Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Cyproconazole (ISO); (2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol

EC Number: -
CAS Number: 94361-06-5

CLH-O-0000001412-86-73/F

Adopted

11 September 2015

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON CYPROCONAZOLE (ISO)(2RS,3RS;2RS,3SR)-2-(4-CHLOROPHENYL)-3-CYCLOPROPYL-1-(1H-1,2,4-TRIAZOL-1-YL)BUTAN-2-OL

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in this table as submitted by the webform. Please note that some attachments received may have been copied in the table below. The attachments received have been provided in full to the dossier submitter and RAC.

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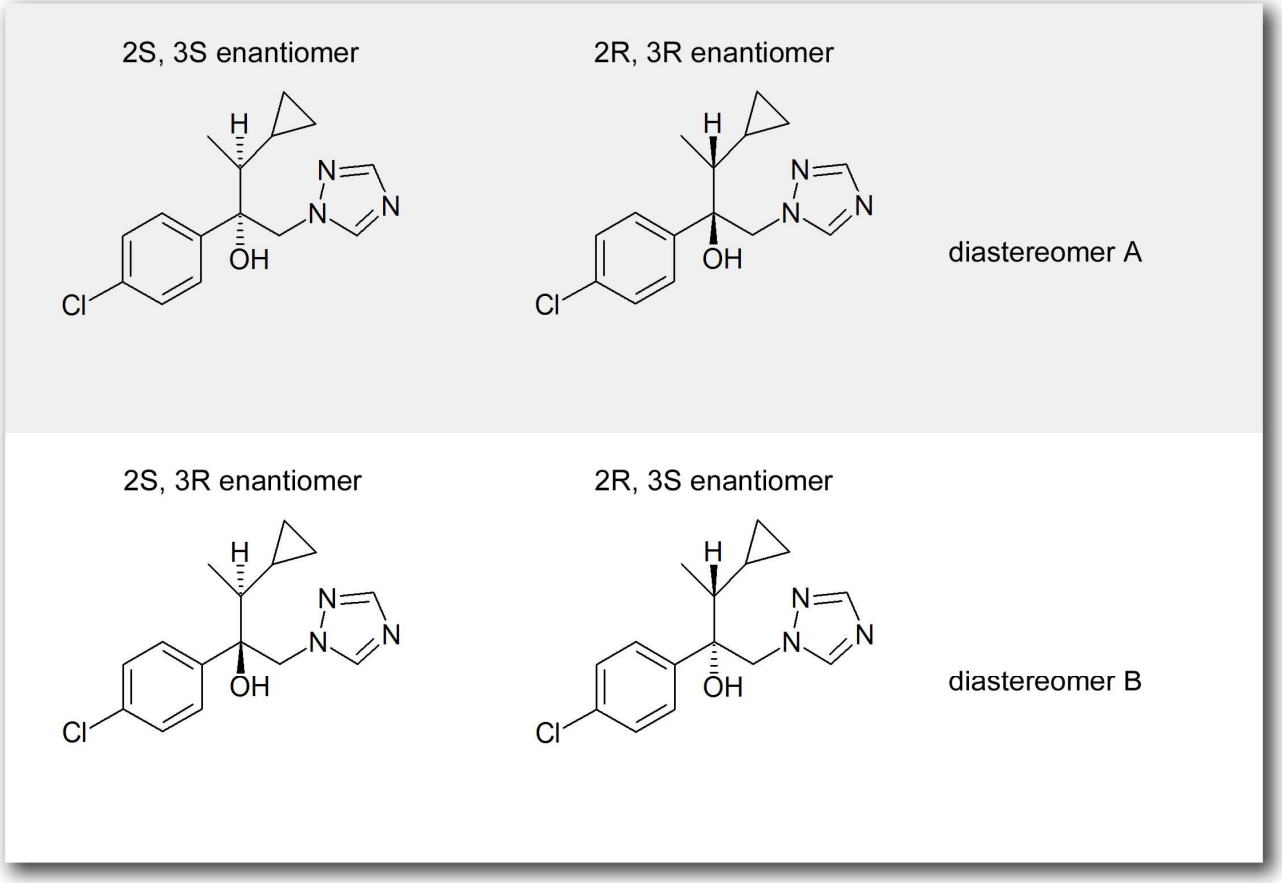
Substance name: cyproconazole (ISO); (2RS,3RS;2RS,3SR)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol
CAS number: 94361-06-5
EC number: -
Dossier submitter: Ireland

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	Germany		MemberState	1
Comment received				
Substance ID: In chapter 1.4 Analytical information of the IUCLID file it is stated that "Cyproconazole is a chiral compound with two chiral centres and has two diastereomers having a 1:1 ratio." This information however is not given in chapter 1.2 Composition of the IUCLID file. Since the composition of a substance needs to be adequately described it is required to add the information about the diastereomers/enantiomers in the IUCLD file chapter 1.2. This includes inter alia the names, EC and CAS numbers and the contents of the isomers. Consequently the substance cannot be considered to be a mono-constituent substance. Since the ratio of diastereomers is 1:1 the substance seems to be a multi-constituent substance. This point should be corrected in the IUCLID dossier. The physico-chemical data are not included in the IUCLID-file.				
Dossier Submitter's Response				
Noted. Thank you for your comments. You are correct, Cyproconazole should be considered a multi-constituent substance. As illustrated under section 1.1 of the CLH report, cyproconazole is a ~1:1 mixture of the two diastereomer pairs (termed A and B, see diagram below), each of which is exactly a 1:1 mixture of two enantiomers. This means that all four stereoisomers are present in similar amounts. In general, enantiomers are chemically and chromatographically indistinguishable and physically identical with the exception that their solutions will rotate plane-polarised light in opposite directions. However, they need not be biologically equivalent, in either their mode of action or in their metabolism. Diastereomers, on the other hand, are different in their chemical and physical properties. Cyproconazole satisfies the definition of a multi-constituent substance, i.e. "… a well-defined substance for which more than one constituent is present at a concentration ≥ 10% and < 80% (w/w)…". The technical active substance actually tested for toxicity comprised these four isomers in approximately equal proportions. This clarification has no impact on the classification proposal for cyproconazole.				

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON CYPROCONAZOLE (ISO)(2RS,3RS;2RS,3SR)-2-(4-CHLOROPHENYL)-3-CYCLOPROPYL-1-(1H-1,2,4-TRIAZOL-1-YL)BUTAN-2-OL



RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	France		MemberState	2

Comment received

MSCA-FR agrees with classification proposal for Carc. 2 H351; Repr. 1B; H360D. Nevertheless, MSCA-FR thinks that the relevance of Phenobarbital-like mechanism in regard to criteria for carcinogenicity classification should be discussed in a general way in order to ensure fair treatment between substances (CLH report of tebuconazole, RCOM - 5 June 2013).
Regarding Acute toxicity, MSCA-FR proposes a classification as Acute Tox 3 – H301 instead of Acute Tox 4 – H302.
MSCA-FR also considers that the statement "(oral)" for STOT RE 2 - H373 should be deleted (table 2 p.7)because hepatotoxicity leading to the classification STOT RE 2 –H373 was observed after oral, inhalation and dermal administrations

We also support the MSCA proposal for environmental hazard but we recommend that Chronic M factors should be of 1 or, please, could you bring further explanations about Acute and Chronic M factors of 10?

Dossier Submitter's Response

Carc: Thank you for your support. The descriptive term "Phenobarbital-like mechanism" is

a historical artefact and is more appropriately referred to as a CAR nuclear receptor mediated mechanism. The relevance of this mechanism in regard to criteria for carcinogenicity classification is of great interest and is a topic of ongoing discussions within ECHA and other Member State Competent Authorities. As such, the relevance of the CAR mediated mechanism with regard to carcinogenicity classification is discussed on a case-by-case by many MSCAs in their role as CLH dossier submitters. The relevance of the CAR mediated mechanism has been discussed in the CLH report under section 4.10.4 and 4.10.5 and in the attached documents accompanying the CLH report ("New MoD studies - Human mouse hepatocytes and HRF analysis 2011.docx"; B.6.8.2.3 C3H Mouse CAR studies with Phenbarbital in "DAR Re-registration addendum Vol 3 B6 (2010).doc"). Please also see comment number 6.
Acute tox: The rationale for classifying in Cat 4 on the basis of the rat and rabbit data and not Cat 3 on the basis of the mouse data were outlined in Chaptor 4.2.4 of the CLH Report. However, it is acknowledged that as the most sensitive species, classification could be based on the LD_{50} of 200 mg/kg bw in the mouse.
STOT RE: The statement 'oral' for STOT RE 2 will be removed.
Ecotox: Agreed, the chronic M factor should be 1 instead of 10. Please see also our comments below.

RAC's response

The comments are noted.
Carcinogenicty and the general approach: RAC has previously decided that when a CAR-mediated MoA is claimed for a substance, RAC would judge on a case-by-case basis whether sufficient and good quality data have been provided showing the presence or absence of key events supporting the proposed MoA.
Acute tox: RAC agreed with the proposal for classification in Category 3.
STOT RE: The data for the dermal and inhalation route would not warrant classification per se due to the reversibility of the effects, or the dose levels at which the effects are observed (above the cut-off values). However, the liver was identified as a targert organ in these studies as well as in the other repeated toxicity studies (in different species) and therefore, based on the consistency of the effects, RAC agreed with the proposal to classify, without indicating the route of exposure.
Ecotox: Chronic M factor of 1 as suggested and agreed by the DS is noted and agreed to by RAC.

