Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

triticonazole (ISO); (*RS*)-(*E*)-5-(4-chlorobenzylidene)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol

EC Number: -
CAS Number: 138182-18-0

CLH-O-0000001412-86-296/F

Adopted

20 September 2019

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON TRITICONAZOLE (ISO); (RS)-(E)-5-(4-CHLOROBENZYLIDENE)-2,2-DIMETHYL-1-(1H-1,2,4-TRIAZOL-1-METHYL)CYCLOPENTANOL

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: triticonazole (ISO); (*RS*)-(*E*)-5-(4-chlorobenzylidene)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-methyl)cyclopentanol
EC number: -
CAS number: 138182-18-0
Dossier submitter: Austria

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
11.02.2019	Germany		MemberState	1
Comment received				
It is not yet fully clear whether the classification as STOT RE 2 is justified (see comment below).				
Dossier Submitter's Response				
CLH proposal has been submitted in order to discuss the appropriatness of STOT RE proposal.				
RAC's response				
RAC agreed with the dossier submitter (DS) and considered that the effects reported in the liver and adrenals observed in rat, mice and dog studies, were not sufficiently severe to conclude on a classification as STOT RE for triticonazole. However, in the developmental toxicity studies in rabbits, mortality after several days of dosing was observed at doses < 300 mg/kg bw/d which is the guidance value (GV) used for a STOT RE 2 classification from a 28-d study. There was no data available to indicate a non-relevance to humans for the mortality reported in the rabbits. Based on this, RAC concluded that classification as STOT RE 2 was justified.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
11.02.2019	Germany		MemberState	2
Comment received				
In the rat study (Augthon 1994), increased incidence of tumours was seen in three organs (pituitary, skin, thyroid). In chapter 10.9.2. of the CLH dossier, only tumours occurring in thyroid are discussed. Even if uncertainties remain for increased incidences of skin and pituitary tumours, these tumours should be also mentioned in chapter 10.9.2.				

Has a trend test been performed for the increased incidence of pituitary adenoma in male rats? Furthermore, please describe 95 percentil of HCD rather than min/max range.
Considering the statistically significant increase in keratoacanthoma in males (without clear dose response), literature search about incidence of keratoacanthoma in CD rats could be performed by the Dossier Submitter.
Is there any data available about about liver enzyme induction and increased turnover of plasma T3 and T4 levels and subsequently stimulation of TSH? This would be a plausible mechanism for the increased incidence of follicular cell adenoma, which is not relevant for humans.
Classification for Cat. 2 should be discussed and based on additional information requested.

Dossier Submitter's Response

All tumours tables/listings/discussions are included in Annes to CLH report and can be seen there, if necessary.

Pituitary adenoma:
While for genotoxicity studies 95th percentiles for HCD are usually presented, this is not the case for the general toxicity studies. In current case, range can only be given. Please consider that very high background incidence for pituitary adenoma and lack of any dose-response (males: 38% (control) - 63% -56% - 58% - 58%; females: 65% (control) – 69% - 77% - 90% - 64%) are arguments aginst any treatment-relation. The trend test revealed no trend in increase of incidences with dosing.

Keratoacanthoma in males:
Keratoacanthoma is one of the most common cutaneous neoplasma in rats. The incidence of keratoacanthomas depends on the system of histologic criteria used for classification and is therefore very variable over the studies. In the long-term study with triticonazole, no dose-response was observed, and the statistically significant findings in males at low (5 animals) and high dose (6 animals), but not at two middle doses (2 animals each), are considered as spontaneous. No findings were observed in females which is in line with information from literature that keratoacanthoma are more common spontaneous findings in males.

Thyroid:
In the CLH report and its Annex it is explained in detail why incidence of follicular cell adenoma in males is not considered treatment-related. No data are available on liver enzyme induction and potential increased turnover of plasma T3 and T4 levels and subsequently stimulation of TSH.

DS is not of the opinion that above mentioned tumours qualify triticonazole for carcinogenicity classification.

