by the dossier submitter. The available data shows that there is reasonable evidence that cymoxanil can impair foetal development. Malformations and variations above the historical control values were demonstrated in two studies in rats and in three out of four studies in rabbits. In a 2-generation study in rats increases in post-implantation loss were reported. These effects were considered not to be related to marked maternal toxicity. Based on the impaired foetal development following exposure to cymoxanil RAC consider that cymoxanil should be classified according to CLP in Repr. 2, H361d ("Suspected of damaging the unborn child"), (DSD, Repr. Cat 3, Xn, R 63 "Possible risk of harm to the unborn child").

Respiratory sensitisation

Summary of the Dossier submitter's proposal

No respiratory tract irritation was observed in acute inhalation toxicity study in rats.

Information received during public consultation

No new information was received during public consultation.

RAC assessment and comparison with the criteria

No irritating effects on respiratory tract were observed in an acute inhalation study with cymoxanil (according to both CLP and DSD).

RAC conclusions

RAC agrees with the dossier submitter that no classification for respiratory sensitisation is proposed.

Other Hazard Classes

Acute toxicity

Summary of the Dossier submitter's proposal

Cymoxanil has moderate oral acute toxicity (oral LD_{50} = 960 mg/kg bw) and low dermal and inhalation toxicity in rats (dermal LD_{50}> 5000 mg/kg bw, LC_{50}> 5.06 mg/l air). The dossier submitter proposes to confirm the minimum classification of Acute Tox. 4 – H302 (CLP) by removing the asterisk and retaining the classification of Xn; R22 (DSD).

Information received during public consultation

No new information regarding acute toxicity was received during public consultation.

RAC assessment and comparison with the criteria

The oral LD_{50} value (960 mg/kg bw) warrants classification under CLP as Acute Tox 4*, H302 (Harmful if swallowed), (DSD Xn, R22, Harmful if swallowed). The LD_{50}

value for acute dermal toxicity and LC_{50} value for acute inhalation toxicity are above the criteria for triggering classification and labelling (both CLP and DSD).

RAC conclusions
RAC agrees with the dossier submitter's proposal to classify cymoxanil for acute toxicity as CLP, Acute Tox 4, H302 (DSD, Xn, R22). The * should be removed from the category since the LD values have been re-evaluated according to the CLP criteria.

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

Summary of the Dossier submitter's proposal

No specific target organ toxicity after single exposure was observed in acute toxicity studies. No classification is proposed.

Information received during public consultation

No new information regarding STOT–SE was received during public consultation.

RAC assessment and comparison with the criteria

No effects observed in acute toxicity studies would trigger criteria for classification and labelling for STOT-SE according to CLP.

RAC conclusions

RAC agree with the dossier submitter that no classification for STOT–SE is warranted.

Skin irritation/skin corrosion

Summary of the Dossier submitter's proposal

According to the results of the rabbit skin irritation study, cymoxanil is not irritating to the intact shaved rabbit skin nor has it any corrosive properties in rabbit skin. No classification is therefore proposed.

Information received during public consultation

No new information regarding skin irritation or skin corrosion was received during public consultation.

RAC assessment and comparison with the criteria

The reported skin irritation scores (0.00) are below the criteria for triggering classification and labelling (according to both DSD and CLP).

RAC conclusions

RAC agrees with the dossier submitter that no classification for irritation or skin corrosion is warranted.

Eye irritation/eye corrosion

Summary of the Dossier submitter's proposal

According to the results of the eye irritation study, cymoxanil is a slight irritant to the rabbit eye; according to classification criteria, classification and labelling is not warranted. Cymoxanil did not show any corrosive properties in rabbit eye and no classification is proposed.

Information received during public consultation

No new information regarding eye irritation/eye corrosion was received during public consultation.

RAC assessment and comparison with the criteria

Estimated eye irritation scores are below the criteria for triggering classification and labelling (according to both CLP and DSD).

RAC conclusions

RAC agrees with the dossier submitter that no classification for eye irritation/eye corrosion is warranted.

Respiratory tract irritation

Summary of the Dossier submitter's proposal

No respiratory tract irritation was observed in an acute inhalation toxicity study in rats. No classification is proposed.

Information received during public consultation

No new information regarding respiratory tract irritation was received during public consultation.

RAC assessment and comparison with the criteria

No irritating effects on respiratory tract were observed in acute inhalation study with cymoxanil.

RAC conclusions

RAC agrees with the dossier submitter that no classification for respiratory tract irritation is warranted.