Date	Country	Organisation	Type of Organisation	Comment number
25.02.2015	Switzerland	Syngenta	Company-Manufacturer	3

Comment received

Syngenta does not agree with the proposed classification of Cyproconazole for Carcinogenicity (Carc. 2 H351), Reproductive and Developmental Toxicity (Repr. 1B H360D) or Specific Target Organ Toxicity: Repeat dose (STOT-RE 2 H373) as contained in the Annex VI Report submitted by Ireland. Detailed comments are provided as separate documents for each of these endpoints.
In addition, comments of correction and clarification are made to the toxicokinetics section and environmental hazard assessment; these have no impact on the classification proposal.

ECHA note: The following attachments were provided with the comment above (Attachments 1 – 3)

1. Cyproconazole Syngenta Public Comments Summary & Corrections
2. Cyproconazole Syngenta Public comments Toxicokinetics
3. Cyproconazole Syngenta Public Comments Environmental Hazard

Dossier Submitter's Response
Editorial and corrections noted. ***Carcinogenicity:*** Noted, see comment number 7 ***Developmental toxicity:*** noted, see response to comment 14 ***STOT RE:*** Noter, see response to comment 28
RAC's response
See response in the corresponding comments (7, 14, 28).

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	Germany		MemberState	4
Comment received				
Chapter 4.10 (p. 53 ff) Considering the increased incidence of liver adenoma and carcinoma observed after long-term treatment in mice and the arguments questioning the proposed MOA framework (based on CAR activation) as presented in the CLH dossier, we support the proposal to classify the substance for carcinogenicity category 2 (H351), since alternative mechanisms such as cytotoxicity could not be excluded.				
Dossier Submitter's Response				
Thank you for your comments and support.				
RAC's response				
The support is noted.				

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	Spain		MemberState	5
Comment received				
There was a clear increase in the incidence of benign and malign liver tumours in male mice at 100 and 200 pm and in females at the top dose level of 200 ppm. In males, the combined incidence of adenoma carcinoma was slightly higher at 15 ppm already and lost statistical significance only upon age-adjusted analysis. Clear evidence of hepatotoxicity was obtained in both sexes at 100 and 200 ppm. There was no carcinogenicity in the rat study, but dose levels in the rat carcinogenicity study may be inadequate for the assessment of carcinogenicity in this specie. The available mechanistic studies suggest a mode of action in mice with effects very similar to those of Phenobarbital (PB) with activation of the CAR receptor being a key event. However some differences in how cyproconazole (CCZ) treatment affects liver parameters compared to PB were observed that show a possibly wider range of liver effects with CCZ than PB alone. The CAR-null genotype negates some of the effects of CCZ implying that initial activation of the CAR receptor is required for some of the effects promoted by CCZ exposure. However some other responses imply that not all the effects of CCZ are wholly mediated by CAR activation. The results obtained with the mouse models clearly indicate a crucial role of CAR. However, the molecular mechanism of CAR-mediated signal transduction and the relative contribution				

of CAR to the total CCZ effect on gene expression remain enigmatic. There are some clues in the direction that CCZ might change gene expression through additional mechanisms apart from the activation of PXR and CAR. There could be more biochemical events associated with the control of gene expression and protein translation that are not addressed by these studies and that other events downstream of this system and CAR-independent events influenced by CCZ exposure may also be operating and perhaps account for some of the responses in the CAR null mice.

On revision of the whole data, it cannot be considered as proven that the activation of the CAR receptor was the only mechanism and it could involve cytotoxicity (relevant to humans) and/or species specific CAR/PXR downstream events (with questionable relevance to man).

Therefore, in our opinion the results from the supplementary studies are not sufficient to eliminate the concern for the relevance of these effects seen in mice to humans. Given the uncertainties, we consider it is justified to classify cyproconazole as Carc; H351.

Dossier Submitter's Response
Thank you for your comments and support.
RAC's response
The support is noted.

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	France		MemberState	6

Comment received

General comment on the relevance of Phenobarbital-like mechanism for carcinogenicity classification:

As already commented by France on the CLH report of tebuconazole (RCOM - 5 June 2013), we think that the relevance of Phenobarbital-like mechanism in regard to criteria for carcinogenicity classification should be discussed in a general way in order to ensure fair treatment between substances.

Dossier Submitter's Response

Noted. It is important to clarify the role of phenobarbital in this area and how CAR is activated. The primary mechanism is a CAR-mediated one and not a phenobarbital like response as can often be read in the literature and mechanistic studies that accompany active substance dossiers. The point about Phenobarbital is that it is a useful positive control for CAR (and PXR) activation but the mechanism of CAR activation can be substantially different for xenobiotics like pesticides. Phenobarbital acts extra-cellularly and causes an indirect activation of CAR due to competition at the EGF receptor site. Most pesticides are assumed to act intra-cellularly and bind with CAR directly, when they act as activating ligands, migration of the activated CAR complex proceeds into the nucleus where transcriptional control is exercised. Also it can be difficult to separate CAR activities from PXR activities due to significant cross talk, this must be especially taken into account when it comes to investigating enzyme activities and mRNA transcripts of key genes such as CYP2B and CYP3A. Complex humanised gene knock in transgenic animal models and gene knock out test systems like CAR-KO, PXR-KO and combined CAR/PXR KO in mice and investigations on rat and human hepatocytes are required as are tests designed to eliminate other nuclear receptors of importance such as the AhR and PPARα.

Elcomb et al., (2014) [Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: A case study with phenobarbital as a model constitutive androstane receptor (CAR) activator. Crit Rev Toxicol. 2014 January ; 44(1): 64–82] published an informative paper on the role of phenobarbital in producing liver tumors in rodents and commented on the MoA with respect to it's relevance in humans.

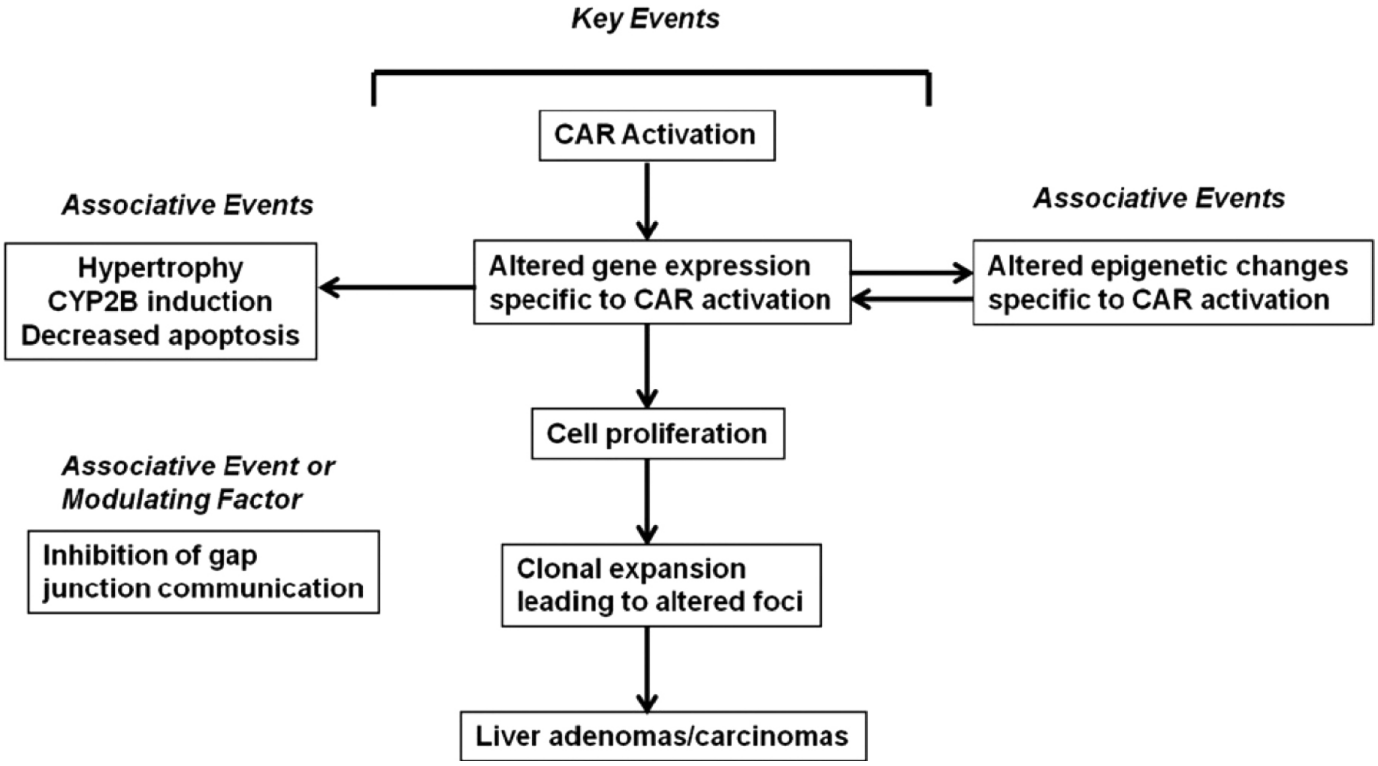


Figure 1 from Elcomb et al (2014) MoA for Phenobarbital-induced rodent liver tumour formation. Proposed key events, associative events and modulating factors for the mode of action (MOA) for PB-induced rodent liver tumor formation. The initial key event is CAR activation which results in altered gene expression, increased cell proliferation, and presumed clonal expansion, leading to altered foci and subsequently to the formation of liver tumours. Associative events which can serve as reliable biomarkers of key events include epigenetic changes, induction of CYP2B enzymes and liver hypertrophy and decreased apoptosis.