RAC's response

RAC discussed the induced pituitary adenoma, keratoacanthoma in the skin and thyroid follicular cell adenoma in the rat carcinogenicity study. In the mice carcinogenicity study no tumours were reported. Therefore, based on the results from the two carcinogenicity studies and using a weight of evidence assessment of the two studies, RAC supported the DS and concluded that no classification for carcinogenicity following exposure to triticonazole was justified.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
11.02.2019	Germany		MemberState	3

Comment received

In CA assay (Dance 1992), an increased polyploidy was seen at 250 and 500 µg/ml (above HCD). As discussed in Volume 1.2.5., no such effect was seen in another, comparable in vitro study nor in vivo. However, it should be noted that exposure of bone marrow was not proven in the in vivo MN test (Edwards 1992). In vivo MN assay is of limited sensitivity for aneugenic effects, requiring sufficient bone marrow exposure over prolonged duration covering more than one cell cycle.
In the CA assay (Marshall 1997), the results are not negative, but should be considered as equivocal (same conclusion was reached by EPA). In the first trial, an increase in cells with aberrations was noted (dose-dependently). This indicates that the test material is possibly genotoxic. The same conclusion was reached by EPA. Bone marrow exposure in vivo has to be demonstrated to rebut this finding.
In addition, an alert for in vivo clastogenicity is found (DE analysis Toxtree, alert QSA34).

Dossier Submitter's Response

DS acknowledges that results observed in Marshall 1997 could be also considered as equivocal, although weight of evidence would speak for negative. Originaly the negative conclusion by RMS was based on the fact that equivocal results observed in the first experiment (positive only in one of two replicates at two concentrations) were not confirmed throughout the second experiment.
However, even if the results of this study would be considered equivocal, second *in vitro* study (Dance, 1992) and *in vivo* MN assay (with prolonged sampling time of 72 hours, which also covers aneugenicity requirement) were negative, proving lack of clastogenicity/aneugenicity potential of triticonazole.

Polyploidy is a common finding in chromosome aberration assays. Although it is recognised that aneugens can induce polyploidy, polyploidy alone does not indicate aneugenic potential and can indicate just the cell cycle perturbation. It is also commonly associated with increasing cytotoxicity. According to ICH S2(R1) (*Genotoxicity testing and data interpretation for pharmaceuticals intended for human use*), if polyploidy, but no structural chromosomal damage is seen in *in vitro* assay, a negative *in vivo* MN assay with assurance of appropraiate exposure would provide sufficient evidence for lack of potential for induction of aneuploidy.

For triticonazole, a well-performed *in vivo* MN assay with even extended sampling time up to 72 hours is available. Although there is currently no official recommendation for the appropriate *in vivo* assay for covering aneugenic effects, it is recognised by experts in field of genotoxicity that a well- performed *in vivo* MN assay with 72 hours sampling time should be sufficient to detect potential aneugens.

In *in vivo* MN assay conducted with triticonazole (Edwards, 1992), clinical signs were observed in all mice treated at 125 and 625 mg/kg (transient overactivity after dosing). In addition, some mice treated at 625 mg/kg showed transient piloerection and hunched posture. There was also a slight body weight loss in six of 10 mice treated at 625 mg/kg and sacrificed after 24 hours.

From short-term and long-term studies conducted in rats and mice it is obvious that in mice studies at least the same, if not lower, NOAEL, has been derived. From the whole data package for triticonazole there is no indication for potential different ADME behaviour between rats and mice.

RMS would consider that sufficient information is available to support the evidence of bone marrow exposure in mice and to extrapolate this to rats.
RAC's response
No germ cell mutagenicity studies in animals were available with triticonazole. Results from studies in somatic cells *in vitro* or *in vivo* did not give any indication for potential mutagenicity. RAC agreed with the DS and concluded that no classification for mutagicity was justified.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
11.02.2019	Germany		MemberState	4
Comment received				
In the 2 generation study in rats (Henwood 1993), mean body weights of F1 females were statistically significant decreased in week 0 compared to control group. Did the Dossier Submitter check the individual data? When exactly did treatment start? Classification for Reproductive toxicity Category 2 H361f should be discussed. Based on hydronephrosis and skeletal findings, Classification for Reproductive toxicity Category 2 H361d should be also discussed.				
Dossier Submitter's Response				
The treatment of F1 animals is a continuum. Young born rats are lactated for around first 10 days to 2 weeks. They start feeding around week two after the gestation, when they start to open their eyes. The eyes are fully open around week 3 post-gestation. All classification classes are often for commenting and discussion. DS has included all available information into the CLH report and Annex to CLH report. RAC should discuss and decide upon the appropriate classification.				
RAC's response				
In the two-generation study a statistically significant reduction in the mating and fertility index, that was outside the historical control data (HCD), was reported in the F1 generation in the high dose group, but not in the F0 generation. This effect was not solely considered as secondary consequences of parental toxicity. Effects were only reported in the high dose group, but is should be noted that a very large dose space was used in the study between the high dose and the next lower dose (5000 ppm vs 750 ppm) complicating the observation of a dose-response for the effect. RAC concluded on classification as Repr. 2; H361f. For developmental toxicity, clear maternal toxicity was reported in the developmental toxicity studies in rat and rabbit, including death in rabbit dams in the presence of developmental toxicity. RAC therefore considered that the effects were not relevant for classification for developmental toxicity. RAC agreed with the DS that classification for developmental toxicity was not justified.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
11.02.2019	Germany		MemberState	5