Skin sensitisation

Summary of the Dossier submitter's proposal

With respect to skin sensitisation following exposure to cymoxanil, three maximisation tests have been submitted. These studies have been conducted according to OECD Guideline 406 and meet the GLP criteria; regarding the study design; all studies are comparable, valid and differ only in the concentration of cymoxanil used in the topical induction phase in the positive study (40% versus 25%), vehicle used and in small differences in purity grade of cymoxanil. The results of two studies indicate no skin sensitising effects of cymoxanil. However, in the third study (*Allan, 1994*), in all test animals (100%) dermal reactions have been observed after challenge (slight to moderate erythema and slight to well defined oedema). No differences between the studies could be identified which

could explain the different results. Based on these results, the possibility of skin sensitization following exposure to cymoxanil cannot be excluded.

Cymoxanil is already classified under CLP as Skin Sens. 1 – H317 (DSD, R43) on Annex VI to the CLP regulation. The dossier submitter proposes to retain the classification under DSD and adapt the classification under CLP to Skin Sens. 1A – H317, to account for the changes introduced with the 2nd ATP to the CLP Regulation.

Information received during public consultation

No new information on skin sensitisation following exposure to cymoxanil was received during public consultation. Two Member States were in agreement and one Member State suggested not to add the subcategory 1A but to retain the classification of Skin Sens. 1 – H317 due to the uncertainty arising from the conflicting studies.

RAC assessment and comparison with the criteria

In one of the three skin sensitisation studies (Magnusson-Kligman Maximisation Test; *Allan, 1994)* on guinea pigs, 100% of the animals showed a skin reaction with 1% test article following intra-dermal induction. This observation triggers a classification according to the CLP (2ndATP) criteria as Skin Sens. 1A, H317 (DSD, Xi, R43 with a SCL at 0.1%). However, two of the three skin sensitisation studies were negative and no major differences between the studies could be identified which could explain the different results.

All test results regarding the skin sensitising potential of cymoxanil are taken into account for classification, i.e. two negative studies and one positive study. According to CLP 3.4.2.2.1.1 skin sensitisers shall be classified in Category 1 where data are not sufficient for sub-categorisation. However, according to CLP 3.4.2.2.1.2, where data are sufficient a refined evaluation allows the allocation of skin sensitisers into sub-category 1A, strong sensitisers, or sub-category 1B for other skin sensitisers as follows:

- 1A: Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered.

- 1B: Substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans. Severity of reaction may also be considered.

No data on humans regarding skin sensitisation is available for cymoxanil. Since the data on skin sensitisation in guinea pigs following exposure to cymoxanil is considered not to be sufficient for a sub-categorisation, cymoxanil should be classified according to the criteria under CLP as Skin Sens. 1, H317 (DSD, Xi, R43).

RAC conclusions

RAC concludes that the animal data on cymoxanil for skin sensitisation is not sufficient for a sub-categorisation and cymoxanil should be classified according to the CLP criteria as Skin Sens. 1, H317 (DSD, Xi, R43). This classification is included in CLP Annex VI.

Repeated dose toxicity / Specific target organ toxicity - repeated exposure (STOT-RE)

Summary of the Dossier submitter's proposal

Based on the results of all sub-chronic and chronic toxicity studies, effects on testes/epididymis caused by cymoxanil are evident in rats, mice and dogs. Further information from these studies is included in the section describing "effects on sexual function and fertility".

The dossier submitter proposes classification for cymoxanil as CLP STOT-RE 2 – H373 (DSD, Xn; R48/22) for effects seen on testes. The dossier submitter acknowledges that the effects seen on male reproductive organs in the repeated dose studies could warrant classification for reproductive toxicity (sexual function and fertility) and requested that RAC draw a conclusion on this.

Information received during public consultation

No new information was received during public consultation. On the other hand some MSCA commented that the adverse effects on testes and epididymis reported in the repeated dose toxicity studies in rats, mice and dogs provides evidence of an effect on fertility and a classification for effects on sexual function and fertility, and should not be used for a classification for STOT-RE according to CLP or repeated dose toxicity according to DSD. However, other effects were also reported in the repeated dose toxicity studies that may be relevant for classification for STOT-RE (CLP) or repeated dose toxicity (DSD).