The above diagram is a simplified representation of what <u>may</u> occur *in vivo*. What makes carcinogenesis so difficult to understand (and predict) is that key events as outlined above, do not operate in isolation. A myriad of control signals, cellular defence and repair mechanisms, species differences in molecular machinery and the ability of recognition sequences to interact with numerous ligands and other unknown factors can all subtly influence the outcome of individual known key events and ultimately tumourigenic potential.

RAC's response
RAC has previously decided that when a CAR-mediated MoA is claimed for a substance, RAC would judge on a case-by-case basis whether sufficient and good quality data have been provided showing the presence or absence of key events supporting the proposed MoA.

Date	Country	Organisation	Type of Organisation	Comment number

25.02.2015	Switzerland	Syngenta	Company-Manufacturer	7

Comment received

Syngenta disagrees with the proposal for cancer classification (Category 2 H351) based on an increased incidence of liver tumours in the mouse only, due to supporting data to demonstrate a human non-relevant mode of action via CAR-activation. Since November 1997, when the final conclusion for cyproconazole on EU classification under Annex VI was reached, no new data demonstrating an increased risk of tumours from administration of cyproconazole have been generated. Therefore, the prior decision of the European Chemicals Bureau (ECB, 1997) that no classification for cancer is needed for cyproconazole is still warranted. In addition, further data have been generated, which strengthens the Mode of Action case for cyproconazole and non-relevance to humans. Syngenta disagrees with the proposal that the tumour mode of action could involve cytotoxicity (relevant to humans) and additional information is provided to support this. Further information to support this position is provided in a separate document.

ECHA note: The following attachment was provided with the comment above (Attachment 4)

4. Syngenta Position on Mode of Action and Human Relevance of Cyproconazole Induced Liver Tumours in the Mouse

Dossier Submitter's Response

The DS welcomes Syngenta's document which seeks to address concerns of cytotoxicity as a complementary mode of action with that of CAR activation. It remains the DS's position that cytotoxicity working in concert with a CAR-mediated mode of action is plausable within the mouse exposed to high levels of cyproconazole and unlike exposure to CCl_4, the effects are subtle but significant over a lifetime study. The DS does not refute the involvement of CAR/PXR in liver upon treatment with cyproconazole but does question whether this mechanism is the primary or sole cause of carcinogenesis in the mouse 18 month study. The evidence for hepatocyte cytotoxicity with high concentrations of cyproconazole is a confounding factor and may work in concert with a CAR-mediate MoA to drive tumour promotion. There was no cytotoxicity associated with phenobarbital in the *in-vitro* cultured hepatocyte assays (up to 1mM concentration) whereas 125µM cyproconazole was clearly cytotoxic to human hepatocytes and a higher dose of 500µM cyproconazole was clearly cytotoxic to rat hepatocytes. Does this fact mean human liver cells are more susceptible to the cytotoxic actions of cyproconazole? We don't know. The assay performed with human liver cells derived material from one human donor, it is indicative at best. These points have already being discussed in the CLH report and supporting documents and Syngenta has not provided any new or additional information which has not already been assessed by the DS. The prior decision of the European Chemicals Bureau (ECB, 1997) that no classification for cancer is needed for cyproconazole has been considered in an overall weight of evidence approach to the carcinogenic assessment of cyproconazole by the DS. Similarly the DS has assessed the data from the various MoA studies that have been submitted by the company. The fact remains that at doses of 200ppm or 33 - 43 mg/kg bw/day (M – F), there is an increased incidence in liver tumours in mice associated with a CAR-mediated MoA in the presence of hepatocyte cytotoxicity. The lack of tumours in the rat Liver may be a consequence of inadequate dosing (top dose 15.6 – 21.8 mg/kg bw/day, M - F).

The Tamura et al., (2015) paper quoted by Syngenta is an interesting one where they investigated the involvement of CAR in the subacute effects of cyproconazole (up to 27 weeks) using CAR-knockout (CAR-KO) mice and wild type (WT) genotypes exposed to a tumour initiating dose of diethylnitorosamine (DEN). The study confirms CAR involvement

in cyproconazole induced liver hypertrophy and events such as enzyme induction and proliferation. The authors also comment on the fact that in contrast to the triazoles they tested, a study by Sakamoto et al., (2013) showed that PB activated only CAR in WT mice and did not induce liver hypertrophy and tumours in CAR-KO mice. In the Tumura et al., (2015) study CAR-KO mice still showed a variety of responses to cyproconazole albeit attenuated compared with the cyproconazole treated WT genotype, but elevated relative to the untreated CAR-KO controls. Proliferative lesions were also investigated in the Tamura paper where cyproconazole treatment still resulted in an elevated number of eosinophilic altered foci and adenomas in treated CAR-KO relative to untreated CAR-KO controls but these results were generated against a background of DEN initiation so it is not certain as to what relevance they are with respect to the studies in the dossier for cyproconazole.

Similar but short-term studies investigating phenobarbital and cyproconazole and without using the tumour initiation and promotion rodent model were performed by Milurn (2006), see DAR re-registration addendum Vol3 B6 (2010). These studies supported the involvement of CAR in mediating some of the effects of cyproconazole – there was upregulation of some of the genes known to be targets of the CAR regulatory system (such as CYP2b), increases in liver weight and hypertrophy. However, differences are also apparent (little change in Mdm2 mRNA levels, increased cell proliferation independent of CAR, increased CYP 2a activity independent of CAR) relative to those effects expected from CAR activation or phenobarbital (PB) exposure.

Syngenta in their response have also commented extensively on the type of proliferative and injury response exhibited by the liver after exposure to xenobiotics. Both cytotoxicant and mitogenic proliferation in the liver is discussed with comparisons made between CCl_4 and cyproconazole as a model hepatic cytotoxicant and mitogen respectively. The comparison with CCl_4 is an extreme one, the effects of cyproconazole are much more subtle. For example, when cyproconazole was administered to CAR KO mice, effects on enzymatic markers of liver damage (AST, ALP but not ALT) were increased compared to KO controls at 7 days (Milburn, 2006). The DS contends that subtle cytotoxic effects operate in conjunction with CAR activation to promote liver tumours and that classification with Carc 2 – H351 is appropriate considering the limited evidence of carcinogenicity in both sexes of a single species – mouse. CAR activation has been shown to be an important event with strong contributions by PXR and there are many similarities with the PB response. Differences exist and CAR-KO genotypes do not eliminate all the effects of cyproconazole exposure.

RAC's response

The detailed response by Industry is appreciated and has been taken into consideration by RAC in its assessment of the data, although RAC agreed with the proposal of the DS to classify. RAC noted that the authors of the quoted Tamura *et al.* study concluded that not only a CAR-mediated MoA was involved in the induction of tumours. This key aspect is further developed in the opinion.

Date	Country	Organisation	Type of Organisation	Comment number
20.02.2015	Netherlands		MemberState	8

Comment received

The NL CA agrees with the Carc. 2 (H351) classification based on 1) the significant increase in the incidence of hepatic adenomas and carcinomas in female and male mice (p. 61, CLH Report), while no tumors were observed in rats; 2) the limited mechanistic information with regards to the human relevance (cytotoxicity and/or species/specific CAR/PXR downstream

events) of cyproconazole-induced hepatic tumors; and 3) lack of genotoxicity.
Dossier Submitter's Response
Agreed. Thank you for your comments and support.
RAC's response
The support is noted.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	Germany		MemberState	9
Comment received				
Chapter 4.9 (p. 51 ff) Considering the presented study results, we support the proposal not to classify for mutagenicity.				
Dossier Submitter's Response				
Thank you for your support				
RAC's response				
The support is noted				

Date	Country	Organisation	Type of Organisation	Comment number
20.02.2015	Netherlands		MemberState	10
Comment received				
The NL CA agrees with no classification for Mutagenicity. Even though there was one in vitro mammalian cytogenetic test in CHO cells which had weakly clastogenic results at 100-200 µg/ml, a re-evaluation of the slides and a similar study at concentrations of 100-800 µg/mL gave negative results (Table 31, p. 51-52 CLH Report). The negative findings were also supported by negative results in the in vivo micronucleus test in bone marrow and chromosome aberrations test (Table 31, p. 52 CLH Report).				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
The support is noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	Germany		MemberState	11
Comment received				
Chapter 4.11 (p. 88 ff) Considering the presented study results (malformations and post implantation loss in two species which were reproducible in several studies and observed in several dose levels), we support the proposal to classify for reproductive toxicity category 1B (H360D).				

Dossier Submitter's Response
Thank you for your support.
RAC's response
The support is noted.

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	Spain		MemberState	12
Comment received				

The study results on azole and triazole compounds with same mode of action and the critical role of several CYP enzymes in reproduction support the classification of cyproconazole for development (Cat 1B H360 D) and for fertility (Repr. Cat 2 H361f).

Fertility

In the long-term mouse study, testicular germinal epithelial deficit and aspermia were found at and above 13.2 mg/kg bw/day (NOAEL = 1.84 mg/kg bw/day). Besides, effects on adrenals (rats), ovaries (decrease relative weight, decreased follicular activity in dogs), utero (inactive endometrium in dogs), pituitary (rats) and thyroids (rats) were observed in subchronic studies at similar doses than liver effects suggesting disrupted hormonal balance.

In the two-generation rat study there was some effect on fertility no statistically significant. The NOEL for fertility was 1.7 mg/kg bw/day based on slightly increased gestation length and reduced implantations in F0 generation at 8-13 mg/kg bw/day. There was also a decreased litter size at birth at this dose (-12% than controls). The highest dose level in the study was only 8-13 mg/kg bw/day and minimal parental toxicity was recorded in F0 males only. However, this study was conducted using too low dose levels and its acceptance could be questioned.

