Committee for Risk Assessment

RAC

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

20 September 2019

CLH-O-0000001412-86-296/F

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

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

Chemical name: **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**

The proposal was submitted by **Austria** and received by RAC on **31 October 2018.**

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

PROCESS FOR ADOPTION OF THE OPINION

Austria has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **3 December 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **15 February 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Christine Bjørge**

Co-Rapporteur, appointed by RAC: **João Carvalho**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **20 September 2019** by **consensus**.

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

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	613-282-00-0	triticonazole (ISO); (*RS*)-(*E*)-5-(4-chlorobenzylidene)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-methyl)cyclopentanol	603-543-7	131983-72-7	Aquatic Chronic 2	H411	GHS09	H411			
Dossier submitter's proposal	613-282-00-0	triticonazole (ISO); (*RS*)-(*E*)-5-(4-chlorobenzylidene)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-methyl)cyclopentanol	-	138182-18-0	**Add** STOT RE 2 Aquatic Acute 1 **Modify** Aquatic Chronic 1	**Add** H373 H400 **Modify** H410	**Add** GHS08 Wng **Retain** GHS09	**Add** H373 **Modify** H410		**Add** M=1 M=1	
RAC opinion	613-282-00-0	triticonazole (ISO); (*RS*)-(*E*)-5-(4-chlorobenzylidene)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-methyl)cyclopentanol	-	138182-18-0	**Add** Repr. 2 STOT RE 2 Aquatic Acute 1 **Modify** Aquatic Chronic 1	**Add** H361f H373 H400 **Modify** H410	**Add** GHS08 Wng **Retain** GHS09	**Add** H361f H373 **Modify** H410		**Add** M=1 M=1	
Resulting Annex VI entry if agreed by COM	613-282-00-0	triticonazole (ISO); (*RS*)-(*E*)-5-(4-chlorobenzylidene)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-methyl)cyclopentanol	-	138182-18-0	Repr. 2 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H361f H373 H400 H410	GHS08 GHS09 Wng	H361f H373 H410		M=1 M=1	

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity

The dossier submitter (DS) included one oral acute toxicity study in CD Sprague Dawley rats (5 rats/sex/group) performed according to OECD TG 401 and GLP (Cummins, 1990). Only one dose of triticonazole was included in the study (0 and 2000 mg/kg bw). No mortality was reported and the LD_{50} value was > 2000 mg/kg bw.

In a rabbit range-finding developmental study morbidity was reported (animals were terminated 1-2 days after beginning of exposure) (Bailay, 1990). However, the DS did not consider the results of this study as appropriate to decide upon acute toxicity, which was considered to be observed after single exposure.

The DS concluded that no classification for acute oral toxicity according to Regulation (EC) 1272/2008 (CLP Regulation) was justified.

Acute dermal toxicity

The DS included one dermal acute toxicity study in CD Sprague Dawley rats (5 rats/sex/group) performed according to OECD TG 402 and GLP (Johnson, 1991). Only one dose of triticonazole was included in the study (0 and 2000 mg/kg bw). No mortality was reported and the LD_{50} value was > 2000 mg/kg bw.

Acute inhalation toxicity

The DS included three acute inhalation toxicity studies performed according to OECD TG 403 and GLP with concentrations of triticonazole up to 5.61 mg/L (Cracknell, 1991 and Bennick, 1998). No deaths were reported in the studies. Therefore, the DS concluded that no classification for acute inhalation toxicity according to the CLP Regulation was justified.

Comments received during public consultation

No comments on acute toxicity were received during the public consultation. However, one Member State Competent Authority (MSCA) commenting on the classification as STOT RE considered that the mortality/morbidity reported in the range-finding developmental toxicity study in rabbits should be discussed for classification for acute toxicity.

Assessment and comparison with the classification criteria

Acute oral toxicity

One oral acute toxicity study in CD Sprague Dawley rats (5 rats/sex/group) according OECD TG 401 was assessed (Cummins, 1990). Only one dose of triticonazole was included (0 and 2000 mg/kg bw). No mortality was reported and the LD_{50} value was > 2000 mg/kg bw. This value falls outside the cut-off value for acute oral toxicity Category 4 under CLP (> 2000 mg/kg bw).

In a rabbit range-finding developmental toxicity study (exposure during gestation day (GD) 6-19) all females in the top dose of 150 mg/kg bw/d were terminated prematurely on GD 8/9 after insemination due to animal welfare reasons (Bailay, 1990). RAC agreed with the DS and considered that the results of this study should not be used for a classification as oral acute toxicity since according to the CLP criteria death should be observed after single exposure.

No human data was available.

RAC concluded that **no classification for acute oral toxicity** is justified.

Acute dermal toxicity

One dermal acute toxicity study in CD Sprague Dawley rats (5 rats/sex/group) was assessed (Johnson, 1991). Only one dose of triticonazole was included (0 and 2000 mg/kg bw, 24 hours exposure). No mortality was reported and the LD_{50} value was > 2000 mg/kg bw. This value falls outside the cut-off value for acute oral toxicity Category 4 under CLP (> 2000 mg/kg bw).

No human data was available.

RAC concluded that **no classification for acute dermal toxicity** is justified.

Acute inhalation toxicity

Three acute inhalation toxicity studies were included by the DS. The first study was in CD Sprague Dawley rats (5 rats/sex/group) with one dose of 1.4 mg/L triticonazole in air (Cracknell, 1991). No deaths were reported and the LC_{50} value was > 1.4 mg/L.

The second and third study was in HSD Sprague Dawley rats (5 rats/sex/group) with one dose of 2.63 mg/L in the second and 5.61 mg/L in the third study (Bennick, 1998). No deaths were reported and the LC_{50} value was > 5.61 mg/L from both studies.

The cut-off value for acute inhalation toxicity (dusts/mists) according to the CLP criteria for category 4 is > 5 mg/L.

No human data was available.

RAC concluded that **no classification for acute dermal toxicity** is justified.

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

Summary of the Dossier Submitter's proposal

For the assessment of STOT SE the DS evaluated one acute oral neurotoxicity study performed according to OECD TG 424 and GLP. In addition, the acute toxicity studies in rats following oral, dermal and inhalation exposure reported in the acute toxicity section were assessed.

In the acute oral neurotoxicity study, Crl:CD rats (10/sex/group) were exposed to a single dose of 0, 80, 400 and 2000 mg/kg bw triticonazole (Weiler, 1997), respectively. The observation period was 14 days. No neurotoxic effects were reported in the study and the NOAEL was > 2000 mg/kg bw.

In the acute oral and dermal toxicity studies there were no indication that triticonazole induced toxicity to specific organs after a single exposure. In the acute inhalation toxicity studies there were no clinical signs of respiratory tract irritation following inhalation exposure to triticonazole. No human data were available.

In conclusion, from the available acute studies there was no evidence that a single exposure to triticonazole resulted in toxicity to specific organs, and thus a classification in STOT SE 1 or 2 is not justified.

As regards classification as STOT SE 3, there were no indications of triticonazole having a narcotic effect, and in the nose-only inhalation studies in rats, no clinical signs were observed which would indicate respiratory irritation.

Overall, the DS concluded that there is no evidence for a classification of triticonazole as STOT SE.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

Based on the results of the studies included by the DS on the CLH report, RAC concluded that classification as STOT SE 1 or 2 is not justified base on the absence of toxicity to specific organs after single exposure to triticonazole. Further, classification as STOT SE 3 is not justified since no respiratory tract irritation were reported in the acute inhalation toxicity studies and there were no indications of triticonazole having transient narcotic effect.

In conclusion, RAC agreed that based on the data available, **no classification of triticonazole for STOT SE** is justified.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS included one skin corrosion/irritation study performed according to OECD TG 404 and GLP in three male New Zealand White (NZW) rabbits and one acute dermal acute toxicity study in CD Sprague Dawley rats (Johnson, 1991). In the skin corrosion/irritation study, rabbits were treated with 0.5 g triticonazole moistened with 0.2 mL water for 4 hours (semi-occluded). No oedema or erythema was observed, and the primary irritation score was 0.0.

In the acute dermal toxicity study, one dose of 2000 mg/kg bw was applied on the skin (5 rats/sex/group). Local signs of irritation (very slight to well defined erythema and eschar formation) at the site of administration were observed in 2 females from day 3 to 10 after treatment. One of these two rats also showed loss of skin flexibility (days 3 - 6) and sloughing (days 7 - 10). The other rats that were affected demonstrated slight exfoliation. However, the DS found that the effects observed were difficult to interpret since no detailed observations or scores were available.

In a weight of evidence assessment, the DS concluded that since no signs of irritation were observed in the skin irritation study, designed to assess skin irritation/corrosivity, no classification as skin corrosion/irritation was proposed.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

RAC agreed with the DS that the dermal acute toxicity study (occluded conditions, 24-h exposure) was difficult to interpret since no detailed observations or scores were included. Since the primary irritation score from the skin corrosion/irritation study, designed for the assessment of skin corrosion/irritation (semi-occluded conditions, 4 hours exposure), was 0.0, and due to the limited information from the dermal acute toxicity study with a much longer exposure time, RAC concluded that **no classification for skin corrosion/irritation** is justified.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS included two studies in NZW rabbits (6 males/study) performed according to OECD TG 405 and GLP for the assessment of serious eye damage/eye irritation.

In the first study, after a single instillation of 100 mg triticonazole, a slight initial pain response was observed in three animals, with the other three rabbits unaffected (Johnson, 1991). During the first 24 hours following treatment, slight injection of the conjunctival vessels was observed in all rabbits. In addition, a very slight discharge was evident in four rabbits at the 1-h examination. No corneal effects or conjunctival chemosis was noted in any rabbit. Very slight iritis was also observed in two rabbits at the 1- and 2-h examinations. The eyes of five rabbits were apparently normal within 48 hours after treatment.

In 1/6 rabbits, iritis was evident from the 24-h observation through to 14 days, with a crescent shaped lesion in the pupil at 7 and 14 days. This was considered to be iris tissue, adhered to the lens as a result of the iridial congestion. The same animal showed moderate conjunctival redness from 1 hour to day 14, as well as pannus (abnormal layer of fibrovascular tissue or granulation tissue) formation at the day 14 examination.

In the 5/6 animals not showing a severe eye response, the mean scores (24 - 72 hours) were 0.0 (conjunctival chemosis), 0.1 (conjunctival redness), 0.0 (corneal opacity), and 0.1 (iridial lesions), respectively. In the severely affected rabbit, the mean scores were 0.0 for corneal opacity and conjunctival chemosis, 1.0 for iridial lesions and 1.7 for redness of the conjunctiva. Since the findings were considered to be an irreversible lesion of the eye, the animal was subsequently killed. This unexpected reaction in one animal only was considered likely to be idiosyncratic in nature by the study author.

In the second study, after a single installation of 100 mg triticonazole, all six animals showed a slight redness of the conjunctiva one hour after application, accompanied by slight discharge in one animal (Dange, 1997). However, the redness disappeared on day 1. According to the classification criteria, the overall mean scores from the 24, 48, and 72-hour observations for redness, chemosis, corneal opacity and iritis were all 0.0.

The DS concluded that based on the combined results from the two studies, the severe eye lesions in one animal in the first study can be considered to be likely idiosyncratic in nature. Therefore, triticonazole was not considered to meet the criteria for classification for serious eye damage/eye irritation, and no classification was proposed.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

The DS included two studies in NZW rabbits (6/males/study) performed according to OECD TG 405 and GLP for the assessment of serious eye damage/eye irritation.

In the first study, one rabbit showed an irreversible lesion of the eye and was subsequently killed. However, the effect reported in this rabbit was considered by the study author to be idiosyncratic in nature. RAC agreed with this assessment, and concluded that this effect should not be taken into account for classification. In the second study, no effects in the eyes relevant for classification as an eye irritant were reported.

In conclusion, RAC considered that based on the combined results from the two studies, **classification for serious eye damage/eye irritation is not justified**.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

There is no information available on the potential of triticonazole to induce respiratory sensitization. Therefore, no comparison with the CLP criteria can be done. The DS proposed no classification for respiratory sensitization based on the absence of data.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

No information was available to the DS on the potential of triticonazole to induce respiratory sensitisation.