In a 90 days study in dogs, a statistically significant reduction in haemoglobin in males (24%) at 10.56 mg/kg bw/day and females (22%) at 10.51 mg/kg bw/day was reported. In one 90 day study in dogs a dose dependent increase in atrophy of the thymus was reported from 10 mg/kg bw/day in males and females.
Effects on eyes were reported in a two years study in rats. Histological evaluation showed statistically significant retina degeneration in males from 30.3 mg/kg bw/day and in females from 38.4 mg/kg bw/day. In a 52 weeks study in dogs lenticular degeneration in both eyes of one male was observed at 5.6 mg/kg bw/day. This effect may occur in untreated Beagle dogs at very low incidences, therefore a relationship to treatment cannot be excluded.
Effects were also reported on the sciatic nerve in a two years study in rats as an increase of axon/myelin degeneration of the sciatic nerve without clinical signs in females at 38.4 mg/kg bw/day, indicative of peripheral neuropathy.
Industry commented during public consultation that a classification of cymoxanil with STOT-RE 2 is not scientifically justified since no treatment related effects of cymoxanil on testes or epididymis were considered to be "significant" or to constitute "serious damage" at dose levels relevant for classification. Effects seen at higher dose levels were considered to be without functional consequences based on the absence of reproductive toxicity in two multi-generation studies. Therefore industry considered that the classification suggested in STOT-RE 2 based on effects on male reproductive organs is not appropriate.

RAC assessment and comparison with the criteria

As regards the adverse effects on testes and epididymis reported in the repeated dose toxicity studies, especially in rats, a classification for fertility is considered more appropriate than a classification for repeated dose toxicity/STOT-RE. This is in accordance with the CLP criteria for reproductive toxicity: *"Adverse effects on sexual function and fertility includes alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behavior, fertility, parturition,*

pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive system". This indicates that a classification for fertility is justified based on the adverse effects on testes and epididymis in the repeated dose toxicity studies in rats, mice and dogs. See further discussion under the section Reproductive toxicity, Effects on sexual function and fertility.

However, effects were also reported in other organs than the reproductive system in rats and dogs following sub-chronic or chronic exposure to cymoxanil that may be relevant for a classification for STOT-RE (CLP) or repeated dose toxicity (DSD).
Haematology: In a 90 days study in dogs (Tompkins, 1993) a statistically significant reduction in haemoglobin in males (24%) at 10.56 mg/kg bw/day and females (22%) at 10.51 mg/kg bw/day was reported. This effect was dose depended; 16.6, 14.2, 13.0 and 11.8 g haemoglobin/dl in the controls, 3.13, 5.13 and 10.56 mg/kg bw/day dose group (male) and 14.9. 15.5, 13.0 and 11.6 g haemoglobin/dl in the controls, 3.0, 5.27 and 10.51 mg/kg bw/day dose group (female). Erythrocytes count showed also a dose-depended decrease that reached statistically significance in males at 5.13 mg/kg bw/day and female at 10.51 mg/kg bw/day. In this study a statistically significant decrease in body weight at 10.50 mg/kg bw/day was reported (11987, 11940, 11963 and 8209* g in males and 11389, 9970, 9094 and 6615* g in females at 0, 3.10, 5.20 and 10.50 mg/kg bw/day). Macroscopic examination of one female in the high dose group euthanized in extremis showed dark red contents and reddened mucosa throughout the gastrointestinal tract. In male dogs at scheduled necropsy, no macroscopic or histopathologic changes were reported in the gastrointestinal tract. In a second 90 days study in dogs (*Venugopala, 1999*) a dose-related reduction in haemoglobin in male and female was also reported (151, 149, 138 and 138 g/l in males and 158, 150, 142 and 136 in females at 0, 5 10 and 15 mg/kg bw/day, respectively) that reached statistical significance at 15 mg/mg/kg bw/day in female dogs. No effects were reported on body weight in males and females, however, a statistically significant reduction in body weight gain was reported from 10 mg/kg bw/day. No treatment related effects were reported by macroscopic examination. Histopathology showed a dose-related increase in thymus atrophy in males and females. The decrease in haemoglobin is considered treatment related and not related to the decreased body weight gain.
Thymus atrophy: In one 90 day study in dogs (*Venugopala, 1999*) a dose dependent increase in lymphoid atrophy of the thymus was reported from 10 mg/kg bw/day in males and females. No atrophy was reported in control animals and low dose animals. Animals affected were 2/4 males and 2/4 females at 10 mg/kg bw/day, and 3/4 males and 4/4 females at 15 mg/kg bw/day with increasing severity indicating a dose relationship. A statistically significant reduction in body weight gain was reported from 10 mg/kg bw/day (males: 0.7, 0.1, -1.6* and -3.7*, females: 0.2, 0.0, -1.5* and -3.0* kg in controls, 5, 10 and 15 mg/kg bw/day dose group) as well as a decrease in food consumption (males: 358, 343, 346 and 201*g, females: 262, 293, 267 and 183 g in controls, 5, 10 and 15 mg/kg bw/day dose group). In a one year study in dogs a dose-related decrease in relative thymus weight was reported in male and females dogs from 1.3 (males) and 0.8 (females) mg/kg bw/day (Teunissen, 2003).
In Greaves (2012[1]) it is noted that it can be difficult to distinguish between substance induced thymus weight loss and atrophy, and substances which produce similar changes as a result of generalised high-dose stress response. However, the dose-response relationship may indicate whether the effect is a non-specific stress related effect or is a substance-specific effect. A substance-specific effect is considered to appear in a dose-related manner with decreased thymus weigh and atrophy starting at non-toxic dose-levels. However, non-specific thymus atrophy as