Comment received

A "cut-off value" for STOT RE 2 of 300 mg/kg/d is given in table 50 (page 55 of the CLH-report) for the relevant studies (Bailey 1990, Burns 1991). This cut-off value is applicable for 28-d-studies, however the number of days with dosing was smaller in these studies. When the dosing began on day 6 after insemination ca. 2-3 days of exposure occurred presumably in the study of Bailey 1990 and ca. 7-12 days in the study of Burns 1991. For such cases the guidance on the application of the CLP criteria (Version 5.0, 2017, p. 464) recommends a further modification of the cut-off value. An example is given there: "For example, the effects in an oral range-finding study of 9 days or less should be compared with a guidance value of 1000 mg/kg bw/day for STOT-RE Category 2." Thus, it is recommended to check the days of exposure and dosing and to modify the cut-off value/guidance value accordingly. If e. g. a guidance value of 1000 mg/kg bw/day would be applicable for STOT RE 2 (100 mg/kg for STOT RE 1) the lethal dose of 75 mg/kg in the study of Burns 1991 might justify STOT RE 1.
Furthermore it is stated in the guidance (chapter acute toxicity, p. 236): "Mortalities during the first 72 h after first treatment (in a repeated dose study) may also be considered for the assessment of acute toxicity." This might affect the study of Bailey 1990 with a very small number of exposure days. However a double classification should be avoided.
In Table 7, the proposal should be made accordingly. At the moment, it is written "conclusive, but not sufficient for classification".

Dossier Submitter's Response

It is acknowledged that STOT RE "cut off values" might be adapted for shorter exposure. As rabbits were exposed on days 6 to 19, STOT RE "cut off" of 75 mg/kg bw/d (STOT RE 1) and 750 mg/kg bw/d (STOT RE 2) would be more applicable. The rabbit mortality/morbidity occurs both at doses below the guidance values originally derived in the CLH report (30 mg/kg bw/d for STOT RE 1 and 300 mg/kg bw/d for STOT RE 2), as well as below guidance values adapted for shorter duration (75 mg/kg bw/d for STOT RE 1 and 750 mg/kg bw/d for STOT RE 2).

RAC members are kindly asked to discuss which (and if any) classification is appropriate.

RAC's response

RAC agreed with the DS and considered that the results of the rabbit range-finding study should not be used for classification as oral acute toxicity since according to the CLP criteria death should be observed after single exposure.

As regards classification as STOT RE 2, RAC agreed with the DS and considered that the effects reported in the liver and adrenals observed in rat, mice and dog studies, were not sufficiently severe to justify classification as STOT RE for triticonazole. However, in the developmental toxicity studies in rabbits, mortality was observed after several days of dosing at doses < 300 mg/kg bw/d (GV for a STOT RE 2 classification from a 28-day study). There was no data available to indicate non-relevance to humans for the mortality reported in the rabbits. RAC concluded on classification as STOT RE 2.