Besides, cyproconazole may potentially impair fertility because of its mode of action. Cyproconazole and other triazole fungicides exert their fungicidal effect by inhibiting the cytochrome P450, enzyme 14α-lanosterol demethylase (CYP51) that result in the inhibition of the biosynthesis of ergosterol. In humans CYP51 is important for the conversion of lanosterol to cholesterol. Thus, inhibition of human CYP51 may disturb steroid synthesis. Besides, CYP51 has important role in meiosis in human gametes. It catalyses the reaction from lanosterol to sterols that activate meiosis and modulate development of male and female germ cells. Deficient germ cell development may lead to reduced fertility. The azole compounds are not selective for CYP51 and a range of other cytochrome P450 enzymes, including aromatase (CYP19) and other CYP enzymes involved in steroidogenesis are also affected by azole compounds. Hence, the result would be the reduction of testosterone and estradiol synthesis and the increased progesterone production. The disturbance of the balance between estrogens and androgens may potentially affect fertility.

Therefore, based on mode of action of cyproconazole, suggestions of impaired fertility in the two generation study, and the testicular effects observed in long-term mouse study classification for fertility (Repr. Cat 2 H361f) may be considered in absence of an adequate fertility study.

Developmental toxicity

Cyproconazole caused embryo/fetal toxicity (increased post implantation loss) and malformations (cleft palate, hydrocephalus and ureterohydronephrosis) in rats at maternally toxic dose (reduced maternal body weight gain in early treatment period) and in rabbits (malrotated hind limb) without maternal toxicity. Similar malformations were induced by other (con)azole compounds.

A number of compounds in the triazole group appear to have a common intrinsic teratogenic activity. The mechanism of the teratogenic effect has been hypothesized to be related to the capability of these substances to alter embryonic retinoic acid catabolism. Retinoid acid is a well-known morphogen in vertebrate and invertebrate embryos. Triazole-related abnormalities are confined to structures controlled by retinoic acid, especially the neural crest cells, hind brain, cranial nerves, and craniofacial structures.

An important role of some CYP isoforms (CYP26 isoforms) expressed during mammalian development is the catabolism of retinoic acid. The suggested mechanism for the teratogenic effects involves the inhibition of CYP 26, which means increased concentrations of retinoic acid (Menegola et al., 2006).

Besides, the interference with key enzymes involved in steroid hormone synthesis and the aromatase (CYP19) inhibition disturb the balance between estrogens and androgens and thus may potentially affect embryonic development. Post-implantation losses could be secondary to endocrine disruptive effects of aromatase inhibition in the dams via oestradiol.

The specificity and the spontaneous infrequence of some malformations (i.e cleft palate) otherwise commonly seen with triazoles, indicates that they cannot be considered secondary to maternal toxicity.

Therefore, we consider that there is sufficient evidence for classifying cyproconazole as Cat 1B H360 D, based on the severe embryotoxicity (decrease foetal bw, resorptions and malformations) not considered secondary to maternal toxicity in two species (rat and rabbit).

Dossier Submitter's Response

Thank you for your detailed comments.

Fertility

MOA: Cyproconazole belongs to the class of aromatase inhibiting triazole substances as pointed out and many class effects are held in common by these substances. However, there is significant differences in both potency and effect, depending on the individual substance functional groups, and classification based on each substance data base is preferable to a chemical group classification. Many of the effects seen with cyproconazole may be linked to inhibition of aromatase and are considered relevant to human health and important for classification.

2-gen study: The dose levels in the 2-generation study were lower than required as minimal parental toxicity was seen (increased relative liver weight and fatty change at 120 ppm in F_0 males only). It could be argued that this endpoint was not sufficiently explored as the doses used caused no effect or minimal effect in parents. Some fertility related parameters were affected slightly in a single generation only (marginal decrease in implantation sites (11.6 vs 12.5 in controls) in F_0 high dose females only which was within the historical range (9.8 – 13.00); mean pregnancy duration (F_0 high dose only and NS). In the F_0 generation there was a slight, statistically non-significant decrease in the number of implants in the high-dose group. In addition, there was a dose-related increase in pre/perinatal mortality in the high-dose group in the F_0 and F_1 generation (16.3% and

12.6%, respectively). There was a corresponding slight increase in postnatal mortality (days 0 – 21 p.p) in the high-dose group of the F_0 and F_1 (8.1% and 7.6%, respectively. These effects are considered related to treatment. This effect on foetal/pup mortality is considered to be indicative of developmental toxicity rather than an effect on fertility and therefore supportive of the developmental toxicity classification proposal.
Testicular effect: In the long-term mouse study, testicular germinal epithelial deficit and aspermia were found at and above 13.2 mg/kg bw/day (NOAEL = 1.84 mg/kg bw/day). In the 90-day mouse study, doses up to 88.8 mg/kg bw were without apparent effect on the testis or epididymis. The testis was not affected in the dog 90-day or 1-year study. A slight statistically significant increase in relative testis weight in the 90-day rat study was without histological correlate, while the testis was not targeted up to 40 mg/kg bw in the 2 year rat dietary study. The effect in the long term mouse study appears treatment-related and could be considered relevant to fertility classification. However, the overall weight of evidence was not considered to support classification for this effect
Developmental tox
Thank you for your support.
There is no specific mode of action information for developmental toxicity of cyproconazole other than the general data relating to closely related substances in the triazole group.

- Fungicidal activity occurs through inhibition of cyt P450 (CYP-51 encoded) sterol 14α-demethylase which converts squalene to ergosterol . This enzyme is unusual in having catalytically identical orthologues in different biological kingdoms. In mammals it converts lanosterol into meiosis activating sterols which are precursors of cholesterol (meiosis-activating sterols recently shown to modulate germ cell development).
- In mammals, the CYP51 reaction is part of the pathway leading to biosynthesis of cholesterol (cholesterol is a primary sterol in cell membrane of mammalian cells and also required to make sex steroid hormones.
- Aromatase (CYP19 encoded) which is responsible for the physiological balance of androgens and estrogens, is another important target of triazoles. A number of commonly used triazole fungicides have been shown to inhibit aromatase.
- Considerable experimental evidence (Mengola, E, 2006) for inhibition of embryonic CYP 26 (which regulates retinoic acid concentration, acting as a specific retinoic acid hydroxylase) thereby regulating retinoic acid dependent gene expression controlling antero-posterior patterning (head development) and this may be the mechanism behind cranial malformations documented for a number of triazoles (cleft palate), including cyproconazole.

Exposure of rodents to triazole fungicides has been documented to affect multiple toxic endpoints including, organ toxicity such as liver, carcinogenicity, reproductive and developmental endpoints and endocrinological effects.
These effects may be due to the fact that triazoles can alter the expression and activity of a number of CYP enzymes. They can act as both inducers and inhibitors, dependent on the tissue and the specific triazole in question., eg., some have been developed to selectively inhibit CYP 19 aromatase for the treatment of breast cancer and testicular cancer.

RAC's response

The support for developmental classification is noted.
For fertility effects, the comment is interesting and has been taken into account in the assessment. However, even though cyproconazole belongs to the class of aromatase inhibiting triazole substances with common class effects, as there are significant differences in both potency and effect depending on the individual substance functional groups, RAC agrees with the DS that classification shall be based on data for the individual substance since data are available. This has already been done for other triazole substances. The conclusion on the classification is further developed in the opinion.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON CYPROCONAZOLE (ISO)(2RS,3RS;2RS,3SR)-2-(4-CHLOROPHENYL)-3-CYCLOPROPYL-1-(1H-1,2,4-TRIAZOL-1-YL)BUTAN-2-OL

Date	Country	Organisation	Type of Organisation	Comment number
26.02.2015	Sweden		MemberState	13
Comment received				
The Swedish CA supports classification of Cyproconazole (Cas No 94361-06-5) as Repr. 1B and agrees with the rationale for classification into the proposed hazard class and differentiation. The increase in resorptions and occurrence of malformations in the developmental toxicity studies in both rats and rabbits supports the classification as Repro 1B.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
The support is noted.				