[1] *"Histopathology of preclinical toxicity studies, interpretation and relevance in drug safety evaluation, Fourth edition, Peter Greaves, 2012*

a stress response is usually limited to high doses where other toxic effects are reported such as significant weight loss or other severe toxic effects.
Since thymus atrophy and decreased thymus weight were reported following exposure to cymoxanil at doses with significant body weight loss, it was argued during the targeted expert consultation for cymoxanil that the effects reported on thymus may be a stress related rather than a substance-specific effect on thymus induced by cymoxanil.
Eye effect: Effects on eyes were reported in a two years study in rats (Cox, 1994a). Histological evaluation showed statistically significant retina degeneration in males from 30.3 mg/kg bw/day (10/45, 18/46, 19/46, 35/46* and 52/54* at 0, 2.0, 4.1, 30.3 and 90.1 mg/kg bw/day) and in females from 38.4 mg/kg bw/day (33/55, 34/54, 28/48, 47/52* and 54/55* at 0, 2.7, 5.4, 38.4 and 126.0 mg/kg bw/day).In a 52 weeks study in dogs, lenticular degeneration in both eyes of one male was observed at 5.6 mg/kg bw/day (Teunissen, 2003). However, this effect may occur in untreated Beagle dogs but at very low incidences.
Neuropathy: Effects were also reported on the sciatic nerve in a two years study in rats (Cox, 1994a) as an increase of axon/myelin degeneration of the sciatic nerve without clinical signs in females at 38.4 mg/kg bw/day, indicative of peripheral neuropathy.
Since effects were reported in repeated dose toxicity studies in dogs following oral exposure, oral guidance values should also be considered for dogs. Earlier RAC has considered using the same guidance values for rat and dog studies. The CLP guidance values (dose level of 10 mg/kg/d for the borderline between STOT-RE 1 and RE 2) refer to significant/severe adverse effects in a standard 90-day oral rat study. In the CLP guidance it is outlined as well that this guidance value can be used as a basis to derive equivalent guidance values for toxicity studies of greater or lesser duration of exposure. However, there is no advice as to the use of these rat-specific guidance values for studies with other experimental species (such as dogs). In 2006, The Netherlands presented a corresponding thought starter (ECBI/64/06) with considerations on how to translate guidance values for the rat to guidance values to dogs based on allometric scaling and different life spans of species. However, these preliminary discussions on the use of allometric scaling and different life spans of species for RDT classification have not yet been finalized and the corresponding concepts have not yet been integrated into the CLP guidance. Thus for now RAC prefers to generally start with the guidance values for the 90-day oral rat study, to adapt these 90-day rat guidance values for different durations of exposure to rats according to Haber's rule and then to use the original or duration-adjusted rat guidance values without further changes for test results with other animal species.

Table 1: Guidance values for oral repeated dose studies, adjusted for duration of exposure (units mg/kg bw/day).

Study type	CLP	DSD
28 day rat/dog	STOT RE 1: C ≤ 30 STOT RE 2: 30 < C ≤ 300	T; R48/25: C ≤ 15 Xn; R48/22: 15< C ≤ 150
90 day rat/dog	STOT RE 1: C ≤ 10 STOT RE 2: 10 < C ≤ 100	T; R48/25: C ≤ 5 Xn; R48/22: 5< C ≤ 50
1 year rat/dog	STOT RE 1: C ≤ 2.5 STOT RE 2: 2.5< C ≤ 25	T; R48/25: C ≤ 1.25 Xn; R48/22: 1.25< C ≤ 12.5
2 year rat/dog	STOT RE 1: C ≤ 1.25	T; R48/25: C ≤ 0.625 Xn; R48/22: 0.625< C ≤

	STOT RE 2: 1.25< C ≤ 12.5	6.25

Table 2: Summary of effects other than on the reproductive organs from repeated dose toxicity studies and possible classification.