In conclusion, RAC considered that **'no classification' for respiratory sensitization** is justified, **based on the absence of data.**

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS included three studies for the assessment of skin sensitisation, one Guinea Pig Maximisation Test (GPMT) with 10 animals/sex/group, one modified Buehler assay with 10 animals/sex/treated group and 5 animals/sex/control group and one Buehler assay with 10 animals/sex/group. All studies were performed in albino Dunkin Hartley Guinea pigs, according to OECD TG 406 (however, with some modifications) and GLP. No human data was available.

In the GPMT assay, triticonazole did not cause delayed contact hypersensitivity in Guinea pigs (Johnson, 1993). Mild to moderate skin reactions were observed in most animals (test and controls) following intradermal injection (two sites with 0.1 mL/injection of A: Freunds Complete Adjuvant (FCA), B: 5% w/v triticonazole in propylene glycol (PG), C: 5% w/v in a mixture of FCA and PG). After topical induction (50% of triticonazole in PG), mild skin irritation and exfoliation was also evident in almost all animals (test and control animals). Following topical challenge (50% and 10% triticonazole in PG) erythema was noted in three males and three females in the control

groups (barely perceptible or slight) and in two males and two females in the 50% groups (barely perceptible). In addition, exfoliation was evident in three males and five females in the controls compared with five males and eight females in the 50% group. Following topical challenge with 10% triticonazole in PG, a barely perceptible erythema was noted in one female in the test group. In addition, exfoliation was noted in three control females and one male, and one female from the test groups. Topical challenge with PG produced no skin reactions. No positive control was included in the study.

In the modified Buehler study, repeated occluded dermal application of triticonazole did not cause delayed contact hypersensitivity in Guinea pigs. During the induction phase (50% triticonazole w/v in PG), very faint erythema at the application site was observed in one male during the first week of induction, and in most animals during the second and third week. No reaction was observed in the control animals. After challenge application (50% w/v in PG), no dermal reactions were observed in treated or control animals. Challenge application of 10% w/v produced very faint erythema in one female from the test group and no reactions amongst controls. Challenge with PG did not cause any dermal reactions. No positive control was included in the study.

In the Buehler assay, after the first induction at day 0 (50% w/v triticonazole in PG), no skin irritation was observed in any animal of the control or test group. The second (day 7) and third (day 14) induction caused discrete or patchy to moderate and confluent erythema in several animals of the control and test group. After challenge (25 or 50% w/v triticonazole in PG), discrete or patchy or moderate and confluent erythema was noticed at the application sites of the 50% triticonazole group in two animals (10%) and in one animal of the control group (5%). No skin reactions were noticed in any application sites of the 25% triticonazole group nor of the vehicle PG. Thus, 10% of Guinea pigs revealed skin reaction after challenge, which is below the value of 15% that trigger classification for skin sensitisation. In the positive control group (α-hexylcinnamaldehyde (HCA) technical), 85% of the animals showed positive reactions to the challenge indicating that the Guinea pig strain used was sufficiently sensitive for detection of skin sensitizing compounds in the Buehler test.

The DS summarised that the results from the GPMT, modified Buehler test and Buehler test do not indicate skin sensitisation according to the CLP Regulation, although the reliability of the GPMT and the modified Buehler test cannot be completely confirmed since no positive control was included in these two studies. Further, the sensitivity of the 3-inductions Buehler assay is limited. The DS concluded that triticonazole does not meet the classification criteria for skin sensitisation.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

The DS included three studies for the assessment of skin sensitisation, one GPMT with 10 animals/sex/group, one modified Buehler assay with 10 animals/sex/treated group and 5 animals/sex/control group and one Buehler assay with 10 animals/sex/group. All studies were performed in albino Dunkin Hartley Guinea pigs and according to OECD TG 406 and GLP. No human data was included.

***Table**: the CLP criteria for classification as Skin Sensitisation based on data from GPMT and Buehler test*

Sub-category 1A	
Assay	**Criteria**
GPMT	≥ 30% responding at ≤ 0,1% intradermal induction dose or ≥ 60% responding at > 0,1% to ≤ 1% intradermal induction dose
Buehler assay	≥ 15% responding at ≤ 0,2% topical induction dose or ≥ 60% responding at > 0,2% to ≤ 20% topical induction dose
Sub-category 1B	
GPMT	≥ 30% to < 60% responding at > 0,1% to ≤ 1% intradermal induction dose or ≥ 30% responding at > 1% intradermal induction dos
Buehler test	≥ 15% to < 60% responding at > 0,2% to ≤ 20% topical induction dose or ≥ 15% responding at > 20% topical induction dose

Under the conditions of the GPMT assay and the modified Buehler assay, triticonazole did not cause delayed contact hypersensitivity in Guinea pigs. However, these two studies have some limitations since no positive control was included.

In the Buehler assay following the first induction, no skin irritation was observed in any of the animals of the control and triticonazole treated groups (25 and 50%). The second and third induction caused discrete or patchy to moderate and confluent erythema in several animals from the control and the triticonazole groups. After the challenge, discrete or patchy or moderate and confluent erythema was noticed at the application sites of the 50% triticonazole group in two animals (10%) with no reactions in the PG treated group. No skin reactions were noticed in any application sites of the 25% triticonazole group or the PG treated group.

According to the CLP criteria, for the Buhler test, classification as Skin Sens. 1B requires responses in 15% of the animals at > 20% topical induction dose. Following the 50% triticonazole induction dose, 10% of the animals showed skin reactions after challenge, which was below the value of 15% that would trigger a classification as Skin Sens. 1B. In the positive control group treated with HCA technical, 85% of the animals showed positive reactions to the challenge indicating that the Guinea pig strain used was sufficiently sensitive for detection of skin sensitizing compounds in the Buehler test. However, positive control data are only available for the Buehler Test.

In summary, the results from the GPMT, modified Buehler test and Buehler test did not indicate skin sensitisation, although the reliability of the GPMT and the modified Buehler test cannot be completely confirmed since no positive control was included in these two studies. Further, the sensitivity of the Buehler assay with three induction doses is considered as limited.

In conclusion, based on the data available, although not including a valid study for the assessment of skin sensitisation, RAC agreed with the DS that **classification for skin sensitisation is not justified**.

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

Summary of the Dossier Submitter's proposal

The DS assessed several repeated dose toxicity studies in rats, mice, dogs and rabbits (Rat: 28-d, 90-d and 2-y oral studies, 90-d oral study with ACHT challenge and 21-d dermal study. Mouse: 6-w, 90-d and 1,5-y oral studies. Dog: 28-d and 1-y oral studies. Rabbit: Tolerance study, range-finding teratology study and teratology study). In table 50 of the CLH report, information regarding the studies are included.

From the assessment of all the studies in rat, mouse and dog, the liver and adrenals were shown to be the target organs following exposure to triticonazole. The DS assessed the severity of the effects and if the relevant effects reported in the liver and adrenals were above or below the guidance values (GV) for a STOT RE classification according to the CLP criteria. In addition, maternal mortality in the rabbit teratology studies was assessed for a STOT RE classification.

Adrenals: The adrenal was shown to be a target organ following exposure to triticonazole. However, only in the 1-y dog study exposed to 0, 2.5, 25 and 150 mg/kg bw/d of triticonazole, histopathological effects (slight vacuolation of zona fasciculata in 3/4 males and moderate vacuolation in 1/3 females) were observed in the 25 mg/kg bw/d dose group (Broadmeadow, 1993). In the other repeated dose toxicity studies (21-d rat dermal, 28-d rat and dog oral, 6-w mouse oral, 90-d rat and mouse oral, 1,5-y mouse oral and 2-y rat oral) no effects or only slight effects were reported in the adrenals at doses above the GV for STOT RE classification. The DS concluded that the severity and nature of effects in the adrenals in dogs after 1 year of exposure to triticonazole, at the GV for a STOT RE 2 classification (25 mg/kg bw/d), did not reflect significant organ damage. Therefore, classification as STOT RE for effects on adrenals was not considered justified.

Liver: In the liver, no severe effects were reported below the GV values for a STOT RE classification in the short and long-term studies in rats, mice and dogs. The only two studies where effects in the liver were reported below the GVs were the 6-w study in mouse (Aughton, 1991) and 28-d study in dog (Broadmeadow, 1991).

The effects reported below the GV were increased liver weight (male and female mice, male dog), increased incidence of inflammatory cells (male mice), increased incidence of hepatocyte hypertrophy (female mice) and increased incidence of peri-acinar hypertrophy of hepatocytes associated with fatty vacuolation (male dogs). None of these effects were observed below the GV in the studies of longer duration. When taking the effects reported in the liver in the mice and dog studies into account, the severity and nature of effects in the liver at the respective GV did not reflect significant organ damage. Therefore, a proposal for STOT RE classification based on effects in the liver was not considered justified by the DS.

Mortality: In the rabbit developmental toxicity studies, treatment-related mortality was reported following repeated exposure to triticonazole. In the preliminary rabbit study (exposure during GD 6-19), all 8 animals at 150 mg/kg bw/d were sacrificed in extremis between GD 8 and 9 following weight losses, reduced food and water intakes and reduced faecal output. No mortalities were observed in the 0, 5, 15, 50 and 75 mg/kg bw/d dose groups.

In the main rabbit study (exposure GD 6-19) at 75 mg/kg bw/d, 6 out of 20 dams were sacrificed in extremis between GD 13 and 18 following marked weight loss, reduced food intake and reduced faecal output, reduced body temperature and red staining in the cage undertray. The same was the case for one female treated with 50 mg/kg bw/d.

The DS excluded some explanations for the mortality reported in the rabbits:

- There was no indication of any misgavage or misdosing
- There was no indication of infections or diarrhoea based on irritation by triticonazole
- Triticonazole is well absorbed and metabolised (see studies on absorption, distribution, metabolism and excretion of triticonazole (ADME) in section 9 of the CLH report) so it can be excluded that there was a recycling of triticonazole by caecotrophy, and therefore a higher exposure to triticonazole.

The DS assessed the individual data on the sacrificed animals and the necropsy findings in the gastro-intestinal tract in the sacrificed rabbits are included in table 3.10.1.6-3 in Annex I to the CLH report.

The most prominent symptom in the sacrificed rabbits in the preliminary and the main teratogenicity study was loss of weight. Necropsy findings in thoracic cavity of sacrificed rabbits were inconsistent. Further, some sacrificed rabbits did not show signs of gastro-intestinal (GI) disorder.

Based on the findings in the sacrificed rabbits, the DS could not conclude that the rabbit mortality reported in the teratogenicity studies at doses below the GV for STOT RE 2 (30-300 mg/kg bw/d based on a 28-d study) was solely due to disorders in the rabbit GI-tract. The animals were sacrificed based on their bad health conditions. At higher doses of triticonazole (above the GV for STOT RE 2 classification), the most prominent effect in the exposed rabbits was that they either stopped feeding or markedly reduced their food and water consumption and finally lost weight significantly.

Based on the results in the rabbit teratogenicity studies, the DS assumed that the rabbit mortality/morbidity in the teratogenicity studies was explained by a very high sensitivity of this species to triticonazole. Considering the limited effects on the rabbit pups (mostly skeletal variations), there was no reason to believe that the mortality was specific for pregnant rabbits, but rather was a general effect of triticonazole in rabbits.

In conclusion, according to the CLP criteria the GV for a STOT RE 2 classification (28-d study) is 30-300 mg/kg bw/d. The rabbit mortality/morbidity was considered as 'clearly severe' by the DS and occurred at doses below the relevant GV. The DS concluded that a classification with STOT RE 2 (H373) was warranted.

Comments received during public consultation

Two comments were received; one from an MSCA and one from a Company/Manufacturer.

The MSCA commented on the GV used by the DS for the teratogenicity studies and considered that they should reflect the actual exposure days i.e. exposure during GD 6-19 (GV 75-750 mg/kg bw/d). The MSCA also considered that since mortality/morbidity was observed at GD 8-9 (following 2-3 days of exposure) at a dose of 75 mg/kg bw/d in rabbits, classification for acute toxicity should be assessed. They referred to the Guidance on the application of the CLP criteria (CLP Guidance; version 5.0, section 3.1.1) where it says that "mortalities during the first 72h after treatment (in a repeated dose study) may also be considered for the assessment of acute toxicity". However, the MSCA also noted that a double-classification for the mortality/morbidity should be avoided.