Date	Country	Organisation	Type of Organisation	Comment number
25.02.2015	Switzerland	Syngenta	Company-Manufacturer	14
Comment received				
A classification for developmental toxicity category 1B is considered not required by Syngenta. Cyproconazole is currently classified for developmental toxicity as Category 2 H361 (Annex of EU Dir 67/548 (26th ATP)) and although it is acknowledged that the second rabbit study (Muller, 1991) may not have been considered as part of the data on which the current classification was agreed, this study is considered to add no significant new information. Syngenta considers that the combined data are insufficient to trigger a change to a H360D classification. Further information to support this position is provided in a separate document. *ECHA note: The following attachment was provided with the comment above (Attachment 5)* 5. Syngenta position on Developmental Toxicity				
Dossier Submitter's Response				
The rationale for the proposal of Cat 1B for developmental toxicity is fully described in the CLH report. It is noted that the second rabbit study (Muller, 1991) was not included in the dossier on which the current 26th ATP classification (C&L 1993-1997) was agreed. The DS believes that this study further supports the proposal to classify as Cat 1B. *The data:* Classification is proposed on the basis of evidence in the rat and the rabbit summarised as follows: Rat 1; 2-generation study 4, 20,120 ppm (0.3-0.5, ≈2, ≈10 mg/kg bw) Marginal parental tox (↑rel liver weight with some fatty change) There was a treatment-related increase in pre/perinatal mortality in both F_0 and F_1 foetuses at the high dose (120 ppm) in the absence of significant parental toxicity. The treatment-related adverse effects on foetal mortality pre- and post-natal at 120 ppm are considered by the RMS as supporting evidence for developmental toxicity. Rat 2; Becker range-finding 7.5, 30, 75, 120 mg/kg -significant reduction in maternal body weight gain in early days of treatment from 75				

mg/kg bw.
- Weight loss relates to foetal loss as ↑↑early resorptions from 30 mg/kg
↑↑ post-implantation loss from 30 (early resorptions)
-Cleft palate 1@30, 1@75 and 10/11@120
Rat 3 Becker main study
(0, 6, 12, 24, 48 mg/kg bw/day)
Increased incidences (treatment and dose-related) of malformations and significant embryo-foetal toxicity were seen at doses causing some early weight gain reduction in the rat.
- 24 mg/kg - 1 hydrocephaly, ↑skeletal variations
- 48 mg/kg - 2(2) cleft palate , 2(2) hydrocephaly.
Rat 4 Machera
(20, 50 and 75 mg/kg bw/day)
Treatment and dose-related severe embryotoxicity and teratogenicity were seen at doses causing early reduced body weight gain in dams.
-cleft palate; 0, 2(2), 11(5), 9(4)
-hydrocephaly(moderate); 0, 5(3), 4(3), 10(9)
-hydrocephaly (full); 0,0,6(3), 6(6)
-ureter hydronephrosis; 1(1), 2(2), 7(3), 2(2)

Rabbit 1
(0, 2, 10, 50 mg/kg)
Increased total resorptions were seen from 10 mg/kg bw/day in the rabbit at doses not causing maternal toxicity. This study is regarded as *supplementary* due to technical difficulties with the dose suspension analysis
Maternal weight ↓ at 50 mg/kg (GD 6-8)
-Internal hydrocephaly – 0, 1, 1, 1
-↑ post implantation loss from 10 (both early and late resorptions)
Rabbit 2
(0, 2, 10, 50 mg/kg).
↓Maternal body weight from days 6-9 of gestation at 50 mg/kg.
-foetal malformations were seen at 50 mg/kg (Table 1 (DAR B6.6.2.3b-3)), including a rare malformation (malrotated hindlimbs 4(4)), all 4 foetuses had multiple malformations. There was also a single incidence at the mid dose, at which there was no evidence of maternal toxicity. Whether or not the incidence at the mid dose is related to treatment is difficult to conclude as additional information on historical control data were not considered reliable.

Table 1 Summary of foetal abnormalities (Muller, 1991)

Parameter	**0 mg/kg**	**2 mg/kg**	**10 mg/kg**	**50 mg/kg**
Number of foetuses examined externally (no. of dead foetuses)	110 (1)	90	75 (2)	60 (1)
Number of foetuses examined viscerally	110	90	73	59
Number of foetuses examined skeletally	110	90	73	59
Malformations				
No. of foetuses with **external/visceral malformations** (no. litters affected)	2 (2)	1 (1)	2 (1)	7 (5)

No. of foetuses with **skeletal malformations** (no. litters affected) *Including:*	1 (1)	0 (0)	3 (3)	13 (7)
Tail redued/rudimentary	-	-	-	*3()*
Malrotated hind limbs	-	-	*1(1)*	*4(4)*
Lumbar vertabre absent	-	-	-	*3()*
Total no. of foetuses with **malformations -**(no. litters affected)	3 (3)	1 (1)	5 (3)	15 (7*)
-Average % malformed foetuses	2.7	1.1	6.1	25
-% of litters affected	18.8	9.1	21.4	70
Variations				
No. of foetuses with **external/visceral variations** (no. litters affected)	3 (2)	3 (1)	3 (2)	2 (2)
No. of foetuses with **skeletal variations** (no. litters affected)	110 (16)	88 (11)	72 (14)	58 (10)

*) $P \leq 0.05$

Consistency: Both rabbit studies show evidence of an adverse effect on development. In the first study (Becker, 1986) there was increased implantation loss (early and late resorptions) from 10-50 mg/kg bw. Maternal weight gain was reduced from days 6-11 at 50 mg/kg only. In the second rabbit study (Muller, 1991), there was a significant reduction in maternal body weight gain at 50 mg/kg bw from days 6-9 only. The foetal data (Muller, 1991) are summarised above in Table 1 (DAR B6.6.2.3b-3).
The findings in the two studies were *not consistent* as pointed out, however an adverse effect was seen in both. These studies were conducted in different laboratories, at different times, using different strains of rabbit, therefore it may not be unreasonable to have different specific results.

Different species may be effected differently: The fact that two different malformations (cleft palate in rats and malrotrated hindlimbs in rabbits) were each seen in only 1 species does not weaken the evidence. It is not unusual for teratogenic substances to elicit different effects in different species (Schardein, JL, et al, Environ Health Perspect. 1985 Sep; 61: 55–67). Thalidamide is a classic example.

RAC's response

RAC considered that the second rabbit study, which was not available when the first assessment of classification was conducted, provides useful information, since severe malformations were reported, and these were dose-related and at doses without any maternal effects. This means cyproconazole can be considered as a teratogenic substance in two species. The general argumentation for classification as Repr. 1B by the DS is supported.

Date	Country	Organisation	Type of Organisation	Comment number
20.02.2015	Netherlands		MemberState	15

Comment received

Effects on fertility
The NL CA agrees with no classification for fertility. However, the dose levels in the 2-generation study were low compared to the 90-day studies (10 times lower) and induced only minimal maternal toxicity. Is there data available from a range-finding study with higher dose levels justifying these low dose levels and were reprotoxic effects observed in this range-finding study? Further, an increase of effects on testis and epididymis was

observed in the 88-week mouse study. Would this affect the conclusion on fertility?

Effects on development
The Netherlands agrees with the Repro.1B (H360D) classification based on the palatoschisis observed in rats even at the lowest dose of 30 mg/kg (p. 91, CLH Report), decreased total number of live fetuses per dam, increased number of early and late resorptions, and increased hydrocephalus in two rat studies (p. 94-97, CLH Report), and fetal abnormalities in rabbits (p. 97-99, CLH Report). In most studies, effects were observed in the presence of limited maternal toxicity. In addition, palatochisis and implantation loss is observed also with other triazole fungicides like epoxyconazole. Mechanistic information is available in the CLH dossier of epoxyconazole. It is shown that cyproconazole inhibits aromatase (http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?sid=144211502).
No increase in placental weight is reported although this is an effect often observed for this class of substances. Was placental weight determined in the rat developmental studies?

In table 2 (p. 7), the current Annex VI classification should be corrected to Repr. 2 (H361)*** instead of **. Also, please include the asterisks for the current classification column in section 3.7 of Table 1 (p.10, CLH Report).
Effects on lactation were not evaluated in the CLH Report.

Dossier Submitter's Response

Fertility: The dose levels in the 2-generation study were lower than required as minimal parental toxicity was seen (increased relative liver weight and fatty change at 120 ppm in F_0 males only). There are no range-finding data available. It could be argued that this endpoint was not sufficiently explored as the doses used caused no effect or minimal effect in parents. Some fertility related parameters were affected slightly in a single generation only (marginal decrease in implantation sites (11.6 vs 12.5 in controls) in F_0 high dose females only which was within the historical range (9.8 – 13.00); mean pregnancy duration (F_0 high dose only and NS).
In the long-term mouse study, testicular germinal epithelial deficit and aspermia were found at and above 13.2 mg/kg bw/day (NOAEL = 1.84 mg/kg bw/day). In the 90-day mouse study, doses up to 88.8 mg/kg bw were without apparent effect on the testis or epididymis. The testis was not affected in the dog 90-day or 1-year study. A slight statistically significant increase in relative testis weight in the 90-day rat study was without histological correlate, while the testis was not targeted up to 40 mg/kg bw in the 2 year rat dietary study. The effect in the long term mouse study appears treatment-related and could be considered relevant to fertility classification. However, the overall weight of evidence was not considered to support classification for this effect.

Dev tox: Thank you for your support.

RAC's response

The rationale proposed by the DS is supported.

RESPIRATORY SENSITISATION

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	Germany		MemberState	16

Comment received

Chapter 4.6 (p. 32 ff)

According to the CLH dossier, the relevant data are lacking to judge for this hazard.

Dossier Submitter's Response
Noted.
RAC's response
Noted.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	Germany		MemberState	17
Comment received				
Chapter 4.2 (p. 24 ff) In the dossier it is proposed to classify cyproconazole into acute toxicity category 4 for oral toxicity (H302). This proposal is based mainly on the results gathered in rats. However, the results obtained with mice might lead to a classification into category 3 (H301). The GD explains (Version 4.0, November 2013, chapter 3.1.2.3.2): "In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested." Pending clarification, we would propose to classify the substance into category 3 (H301) for acute oral toxicity based on the results in mice. Considering the presented study results after dermal or inhalation exposure, we support the proposal not to classify for acute dermal or inhalation toxicity.				
Dossier Submitter's Response				
The rationale for classifying in Cat 4 on the basis of the rat and rabbit data and not Cat 3 on the basis of the mouse data were outlined in Chaptor 4.2.4 of the CLH Report. However, it is acknowledged that as the most sensitive species, classification could be based on the LD_{50} of 200 mg/kg bw in the mouse.				
RAC's response				
RAC agreed with the proposal for Category 3.				