Study type	STOT RE 1-T;R48/25	STOT RE 2 – Xn; R48/22	No classification
90 day rat/dog	↓ Haemoglobin : 10.5 mg/kg bw/day Atrophy of thymus: 10 mg/kg bw/day	↓Haemoglobin : 10.5 mg/kg bw/day Atrophy of thymus: 10 mg/kg bw/day	
1 year rat/dog		Lenticular degeneration: 5.6 mg/kg bw/day	
2 year rat/dog			Retina degeneration: 35.0 mg/kg bw/day Sciatic nerve effect: 38.4 mg/kg bw/day

In a 90 days study in dogs a statistically significant reduction in haemoglobin in males (24%) at 10.56 mg/kg bw/day and females (22%) at 10.51 mg/kg bw/day was reported. This effect was supported in a second 90 day study in dogs. According to the CLP guidance a reduction in the haemoglobin ≥ 20% is considered an adverse effect on haematology and should be considered in the classification for STOT-RE. A dose-dependent increase in thymus atrophy was reported in a 90 day study in dogs from 10 mg/kg bw/day.
The effects on haematology and the thymus atrophy reported in dogs were on the border between the guidance values for a classification in STOT-RE 1 and RE 2. Furthermore, the effects were not reported in all sub-chronic or chronic dog studies available for evaluation. Therefore, it is considered that the effects reported are in accordance with a classification of cymoxanil in STOT-RE 2 (CLP). The classification in STOT-RE 2 is also in accordance with the classification according to DSD in Xn; R48/22.

RAC conclusions

RAC considers that the adverse effects reported in the repeated dose toxicity studies on male reproductive organs should be considered for a classification for effects on sexual function and fertility (see section on reproductive toxicity).

However, effects were reported on blood parameters and the thymus in dogs following sub-chronic or chronic exposure to cymoxanil that are relevant for a classification for STOT-RE (CLP) or repeated dose toxicity (DSD).
Based on the above, RAC concludes that cymoxanil should be classified according to CLP as STOT-RE 2 – H373 (blood, thymus), (DSD, R48/22)

Other effects

Summary of the Dossier submitter's proposal

With respect to the possible neurotoxicological potential of cymoxanil, a sub-chronic

neurotoxicity study as well as a developmental neurotoxicity study in rats were submitted.

Based on the results of a 90 day neurotoxicity study in rats (the study is designed as a sub-chronic study as well as a study on neurotoxicity) no treatment related effects with respect to neurotoxicity were observed; the NOAEL for neurotoxic effects can be set to be higher than the highest dose administered, i.e. 3000 ppm (corresponding to 224 – 333 mg/kg bw).
In the developmental neurotoxicity study, parental female rats showed statistically significant reduced body weight gain and feed consumption at 50 mg/kg bw/day. Therefore, the maternal NOAEL was considered at the next lower dose of 5 mg/kg bw/day. With respect to litter observation/reproductive parameters, the number of pups found dead/cannibalized, the viability index, the lactation indices, the number of surviving pups and the live litter size were considered to be treatment related altered in the high dose group (100 mg/kg bw/day). In addition, body weight reduction of male and female pups together with clinical observations ("cold to touch", not nursing and nesting) were evident in this dose group as well. Therefore, the developmental NOAEL was 50 mg/kg bw/day. The observation of the pups with respect to possible developmental neurotoxic effects (neurohistological evaluation, passive avoidance testing, water maze performance, motor activity testing, auditory startle response) showed no treatment related changes even at the highest dose tested. Based on the findings, the test substance has no developmental neurotoxic potential.
Concerning immunotoxic effects of cymoxanil, 2 studies have been provided.
In the *28-day study in rats*, no effects on immunotoxicity could be observed. However, general toxicity (decreased body weight gain and body weight) was evident at the two highest dose groups. Based on these alterations, the NOAEL was set at 800 ppm (equivalent to 53.9 mg/kg bw) in males and 400 ppm (equivalent to 31.3 mg/kg bw) in females. The NOAEL for immunotoxicity was established at >1600 ppm (equivalent to 107.7 mg/kg bw in male rats and 117.4 mg/kg bw in female rats).
In *28-day study in mice,* again, no effects on immunotoxicity (thymus and spleen weight; humoral immune function) were seen. Findings of general toxicity (decreased body weight gain) were evident at the highest dose group females. Based on these findings, the NOAEL was set at 1200 ppm (equivalent to 218.4 mg/kg bw in males and 268.5 mg/kg bw in females), and the NOAEL for immunotoxicity could be established at >1200 ppm (equivalent to 218.4 mg/kg bw) in males and >2400 ppm (equivalent to 552.4 mg/kg bw) in females, i.e. the highest dose tested.