The Company/Manufacturer disagreed with the STOT RE classification based on mortality in the rabbit teratogenicity studies. Their main argument was that the mortality was related to effects in the GI-tract in the rabbits following exposure to triticonazole. They stated that in the prenatal

rabbit study several indications of effects in the GI-tract were reported, including reduced food consumption (between days 6 and 12 reduced by 21.7%, and between days 13 and 19 reduced by 17.6%) at doses ≥ 50 mg/kg bw/d, and reduced defecation, indicating a rabbit specific, and hence non-human relevant, effect on the GI-tract. The Company/Manufacturer described that the data on maternal toxicity in the rabbit developmental toxicity study and the range-finding study were limited, acknowledging the lack of a full histopathologic investigation of the intestinal tract and measurement of the time of intestinal passage of the faeces, the caecal microbiome or the fibre content of the faeces of all animals. They considered that the lack of this information precludes an in-depth investigation of alimentary disorder of the dams. Consequently, even though the exact type of intestinal disorder could not be shown, and several severe signs of intestinal disorders have not been seen, they considered that there is significant evidence that treatment with triticonazole resulted in rabbit specific toxic effect on the GI-tract. Therefore, they considered that classification for STOT RE is not warranted.

Assessment and comparison with the classification criteria

From the assessment of the repeated dose toxicity studies included by the DS in the CLH report, RAC agreed with the DS that the liver and adrenals were target organs following exposure to triticonazole. Further, the mortality reported in the rabbit teratogenicity studies should also be assessed for a classification as STOT RE.

Adrenals

Effects were reported in the adrenals only in the 1-y study in dogs (4/sex/group) exposed to 0 (empty capsule), 2.5, 25 and 150 mg/kg bw/d of triticonazole in gelatine capsule (Broadmeadow, 1993). The effects reported included histopathological changes in the adrenals evident as slight vacuolation of Zona fasciculata, with most animals affected at 150 mg/kg bw/d. This exposure was above the GV at 25 mg/kg bw/d for a STOT RE 2 classification based on a 1-y study (see table below). The incidence of vacuolation of the Zona fasciculata was reassessed in 2015 by an external pathologist which concluded that the results were very similar, and that the differences in incidence and severity between the original data and the re-examination largely was due to variation in the level of reporting between the pathologists. The results of the slide review confirmed that there was a greater incidence and/or severity of multifocal vacuolation in the Zona fasciculata of both sexes exposed to 150 mg/kg bw/d compared to controls. Historical control data (HCD) from the same laboratory from 8 studies in the period from 1987 to 1992, showed vacuolation in the Zona fasciculata in two males and two females (no discrimination between the severity was provided). In the 28-d study in dogs, no effects on the adrenals were reported at higher doses (300 mg/kg bw/d) (Broadmeadow, 1991). RAC considered that when taking into account the effects reported in the adrenals in the 1-y dog study, they were seen above the GV for classification as STOT RE 2, and that classification based on adrenal effects was not justified.

***Table**: Group incidences of histopathological findings in the adrenals from the 1-y repeated dose toxicity study in dogs*

	Dose group level (mg/kg bw/d)							
	Males				Females			
	0	**2.5**	**25**	**150**	**0**	**2.5**	**25**	**150**
Adrenals (Broadmeadow, 1993) Vacuolation of Zona fasciculata • minimal • slight	 1/4 0/4	 0/4 1/4	 1/4 1/4	 1/4 3/4	 0/4 0/4	 0/4 0/4	 0/3 1/3	 0/4 3/4
Adrenals (Millar, 2015, re-examination) Vacuolation of Zona fasciculata • minimal • slight • moderate	 2/4 0/4 0/4	 1/4 1/4 0/4	 1/4 3/4 0/4	 1/4 1/4 2/4	 2/4 1/4 0/4	 2/4 0/4 0/4	 2/3 0/3 1/3	 0/4 2/4 2/4

Liver

From the available repeated dose toxicity studies in rats, mice and dogs, two studies reported effects in the liver that were below the GV for STOT RE 2. The first study was the 6-w study in mouse (GV 225 mg/kg bw/d for STOT RE 2 classification) (Aughton, 1991) and the second the 28-d study in dog (GV 300 mg/kg bw/d for STOT RE 2 classification) (Boradmeadow, 1991).

In the 6-w study, mice (12/sex/group) were exposed in the diet to 0, 500, 1500, 5000, 15000 or 50000 ppm triticonazole, equivalent to 0, 77, 233, 851 and 3270 mg/kg bw/d in males and 0, 98.8, 286, 982 and 4091 mg/kg bw/d in females. Effects reported in the liver are shown in the table below and included a significant increase in the relative liver weight from 77/99 mg/kg bw/d in males/females, and significant increase in histopathological findings from 851/982 mg/kg bw/d in males/females:

***Table**: Liver effects reported in the 6-w dietary repeat dose study in mice.*

Finding description	Dose group level (mg/kg bw/d)									
	Males					Females				
	0	**77**	**233**	**851**	**3270**	**0**	**99**	**286**	**982**	**4091**
Terminal bw (g) (% of controls)	35.7	34.5 (96)	34.5 (96)	32.4 (90)	19.1 (53)	26.1	24.6 (94)	26.1 (100)	25.0 (95)	19.0 (72)
Relative liver weight (% of control)	5.29	6.02* (113.8)	7.99* (151.0)	12.86* (243.1)	16.85* (318.5)		6.02* (110.3)	6.90* (126.4)	11.22* (205.5)	16.19* (296.5)
Enlarged liver	0/12	0/12	1/12	5/12[a]	7/11[b]	0/12	0/12	0/12	4/12	2/2
Hepatocyte hypertrophy	0/12	0/12	0/12	12/12[c]	11/11[c]	0/12	0/12	2/12	10/12[c]	0/2
Fatty vacuolation	1/12	1/12	1/12	2/12	11/11[c]	0/12	0/12	0/12	6/12[a]	0/2
Increased ploidy	0/12	0/12	0/12	2/12	11/11[c]	0/12	0/12	0/12	9/12[c]	0/2
Inflammatory cells	0/12	2/12	4/12	3/12	5/11[a]	0/12	1/12	0/12	0/12	0/2
Focal mineralisation	0/12	0/12	0/12	2/12	4/11[a]	0/12	0/12	0/12	0/12	0/2

* ($p \leq 0.05$); b ($p \leq 0.01$); significantly different from controls (Dunett's test).
a ($p \leq 0.05$); b ($p \leq 0.01$); c ($p \leq 0.001$) significantly different from controls (Fisher's exact test)

In the 28-d study in dogs (2/sex/group) the animals were exposed to 0 (empty capsule), 10, 30, 100 and 300 mg/kg bw/d of triticonazole in gelatine capsules. Effects were mainly reported in the high dose group and included: a decreases in body weight gain when compared to control animals (males: 63% of controls, females: 40% of controls). A decrease in food consumption in females (92% of controls). Treatment-related effects in the liver was evident as a slight increase in relative liver weight in males (+18.9% compared to control) and in females receiving 100 (+9.7%) and 300 (+8.5%) mg/kg bw/d. Upon histopathological examination, treatment-related findings were limited to periacinar hypertrophy of hepatocytes with associated fatty vacuolation in both males in the high dose group. It should be noted that no effects on the liver were reported in the 1-y study in dogs with doses up to 150 mg/kg bw/d.

In the other repeated dose toxicity studies in rats, mice and dogs, no effects in the liver relevant for classification as STOT RE were reported. RAC considered, when taking into account the effects reported in the liver in the mice and dog studies that the severity and nature of effects in the liver at the respective GV do not reflect significant organ damage and classification as STOT RE based on liver effects was not considered justified.

In conclusion, RAC agreed with the DS that based on the severity of the effects reported in the liver and adrenals at doses ≤ the GV, no classification as STOT RE was justified based on the effects in these organs.

Mortality

In the repeated dose toxicity studies in rats, mice (mortality reported at doses above 3000 mg/kg bw/d) and dogs, no mortality was reported at doses relevant for classification as STOT RE. However, in the rabbit developmental toxicity studies, treatment related mortality/morbidity was reported following repeated exposure to triticonazole. In the range-finding rabbit study (exposure during GD 6-19, 8 rabbits/group; Bailey, 1990), all 8 animals in the 150 mg/kg bw/d dose group were sacrificed in extremis between GD 8 and 9 following weight losses, reduced food and water intake and reduced faecal output. No mortalities were reported in the 0, 5, 15, 50 and 75 mg/kg bw/d dose groups.

In the main rabbit teratogenicity study (exposure on GD 6-19, 20 rabbits/group; Burns, 1991) 6 out of 20 rabbits in the 75 mg/kg bw/d dose group were sacrificed in extremis between GD 13 and 17 following marked weight loss, reduced food intake and reduced faecal output, reduced body temperature and red staining in the cage undertray. The same was the case for one female exposed to 50 mg/kg bw/d that was killed in extremis on GD 17. In the table below, the individual findings at necropsy in the 7 rabbits sacrificed in extremis is included.

***Table**: Individual observations at necropsy in animals killed in extremis (modified from table 3.10.1.6-6 in Annex I to the CLH report)*

Group (mg/kg bw/d)	Animal number	History and circumstances of death	Summary of necropsy findings
50	89FR515	Weight loss 660 g from day 6. Reduced food intake and faecal output. Animal thin with apparent reduction in body temperature. Red staining in undertray. Killed *in extremis* **GD 17.**	Abdominal cavity: Stomach contents reduced. Large amount of yellow mucoid material in small intestine. Pregnant, all implantations resorbing.
75	89FR445	Weight loss 410 g from day 6. Reduced food intake and faecal output. Apparent reduction in body temperature; hunched posture and thin. Red liquid in undertray. Killed *in extremis* **GD 13.**	Abdominal cavity: Liver friable with pale areas and lobular pattern accentuated. Stomach contents reduced, GI-tract gaseous, caecal contents dehydrated. Pregnant, all implantations early resorptions.

75	89FR448	Weight loss 500 g from day 6. Reduced food intake and faecal output. Lethargic, apparent reduction in body temperature. Red liquid in undertray. Killed *in extremis* **GD 13.**	Abdominal cavity: No abnormality detected. Pregnant, all implantations early resorptions.
75	89FR455	Weight loss 530 g from day 6. Reduced food intake and faecal output. Apparent reduction in body temperature. Killed *in extremis* **GD 14**.	Abdominal cavity: Liver lobular pattern slightly accentuated. Stomach contents compacted. Pregnant, one early resorption, remainder of implantations grossly normal.
75	89FR488	Weight loss 680 g from day 6. Reduced food intake and faecal output. Increased respiration rate and apparent reduction in body temperature. Red liquid in undertray. Killed *in extremis* **GD 13**.	Abdominal cavity: Liver lobular pattern slightly accentuated, irregular dark striations on periphery of left liver lobe. Stomach contents compacted with fur and food material. Caecal contents dark and reduced. Remainder of GI-tract generally devoid of content. No faecal pellet formation in rectum. Pregnant, all implantations resorbing.
75	89FR521	Weight loss 590 g from day 6. Reduced food intake and faecal output. Red liquid in undertray. Killed *in extremis* **GD 15.**	Abdominal cavity: Stomach contents compacted. Pregnant, all implantations grossly normal.
75	89FR541	Weight loss 710 g days 6-15. Reduced food intake and faecal output. Red staining in undertray. Apparent reduction in body temperature. Killed *in extremis* **GD 16**.	External: animal thin Abdominal cavity: Stomach contents compacted with fur and food material. Caecal contents reduced and dehydrated. Remainder of gastro-intestinal tract contents reduced. Pregnant, all implantations resorbing.

RAC agreed with the DS that some explanations for the mortality reported in the rabbits could be excluded (mis-gavage/mis-dosing, no infections or diarrhoea due to irritating properties of triticonazole and no recycling of triticonazole due to caecotrophy).

The main symptom in all the sacrificed rabbits in the preliminary and the main teratogenicity study was loss of weight, and that the animals had a bad health condition. Among the sacrificed rabbits, some did not show any signs of GI disorder. No changes in the thoracic cavity was reported in any sacrificed rabbits. It should be noted that in the ECHA poster "Rabbit gastrointestinal toxicity in prenatal developmental toxicity studies" by Reuter *et al.* (2018), it was stated that some substance properties may contribute to rabbit specific GI-toxicity; irritating/corrosive properties, low water solubility > 1 mg/L (triticonazole from 7.7 to 9.3 mg/L) and bactericide. As triticonazole did not have these properties, RAC considered the mortality/morbidity reported in the rabbit teratogenicity should be taken into account for a classification as STOT RE.