Date	Country	Organisation	Type of Organisation	Comment number
14.02.2019	Germany	BASF SE	Company-Manufacturer	6

Comment received

In summary, BASF disagrees with the STOT RE 2 based on mortality in the rabbit study. Shown in a poster from ECHA at the EUROTOX 2018 (Reuters et al. 2018) and also discussed in the supplementary renewal dossier of Triticonazole for the AIR 3 process, the rabbit is known to be susceptible for gastrointestinal imbalances (GI). These GI can result in abortion, fetal resorption and maternal death. The discussed prenatal rabbit study shows several indications for GI: Reduced food consumption (between day 6-12 reduced by 21.7% and between day 13-19 reduced by 17.6%) at doses ≥50 mg/kg bw/day and reduced defecation indicate a rabbit specific and hence non-human relevant effect on the gastrointestinal tract. Therefore, classification with STOT RE 2 is not warranted.
Please see more details on the argumentation below:
The RMS lists typical findings that are associated with gastrointestinal disorders in the rabbit. The RMS doesn't consider mucoid enteropathy as an effect, because anorexia, lethargy, crouched stance, diarrhea, succession splash, teeth grinding, cecal impaction and mucous production is only partially observed in the study (Findings described in Percy and Barthold, 2007). However, it is stated in the publication that these findings are often, but not always combined with these effects. There was no indication for infection, diarrhea or irritation, which are all common causes of gastrointestinal disorders, and associated with the clinical symptoms indicated by the RMS.
The data for maternal toxicity listed in the respective description for the rabbit developmental toxicity study and the range finder are limited. BASF acknowledges that the lack of a full histopathologic investigation of the intestinal tract and measurement of the time of intestinal passage of the faeces, the cecal microbiome or the fibre content of the faeces of all animals precludes an in-depth investigation of alimentary disorder of the dams.
However, there are a number of findings in animals killed in extremis, and the other does, that indicate gastrointestinal issues:
• At 50 mg/kg bw and above, food consumption and body weight are significantly impaired starting from gestational day 6 to 19. During this time period, reduced faeces under tray is a seen in in 0, 3, 6, 7 and 15 does at 0, 5, 25, 50 and 75 mg/kg bw/day, respectively. Furthermore, all animals killed in extremis displayed red staining under tray, which might be indicative of intestinal bleeding.
• As indicated in Table 10.12.1-1, of the CLH report listing clinical and necropsy findings of rabbits prematurely sacrificed, several other indications of intestinal problems were identified in individual animals killed in extremis.
Consequently, albeit the exact type of intestinal disorder could not be shown, and several severe signs of intestinal disorders have not been seen, there is significant evidence that treatment with Triticonazole resulted in rabbit specific toxic effect on the intestinal tract, which is not human relevant. Therefore, classification with STOT RE is not warranted.

Dossier Submitter's Response

DS considered every single sacrificed animal and listed individually the observed necropsy findings in gastro-intestinal tract in the sacrificed rabbits (please see summaries of the two rabbit developmental studies).
According to the literature data and knowledge on rabbit physiology, the most common symptoms of gastrointestinal disorders, among others mucous enteropathy, in rabbits, are anorexia, lethargy, crouched stance, diarrhea, teeth grinding, cecal impaction, accumulation of large quantities of clear gelationous mucous in the colon. At necropsy, the stomach may be distended with fluid and gas. The jejunum is frequently distended

with translucent, watery fluid. Most of the gastro-intestinal disorders in rabbits come from wrong nutrition, causing dysbiosis in the very fragile rabbit digestive system. DS acknowledges that most of rabbit gastro-intestinal disorders can hardly be extrapolated to humans, based on very specific gastro-intestinal properties of rabbits. The most prominent symptom in sacrificed rabbits in the preliminary and the main study was loss of weight. Necropsy findings in thoracic cavity of sacrificed rabbits were inconsistent. Some sacrificed rabbits did not show signs of gastro-intestinal disorder. By balancing the available data DS cannot conclude that rabbit mortality at doses below the cut-off values for STOT RE 2 was solely due to disorder in rabbits gastro-intestinal tract. The animals were sacrificed based on their bad health conditions. The most prominent effect of higher doses of triticonazole on rabbits was that they either stopped feeding or markedly reduced their food and water consumption and finally lost weight remerkably. The reason for reducing food and water consumption cannot be clarified. It cannot be disregarded that this could be a result of toxicity of triticoazole to rabbits. Systemic toxicity in developmental studies is measured in very limited set of parameters. While for other species (rat) also other studies with much more extensive data set are available, this is not the case for rabbits. Therefore, rabbit developmental studies have to be taken for themselves alone. Based on the results od the rabbit studies it is assumed that the rabbit mortality/morbidity can be explained by a very high sensitivity of this species. Considering the limited effects on the rabbit pups (mostly skeletal variations), there is no reason to believe that the mortality is specific for pregnant rabbits, but rather is a general effect of triticonazole on rabbits.
RAC's response
RAC agreed with the DS and considered that the rabbit mortality reported in the developmental toxicity studies, that were observed after several days of dosing, at doses < 300 mg/kg bw/d (GV used for a STOT RE 2 classification from a 28-day study) was relevant for classification as STOT RE and not considered to be due to a rabbit specific effect on the GI tract. There was no data available to indicate non-relevance to humans for the mortality reported. RAC concluded on classification as STOT RE 2.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
15.02.2019	France		MemberState	7
Comment received				
FR agrees with the proposal of classification for environmental hazards and the proposals of acute and chronic M factors.				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
13.02.2019	Germany	BASF SE	Company-Manufacturer	8
Comment received				
Table 73: Summary of relevant information on bioaccumulation				