Information received during public consultation

No information regarding neurotoxic effect, developmental neurotoxicity or immunotoxic effects following exposure to cymoxanil was received during public consultation.

RAC assessment and comparison with the criteria

According to the available studies, there was no indication of a neurotoxic or immunotoxic potential of cymoxanil.

RAC conclusions

RAC supports the dossier submitter's assessment that there was no indication of a potential neurotoxic, developmental neurotoxic or immunotoxic effect of cymoxanil.

Environmental hazard assessment

Summary of the Dossier submitter's proposal

Degradation
In the original CLH report, the dossier submitter considers cymoxanil as not readily biodegradable according to Directive 67/548/EEC and rapidly degradable according to Regulation EC 1272/2008.
With respect to rapid degradation, hydrolysis and photolysis data show that cymoxanil undergoes rapid degradation. Hydrolysis half-life times of cymoxanil at pH 5, 7 and 9 were 144, 1.1 and 0.02 days at 25 °C, respectively. Aquatic photolysis half-life times correspond to 4.3 and 12.1 days under environmental conditions (mid summer day, approx. 40 °N). Concerning soil photolysis, under irradiation cymoxanil degraded with a DT_{50} of 15.1 days, while under non-irradiated conditions the DT_{50} was 37.3 days.
Regarding simulation tests, the DT_{50} (whole system) in a water sediment study is 0.3 days.
According to these data, the dossier submitter concludes that cymoxanil undergoes rapid primary degradation under environmental conditions.

The information on metabolites is summarized in table 3.

Table 3.Summary of the maximum occurrence, degradation and availability of ecotoxicological data for all identified degradation products of cymoxanil.

Compound	Photolysis		Hydrolysis						Water-sediment study		Aquatic toxicity data available
	Max. Occurence % (day)	Half-life time	Max. Occurence (day)			Half-life time (max. values)			Max. Occurence (day)	DT50	
			pH 5	pH 7	pH 9	pH 5	pH 7	pH 9			
IN-U3204	0.6 (7)	nd	9.1 (7)	52.7 (2)	60.8 (0.2)	25.8	2.6	0.5	24.7 (0.1)	0.4	Y
IN-JX915	52.6 (6)	21.2	1.8 (7)	7.2 (3)	11.0 (0.13)	-	1.1	1.7	8.5	1.7	N
IN-T4226	6.7 (15)	nd	0.0	5.4 (10)	9.8 (1)	-	7.2	2.0	12.0 (3)	4.6	Y
IN-W3595	-	-	2.3 (30)	22.6 (13)	41.5 (2/30)	-	Stable	Stable	27.5 (0.3)	3.0	Y
IN-KP533	7.9 (15)	nd	0.8 (10)	57.4 (30)	34.4 (13)	-	Stable	Stable	26 (10)	2.6	N
IN-R3273	35.4 (15)	4.7	0.9 (30)	10.2 (15)	7.2 (7)	-	Stable	Stable	5	6.3	N
IN-KQ960	-	-	nd	9.0 (30)	14.1 (21)	-	Stable	Stable	14.3 (3)	47.4	Y
Metabolite fraction M5	-	-	-	-	-	-	-	-	22.9 (1)	1.4	N

After the Public Consultation the dossier submitter concluded that cymoxanil is not rapidly degradable based on the following argumentation:
- Ultimate degradation could not been shown in biotic and abiotic degradation studies. In a water/sediment study cymoxanil is rapidly degraded with a DT_{50} (geometric mean, whole system) of 0.3 d leading to the formation of numerous metabolites. The mineralization to CO_2 was too slow to consider the substance to be ultimately degraded (41–82% CO_2 at day 99/102) indicating that cymoxanil is susceptible to primary degradation.
- Some of the degradation products from the water/sediment and hydrolysis tests are rather stable (IN-KP533, IN-R3273, IN-KQ960 and IN-W3595);
- For two of these stable metabolites (IN-KP533 and IN-R3273), no aquatic toxicity data are available. Also there is no information on the toxicity for metabolite IN-JX915 and for the metabolite fraction M5. Therefore it cannot be shown that the degradation products are not classifiable.

Acute (short-term) aquatic toxicity

10 short-term aquatic toxicity studies for the assessment of cymoxanil were submitted, covering fish, crustaceans and micro-algae. The results are summarized in the table below. According to these studies, Cymoxanil is of high acute toxicity to algae (*Anabaena flos-aquae*) with an E_rC_{50} = 0.254 mg/l.