Based on the findings in the sacrificed rabbits, RAC agreed with the DS and concluded that the rabbit mortality reported in the teratogenicity studies at doses below the GV for STOT RE 2 (30 to 300 mg/kg bw/d for 28 days of exposure) was considered severe, and was not solely due to disorder in the GI-tract in the rabbits. The morbidity of the rabbits in the teratogenicity studies occurred after repeated dosing to triticonazole, i.e. at 50 mg/kg bw/d after 11 days of dosing, at 75 mg/kg bw/d after 7-11 days of dosing, and 150 mg/kg bw/d after 2-3 days of dosing. The

morbidity is therefore not considered to be an acute effect of triticonazole following single exposure.

Further, RAC agreed that the mortality was not specific to pregnant rabbits, but rather a general effect of triticonazole in rabbits.

According to the CLP Regulation, Annex I, Section 3.9.2.7.3 "morbidity or death resulting from repeated dose or long-term exposure" is considered relevant for classification as STOT RE.

In summary, RAC agreed with the DS and considered that the effects reported in the liver and adrenals observed in rat, mice and dog studies, discussed above, were not sufficient for classification as STOT RE. However, in the developmental toxicity studies in rabbits, mortality was observed at doses < 300 mg/kg bw/d, which is the GV for STOT RE 2 from a 28-d study. There is no data available to indicate a non-relevance to humans for the mortality reported in the rabbits.

RAC concluded that **classification as STOT RE 2** according to the CLP criteria, based on the mortality/morbidity reported in the rabbit studies, is justified.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Triticonazole was tested in six *in vitro* and one *in vivo* mutagenicity assays measuring different endpoints of potential mutagenicity such as gene mutation in bacteria and in mammalian cells, chromosomal aberration (CA), and Unscheduled DNA Synthesis (UDS) and micronucleus (MN) in somatic cells. All the studies were performed according to OECD TG and GLP.

Results from these studies showed that triticonazole did not induce gene mutation in two AMES tests, or in one gene mutation assay in cultured mammalian cells (CH-V79 assay). Further, no potential for clastogenicity was observed in the *in vitro* CA assay in human lymphocytes (2 studies) or in an *in vitro* UDS assay in rat hepatocytes.

The only indication of a genotoxic response was an increase in the incidence of polyploid cells in one of the *in vitro* assays with human lymphocytes in the presence of exogenous metabolic activation system (S-9 fraction) (Dance, 1992). However, there was no clear dose response, and no effect on the mitotic index was observed in the study. Moreover, no effect on numerical aberrations was evident in the second, more recent, CA study in human lymphocytes with a comparable concentration range tested.

In the *in vivo* mouse MN assay with exposure to 0, 25.0, 125.0 and 625 mg/kg triticonazole, a clear negative result was obtained. Based on ADME studies with triticonazole and the observed toxicity in the MN assay including clinical signs (transient over activity after dosing in the mid- and high dose group, and transient piloerection and hunched posture in the high dose group), it can be concluded that triticonazole reached the bone marrow.

In summary, the results from the *in vitro* and *in vivo* studies in bacterial and mammalian cells do not give any indication of a potential mutagenic activity following exposure to triticonazole. Further, results from QSAR analysis of structurally similar metabolites do not give any alert for mutagenicity. The DS concluded that triticonazole does not meet the criteria for classification as a germ cell mutagen.

Comments received during public consultation

One MSCA commented on the assessment of mutagenicity. The MSCA considered that the in the CA *in vitro* assay (Dance, 1992) an increase in polyploidy was reported at 250 and 500 µg/mL (above HCD); however, no such effects were reported in another comparable *in vitro* and *in vivo* study. Further, the MSCA commented that exposure of the bone marrow was not proven in the *in vivo* MN test (Edwards, 1992). The MSCA also noted that in another *in vitro* CA assay (Marshall, 1997) the results were not negative, but should be considered as equivocal. The MSCA considered that triticonazole could possibly be genotoxic.

Assessment and comparison with the classification criteria

Triticonazole was tested in six *in vitro* and one *in vivo* genotoxicity/mutagenicity assays measuring different end-points of possible mutagenicity. These included gene mutation in bacteria and in mammalian cells, CA, UDS and MN in somatic cells. All the studies were performed according to OECD TG and GLP. Triticonazole was not tested in germ cell mutagenicity tests. Further, no human epidemiological studies assessing the mutagenic potential of triticonazole was included by the DS. The *in vitro* data in bacterial and mammalian cells are shown in the table below.

***Table**: Summary of the mutagenicity studies with triticonazole*

Type of study	Dose range	Results	Reference
Reverse mutation assay (OECD TG 471) (*S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100)	0, 25, 79, 250, 790 and 2500 µg/plate (dissolved in DMSO)	negative (+/- S-9 mix)	May, 1991
Reverse mutation assay (OECD TG 471) (*S. typhimurium* TA 1535, TA 1537, TA 98, TA 100 and *E. coli* WP2 uvrA)	0, 33, 100, 333, 1000, 2750 and 5500 µg/plate (dissolved in DMSO)	negative (+/- S-9 mix)	Woitkowiak, 2014
Chinese Hamster V79 cell/HGPRT locus gene mutation assay (OECD TG 476)	0, 62.5, 125, 250, 500 and 1000 µg/ml (dissolved in DMSO)	negative (+/- S-9 mix)	Lloyd, 1991
CA assay in cultured human lymphocytes (OECD TG 473)	0, 10, 20, 40, 50 and 60 µg/ml (- S-9) 0, 125, 250 and 500 µg/ml (+ S-9) (dissolved in DMSO)	no structural aberrations (+/- S-9 mix) increased polyploidy at 250 and 500 µg/ml (+ S-9) [questionable significance.]	Dance, 1992

Type of study	Dose range	Results	Reference
CA assay in cultured human lymphocytes (OECD TG 473)	0 – 800 µg/L (dissolved in DMSO) evaluation performed at: (I) 274.4, 392, 560 µg/ml (+/- S-9) (II) 253.1, 337.5, 450 µg/ml (- S-9) 337.5, 450, 600 µg/ml (+ S-9)	(I) Equivocal[1] (II) negative (+/- S-9 mix)	Marshall, 1997
UDS assay in rat hepatocytes (OECD TG 474)	0, 7.81, 15.6, 31.3 and 62.5 µg/mL (dissolved in DMSO)	negative	Foster, 1992

1 absence of S-9 resulted in statistically significant increased frequencies of cells with structural aberrations at 392 µg/mL and 560 µg/mL, however, with 50% cytotoxicity at 560 µg/mL. No increase was reported in the presence of S-9.

The results from these studies showed that triticonazole did not induce gene mutation in two AMES tests, or gene mutation in mammalian cells in culture (CH-V79 assay). No potential for clastogenicity was observed in the *in vitro* CA assay in human lymphocytes (two studies) or in the *in vitro* UDS assay in rat hepatocytes.

The only indication of genotoxicity was an increase in the incidence of polyploid cells in one of the *in vitro* assays with human lymphocytes in the presence of S-9 activation (Dance, 1992). However, in the study there was no clear dose response in polyploid cells in the absence of any effect on the mitotic index, see table below. Moreover, no such effect on numerical aberrations was evident in the second, more recent CA *in vitro* study in human lymphocytes with a comparable concentration range tested (Marshall, 1987). In addition, no indications of numerical aberrations were evident in the *in vivo* mouse MN assay.

***Table**: Mitotic indices and mean % cells with CA in cultured lymphocytes treated with triticonazole (Dance, 1992)*

Treatment (µg/ml)	without S-9				with S-9			
	Mitotic index [1] (mean)	mean % cells with aberrations		number of polyploid cells [2]	Mitotic index [1] (mean)	mean % cells with aberrations		number of polyploid cells [2]
		incl. gaps	excl. gaps			incl. gaps	excl. gaps	
Control	13.9	1.7	0.3	4, 0, 0	12.1	1.7	0.0	0, 0, 0
Triticonazole 40	11.9	2.7	0.0	0, 1, 0	-	-	-	-
50	10.2	2.3	2.0	2, 0, 1	-	-	-	-
60	6.9	3.7	0.7	1, 1, 0	-	-	-	-
125	-	-	-	-	17.5	0.7	0.7	**0, 0, 0**
250	-	-	-	-	13.0	3.0	1.0	**9, 1, 7**
500	-	-	-	-	11.6	4.0	1.0	**0, 3, 5**
Positive control Chlorambucil	12.3	35.0***	27.3***	1, 1, 0	-	-	-	-
Positive control Cyclophosphamide	-	-	-	-	13.1	22.3***	17.0***	2, 0, 0

1 mitotic index = $\frac{\text{number of metaphases}}{\text{number of lymphocytes}} \times 100$

2 number of polyploid cells per culture (three cultures examined) when scoring 100 metaphases

*** = ($p \leq 0.001$) significantly different from controls

In vivo study

In the *in vivo* mouse MN in CD-1 mice exposed to 0, 25, 125 and 625 mg/kg bw triticonazole in a single dose by gavage, a clear negative result was observed with no MN formation in polychromatic erythrocytes at doses up to 625 mg/kg bw (Edwards, 1992). Based on toxicokinetic studies with triticonazole and the observed toxicity in the MN assay it was concluded that triticonazole reached the bone marrow. For further information, see "Additional Key elements".

Comparison with the CLP criteria

No human epidemiological data was available assessing germ cell mutagenicity following exposure to triticonazole. RAC therefore concluded that classification as Muta. 1A is not justified.

No germ cell mutagenicity studies in animals were available with triticonazole. Results from studies in somatic cells *in vitro* or *in vivo* do not give any indication for potential mutagenicity. RAC therefore concluded that classification as Muta. 1B or Muta. 2 is not justified.

In conclusion, RAC agreed that **no classification for germ cell mutagenicity** is justified.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS included two carcinogenicity studies, one in CD rats and one in CD-1 mice performed according to OECD TG 451 and GLP, see table below. No human data were available.

***Table**: Carcinogenicity study in CD-rats and CD-1 mice following exposure to triticonazole*

Study	Doses and duration of exposure	Results	Reference
OECD TG 451 (1981) CD rats 50 males/females per group	Triticonazole, purity 97% 0, 5, 25, 750 and 5000 ppm (equivalent to 0, 0.2, 1.0, 29.4 and 203.6 mg/kg bw/d males and 0, 0.3, 1.3, 38.3 and 286.6 mg/kg bw/d females 99 weeks (males), 100 weeks (females) Additional 15 animals/sex and dose group were sacrificed after 26 and 53 weeks of treatment.	The NOAEL was 750 ppm based on decreased bodyweight gain and significant histopathological findings in the liver and adrenals at 5000 ppm. Slightly increased incidence (above HCD at 5000 ppm) of thyroid follicular cell adenoma in males at 5000 ppm (3/47 (6%), 1/30 (3%), 0/34 (0%), 0/39 (0%), 7/49 (14%) at 0, 5, 25, 750 and 5000 ppm, resp.) was considered to reflect spontaneous occurrence of this finding. HCD, 104 weeks studies 1987-1993:	Aughto, 1994

		males: 2-10%; females: 0-4.2%	
OECD TG 451 (1981) CD-1 mice 52 males/females per group	Triticonazole, purity 96.4 – 97.1% 0, 15, 150 and 1500 ppm (equivalent to 0, 1.8, 17.4 and 202.2 mg/kg bw/d (males) and 0, 2.1, 20.1 and 209.5 mg/kg bw/d (females) 78 weeks Additional 16 animals/sex and dose group were sacrificed after 26 weeks of treatment.	The NOAEL was 150 ppm based on reduced body weight gains and clear effects on the liver (including increased organ weights and histopathological findings at 1500 ppm). No evidence on any treatment-related increase in the type or incidence of neoplastic findings in this study suggestive of a carcinogenic effect.	Eddie, 1994

In the CD-1 mice carcinogenicity study, no increase in neoplastic lesions were reported. In the CD-rat carcinogenicity study a slightly increased incidence of benign follicular cell adenomas in the thyroid was reported in males in the high dose group (5000 ppm), above the HCD. However, this finding was considered by the DS to not be treatment-related based on a weight of evidence assessment:

1. *Tumour type and background incidence:* The incidence of thyroid follicular adenomas in male rats was only slightly above the HCD and not statistically significant different from controls, and dose-response was observed (no positive trend in the trend-test).
2. *Multi-site responses:* Effects were considered treatment-related in any other organ; hence, no multi-site response was observed.
3. *Progression of lesions to malignancy:* Only benign tumours were observed.
4. *Whether responses are in a single or in both sexes:* Only males in the high dose group were affected, slightly above the HCD.
5. *Whether responses are in single species or several species:* Increased incidence of thyroid follicular cell adenoma was observed only in male rats, and not in female rats or mice.
6. Pattern from HCD (higher incidence in males than in females) was reflected in the study results.