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	Spain		MemberState	18
Comment received				
According to the Guidance on the Application of the CLP Criteria (November 2013), when there are data from several species, "classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested". Consequently, considering the most sensitive specie we regard the LD50 values in mice (> 50 < 300) to propose a classification as Acute tox. 3 - H301.				
Dossier Submitter's Response				
The rationale for classifying in Cat 4 on the basis of the rat and rabbit data and not Cat 3 on the basis of the mouse data were outlined in Chaptor 4.2.4 of the CLH Report. However, it is acknowledged that as the most sensitive species, classification could be based on the LD_{50} of 200 mg/kg bw in the mouse.				
RAC's response				

RAC agreed with the proposal for Category 3.

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	France		MemberState	19
Comment received				
Acute toxicity by oral route: According to the guidance on CLP criteria, although the preferred test species for evaluation of acute toxicity by the oral route is the rat, it is noted that when experimental data for acute toxicity are available in several animal species, scientific judgement shall be used in selecting the most appropriate LD50 values. In the specific case of cyproconazole, mouse is clearly the most sensitive species. Therefore, a classification Acute Tox 3 – H301 instead of Acute Tox 4 – H302 is considered relevant.				
Dossier Submitter's Response				
The rationale for classifying in Cat 4 on the basis of the rat and rabbit data and not Cat 3 on the basis of the mouse data were outlined in Chaptor 4.2.4 of the CLH Report. However, it is acknowledged that as the most sensitive species, classification could be based on the LD_{50} of 200 mg/kg bw in the mouse.				
RAC's response				
RAC agreed with the proposal for Category 3.				

Date	Country	Organisation	Type of Organisation	Comment number
20.02.2015	Netherlands		MemberState	20
Comment received				
The NL CA disagrees with the classification for Acute Tox. 4 (H302) because there is no evidence provided as to why the oral rat study with LD50 of 350 mg/kg bw/day is more relevant to humans than the mouse oral study with LD50 200 mg/kg bw/day. According to Annex I: 3.1.2.2.1 'The preferred species for evaluation of acute toxicity by oral and inhalation routes is the rat…. When experimental data for acute toxicity are available in several animal species, scientific judgment shall be used in selecting the most appropriate LD50 value from among valid, well-performed tests'. According to the CLP guidance: "In general, classification is based on the lowest ATE value available i.e. the lowest ATE in the most sensitive appropriate species tested. However, expert judgement may allow another ATE value to be used in preference, provided this can be supported by a robust justification. If there is information available to inform on species relevance, then the studies conducted in the species most relevant for humans should normally be given precedence over the studies in other species." Extrapolation of the LD50 used for the DSD classification to CLP is not justified because the DSD criteria were only based on rat LD50 values whereas CLP also requires other species to be taken into account. Therefore, Acute Tox. 3 (H301) should be considered. Please include the asterisks for the current classification column in section 3.1 of Table 1 (p.9, CLH Report) and in section 2.3.1 under 'Current classification and labelling'.				
Dossier Submitter's Response				
The rationale for classifying in Cat 4 on the basis of the rat and rabbit data and not Cat 3 on the basis of the mouse data were outlined in Chaptor 4.2.4 of the CLH Report. However, it is acknowledged that as the most sensitive species, classification could be based on the LD_{50} of 200 mg/kg bw in the mouse.				

RAC's response
RAC agreed with the proposal for Category 3.

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	Germany		MemberState	21
Comment received				
Chapter 4.4 and 4.5 (p. 29 ff) Considering the presented study results, we support the proposal not to classify for skin corrosion/irritation.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
The support is noted.				

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	Germany		MemberState	22
Comment received				
Considering the presented study results, we support the proposal not to classify for serious eye damage/eye irritation.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
The support is noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	Germany		MemberState	23
Comment received				
Chapter 4.6 (p. 32 ff) Considering the presented study results, we support the proposal not to classify for skin sensitisation.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
The support is noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment

				number
27.02.2015	Germany		MemberState	24
Comment received				
Chapter 4.3 (p. 29 ff) Considering the presented study results, we support the proposal not to classify for STOT-SE.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
The support is noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	Germany		MemberState	25
Comment received				
Chapter 4.7 (p. 34 ff) Considering the increased incidence of severe liver findings (such as single cell necrosis) observed after repeated-dose treatment in rats, mice and dogs as presented in the CLH dossier, we support the proposal to classify the substance for STOT-RE 2 (H373).				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
The support is noted.				

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	Spain		MemberState	26
Comment received				
Evidence of significant hepatotoxicity was seen at dose levels below the cut-off levels in all oral, dermal and inhalation repeated dose toxicity studies in the three species tested. Increases in relative liver weight were seen with histopathological change such as hepatocellular hypertrophy, vacuolation, fatty change, and single cell necrosis. It was also seen altered clinical chemistry and marker enzymes. Therefore, the Spanish CA supports the proposed classification of cyproconazole as STOT RE 2 H373 (May cause damage to organs (liver) through prolonged or repeated exposure).				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
The support is noted.				

Date	Country	Organisation	Type of Organisation	Comment number

27.02.2015	France		MemberState	27
Comment received				
Hepatotoxicity leading to the classification STOT RE 2 – H373 was observed after oral, inhalation and dermal administrations. Therefore, we consider that the statement "(oral)" should be deleted from table 2 page 7.				
Dossier Submitter's Response				
Thank you for your support. The statement 'oral' will be removed.				
RAC's response				
The data from the dermal and inhalation route would not warrant classification per se based on the reversibility or the dose levels at which the effects are observed (above the cut-off values). However, the liver was identified as a target organ in these studies as well as in the other repeated toxicity studies (in different species) and therefore based on the consistency of the effects, RAC supported to classify without indicating the route.				

Date	Country	Organisation	Type of Organisation	Comment number
25.02.2015	Switzerland	Syngenta	Company-Manufacturer	28
Comment received				
A classification for STOT-RE category 2 is considered not required by Syngenta. Although it is acknowledged that the target organ in all the mammalian toxicology species is the liver and effect levels are within the 'Guidance Values' for classification, the effects reflect adaptive responses due to xenobiotic metabolism and are not of toxicological concern; thus these findings in the liver do not meet the criteria triggering STOT-RE classification. Further information to support this position is provided in a separate document *ECHA note: The following attachment was provided with the comment above (Attachment 6)* 6. Cyproconazole position on STOT-RE in response to CLH dossier				
Dossier Submitter's Response				
The effects of cyproconazole on the liver of the rat, mouse and dog following 28-, 90- and 1 year (dog) are clearly outlined in the CLH report and further reiterated in the position paper submitted by the industry (Tables 1 and 2). There is no difficulty in recognising that there are clear effects in the liver in all three species *via* all routes at dose levels consistant with classification for STOT RE. The question raised is whether these documented effects are adaptive or toxic in nature. Findings considered adaptive in the position paper include; fatty change/hepatocellular vacuolation and single cell necrosis (mouse), single hepatocyte degeneration (dog). In most cases this was accompanied by altered clinical chemistry markers of toxicity and/or functional change to a greater or lesser degree. Fatty change or vacuolation may be associated with toxicity, altered lipid metabolism and sometimes with metabolism of xenobiotic substances and can occur either with or without cellular necrosis. Cytochemical and ultrastructural analysis would be required to interpret whether such findings are related to specific toxicity or adaptive in nature. This is not available for cyproconazole. The picture is not consistant in detail across the species and routes, which is perhaps not surprising, but the DS believes that the balance of evidence supports classification for sub-chronic hepatotoxicity.				
RAC's response				
The detailed response by Industry is appreciated and has been taken into consideration by RAC in its assessment of the data. However, RAC supported the proposal of the dossier				

submitter that the overall picture showed that the adaptive capacity of the liver was overwhelmed by cyproconazole, leading to liver damage, at doses well below the guidance value for classification.

Date	Country	Organisation	Type of Organisation	Comment number
20.02.2015	Netherlands		MemberState	29
Comment received				
The NL CA agrees with the STOT RE 2 (H373, liver) classification. However, the STOT RE classification is not consistent within the document as a limitation to the oral route is included in table 2. Please provide an argumentation for using a route restriction or not. The Netherlands would disagree that effects are limited to only oral exposure, given that hepatotoxicity effects were also observed in the 16-day rat inhalation study (p. 46, CLH Report) at 1 mg/L and in the 28-day dermal rat study at 100 mg/kg bw/day (p. 47-48, CLH Report).				
Dossier Submitter's Response				
Thank you for your support. 'Oral' will be removed.				
RAC's response				
The support for classification is noted. As regards the route of exposure, the data from the dermal and inhalation route would not warrant classification per se based on the reversibility or the dose levels at which the effects are observed (above the cut-off values). However, the liver was identified as a target organ in these studies as well as in the other repeated toxicity studies (in different species) and therefore based on the consistency of the effects, RAC agreed with the proposal to classify without indicating the route.				

OTHER HAZARDS AND ENDPOINTS – Aspiration Hazard

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	Germany		MemberState	30
Comment received				
According to the CLH dossier, the relevant data are lacking to judge for this hazard.				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	Germany		MemberState	31
Comment received				
Degradation: Section 5.1: The abiotic degradation in air is missing. Please insert the missing section. Section 5.1.3, page 110: please correct in last paragraph: The geomean DT50 lab calculated for 12°C is 298 d. Section 5.1.3, page 111: please insert ">" at the end of the first paragraph: " > 1,000 days (DFOP)". At the end of the second paragraph please add the corresponding value at 12°C:				

"At 12°C the geomean DT50 was 18.09 d".