BASF: The RMS states that the study used for the derivation of the BCF (L. macrochirus) is valid with restrictions, as the results of the study indicate uncertainties and some information is missing in the study report (lipid content of fish, TOC, testing of a second concentration)."
BASF believes it would not be justified to request a new study, due to the low experimental and calculated BCFs as well as animal welfare concerns (over 200-300 fish are used for a single BCF study).
The RMS stated that "the results of the study indicate some uncertainties as the bioconcentration factor seems to first decrease and then increase again." In the study report, BCFs were calculated on days 1, 3, 7, 14, 21, and 28. The following BCFs in whole fish correspond to those obtained on sequential days: 28, 53, 68, 87, 94, and 65. Therefore, the bioconcentration factor increases steadily throughout the uptake phase but also decreases at the last time point of the uptake phase (28 days). Due to the decrease at the last sampling point one could assume that steady state is reached. As a conservative approach the highest BCF measured in the uptake phase could be used (i.e. 94 L/kg) or alternatively, as suggested in OECD 305 (cf. paragraph 38) (2012), the kinetic BCF. The BCFk(TRR) (i.e. 72.55L/kg) calculated here is appropriate and serves as a reliable endpoint value.
Regarding lipid content of fish, BASF collected historic lipid data from 14 bluegill BCF studies from different CROs done prior to 2015. These studies included lipid content measurements throughout the study period, and a mean lipid content across all studies was calculated for this species. Based on this approach, the average lipid content was determined to be 5.5%. Using the kinetic BCF of 72.55, this would result in a lipid-normalized kinetic BCF (BCFKL(TRR)) of 66.0 L/kg, a value even lower than the initially reported kinetic BCF. This value further supports the low bioaccumulation potential of triticonazole.
Regarding the TOC measurements, charcoal-filtered dechlorinated tap water was used for the bluegill BCF study. As normal tap water has a TOC <2 mg/L, it is likely that the treated tap water used in this study had <2 mg/L TOC at test initiation. However, this is only a minor deviation from the study guideline, which does not justify a request for the study to be repeated.
Regarding the uncertainty of testing two concentrations, OECD 305 states that "The testing of only one test concentration can be considered sufficient, when it is likely that the bioconcentration factor (BCF) is independent of the test concentration." OECD 305 also states that: "The test was originally designed for non-polar organic substances. For this type of substance, the exposure of fish to a single concentration is expected to be sufficient, as no concentration effects are expected." Prior to the bluegill study, a study with rainbow trout had also been completed. This study used two concentrations (nominal = 100 and 400 µg a.i./L, measured = 90 and 160 µg a.i./L), and showed no significant difference in BCF (11.67 and 12.75, respectively) based on concentration. Although the rainbow trout study was considered invalid by the RMS (Vol. 3CA. B.9.2.4) due to lack of oxygen measurements and failure to maintain concentration of the substance in the chambers is within ±20% of the mean measured values during the uptake phase at the highest dose only, there was no significant difference in experimental BCFK (11.67 and 12.75, respectively). Therefore, based on these results that show concentration effects are not expected and to reduce the use of animals and/or resources, the use of a single concentration is justified in the bluegill study.
In addition, an extensive data review of Creton et al. (2013) supports the use of only one test concentration in BCF studies specifically for plant protection products. The researchers reviewed 55 active substances with a wide log Kow range (-0.81 to 6.9) and various modes of action. They compared BCF values from low and high test concentrations (generally a factor of 10 apart) and found a linear relationship for all