7. Thyroid was not a target organ following triticonazole exposure in any of the triticonazle studies.
8. No effects on thyroid hormone receptors and no inhibition of TPO or deiodinase type 1 enzyme activity was observed in the US EPA ToxCast screening programme.

Based on the results from the carcinogenicity study in rats and mice, the DS concluded that triticonazole does not meet the CLP criteria for a classification for carcinogenicity.

Comments received during public consultation

Comments were received from one MSCA. The MSCA asked for more information regarding the incidence of tumours in the pituitary and skin, since only information for the induction of thyroid tumours was included in the CLH report. The MSCA also asked for more information regarding the liver enzyme induction and increased turnover of plasma T3 and T4 levels and subsequently stimulation of TSH, since this could be a plausible mechanism for the increased incidence of follicular adenoma, which is not considered relevant for humans. The MSCA considered that a classification as Carc. 2 should be discussed.

Assessment and comparison with the classification criteria

The DS included two carcinogenicity studies, one in CD rats and one in CD-1 mice performed according to OECD TG 451 and GLP, see table above. No human data was available.

Rat study

In the CD-rat carcinogenicity study the following doses were assessed: 0, 5, 25, 750 and 5000 pmm (corresponding to 0, 0.2, 1.0, 29.4 and 203.6 mg/kg bw/d in males and 0, 0.3, 1.3, 38.3 and 286.6 mg/kg bw/d in females) for two years (Aughton, 1994). Additionally, 15 animals/sex/dose group were sacrificed after 26 and 53 weeks of treatment. General toxicity in this study was evident as a decreased body weight gain that was more pronounced in female rats, and significantly increased histopathological findings in the liver, adrenals and lungs at 5000 ppm, see tables below.

***Table**: Number of surviving animals, body weight gain and final body weight*

	0 ppm		**5 ppm**		**25 ppm**		**750 ppm**		**5000 ppm**	
	males	**females**	**males**	**females**	**males**	**females**	**males**	**females**	**males**	**females**
Surviving animals	14/50	19/50	23/50	14/50	14/50	16/50	17/50	18/50	14/50	19/50
(%)	(28)	(38)	(46)	(28)	(28)	(32)	(34)	(36)	(42)	(56)
Body weight gain										
Week 0-1										
No. of animals	80	80	80	80	80	80	80	80	80	80
bw gain (g)	64	31	65	29	65	29	64	31	51**	22**
(% of control)			(102)	(94)	(102)	(94)	(100)	(100)	(80)	(71)
Week 0-76 (♂) and 0-88 (♀)										
No. of animals	32	29	39	31	39	29	40	29	42	38
bw gain (g)	798	465	781	456	833	396	797	412	738	367**
(% of control)			(98)	(98)	(104)	(85)	(100)	(89)	(92)	(79)

	0 ppm males	females	5 ppm males	females	25 ppm males	females	750 ppm males	females	5000 ppm males	females
Surviving animals	14/50	19/50	23/50	14/50	14/50	16/50	17/50	18/50	14/50	19/50
(%)	(28)	(38)	(46)	(28)	(28)	(32)	(34)	(36)	(42)	(56)
Week 0-98 (♂) and 0-100 (♀)										
No. of animals	14	19	23	14	16	16	17	18	22	28
bw gain (g)	698	404	732	423	748	417	771	472	690	363
(% of control)			105	105	107	103	110	117	99	90
Final body weight										
Week 98 (♂) and 100 (♀)										
bw (g)	877	558	913	571	930	570	946	623	866	515
(% of control)			104	102	106	102	108	112	99	92

*** = ($p \leq 0.01$) significantly different from controls

***Table**: Non-neoplastic findings at the interim and terminal sacrifice (scheduled and unscheduled sacrifice*

Findings	Dose level (ppm) Males					Females				
	0	**5**	**25**	**750**	**5000**	**0**	**5**	**25**	**750**	**5000**
Adrenals										
(26 weeks)										
multinucleated cells	0/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15	0/15	**9/15*** **
cortical fatty vacuolation	4/15	2/15	2/15	3/15	7/15	0/15	0/15	1/15	0/15	1/15
(53 weeks)										
multinucleated cells	0/14	-	-	-	0/14	0/15	0/15	0/14	0/14	3/14
chronic inflammation	0/14	-	-	-	0/14	0/15	0/15	0/14	0/14	**4/14***
cortical fatty vacuolation	1/14	-	-	-	3/14	1/15	0/15	0/14	0/14	0/14
(terminal)										
multinucleated cells	0/50	0/35	0/42	0/40	0/50	0/50	0/46	0/48	0/46	3/50
cortical fatty vacuolation	11/50	10/35	7/42	10/40	13/50	8/50	4/46	4/48	6/46	11/50
Liver (terminal)										
centriacinar fatty vacuolation	6/50	3/50	5/50	5/50	9/50	16/50	15/50	11/50	23/50	**33/50****
Lungs (terminal)										
accumulation of alveolar macrophages	2/50	1/50	0/50	1/50	2/50	0/50	3/50	1/50	1/50	**7/50***

* = ($p \leq 0.05$); ** = ($p \leq 0.01$); *** = ($p \leq 0.001$) significantly different from controls (Fisher's exact test)

Neoplastic lesions included, in male rats, a statistically significant increased incidence of pituitary adenomas at 5000 ppm. However, the incidence was within the HCD, and this tumour type is considered to be a spontaneous age-related lesion in rats and therefore of limited biological relevance. Further, the increase was clearly not dose related. In male rats, a statistically significant increase in skin keratoacanthoma was reported in the low and high dose group. Due to the absence of a dose-response, this tumour was not considered related to treatment by RAC. It was reported by the DS (see response to comment 2 in RCOM) that this tumour type is a common cutaneous neoplasm in rats, especially in male rats. In the thyroid, a slightly increased incidence of thyroid follicular cell adenoma in males, outside the HCD, in the high dose group was reported. No information was available in the study report regarding liver enzyme induction and increased turnover of plasma T3 and T4 with a following stimulation of TSH (see RCOM); this

to explain a plausible mechanism of the increased follicular cell adenomas. RAC considered that the incidence of thyroid adenomas in male rats reflected the spontaneous occurrence of this finding based on a weight of evidence assessment included in the end of this section.

***Table**: Group incidences of relevant neoplastic findings at the terminal phase*

Findings	Dose level (ppm)									
	Males					Females				
	0	**5**	**25**	**750**	**5000**	**0**	**5**	**25**	**750**	**5000**
Pituitary (animals killed or dying during the treatment period)										
number examined	38	30	38	36	30	32	38	32	33	23
- Adenomas	13	16	19	18	17	21	25	23	**29***	16
- Carcinomas	0	1	0	2	0	3	5	1	1	2
Pituitary (all animals)										
number examined	50	38	43	43	50	49	45	44	44	50
- Adenomas	19	24	24	25	**29***[,ii]	32	31	34	40	32
(%)[1]	(38)	(63)	(56)	(58)	(58)	(65)	(69)	(77)	(90)	(64)
- Carcinomas	0	1	2	2	0	3	5	1	1	2
Skin (all animals)										
number examined	20	22	21	24	27	9	11	10	10	8
- Papilloma	4	2	1	1	3	0	1	1	0	0
- Keratoacanthoma	0	**5***	2	2	**6***	0	1	0	0	1
(%)		(23)			(22)					
Thyroid (all animals)										
Number examined	47	30	34	39	49	49	38	34	35	50
Follicular cell adenoma	3	1	0	0	7[i]	1	0	1	1	1
(%)[2]	(6.4)	(3.3)	(0)	(0)	(14.3)	(2.0)	(0)	(2.9)	(2.9)	(2.0)

1 HCD 104-w studies 1987–1993: males: 30.7-60.2%. No information on the 95th percentiles of HCD was available.
2 HCD 104-w studies 1987–1993: males: 2–10%, females: 0–4.2%
* = ($p \leq 0.05$) significantly different from controls (Fisher's exact test)
i = neither statistically significantly different to control (Fisher's exact test) nor any positive trend in (one-sided) Cochran-Armitage Trend-test using STATXAC. ii = information from the DS that no trend in the increase of incidence with dosing.

Mice study

In the CD-1 mice study, animals were exposed to 0, 15, 150 and 1500 ppm (equivalent to 0, 1.8, 17.4 and 202.2 mg/kg bw/d (males) and 0, 2.1, 20.1 and 209.5 mg/kg bw/d (females)). General toxicity in this study was evident as reduced body weight gains in the high dose group (25% (males) and 31% (females) at week 52 and 85% (males) and 77% (females) for the entire dosing period compared to controls). In the liver, statistically significant weight changes were reported as well as non-neoplastic lesions, see tables below.

***Table**: Weight changes in the liver (mean group values)*

Parameter	Dose group level (ppm) Males				Females			
	0	**15**	**150**	**1500**	**0**	**15**	**150**	**1500**
Terminal phase (78 weeks)								
Liver								
absolute weight (g)	2.88	2.46*	2.58	3.36*	1.90	1.95	1.95	2.19**
(% control)		(85)	(90)	(117)		(103)	(103)	(115)
relative weight (%)	6.05	5.40	5.31	7.65**	4.84	5.12	4.97	6.23**
(% control)		(89)	(88)	(126)		(106)	(103)	(129)

* ($p \leq 0.05$); ** ($p \leq 0.01$); significantly different from controls (Student's t-test)

***Table**: Group incidences of non-neoplastic findings*

Parameter	Dose group level (ppm) Males				Females			
	0	**15**	**150**	**1500**	**0**	**15**	**150**	**1500**
Terminal phase (78 weeks)								
Liver								
centriacinar fatty vacuolation	1/52	0/52	0/52	**12/52****	0/52	1/520	0/52	**8/52****
periacinar hepatocyte hypertrophy	0/52	0/52	0/52	0/52	0/52	0/52	0/52	1/52
Mesenteric lymph nodes								
Parafollicular hyperplasia								
• terminal sacrifice only	0/31	1/4	1/5	**4/29****	1/38	0/7	0/3	1/44
• all animals of terminal phase	1/49	1/24	1/28	4/46	1/52	0/21	0/16	1/49

* ($p \leq 0.05$); ** ($p \leq 0.01$); significantly different from controls (Fisher's exact test)

No evidence on any treatment-related increase in neoplastic lesions was reported in the mice carcinogenicity study (Eddie, 1994).

In summary, the only neoplastic findings reported above the HCD was an increased incidence of benign follicular cell adenomas in the thyroid in male rats in the high dose group (5000 ppm). A table including a weight of evidence assessment of the reported findings in the rat and mouse carcinogenicity studies is seen below.

***Table**: A weight of evidence assessment of the rat and mice carcinogenicity study*

Species	Tumour type and background incidence	Multisite responses	Progression to malignancy	Reduced tumour latency	Responses in single or both sexes	Confound-ing effect by excessive toxicity	Route of exposur e	MoA and relevance to humans
CD-rats	Benign follicular cell adenomas in thyroids of males at 5000 ppm (14.3%) HCD (males): 2-10%	No	Only benign tumours were reported	Observed at terminal phase	Only males	Not known	Oral	Findings considered incidental
CD-1 mice	No evidence of treatment-related increase in neoplastic findings.	-	-	-	-	-	-	-

In conclusion, based on the results from the carcinogenicity study in rats and mice performed according to OECD TG and the weight of evidence assessment RAC supported the DS and concluded that **no classification for carcinogenicity** following exposure to triticonazole is justified.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Adverse effects on sexual function and fertility

For the assessment of adverse effects on sexual function and fertility, a two-generation reproductive toxicity study in rats performed according to GLP and OECD TG 416, with some deviations, see table below, were included. Several *in vitro* studies to assess relevant toxicity on sexual function and fertility following exposure to triticonazole was also included, see CLH report and Annex 1 to the CLH report for detailed information. No epidemiological data was available.