Aquatic toxicity:
Section 5.4, Table 50, page 118: Fish Fathead minnow Pimephales promelas 357 days (Williams, T.D.; 2001) please add the NOEC of 0,125 mg a.s./L for egg production.
Section 5.4.3: Please add the study on aquatic macrophytes: according to our information a study on aquatic macrophytes is available (Everett, C.J., Wyeth, K. and Powley, W. (2007): Cyproconazole (SAN619) – Growth inhibition to Lemna gibba under semi static conditioned Amendment 21 February 2007, Report No. T002449-06-REG) Please include this study in the aquatic section for completeness.
Section 5.4.3.1, page 153: "Acute toxicity of SAN 619F to Scenedesmus subspicatus (OECD: Algae Growth Inhibition Test);
Ellgehausen H. November 1986(a"): The relevant parameter from an algae growth inhibition test is growth rate, not biomass. Please give also the growth rate related effect values (ErC50/NOErC). The environmental classification should be based on the growth rate related effect values. According to the CAR for cyproconazole the 96h-ErC50 is 0.12 mg/L based on nominal concentrations. Please give the ErC50 based on mean measured concentrations and use this value for the environmental classification.
The NOEC is given as 0.021 mg/L. It is not quite clear where this value comes from. From table 85 it seems that the NOEC was the lowest tested concentration. The mean measured concentration however is given as 0.028 mg/L, not 0.021 mg/L. Please clarify.
Section 5.5, page 158: The EbC50 from the algae study should not be used for classification. Instead, the ErC50 related to measured concentrations should be used.
For chronic classification also the 21d-NOEC of 0.023 mg/L obtained for Daphnia magna should be considered, if the NOEC for green algae is not correct.

M-Factor
Section 5.6, page 158 and table 2 page 8:
We agree with the classification as acute 1 and chronic 1 with the hazard statements H400 and H410. However, the M factor for chronic toxicity seems wrong. The lowest endpoint was the NOEC of 0.021 mg/L. As this NOEC is between 0.01 and 0.1 mg/L and the substance is not rapidly degradable, the chronic M factor should be 1 instead of 10.

Dossier Submitter's Response

Response to:

Section 5.1:

Abiotic degradation in air

The rate of the photochemical oxidative degradation of cyproconazole in the atmosphere was estimated using the Atmospheric Oxidation Program (version 1.5, Syracuse Research Corporation, USA). The estimations are based on the structural activity relationships developed by Atkinson *et al*. A rate constant for reactions with hydroxyl radicals was calculated. No reaction through tropospheric ozone could be calculated because cyproconazole contains no olefinic double or triple bonds as structural elements. The calculations provided by the applicant were not in accordance with the TGD (Section 2.6.3). The CA generated new calculations. The rate constant for hydroxyl radical reactions was calculated as 16.18×10^{-12} cm^3 $molec^{-1}$ s^{-1}. This represents an atmospheric half-life of ~1 days (24 hour day).

Table 4.1.1-6-1. Photodegradation of cyproconazole in air

Guideline /Test method	Initial TS concentration, C_0	Total recovery of TS	Specific degradation rate constant with OH	DT_{50}(d) reaction with OH	DT_{50} reaction with	Ref.

			radicals (cm^3 $molec^{-1}$ s^{-1})	**radicals**	**ozone.**	
AOPWIN Based on QSAR	Not applicable	Not applicable	16.18×10^{-12}	~1 d*	---	IIIA 7.3/1

* 24 hr time period
The concentration of OH radicals in the atmosphere was assumed to be 5×10^5 molec cm^{-3}

Section 5.1.3: Agree.

Section 5.1.3, page 111: Agree.

Section 5.4, Table 50, page 118: Agree. The NOEC of 0.125 mg a.s./L for egg production to be inserted into Table 50.

Section 5.4.3: Agree. The study: Cyproconazole (SAN619) Growth inhibition to Lemna gibba under semi static conditioned (Amendment 21 February 2007, Report No. T002449-06-REG) Everett, C.J., Wyeth, K. and Powley, W. (2007) should be included in the aquatic section.

Section 5.4.3.1, page 153: (a)This study did not report growth rate, the peer review of cyproconazole for plant protection and as a biocide discussed this matter. The only available information, subsequently submitted by the applicant was an estimation of the 96 h E_rC50, by fitting a probit model to the data, is the given nominal concentration of 0.12 mg/L.

(b) The Table is misleading, the value of 0.028 is a calculated 'measured concentration' based percentage of nominal concentration. The actual definitive value used to derive the NOEC of 0.21 mg a.i./L is a measured value. This value is based on biomass. No corresponding NOEC for rate is available from the study.

Section 5.5, page 158: As discussed above there is no measured E_rC50 for algae available from this study. In previous discussions about this issue with ECHA and EFSA during peer review of cyproconazole the E_bC50 value was accepted in the absence of the E_rC50.
For chronic classification it is possible to use the higher value of 0.023 mg/L for classification purposes if the NOEC for green algae in not considered acceptable.

M-Factor
Section 5.6, page 158 and table 2 page 8: Agree, the chronic M-factor should be 1.

RAC's response

Editorial information and chronic M-factor noted. As regards acute M-factor, this point is further developed in the opinion.

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	United Kingdom		MemberState	32

Comment received

Table 50 of the CLH Report includes an ErC50 value of 0.12 mg/l (nominal) [Ellgehausen, 1986]. We cannot see this value in the study report and it is unclear whether this is a 72 hour or 96 hour value and why nominal is appropriate given other study endpoints are

apparently based on mean measured. In addition, is a NOErC or ErC10 available for the study or was the current NOEC based on biomass alone?

The 2010 pesticides assessment Additional Report and EFSA Peer Review Conclusion for cyproconazole includes a 7 day study on aquatic toxicity to Lemna gibba (Everett, Wyeth and Powley, 2007). The relevant endpoints (EC50 and NOEC / EC10) from this should be confirmed and considered for the aquatic classification.

We feel additional information regarding these key endpoints should be presented before the environmental classifications and acute and chronic M-factors (for a non-rapidly degradable substance) can be agreed.

Dossier Submitter's Response

Table 50 of the CLH Report includes an ErC50 value of 0.12 mg/l (nominal) [Ellgehausen, 1986]. We cannot see this value in the study report and it is unclear whether this is a 72 hour or 96 hour value and why nominal is appropriate given other study endpoints are apparently based on mean measured. In addition, is a NOErC or ErC10 available for the study or was the current NOEC based on biomass alone?"

DS: This study did not report growth rate, the peer review of cyproconazole for plant protection and as a biocide discussed this matter. The only available information, subsequently submitted by the applicant was an estimation of the 72 h E_rC50, by fitting a probit model to the data, is the given nominal concentration of 0.12 mg/L.

No, neither of these values are available from the study.

"The 2010 pesticides assessment Additional Report and EFSA Peer Review Conclusion for cyproconazole includes a 7 day study on aquatic toxicity to Lemna gibba (Everett, Wyeth and Powley, 2007). The relevant endpoints (EC50 and NOEC / EC10) from this should be confirmed and considered for the aquatic classification".

DS: The endpoints from this study on Lemna gibba are as follows: 7 day EC_{50} (frond number)=0.059 mg/L nominal and 7 day NOEC AUC and increase in dry weight 12.5 mg/L nominal. We will include this study in the aquatic section.

"We feel additional information regarding these key endpoints should be presented before the environmental classifications and acute and chronic M-factors (for a non-rapidly degradable substance) can be agreed".

DS: Agreed. However, this will not change the classification.

RAC's response

Comments noted. As regards the acute M-factor, this point is further developed in the opinion.

Date	Country	Organisation	Type of Organisation	Comment number
27.02.2015	France		MemberState	33

Comment received

- For the acute classification of cyproconazole, the algae endpoint calculated from growth rate should be used instead of the endpoint calculated from biomass. This would not modify

the classification proposal (Aquatic Acute Category 1 – H400). - Considering the ErC50 = 0.12 mg/L and the NOEC = 0.021 mg/L from the study of Ellgehausen, H. (1986a) on Scenedesmus subspicatus and that cyproconazole is not rapidly degradable, acute and chronic M factors should be of 1. Could you please bring further explanations about acute and chronic M factors of 10?
Dossier Submitter's Response
- "For the acute classification of cyproconazole, the algae endpoint calculated from growth rate should be used instead of the endpoint calculated from biomass. This would not modify the classification proposal (Aquatic Acute Category 1 – H400)". DS: This study did not report growth rate. During the peer review process of cyproconazole for plant protection and as a biocide this matter was discussed. The only available information, subsequently submitted by the applicant was an estimation of the 96 h E_rC50, by fitting a probit model to the data, is the given nominal concentration of 0.12 mg/L. We agree with the classification as acute 1 and chronic 1 with the hazard statements H400 and H410. - "Considering the ErC50 = 0.12 mg/L and the NOEC = 0.021 mg/L from the study of Ellgehausen, H. (1986a) on Scenedesmus subspicatus and that cyproconazole is not rapidly degradable, acute and chronic M factors should be of 1. Could you please bring further explanations about acute and chronic M factors of 10?" DS: Agree. The M-factor for chronic toxicity should be 1 instead of 10. The M-factor for acute toxicity should remain as 10 as the algal growth inhibition study did not report the ErC50 for this study. In previous discussions regarding this issue with ECHA and EFSA during peer review of cyproconazole, for biocides and PPP, the EbC50 value was accepted in the absence of the ErC50. This is in agreement with Annex I: Table 4.1.0 (Note 2) of the CLP Reg (EU) No. 1272/2008 which states "Classification shall be based on the ErC50 [= EC50 (growth rate)]. In circumstances where the basis of the EC50 is not specified or no ErC50 is recorded, classification shall be based on the lowest EC50 available". In this instance, the experimentally available data should take precedence over model-derived data and the M-factor for acute toxicity should remain as 10.
RAC's response
Chronic M-factor of 1 as suggested is noted and agreed to by RAC. As regards the acute M-factor, this point is further developed in the opinion.