examined dimensions (whole body, edible and non-edible tissue). Among the 55 reviewed active substances, some triazoles were present, e.g. prothioconazole, triticonazole, metconazole and epoxiconazole. The ratio between the 'low-concentration BCF' and 'high-concentration BCF' for triazoles differed only between 0.85 and 1.19. Paragraph 78 from the OECD 305 guidelines defines that a concentration dependence is not indicated if uptake and depuration rate (and therefore also the kinetic BCF as a function of these rate constants) vary by less than 20% from two test concentrations. This is the case for plant protection products and specifically for triazole fungicides.
The review by Creton et al. (2013) demonstrates clearly that no significant difference between the BCF in low and high concentrations can be found for plant protection products, although the data set considered substances with highly differing physico-chemical properties and even different fish species in the tests.
In order to minimize vertebrate testing and since no concentration effect is expected for triticonazole, only one test concentration was chosen to be sufficient for the respective bioconcentration study in fish.
As suggested by the co-RMS, UK, BASF used the EPISuite module BCFBAF to calculate the BCF for triticonazole. The input parameters of log kow = 3.3 and the smiles code for triticonazole (i.e. c1cc(Cl)ccc1C=C2CCC(C)(C)C2(O)Cn3ncnc3) were used. The calculated BCF value was determined to be 69.88. This is in line with the experimental value provided in the BCF study with L. macrochirus, which was 72.55, and within the same order of magnitude as the BCF of 12.8 reported in the invalid rainbow trout study. Therefore, according to both the experimental and calculated BCF values, BASF believes that there is low bioaccumulation potential of triticonazole in fish.
Additionally, the co-RMS also suggested predicting the BCF for metabolite RPA 406203 using EPI Suite. For the metabolite, RPA 406203, a log kow = 3.5 was used for the EPISuite calculation. This resulted in a BCF = 94.7. This value is below the trigger of 2000 and therefore the concern for bioaccumulation is low and a further assessment addressing bioaccumulation is not necessary. This metabolite has also been tested in Daphnia and green algae, and the toxicity values are similar for triticonazole and metabolite RPA 406203.
References:
OECD (2012): Test No. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing
Creton S., Weltje L., Hobson H., Wheeler J.R. (2013): Reducing the number of fish in bioconcentration studies for plant protection products by reducing the number of test concentrations. Chemosphere 90 (2013), 1300–1304.

Table 74: Summary of relevant information on acute aquatic toxicity

The test organism for this study is incorrectly listed. Please update the test organism to correctly state that this study is for bluegill sunfish (Lepomis macrochirus).

Table 75: Summary of relevant information on chronic aquatic toxicity

The survival NOEC is incorrectly reported. Based on the study report, no effects on survival were observed in either generation in the study. Therefore, please update the NOEC to be > 0.0462.

Table 75: Summary of relevant information on chronic aquatic toxicity

Validity of the study is considered borderline by the RMS due to the following open questions (presented below in italics):

- According to OECD 219 quantification of the test substance in overlying water, pore water and sediment with known and reported accuracy and limit of detections should be available. In the study only the quantification of the test substance in overlying water was conducted.
BASF: According to OECD 219 (2004) measurement in sediment might not be necessary when comparable water/sediment partitioning studies are available. As this is the case for triticonazole, measurement of the test concentrations in the water, which is the most relevant matrix, is considered sufficient. It is state-of-the-art to measure test concentrations in water, since the risk assessment for plant protection products according to the EFSA Aquatic Guidance compares endpoints based on the concentrations in water with PECSW values in the water phase (both in µg/L).
-Regarding the quantification of the test substance in pore water and sediment the Notifier argues that a water-sediment study under comparable conditions is available determining the partitioning of the test substance between water and sediment. However, upon consultation with the environmental fate expert, the water/sediment studies were not conducted under comparable conditions as for example the sediment to water ratio was not the same.
BASF: The sediment-to-water ratio in the water/sediment study, which was performed according to OECD TG 308, was between 1:3 and 1:4. In the chronic chironomid study this ratio was 1:8. However, the higher water column and less sediment in relation to water in the chronic chironomid study can be considered as worst-case scenario due to the slower diffusion of triticonazole from the water into the sediment.
- According to OECD 219 the sediment/water ratio should not exceed 1:4. The ratio in the test was 1:8.
BASF: As this is not a validity criterium and only a recommendation, the test can be still considered as valid.
The RMS states that "the available endpoints for daphnids of 0.11 mg ai/L and 0.19 mg ai/L are above the trigger, but only very marginal. Therefore the RMS considers a study on sediment dwelling organisms necessary."
According to the EFSA Aquatic Guidance Document (2013), the trigger for testing of sediment-dwellers is a NOEC (or EC10) of <0.10 mg/L for chronic daphnia tests.
The old agreed endpoint as proposed by the applicant with NOEC= 0.43 mg/L exceeds the trigger clearly. BASF agrees that the recalculated endpoint proposed by the RMS with NOEC= 0.11 mg/L as well as the other daphnid endpoint of NOEC = 0.19 mg ai/L both exceed the trigger and therefore the lowest endpoint does not fulfil the given criteria for testing sediment-dwellers.