***Table**: Overview of two-generation study in rats*

Study design	Doses	Results	Reference
Two-generation reproduction study OECD TG 416 (1983) Deviations from OECD TG 416 (2001): - missing measurements for oestrus cycling, differential ovarian follicle count, sperm morphology and motility, implantation sites, some organ weights, anogenital distance, areola / nipple retention, sperm histopathology, detailed testicular histopathology, examination of intact epididymis, investigation on post-lactational ovary - pairs without progeny not evaluated to determine the apparent cause of the infertility - no re-mating with proven sirs or dams conducted for F1 generation where 8 animals per sex at 5000 ppm did not mate	Triticonazole, purity 97.1% 0, 5, 25, 750 and 5000 ppm in the diet equivalent to 0, 0.34 (M) – 0.32 (F), 1.64 (M) – 1.59 (F), 49.35 (M) – 48.41 (F) and 350.8 (M) – 337.6 (F) mg/kg bw/d	<u>Parental NOAEL (750 ppm):</u> 49.35 (M) – 48.41 (F) mg/kg bw/d <u>Parental effects at LOAEL (5000 ppm)</u>: – mortalities – decreased weight gain – necropsy findings (histopathology and organ weights) in adrenals, ovaries and liver <u>Reproductive NOAEL (750 ppm)</u>: 49.35 (M) – 48.41 (F) mg/kg bw/d <u>Effects on reproduction at LOAEL (5000 ppm)</u>: – decreased mating and fertility indices <u>Offspring NOAEL (750 ppm)</u> : 49.35 (M) – 48.41 (F) mg/kg bw/d <u>Effects on offspring at LOAEL (5000 ppm)</u>: – decreased pup body weight – decreased livebirth and viability indices	Henwood, 1993

In the two-generation study in rats, marked parental toxicity was observed in the high dose (5000 ppm). These included treatment-related premature deaths (F0 females only), significant reduction in bodyweight, bodyweight gain and food consumption, and necropsy findings in adrenals, liver and ovary. Adverse effects on reproductive parameters in the high dose group included a decreased mating and fertility index (F1 generation). These effects were seen in the presence of systemic toxicity rather than related to hormonal disturbance. Further, a significant increase of pregnancy duration in the F0 females was reported in the high dose group. However, it was shown that this effect was mainly due to two females with longer gestation periods, and since the group mean values were in the range of HCD, the effect was not considered by the DS to be treatment-related. Regarding the offspring, increased pup mortality, decreased pup viability and decreased pup bodyweight was observed in both the F0 and F1 generation in the high dose group. Necropsy of dead pups showed "no milk in the stomach" as the only reported finding (F1: 16 (5), 7 (6), 1 (1), 9 (8) and 26 (11), F2: 3 (3), 2 (2), 5 (4), 6 (4) and 13 (5) in foetuses (litters) at 0, 5, 25, 750 and 5000 ppm). Effects observed in the high dose group were considered a consequence of distinct maternal toxicity at this high dose level, exceeding the maximum tolerated dose. There were no significant parental, reproductive or offspring findings in the triticonazole doses below 5000 ppm.

Triticonazole was tested *in vitro* to assess aromatase inhibiting properties to see if this mechanism could be involved in the increased gestation length observed in the F0 and F1 generation. The IC_{50} in rat aromatase was 1.8×10^{-6} M; however, a full inhibition of the aromatase was not seen. The positive controls showed IC_{50} values which were three orders of magnitude lower (Letrozole: IC_{50}: 1.53×10^{-9} M), compared to triticonazole indicating rather low potency of triticonazole to inhibit aromatase in rats.

Several *in vitro* studies were included to assess the endocrine activity of triticonazole. The YAS- and YES-assay (Yeast Androgen Screening (YAS) and Yeast Estrogen Screening (YES) assay), measuring androgen and oestrogen activity showed no increase in androgen and oestrogen activity in genetically modified yeast cells exposed to triticonazole. Predictive endocrine testing in the 21st century using *in vitro* assays of oestrogen receptor responses was negative for oestrogen receptor signalling endpoints with triticonazole. By using ToxCast the AC_{50} concentrations of triticonazole was determined and were relatively high ($>10^{-5}$ M) indicating weak activity. For further details, see Annex I to the CLH report.

Thus, there is no evidence that a specific anti-androgenic or anti-estrogenic mode of action (MoA) has contributed to the observed effects on reproduction in the 2-generation toxicity study. The effects were therefore considered a consequence of general parental systemic toxicity.

In summary, the DS concluded that the data available was not sufficient to conclude that the reduced mating performance observed in the F1 parental animals at the highest dose with triticonazole was a consequence of a substance specific effect on sexual function and fertility. There was no dose response evident for any of the reported effects. Significant systemic toxicity in high dose was reported in both males and females evident as markedly lower body weight and body weight gain compared to control animals and adrenal toxicity as well as death in F0 females. There was no data available with respect to the mechanism of action and cause of the reduced mating performance. The relationship and importance of adrenal insufficiency and hormonal disturbance to reduced mating performance remain speculative since comparison of individual data revealed no correlation. The slight increase in gestational length amongst the F0 and F1 females was not considered as strong evidence in support of classification, and was therefore not identified as being a primary effect of triticonazole in the high dose group (which was considered to be well above the maximum tolerated dose; MTD). Therefore, the DS concluded that the observed findings do not justify classification for adverse effects on sexual function and fertility. They also reported that this is in line with the previous conclusion derived by the European Chemicals Bureau (ECB, 2007).

The DS mentioned that in the DAR (2003) it was concluded that there was evidence that reproductive parameters like female fertility, number of live born pups and pup viability were adversely affected by triticonazole at a very high dose level, exceeding the MTD. However, no effects on reproductive parameters were seen in the absence of maternal toxicity. Therefore, the adverse effects on the reproductive function were considered likely to be a consequence of distinct maternal toxicity, and no classification of triticonazole for reproductive toxicity was considered justified.

Developmental toxicity

For the assessment of developmental toxicity following exposure to triticonazole, a range-finding and a main study in CD Sprague Dawley rats, as well as a tolerance, a range-finding and a main study in NZW rabbits, were assessed. The studies were performed according to OECD TG 414 and according to GLP; however, the tolerance study in rabbits was not according to any test guideline, and the range-finding studies in rats and rabbits were performed with limited number of animals. No human epidemiological studies were available.

Other studies on the mechanism of action were also included (for further details see Annex 1 to the CLH report):

- Chicken embryotoxicity screening test (CHEST)
- Zebrafish developmental screening assay
- Modified zebrafish embryotoxicity test (ZET)
- Gene expression changes in the zebrafish embryo
- Comparison of the mouse embryonic stem cell test, the rat embryo culture and the zebrafish embryotoxicity test
- Yeast Androgen Screening (YAS) and Yeast Estrogen Screening (YES) assay
- Estrogen receptor signalling response assay
- Tox cast data
- Cell stress and cytotoxicity
- *In vitro* high-throughput screening assays for the oestrogen receptor
- A computational approach for the prioritization and screening of chemicals in the endocrine disruptor screening program
- Leydig cell testosterone secretion and androgen receptor activation *in vitro*
- Aromatase inhibition

In the range-finding developmental toxicity study in rats (six per group), the animals were exposed by oral gavage to 0, 50, 250 and 1250 mg/kg bw/d of triticonazole during GD 6-15. In the high dose group, reduced body weight and body weight gain was reported in dams, and bilateral hydronephrosis in foetuses. A dose of 1000 mg/kg bw/d was therefore used as the top dose in the main study.

In the main developmental toxicity in rats (23 per group), the animals were exposed by oral gavage to 0, 40, 200 and 1000 mg/kg bw/d triticonazole on GD 6-15.

Maternal toxicity: At 1000 mg/kg bw/d a reduction in body weight gain (85.5% of control animals on GD 12-16) and food consumption was reported.

Foetal toxicity: Foetal survival and growth was not affected in any dose group. However, there was an increase in the incidence of foetuses with an additional 14th rib or pair of ribs (13/14 or 14/14) at all dose levels, but only outside the HCD at 1000 mg/kg bw/d. This finding was considered related to maternal toxicity. It was concluded that no treatment-related teratogenic effect was observed at any dose level.

In the tolerance study in rabbits (no OECD TG), two rabbits per dose were exposed by oral gavage in three different groups: group 1.) non-pregnant rabbits to 500 mg/kg bw/d for two days; group 2.) non-pregnant rabbits starting with 25 mg/kg bw/d and in the absence of any adverse effects, the dose was doubled every two days until effects were reported at 100 mg/kg bw; group 3.) pregnant rabbits to 50 mg/kg bw/d on GD 6-12. It was concluded from the tolerance study that severe toxicity was reported at doses above 50 mg/kg bw/d.

In the range-finding developmental toxicity study in rabbits (7-8/group) the animals were exposed by oral gavage to 0, 5, 15, 50, 75 and 150 mg/kg bw/d triticonazole on GD 6-19. All females in the 150 mg/kg bw/d group were terminated prematurely due to animal welfare reasons. Maternal toxicity was evident at 15 mg/kg bw/d and included a dose related (slight) body weight loss (days 6 to 8). Foetal effects were reported from 75 mg/kg bw/d and included increased post-implantation loss (above HCD).

In the main developmental toxicity study, rabbits (18 per dose) were exposed to 0, 5, 25, 50 and 75 mg/kg bw/d triticonazole on GD 6-19.

Maternal toxicity: Body weight losses and decreased food consumption were reported at doses ≥ 25 mg/kg bw/d. At 50 and 75 mg/kg bw/d, excessive maternal toxicity was reported and included deaths, abortions, decreased faeces and an increased respiration rate. In the high dose group, 30% maternal mortality was reported.

Foetal toxicity: A slight increase in both pre- and post-implantation losses were observed at 75 mg/kg bw/d, which was considered to be related to maternal toxicity. Increased incidences of precocious ossification of acromion process (elongation of acromion process) were seen at ≥ 25 mg/kg bw/d. The precocious ossification of the acromion process was considered to be of low severity, as this part of the scapula is ossified during development of the offspring and an earlier ossification has no impact on survival or quality of life. In the top dose, increased incidences of variations of the midline anterior cranial bones, rudimentary floating 13th rib, and reduced/incomplete ossification of metacarpals and phalanges (also seen at 50 mg/kg bw/d) were seen at excessive maternal toxic doses. As these variations were reported only in the presence of marked maternal toxicity, they were not indicative of a specific teratogenic response of triticonazole.

In the additional studies, investigating mechanisms of action of triticonazole, no effects on mortality, blood vessel development, and blood vessels discoloration were reported in the CHEST. Reduced embryo development with an incidence of 4% compared to control was slightly above the laboratory HCD (2%) for animals treated with the highest dose.

The literature data showed that triticonazole had a toxic potential on the zebra fish embryo/larvae indicated by non-viable larvae or larvae that did not hatch at concentrations > 20 µM. However, no malformations were observed in the larvae after exposure to triticonazole.

Triticonazole was found to be the least potent triazole with regard to general developmental and to specific teratogenic endpoints in the ZET. In this test, zebrafish embryos were evaluated 72 hours post fertilization. The results showed that triticonazole was non-embryotoxic, based on very slight morphological changes induced in the zebra fish embryos.

The results of the gene expression data and the concentration-response genes correlated to the general morphology score (GMS) suggested that triticonazole has the lowest level of unwanted effects when compared to other tested triazoles, with respect to developmental toxicity.

In the Embryonic Stem Cell Test (EST), triticonazole was shown to be the least potent triazole compared to the other tested triazoles. In the Whole embryo culture (WEC), triticonazole was the second-least potent compound. In summary, the WEC, EST and ZET assays correctly

identified the potency of triticonazole for developmental effects, based on *in vivo* data, as triticonazole did not induce malformations such as cleft palate, renal malformations and hydrocephaly.

Neither in rat nor in the rabbit developmental toxicity studies, effects that would justify classification for developmental toxicity were reported. No effects (variations/anomalies) in foetuses were observed without maternal toxicity and no treatment-related malformations were observed which could support classification in absence of maternal toxicity. The DS therefore concluded that triticonazole should not be classified for developmental toxicity.

Adverse effects on or via lactation

The DS concluded that a lactation effect of triticonazole was not supported by the data. Perinatal maternal toxicity was much more likely since observations on the pups during necropsy ("no milk in stomach") indicated nursing deficits of the female rats.

Comments received during public consultation

One MSCA commented on reproductive toxicity. Comments related to effects on sexual function and fertility included information regarding the age at week 0 for the F1 generation, and information regarding when the exposure of the F1 generation started. The MSCA considered that classification as Repr. 2; H361f should be discussed. As regards developmental toxicity, the MSCA commented that based on the reported hydronephrosis and skeletal findings in rats/rabbits classification as Repr. 2; H361d should be discussed.