Date	Country	Organisation	Type of Organisation	Comment number
26.02.2015	Sweden		MemberState	34
Comment received				
The Swedish CA support the classification of Cyproconazole in Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410) as specified in the proposal. This conclusion is based on the effect of the most sensitive algae Scenedesmus subspicatus and that the substance is not rapidly degradable and has a low bioaccumulation potential. The SE CA do not agree with the rationale for the setting of M-factors for both acute and chronic toxicity for the aquatic organisms. For acute toxicity in algae Scenedesmus subspicatus the 96 h ErC50 = 0.12 mg/l for				

cyproconazole should have been used, instead of the 96 h EbC50=0.077 mg/l proposed by Ireland, implying an M-factor of 1 (0.1<L (E) C50≤1) instead of 10. The endpoint ErC50 of the toxicity test of algae is a more reliable endpoint according to CLP guidelines.
For chronic toxicity in algae Scenedesmus subspicatus the NOEC was 0.021 mg a.i./l, which is implying an M-factor of 1 (0.01<NOEC≤0.1 mg/l) instead of 10 which was proposed by Ireland.

Minor comments:
In general the tables with relevant studies could have been improved to make the reporting of the results more suited for the purpose and to facilitate reading. In addition, it would have been helpful if the reliability of each study was indicated.

Page 105 Table 48. This table would benefit from having the half-life (DT50) degradation rates for the biodegradation test clearly stated.
It is also unclear if there are any ready biodegradable tests carried out, preferable according to OECD guidelines 301 B.

Page 103 Table 45 (photolysis) and page 106 table 48
(anaerobic degradation). Since it is difficult to evaluate photolysis tests and anaerobic degradation tests, they are not used for classification purposes when it comes to rapid and ultimate degradation, and can be excluded in the CLH report.

Page 113 section 5.4. 'Aquatic toxicity'. In some aquatic toxicity studies it is not clear if it is the product SAN 619F or the active substance cyproconazole that has been tested.

Page 113 section 5.4. 'Aquatic toxicity'. Summaries and discussions are missing for every aquatic organism (fish, Daphnia, algae or other aquatic plants and other aquatic organisms).

Page 158 section 5.5. 'Comparison with criteria for environmental hazards'. Comparisons with the classification criteria set in CLP Regulation (EC 1272/2008) for acute and chronic toxicity, degradation and bioaccumulation could be developed further in the report. The limits for the M-factor intervals should also be mentioned where it is appropriate.

Dossier Submitter's Response

- "For the acute classification of cyproconazole, the algae endpoint calculated from growth rate should be used instead of the endpoint calculated from biomass. This would not modify the classification proposal (Aquatic Acute Category 1 – H400)".

DS: This study did not report growth rate. During the peer review process of cyproconazole for plant protection and as a biocide this matter was discussed. The only available information, subsequently submitted by the applicant was an estimation of the 96 h E_rC50, by fitting a probit model to the data, is the given nominal concentration of 0.12 mg/L.

We agree with the classification as acute 1 and chronic 1 with the hazard statements H400 and H410.

- "Considering the ErC50 = 0.12 mg/L and the NOEC = 0.021 mg/L from the study of Ellgehausen, H. (1986a) on Scenedesmus subspicatus and that cyproconazole is not rapidly degradable, acute and chronic M factors should be of 1. Could you please bring further explanations about acute and chronic M factors of 10?"

DS: Agree. The M-factor for chronic toxicity should be 1 instead of 10.

The M-factor for acute toxicity should remain as 10 as the algal growth inhibition study did not report the ErC50 for this study. In previous discussions regarding this issue with ECHA and EFSA during peer review of cyproconazole, for biocides and PPP, the EbC50 value was accepted in the absence of the ErC50. This is in agreement with Annex I: Table 4.1.0 (Note 2) of the CLP Reg (EU) No. 1272/2008 which states "Classification shall be based on the ErC50 [= EC50 (growth rate)]. In circumstances where the basis of the EC50 is not specified or no ErC50 is recorded, classification shall be based on the lowest EC50 available". In this instance, the experimentally available data should take precedence over model-derived data and the M-factor for acute toxicity should remain as 10.

RAC's response

Chronic M-factor of 1 as suggested is noted and agreed to by RAC. As regards the acute M-factor, this point is further developed in the opinion.

Date	Country	Organisation	Type of Organisation	Comment number
24.02.2015	Belgium		MemberState	35

Comment received

Based on the results of the aquatic toxicity test on the most sensitive species (algae Scenedesmus subspicatus with 96hErC50 = 0.12 mg/l (nom) and a 96hNOEbC=0.021mg/l), the fact that the substance is not rapidly degradable it is justified to classify, following the classification criteria of the regulation 1272/2008, as Aquatic acute 1, H400 and Aquatic chronic 1, H410 . Furthermore, it is unlikely that the substance has the potential to bioaccumulate.

We agree with the proposed environmental classification. However we do question the proposed M-factors.

Acute toxicity : In table 50 also a 96hErC50=0.12mg/l (nom) for Scenedemsus subspicatus is reported. We prefer to use this value to decide on the aquatic acute toxicity as the use of growth rate is preferred as endpoint for classification as it is test design independent in comparison to the biomass. So the M-factor should be 1 instead of 10.

Chronic toxicity : The substance is not rapidly degradable and the NOEbC (no NOErC reported) is between 0.01mg/l and 0.1 mg/l, resulting in a M-factor of 1 instead of 10.

Some editorial or/and minor comments :
5.3.1.1. BCF : For substances with high lipophilicity(log Kow >3) the BCF should be lipid normalised.

5.4.2.1 Short-term toxicity to aquatic invertebrates : table 78 with the effects on Daphnia magna is missing

Dossier Submitter's Response

"Based on the results of the aquatic toxicity test on the most sensitive species (algae Scenedesmus subspicatus with 96hErC50 = 0.12 mg/l (nom) and a 96hNOEbC=0.021mg/l), the fact that the substance is not rapidly degradable it is justified to classify, following the classification criteria of the regulation 1272/2008, as Aquatic acute 1, H400 and Aquatic chronic 1, H410 . Furthermore, it is unlikely that the substance has the potential to

bioaccumulate". DS: We agree with the classification as acute 1 and chronic 1 with the hazard statements H400 and H410 "Acute toxicity : In table 50 also a 96hErC50=0.12mg/l (nom) for Scenedemsus subspicatus is reported. We prefer to use this value to decide on the aquatic acute toxicity as the use of growth rate is preferred as endpoint for classification as it is test design independent in comparison to the biomass. So the M-factor should be 1 instead of 10". + DS: The M-factor for acute toxicity should remain as 10 as the algal growth inhibition study did not report the ErC50 for this study. In previous discussions regarding this issue with ECHA and EFSA during peer review of cyproconazole, for biocides and PPP, the EbC50 value was accepted in the absence of the ErC50. This is in agreement with Annex I: Table 4.1.0 (Note 2) of the CLP Reg (EU) No. 1272/2008 which states "Classification shall be based on the ErC50 [= EC50 (growth rate)]. In circumstances where the basis of the EC50 is not specified or no ErC50 is recorded, classification shall be based on the lowest EC50 available". In this instance, the experimentally available data should take precedence over model-derived data and the M-factor for acute toxicity should remain as 10. "Chronic toxicity : The substance is not rapidly degradable and the NOEbC (no NOErC reported) is between 0.01mg/l and 0.1 mg/l, resulting in a M-factor of 1 instead of 10". DS: Agree. The M factor for chronic toxicity should be 1 instead of 10. Some editorial or/and minor comments : 5.3.1.1. BCF : "For substances with high lipophilicity(log Kow >3) the BCF should be lipid normalised". DS: Agreed. 5.4.2.1 "Short-term toxicity to aquatic invertebrates : table 78 with the effects on Daphnia magna is missing" DS: Agreed. The effects on Daphnia magna should be included in the table (Table 78).
RAC's response
Chronic M-factor of 1 as suggested is noted and agreed to by RAC. As regards the acute M-factor, this point is further developed in the opinion.

ATTACHMENTS RECEIVED

The following non-confidential attachments were submitted on 25.02.2015 by Syngenta during the public consultation:

1. Cyproconazole Syngenta Public Comments Summary & Corrections *(Filename: Cyproconazole Syngenta Public Comments Summary & Corrections) [Please refer to comment 3]*

2. Cyproconazole Syngenta Public comments Toxicokinetics *(Filename: Cyproconazole Syngenta Public comments Toxicokinetics) [Please refer to comment 3]*

3. Cyproconazole Syngenta Public Comments Environmental Hazard *(Filename : Cyproconazole Syngenta Public Comments Environmental Hazard.Docx) [Please refer to comment 3]*

4. Syngenta Position on Mode of Action and Human Relevance of Cyproconazole Induced Liver Tumours in the Mouse *(Filename: Cyproconazole Syngenta Public Comments Carcinogenicity) [Please refer to comment 7]*

5. Syngenta Position on Developmental Toxicity *(Filename: Cyproconazole Syngenta Public comments Developmental Toxicity) [Please refer to comment 14]*

6. Cyproconazole position on STOT-RE in response to CLH dossier *(Filename:Cyproconazole Syngenta Public Comments on STOT) [Please refer to comment 28]*