Dossier Submitter's Response

Table 73: Summary of relevant information on bioaccumulation

The reliability of the respective study is expected to be discussed in an expert consultation.

According to OECD 305 steady state is reached when the curve of the test substance in fish (Cf) against time becomes parallel and three successive analyses of Cf made on samples taken at intervals of at least two days are within ± 20% of each other, and there is no significant increase of Cf in time between the first and last successive analysis.

In this study measurements on day 14 (6336 ng equiv/fraction) and day 21 (6769 ng equiv/fraction) for whole fish did not differ more than 20 % For edible parts and unedible parts this was not the case. Furthermore the standard deviation was high 2440 and 1974 for whole fish on day 14 and day 21, respectively.

The applicant argues that the BCF for whole fish decreases at the last sample point of the uptake phase indicating that the steady-state was reached. However, it has to be

mentioned that at this time point the fish were still exposed and it is not clear why a decrease should take place.

Therefore the assumption that the steady state could be reached after 28 days is based on rather uncertain values.

The approach with the averaging of the lipid content from historical data may be acceptable, however, detailed information and verification of the studies has to be provided.

The lack of reporting the TOC is a further uncertainty of this study. However, the RMS considers the argumentation of the applicant reasonable.

Even if the endpoint of this study is not fully reliable it may be used in the risk assessment as the value very low and supported by the modelled BCF.

However, it is noted that a valid model does not necessarily produce a valid prediction. Therefore it is necessary to strictly follow the instructions for QSARs given in the aquatic Guidance - EFSA Journal 2013;11(7):3290.

Table 74: Summary of relevant information on acute aquatic toxicity
The test organism for the study Study No. BASF DocID 2006/1018146 is the Bluegill sunfish (Lepomis macrochirus)

Table 75: Summary of relevant information on chronic aquatic toxicity

The NOEC for survival of the Study No. BASF DocID 2008/1028361 is > 0.0462 mg/L

Table 75: Summary of relevant information on chronic aquatic toxicity
The available study on Chironomus riparius is not considered reliable. It is acknowledged that the uncertainties identified are not responsible for the valitiy of the study, but for the reliability which is not given.
The comparability of the water/sediment study is not given as on the one hand the water/sediment ratio is not the same and on the other hand the organic carbon content is not comparable (approx. 5% in the Chironomus study versus max. 1.4% in the water/sediment study).

In the data requirements it is stated that the impact on a sediment-dwelling organism shall be assessed, if accumulation of an active substance in aquatic sediment is indicated or predicted by environmental fate studies.
The aquatic guidance recommends as trigger for sediment dwelling organism testing a long-term toxicity of < 0.1 mg ai/L for daphnid (or another comparable tested species) long-term toxicity in combination with >10% of applied radioactivity at or after day 14 present in the sediment.
The water/sediment study shows that triticonazole partitioned from water to sediment by more than 50% after 14 days and more than 70% after 105 days. Triticonazole therefore highly accumulates in the sediment.
The available endpoints for daphnids of 0.11 mg ai/L and 0.19 mg ai/L are above the trigger but only very marginal. Furthermore a study with Mysidopsi bahia results in a NOEC of 0.041 mg/L which meets the trigger. Therefore the RMS considers a study on sediment dwelling organisms necessary.