Data element: Acute (short-term) aquatic toxicity of the active substance Cymoxanil

Generally expressed in terms of LC_{50} or EC_{50} (mg/l)

	L(E)C_{50} [mg/l]	Test guideline / design	GLP (y/n)	Reliability
Fish (96 hr LC_{50}):				
Lepomismacrochirus	29	OECD 203, EPA 72-1	y	n
Oncorhynchusmykiss	61	OECD 203, EPA 72-1	y	n
Cyprinodonvariegatus	> 47.5	US EPA 72-3	y	n
Crustacea(48 hr EC_{50}):				
Daphnia magna	27	OECD 202, US EPA 72-2	y	y
Algae and water plants: (E_rC_{50})				
Pseudokirchneriellasubcapitata	2.47	OECD 201, US EPA 123-2	y	n
Pseudokirchneriellasubcapitata	0.63	OECD 202	y	n
Anabaena flos-aquae	**0.254**	US EPA 122-2 and 123-2	y	y
Lemnagibba	> 0.7 (14d)	US EPA 122-2	y	n
Other aquatic organisms (96 hr LC_{50}):				
Mysidopsisbahia	> 44.4	US EPA 72-3(c)	y	n
Crassostreavirginica	> 46.9	US EPA 72-3(b)	y	n

Conclusion: Cymoxanil is of high acute toxicity to algae (*Anabaena flos-aquae*) with an ErC_{50} = 0.254 mg/l

Chronic (long-term) aquatic toxicity

The results of the long-term aquatic toxicity studies are summarized in the table below. Cymoxanil is of high toxicity to all species tested, with a lowest NOEC value of 0.044 for fish (*Oncorhynchusmykiss*).

Data element: Chronic (long-term) aquatic toxicity of the active substance Cymoxanil Generally expressed in terms of NOEC (mg/l)				
	NOEC (mg/l)	Test guideline / design	GLP (y/n)	Reliability
Fish (NOEC):				
Oncorhynchusmykiss	0.22 (21 d)	OECD 204	y	n
Oncorhynchusmykiss	0.12 [a] (97 d)	OECD 210, US EPA 72-4	y	n
Oncorhynchusmykiss	**0.044 (90 d)**	OECD 210, US EPA 72-4	y	y
Cyprinodonvariegatus	0.0942 (36 d)	OECD 210,US EPA 72-4	y	n
Crustacea (21 d NOEC):				
Daphnia magna	0.067	OECD 202, US EPA 72-4	y	n
Algae and water plants (NOEC):				
Anabaenaflos-aquae	NOEC = 0.0652 (96 h)	US EPA 122-2 and 123-2	y	y
Lemnagibba	NOEC = 0.7 (14 d)	US EPA 122-2	y	n
Conclusion: Cymoxanil is of high chronic toxicity to fish (*Oncorhynchusmykiss*) with a NOEC=0.044 mg/l.				

In the CLH report submitted before the public consultation, in which the substance was considered as 'not ready biodegradable' but 'rapidly degradable', the dossier submitter proposed to classify the substance for aquatic acute toxicity category 1 - H400, M-factor = 1 and aquatic chronic category 2 - H411, according to CLP and R50/53 according to DSD.
According to the amendment to the assessment of degradation following the public consultation, in which the substance was considered to be 'not ready biodegradable' and also 'not rapidly degradable', the dossier submitter then proposed to classify the substance for aquatic acute toxicity category 1, M-acute = 1 and aquatic chronic category 1, M-chronic = 1, according to CLP and R50/53 according to DSD.

Information received during public consultation
Some comments were received during public consultation concerning the degradation information in the dossier.
One Member State pointed out that no evidence is provided in the CLH dossier that the degradation products are not classifiable. Therefore the substance cannot be considered to be rapidly degradable according to the CLP criteria.
Another Member State challenged the 'not ready biodegradable' proposal for the substance on the basis of the results of the water-sediment studies providing DT_{50} < 16 days, suggesting that the substance should be seen as "ready biodegradable".

For the full set of comments and responses, see the response to comments (RCOM) in the Annex 2.