Assessment and comparison with the classification criteria

Effects on sexual function and fertility

For the assessment of effects on sexual function and fertility, the main study was a two-generation reproductive toxicity study in Sprague Dawley CRL:CD rats performed according to GLP and OECD TG 416, with deviations as described in "Summary of the DS proposal".

Several *in vitro* mechanistic studies were also included (see "Summary of the DS proposal"). However, as the main effects assessed according to the CLP criteria was reported in the two-generation study, the mechanistic *in vitro* studies were used in the assessment of a potential MoA for the reduction in mating and fertility index reported in the F1 generation in the high dose group (detailed information on these studies are found in Annex 1 to the CLH report). No human epidemiological data was included in the CLH report.

In the two-generation study, rats were exposed to 0, 5, 25, 750 and 5000 ppm triticonazole. It should be noted that the dose spacing between the high dose group and the next lower dose in the study was rather high (5000 to 750 ppm) making the interpretation of the data challenging. The corresponding mean intake of triticonazole during the study is shown in the table below.

***Table**: Mean intake (mg/kg bw/d) of triticonazole in the two-generation study in rats*

Dose level (ppm)	F0	F1	F0	F1	F0	F1
	male/female (pre-mating)		female (gestation)		female (lactation)	
5	0.34/0.37	0.37/0.43	0.32	0.33	0.58	0.46
25	1.64/1.81	1.82/2.14	1.59	1.60	2.97	2.96
750	49.35/54.80	56.18/65.25	48.41	49.10	87.99	93.25
5000	350.8/389.3	445.3/493.8	337.64	339.08	592.99	528.05

General toxicity seen in the high dose group was mortality (only in F0 generation), clinical signs, decreased body weight and body weight gain as well as histopathological changes and organ weigh changes in the adrenals, liver and ovary.

During gestation, in F0 females at 5000 ppm, one female was sacrificed after prolonged parturition and three were sacrificed or found dead during late gestation (day 23) or lactation (day 7 and 9), a period with high intake of triticonazole (approx. 600 mg/kg bw/d). No specific clinical signs were noted before death of these animals; however, the animal that died on lactation day 9 was thin, hunched and passive. At necropsy, histopathological lesions in the adrenal cortex were reported in these four animals, and the study report considered that the four deaths could have been treatment-related. In addition, five premature deaths were reported in the control and exposed animals. However, these deaths were not considered to be treatment-related.

In F0 and F1 females a statistically significant decrease in body weight and body weight gain was reported at 5000 ppm during premating, gestation and lactation, and in males during premating, see tables below:

***Table**: Mean body weights of F0 and F1 females in grams and (% control)*

Week	Treatment group					
	ppm	**0**	**5**	**25**	**750**	**5000**
Week 0	F0	200.11	198.57	195.74	192.64	194.89
	F1[1]	151.48	150.37	148.11	144.14	**102.41**** (67.6)
Week 10 (end of pre-mating)	F0	331.62	322.48	325.81	323.44	**296.57**** (89.4)
	F1	290.71	293.56	294.09	291.13	**239.07**** (82.2)
Overall premating weight gain (0-10)	F0	131.5	123.9	130.1	130.8	**101.7**** (77.3)
	F1	139.2	143.2	146.0	147.0	136.7 (98.9)
GD0	F0	329.35	322.74	326.34	320.35	**293.75**** (89.2)
	F1	290.80	297.14	292.14	296.90	**236.27**** (81.2)
GD21	F0	458.16	463.80	465.68	466.35	**418.83**** (91.4)
	F1	426.97	435.33	418.44	434.01	**335.59**** (78.6)
LD0	F0	363.5	362.3	364.5	364.5	**324.7**** (89.3)
	F1	334.7	346.2	335.6	337.3	**271.0**** (80.9)
LD21	F0	368.7	363.2	363.4	359.2	**328.5**** (89.1)
	F1	334.6	341.2	335.1	338.3	**264.2**** (78.9)

1 Week 0 for F1 is postnatal day 21 (weaning)

**($p \leq 0.01$); significantly different from control

***Table**: Mean body weights of F0 and F1 males in grams and (% control)*

Week	Treatment group					
	ppm	**0**	**5**	**25**	**750**	**5000**
Week 0	F0	217.45	222.26	220.96	222.27	222.08
	F1	193.29	187.30	189.96	179.21* (92.7)	**125.53**** (64.9)
Week 10 (end of pre-mating)	F0	476.82	487.96	479.81	497.41	471.94
	F1	546.35	551.49	537.86	536.80	**429.60**** (78.6)
Week 19 (termination)	F0	549.78	558.00	557.68	577.07	541.21
	F1	658.36	665.25	635.88	641.13	**517.02**** (78.5)
Overall weight gain (0-19)	F0	332.3	335.7	336.7	354.1	319.1
	F1	465.1	478.0	445.9	461.9	**391.9**** (84.2)

***** ($p \leq 0.05$), ****** ($p \leq 0.01$); significantly different from control

A marked decrease in food consumption was reported in F0 females at 5000 ppm throughout gestation, and in F1 females at 5000 ppm throughout premating, gestation and lactation and in F1 males during premating.

Pathological findings in the F0 females of the high dose group included a significant lower absolute and relative left adrenal weight and significantly higher absolute and relative liver weight when compared to controls. Similar findings were reported in F1 females. While in F0 and F1 females a decrease in relative adrenal weight was observed, this was opposite to an increase in the relative adrenal weight in F1 males.

At 5000 ppm in F0 and F1 females, microscopic findings in the adrenals comprised increased incidence and severity of degenerative changes of the adrenal cortex (vacuolation, syncytial giant cell formation, deposition of collagen, large pigment laden cells and minimal inflammation). In F1 females (high dose group), vacuoles and giant cells were present in the ovaries in four females which were considered treatment-related. No similar findings were observed in F0 females, see table below.

***Table**: Group incidences of histopathological changes in F0 and F1 females (modified from table 3.10.1.1-10 in Annex 1 to the CLH report)*

Findings		Doses (ppm)				
		0	5	25	750	5000
Adrenals						
Vacuolation of cortex	F0	0/27	0/28	1/28	1/28	0/24
	F1	0/28	1/27	0/28	0/27	0/28
Degeneration of cortex	F0	0/27	0/28	0/28	0/28	**22/24**
	F1	0/28	0/27	0/28	0/27	**11/28**
Collagen deposition	F0	0/27	0/28	0/28	0/28	6/24
	F1	0/27	0/28	0/28	0/28	0/28
Giant cells	F0	0/27	0/28	0/28	0/28	**16/24**

	F1	0/27	0/28	0/28	0/28	**14/28**
Cortical pigment	F0 F1	0/27 0/28	0/28 0/27	0/28 0/28	0/28 0/27	**6/24** **7/28**
Chronic inflammation	F0 F1	0/27 0/28	0/28 0/27	0/28 0/28	0/28 0/27	2/24 0/28
Ovaries						
Vacuolation	F0 F1	0/27 0/28	-	-	-	0/24 4/28
Giant cells	F0 F1	0/27 0/28	-	-	-	1/24 2/28

F0 generation: There were no treatment-related effects on mating and fertility index, conception rate (no. pregnant animals/no. mated animals) and gestation index (no. live litters born/no. animals pregnant) reported. However, in the high dose group the mean duration of gestation was significantly increased (22.6 days vs. 22.1 days in controls) but was within the laboratory HCD (F0: 21.8 – 22.7 days). It was shown that this effect was mainly due to two females with longer gestation (24 and 25 days) and the effect was therefore not considered to be treatment-related, see table below. The F0 female with a gestation of 25 days had to be sacrificed moribund after prolonged parturition, however, the other female (GD 24) had surviving pups.

F1 generation: The longer gestation seen in the high dose group was not statistically significant increased (22.5 days) and was just at the border of the laboratory HCD (22.0 – 22.5 days). However, it should be noted that in the high dose group a lower number of pregnant females were available due to the impairment of mating performance in F1 (F0: 27 animals, F1: 17 animals at 5000 ppm).

***Table**: Summary of gestation times for the F0 and F1 generations*

Dose group / No. of gestation days	**Number of animals with the respective no. of gestation days**									
	F0					**F1**				
	21	**22**	**23**	**24**	**25**	**21**	**22**	**23**	**24**	**25**
0 (control)	-	20	3	-	-	-	23	3	-	-
5 ppm	3	18	4	1	-	1	21	6	-	-
25 ppm	-	18	4	-	-	2	17	4	-	-
750 ppm	3	19	2	-	-	-	21	3	-	-
5000 ppm	1	11	13	1	1	-	10	5	1	1

Further, in the F1 generation at 5000 ppm a statistically significantly decrease in mating and fertility indices was reported that were outside the HCD, see table below. It should be noted that the pairs without progeny were not evaluated to determine the apparent cause of the infertility, and there were no re-mating of the sirs and dams in the F1 generation that did not mate.

Litter data: Several litter parameters in the high dose group were affected in the F1 and F2 generations, that could be due to a higher intake of triticonazole during lactation in the F0 and

F1 dams in the high dose group compared to the intake of triticonazole in this dose group during gestation. The livebirth and viability index were statistically significantly reduced compared to controls, whereas the total number of stillborn F1 and F2 pups was increased. In addition, total litter death was noted for four F0 females during lactation between days 0 – 4, and the mean number of live pups/litters on days 0 and 4 of lactation were all statistically significantly reduced among F2 litters, see table below. Necropsy of dead pups showed "no milk in the stomach" as the only reported finding (F1: 16 (5), 7 (6), 1 (1), 9 (8) and 26 (11) and F2: 3 (3), 2 (2), 5 (4), 6 (4) and 13 (5) in foetuses (litters) at 0, 5, 25, 750 and 5000 ppm).

***Table**: Summary of study findings on reproductive performance, delivery and litter data (modified from table 3.10.1.1-5 in Annex 1 to CLH report))*

Parameter		Dose level (ppm)				
		0	**5**	**25**	**750**	**5000**
Pre-coital interval (days)	F_0	2.12	2.81	3.32	2.81	2.61
	F_1	3.07	3.18	2.54	3.38	3.30
Number of paired females	F_0	27	28	28	28	28
	F_1	28	28	28	27	28
Total number inseminated	F_0	26	28	28	27	28
	F_1	28	28	26	27	**20****
Total number pregnant (%)	F_0	23 (88)	27 (96)	22 (79)	25 (93)	28 (100)
	F_1	26 (93)	28 (100)	25 (96)	25 (93)	**18**** (90)
Number of dams delivering	F_0	23	27	22	25	27
	F_1	26	28	25	25	17
Gestation length (days) (HCD of laboratory, 1988 – 1993; F0 = 21.8 – 22.7 d; F1 = 22.0-22.5 d)	F_0	22.1	22.1	22.2	22.0	**22.6***
	F_1	22.1	22.2	22.1	22.1	22.5
Mating index (%) (no. animals inseminated/ no. animals paired x 100) (HCD of laboratory, 1988 – 1993; F0 = 90 – 100%, F1 = 81-100%	F_0	96	100	100	96	100
	F_1	100	100	93	100	**71****
Fertility index (%) (no. animals pregnant/ animals paired x 100) (HCD of laboratory, 1988 – 1993; F0 and F1 = 76 – 96%	F_0	85	96	79	89	100
	F_1	93	100	89	93	**64****
Gestation index (%) (% pregnancies yielding live litters)	F_0	100	100	100	100	93
	F_1	100	100	100	100	89
Livebirth index (%) (no. live pups at day 1/no. born pups x 100)	F_1-pups	93	98	99	98	**82****
	F_2-pups	99	98	99	98	**85****

Parameter		Dose level (ppm)				
		0	**5**	**25**	**750**	**5000**
Viability index (%) (no. pups alive on day 4/ no. live born pups)	F_1-pups	92	94	99	97	**82****
	F_2-pups	98	100	99	97	**89****
Weaning index (%) (days 4 - 21)	F_1 pups	95	100	100	100	94
	F_2 pups	100	100	100	100	100
No. stillborn pups/no. pups delivered (%)	F_1 pups	18/335 (5.3%)	7/419 (1.7%)	2/341 (0.6%)	7/411 (1.7)	46/382 (12.04%)
	F_2 pups	4/381 (1.1%)	4/408 (0.98%)	3/343 (0.87%)	7/388 (1.8%)	11/203 (5.4%)
Entire litter died, killed, missing, and/or cannibalised Day 0-4 Day 5-21	F_1 pups	0 0	1 0	0 0	0 0	4 0
	F_2 pups	0 0	0 0	0 0	0 0	1 0
Mean live pups per litter Day 0 Day 4	F_1 pups	13.70 13.00	15.22 14.85	15.32 15.14	16.12* 15.64*	12.35 12.64
	F_2 pups	14.46 14.15	14.32 14.25	13.52 13.36	15.20 14.80	**11.13**** **11.07****

* ($p \leq 0.05$); ** ($p \leq 0.01$); significantly different from controls

Individual data for the eight F1 males and females that did not mate in the high dose group were evaluated (parameters assessed: body weight, adrenal and ovary findings); however, no explanation for the absence of mating was found among those parameters. However, it should be noted that there were no measurements for oestrus cycling, differential ovarian follicle count, sperm morphology and motility, implantation sites, testicular histopathology or examination of intact epididymis.