RAC's response
Regarding the bioaccumulation in fish study: RAC notes that based on the BCFs indicated in the CLH report and its Annex, the whole fish BCF values increased until day 21 of the uptake phase and decreased on day 28. Therefore, based on the available information the whole fish BCF did not first decrease and then increase again as stated by the DS. However, RAC agreeed with the DS that it is not clear whether steady state was reached in the study, and hence, the kinetic BCF should be preferred. Regarding the missing data on lipid content, RAC agreeed with the DS that this leads to some uncertainty regarding the BCF values. RAC considered that the use of historic data on lipid content cannot be used in the lipid-normalisation as the lipid content can be affected by many factors, e.g. test conditions, feeding, fish age. Furthermore, RAC notes that the test concentration used in the BCF study (0.089 mg/L) is above the NOEC of 0.011 mg/L determined for *Pimephales promelas* based on growth. Therefore, although the available NOEC is for another fish species than the one used in the BCF study, it cannot be excluded that the growth, and consequently the lipid content, of the exposed fish in the BCF study were possibly affected by the test substance. As to the lack of information on TOC, since charcoal-filtered dechlorinated tap water was used as dilution water it is expected that the TOC level was not high and it does not affect the reliability of BCF significantly. Regarding the testing of only one concentration, RAC agreed with the DS that there is some uncertainty in the BCF value as only one concentration has been tested. RAC notes that the BCF study with rainbow trout referred to in the comment does not seem valid as the test concentrations were not properly maintained and the oxygen levels were not measured. Furthermore, the test concentrations used in the study (0.090 and 0.160 mg/L measured) are above the 28-d NOEC of 0.01 mg/L determined for the same specie rainbow trout in a fish juvenile growth test included as additional information in the Annex of the CLH report, which adds further uncertainty to the reliability of the study. Therefore, the results from the BCF study with rainbow trout should not be used to justify the use of only one concentration for triticonazole. It is also noted that in the study by Creton et al (2013), the BCF values used for triticonazole seems to come from the same rainbow trout study. In conclusion, RAC agreed with the DS that, although some uncertainty remains regarding the available BCF study, considering that the available BCFs based on total radioactivity are low (below 100) and likely overestimate the BCF of the parent substance, and that rapid elimination of the substance was observed (depuration half-life < 1 day), the study can be used to conclude on the lack of bioaccumulation potential of the substance for classification purposes. *Regarding the available Chironomus riparius study:* RAC agreed with the DS that the study cannot be considered fully reliable. However, this does not affect the classification since valid chronic data is available for aquatic invertebrates, and hence, no further information is needed for classification purposes.

Regarding the remaining issues raised in the comment, RAC agreed with the DS's responses.

Date	Country	Organisation	Type of Organisation	Comment number
15.02.2019	Belgium		MemberState	9
Comment received				
BE CA supports your proposal to classify Triticonazole for the environment with Aquatic Acute 1, H400 (M=1) and Aquatic Chronic 1, H410 (M=1). Some editorial or/and minor comment : p. 112, 11.7.3, 3th bullet point : 192dNOEC=0.0114mg/L (mm, growth) was determined for Pimephales promelas instead of Oncorhynchus mykiss.				
Dossier Submitter's Response				
The 192dNOEC=0.0114mg/L (mm, growth) was indeed determined for *Pimephales promelas* instead of *Oncorhynchus mykiss*.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
05.02.2019	Netherlands		MemberState	10
Comment received				
We agree with the proposed classification. Please do note that with a classification as Aquatic acute 1 or Aquatic chronic 1 the signal word warning (Wng) should also be given on the label. This is currently not indicated in table 6 of the CLH-report.				
Dossier Submitter's Response				
We acknowledge that in table 6 the signal word Warning! is missing				
RAC's response				
Noted. RAC agreed that the signal word Warning should be given in the label.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Ozone Layer

Date	Country	Organisation	Type of Organisation	Comment number
11.02.2019	Germany		MemberState	11
Comment received				
page 3, point 2.1 Proposed harmonised classification and labelling (Table 6): We agree with the proposal of classification for environmental hazards as Aquatic acute 1 (H400), Aquatic chronic 1 (H410) and acute/chronic M-factor of 1.				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted.				