RAC assessment and comparison with the criteria

Endpoint	Classification Criteria(criteria in bold)		Evidence for Cymoxanil
	CLP (2nd ATP)	**DSD**	
Degradation Cymoxanil	Cymoxanil is not readily biodegradable under test conditions within 28 days. Ultimate degradation could not be shown in abiotic and biotic degradation studies. A water-sediment study indicated primary degradation (DT_{50} = 0.3 d) but a low mineralization and due to missing data on aquatic toxicity of degradants it is not possible to show that the metabolites are not classified as hazardous to the aquatic environment. Furthermore, the hydrolysis test showed a rapid degradation of Cymoxanil at pH 7 and 9 but this process lead to the formation of stable metabolites with unknown aquatic toxicity. Therefore, **non ready/ non rapid degradation** is proposed, the substance fulfilled neither the criteria for rapid degradation (Section II.4 of "Guidance on the Application of the CLP criteria) nor ready biodegradation (Annex VI, 5.2.1.3 DSD).		The classification as **R50/53** according to Directive 67/548/EEC. is based on the acute toxicity data and on the fact that the active substance is not considered as ready biodegradable/rapid degradable.
Bio-accumulation Cymoxanil	**Log K_{ow} is < 4** Cymoxanil Log K_{ow} = 0.67 - 0.59	**Log K_{ow} is < 3** Cymoxanil Log K_{ow} = 0.67 - 0.59	The measured log POW is in the range of 0.67-0.59 (at 20 °C) and is below the two classification criteria of 3 and 4, therefore cymoxanil is considered to have **a low bioaccumulation potential.**
Acute aquatic toxicity Cymoxanil	**LC/EC_{50}≤ 1 mg/l**		Cymoxanil is of high acute toxicity to algae (*Anabaena flos-aquae*) with an E_rC_{50} = 0.254 mg/l and it cannot be considered as readily biodegradable. Therefore, it fulfills the criteria for the proposed classification as **R50/53**according to Directive 67/548/EEC. Cymoxanil also meets the criteria for the proposed classification as **H400** according to Regulation EC 1272/2008. A **M-factor of 1** is
	Anabaena flos-aquae LC_{50} = 0.254 mg/l		

		applicable based on 0.1 <L(E)C_{50}≤1 mg/l.

Chronic aquatic toxicity Cymoxanil	**For non rapidly degradable substances: 0.01 <NOEC ≤0.1 mg/l**		3.	Cymoxanil is of high chronic toxicity to fish (*Oncorhynchusmykiss*) with a NOEC= 0.044 mg/l. Therefore Cymoxanil fulfils the criteria for the proposed classification as **H410** according to Regulation EC 1272/2008.A **M-factor of 1** is applicable based on 0.01 < NOEC ≤ 0.1 mg/l.
	Oncorhynchus mykiss	NOEC(90d) = 0.044mg/l		

This opinion is based on the information provided by the CLH report. However, not all the desirable information was provided for some of the reported studies in the CLH dossier.
With respect to algae toxicity studies, after receiving from the DS the original study reports of the tests of Bell, Boire and Hughes on Pseudokirchneriellasubcapitata, more information can be added regarding chronic toxicity. These tests can be considered reliable because the validity criteria of the test, OCDE 201, are fulfilled.
The inappropriate selection of concentration range for Pseudokirchneriellasubcapita test makes it impossible to calculate the NOEC (first tested concentration shows statistically significant difference from the control ($p<0.05$)), but allows the ErC20 calculation which is showed in the table below:

Data element: Chronic (long-term) aquatic toxicity of the active substance Cymoxanil				
	ErC20 (72h) [mg/l]	Test guideline/ design	GLP (y/n)	Reliability
Algae and water plants				
Pseudokirchneriella subcapitata	0.39	OECD 201	y	y
Pseudokirchneriella subcapitata	>0.662	OECD 201, US EPA 123-2	y	y

This data confirms that the lowest chronic toxicity values are those related to the NOEC for fish.

RAC conclusions

RAC supports the dossier submitter proposal with the amendments described in the version resubmitted after the public consultation (version 3).

With respect to degradation, since cymoxanil shows rapid primary degradation in both abiotic and biotic studies but a slow mineralisation in the water-sediment study and it cannot be shown that the degradation products are not classifiable, RAC considers cymoxanil not rapidly degradable.

The proposed harmonised environmental classification for cymoxanil under CLP is aquatic acute 1, H400 and aquatic chronic 1, H410 with an M-factor of 1 in both cases (DSD, N; R50/53).

ANNEXES:

Annex 1	Background Document (BD)[1]
Annex 2	Comments received on the CLH report, response to comments provided by the dossier submitter and RAC (excl. confidential information). The revised CLH report as received after public consultation is included as an appendix to the RCOM for information.
Annex 3	Meeting notes from the Meeting of Experts on Cymoxanil, held on the 11th June 2012.

[1] The Background Document (BD) gives 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.