In summary, effects on sexual function and fertility was only reported in the high dose group. However, it should be noted that the dose spacing between the high dose group (5000 ppm) and the next lower dose (750 ppm) was large. In the high dose group, four effects has to be taken into account for the decision on classification for effects on sexual function and fertility:

1. *Mortality in the F0 females in late gestation/early lactation at 5000 ppm.* It should be noted that the mortality in F0 was seen with no reduction in mating and fertility index, whereas in the F1 females a statistically significant reduction in mating and fertility index were reported at 5000 ppm, outside HCD and in the absence of mortality. However, in the F1 females a more pronounced effect on the reduction in body weight was reported compared to F0 (82% in F1 and 89% in F0) at week 10 just prior to mating. On the other hand, this could be due a higher intake of triticonazole in the F1 generation during the 10-w pre-mating period compared to the F0 generation (F0: 389.3 mg/kg bw/d, F1: 493.8 mg/kg bw/d).
2. *Increase in pregnancy duration in F0/F1 females at 5000 ppm.* This effect was mainly due to two F0 females with longer gestation periods (24 and 25 days). The group mean values (22.6 days for F0 and 22.5 days for F1) were in the range of historical control data (21.8 – 22.7 days for F0 and 22.0 – 22.5 days for F1); the effect was not considered treatment-related.

3. *Reduction in female fertility in the F1 generation at 5000 ppm.* This effect could be related to a decreased receptivity of females since female sexual response in laboratory animals is considered to be regulated by hormones produced by the ovaries and adrenals. The degeneration of the adrenal cortex, as well as the effects reported in the ovary observed in female rats treated with 5000 ppm triticonazole, could be responsible for a reduced secretion of adrenal or ovary hormones. However, when comparing individual data, no clear correlation was found, and it was noted that for quite a number of F0 and F1 animals having these changes, mating was normal. Further, there were no evidence that a specific anti-androgenic or anti-estrogenic MoA contributed to the observed effects on mating and fertility index in the F1 generation.
4. *Increase in stillbirth and neonatal mortality, decrease in the mean number of pups/litter in F0/F1 generation at 5000 ppm.* These effects were considered a consequence of severe maternal toxicity and not a primary effect of triticonazole since it was noted, that in the F0 and F1 dams at 5000 ppm there was a higher intake during lactation compared to the intake during gestation. Furthermore, there was no evidence of malformations in the offspring, even, when parental animals were exposed to 5000 ppm.

Comparison with the criteria

RAC considered that in a weight of evidence assessment, the statistically significant reduction in mating performance and fertility, that were outside the HCD, observed only in the high dose F1 parental animals was of concern and was not considered related to the decrease body weight gain reported in the F1 animals.

Category 1A: Based on evidence from human data. No human epidemiological data was available. Classification as Repr. 1A is therefore not justified.

Category 1B/2: Based mainly on evidence from animal study where the data shall provide clear evidence/some evidence of an adverse effect on sexual function and fertility, in the absence of other toxic effects, or if occurring together with other toxic effects, the adverse effect on reproduction is considered not to be a secondary, non-specific consequence of other toxic effects.

In the two-generation study, a statistically significant reduction in the mating and fertility index, that were outside the HCD, was reported in the F1 generation in the high dose group, but not in the F0 generation. This effect was not considered a secondary consequence 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.

Based on this, RAC considered that **classification as Repr. 2 (H361f) for effects on sexual function and fertility** is justified.

Developmental toxicity

Developmental toxicity following exposure to triticonazole was studied in CD Sprague Dawley rats and NZW rabbits. In rats, a range-finding study and main study was included and in rabbits, a tolerance, a range-finding and a main study was reported. The studies were performed according to OECD TG 414 and GLP, however, the tolerance study in rabbits was not performed according to an OECD TG and the range-finding studies in rats and rabbits were performed with limited number of animals. No human epidemiological studies were available.

Other studies on the mechanism of action were also included (for further details see Annex 1 to the CLH report and "Summary of the DS proposal" above).

In the range-finding developmental toxicity study in rats (6/group), the animals were exposed by oral gavage to 0, 50, 250 and 1250 mg/kg bw/d on GD 6-15. In the high dose group, reduced body weight (90% of controls at GD 14) and body weight gain (73% of controls at GD 14) was reported in dams. Gross necropsy in dams at termination did not reveal any treatment-related findings. In the foetuses, a low but possible dose related increased incidence of hydronephrosis was reported, see table below. A dose of 1000 mg/kg bw/d was therefore used as the highest dose in the main developmental toxicity study in rats.

***Table**: Foetal incidences of hydronehprosis (modified from table 3.10.1.2-5 in Annex I of the CLH report)*

Dose level (mg/kg bw/d)	**0**	**50**	**250**	**1250**
Litters/foetuses evaluated	6/54	6/51	6/60	6/47
- Unilateral hydronephrosis % incidence (no. of litters) HCD: 0.99 (0.0-7.6)	0.0 (0)	0.0 (0)	5.0 (2)	6.4 (3)
Bilateral hydronephrosis HCD: 0.70 (0.0-7.6)	0.0 (0)	3.9 (2)	6.7 (1)	8.5 (2)

In the main developmental toxicity study in rats (23 per group), the animals were exposed by oral gavage to 0, 40, 200 and 1000 mg/kg bw/d triticonazole on GD 6-15.

Maternal toxicity: No mortality or clinical signs were reported. At 1000 mg/kg bw/d, a reduction in body weight gain was seen (85.5% of control animals on GD 12-16) resulting in maternal bw of 96% of control. The food consumption was marginally reduced.

Foetal toxicity: Foetal survival and growth was not affected in any dose group. The incidence of hydronephrosis (unilateral and bilateral) was slightly increased in the high dose group (1.3% unilateral and 0.0% bilateral) compared with controls (0.6% unilateral and 0.6% bilateral) but was within the HCD of 16 studies and 2466 foetuses from the laboratory, conducted approximately up to the time of the present study (0 - 3.6% unilateral and 0 - 1.4% bilateral). Further, there was an increase (not statistically significant) in the incidence of foetuses with an additional 14th rib or pair of ribs (13/14 or 14/14) at all dose levels, but only outside the HCD at 1000 mg/kg bw/d; see table below. RAC considered that the marginally increase in skeletal variations that were outside the HCD at 1000 mg/kg bw/d is not sufficiently severe for classification for developmental toxicity.

***Table**: Incidence (%) of an additional 14th rib or pair of ribs in rats (modified from table 3.10.1.3-6 from Annex I to the CLH report)*

Doses (mg/kg bw/d)	**0**	**40**	**200**	**1000**
13/14th ribs				
Foetal incidence no./% HCD: 0.0 – 15.9[1]	21/11.7	25/13.2	25/13.4	28/**16.9**
Litter incidence	11	18	13	15
14th rib				
Foetal incidence no./% HCD: 0.0 – 10.4[1]	9/5.0	17/9.0	18/9.7	24/***14.5***
Litter incidence	3	6	9	9

1 The HCD was from 16 studies conducted approximately in the time of the present study and 91 studies between 1991 and 1994.

Studies in rabbits

In the rabbit tolerance study (no OECD TG) rabbits (two per dose) were exposed by gavage in three different groups; see 'Summary of dossier submitter proposal". In group 1.) one female was killed in extremis and the second terminated following a marked toxic response, characterised by bodyweight loss and reduced food intake and faecal output. In group 2.) body weight loss and reduced food intake and faecal output was reported following the first dose at 100 mg/kg bw/d. Terminal necropsy of both females showed no macroscopic changes. At doses down to 25 mg/kg bw/d no effects were reported. In group 3.) both pregnant females exposed to 50 mg/kg bw/d on GD 6-12 exhibited timid behaviour for varying durations during the study and showed slight transient body weight loss. No other adverse effects were reported. Both animals were pregnant. One female had one late resorption, but all remaining foetuses from both females appeared normal. It was concluded from this study that in the range-finding developmental toxicity study in rabbits the dose should not exceed 50 mg/kg bw/d, since severe toxicity was reported at doses above 50 mg/kg bw/d.

In the range-finding developmental toxicity study in rabbits (7-8 per group) the animals were exposed by oral gavage to 0, 5, 15, 50 in one study and 0, 75 and 150 mg/kg bw/ in another study on GD 6-19.

Maternal toxicity: All females in the 150 mg/kg bw/d group were terminated prematurely due to animal welfare reasons. In the 75 mg/kg bw/d dose group reduced body weight gain (-62% compared to controls GD 6-18), food consumption (-21% compared to control GD 6-19) and defecation was reported. No macroscopic changes in maternal condition were reported that could be related to treatment.

Foetal toxicity: From 75 mg/kg bw/d an increase in post-implantation losses (35.8% vs. 14.0% in controls and above HCD: 4.8-21.7%) was reported. The percentage of early resorptions was 1.0 or 0.4 in the two control groups, and 0.3, 0.3, 1.5% and 3.1% at 5, 15, 50 and 75 mg/kg bw/d, respectively (HCD: 0.1-1.4%). All other litter parameters were essentially similar in all groups. External examination of the foetuses showed some anomalies in all groups; however, without a dose-response and in most of the cases within the HCD. Internal examination showed one foetus with multiple malformations in the 75 mg/kg bw/d dose group (abnormal heart and major vessels, enlarged left and reduced right ventricles, interventricular septal defects and malpositioned kidney). However, foetuses in the remaining litters showed a low incidence of anomalies, and if reported they were previously reported in this strain of rabbit in the test laboratory. Based on the limited toxicity seen in dams and foetuses following *in utero* exposure to 75 mg/kg bw/d, this dose was selected as the highest dose in the main developmental toxicity study in rabbits.

In the main developmental toxicity study, rabbits (18 per dose) were exposed to 0, 5, 25, 50 and 75 mg/kg bw/d triticonazole on GD 6-19.

Maternal toxicity: Marked maternal toxicity was reported and included deaths in one and six rabbits at 50 and 75 mg/kg bw/d, respectively. Decreased faeces and an increased respiration rate was reported, with increasing incidence with increasing dose. A decrease in body weight gain compared to controls was reported on GD 6-20 (100%, 89.5%, 100%, 42.1% and 15.8% in the 0, 5, 25, 50 and 75 mg/kg bw/d group, respectively). Thereafter, body weight gain was essentially similar to controls in all dose groups. The food consumption was decreased at doses ≥ 25 mg/kg bw/d on GD 6-12 (87.7, 82.1 and 65.4% compared to controls) and at doses ≥ 50 mg/kg bw/d on GD 13-19 (80.8 and 87.8% compared to controls).

Foetal toxicity: A slight increase in the percentage of pre- and post-implantation losses were observed at 75 mg/kg bw/d, but was within the HCD (pre-implantation losses: 28.8% vs. 18.9%

in controls, HCD: 8.9% - 43% and post-implantation losses: 20.7% vs. 13.6% in controls, HCD: 4.8% - 21.7%). All other litter parameters were similar in the control and exposed groups.

Skeletal malformations were reported in the high dose group and included "two or more caudal vertebrae fused and/or reduced, kinky tail" and "frontal bone fusion and other major cranial anomalies" that were above the HCD, see table below. In the assessment of the vertebrae, limbs and girdles, an increased incidence of the variant "precocious ossification of acromion process" (elongation of acromion process in the scapula) was seen at doses ≥ 25 mg/kg bw/d and was above the HCD, but was only statistically significant in the high dose group, see table below. The DS reported that the precocious ossification of the acromion process was considered to be of low severity, as this part of the scapula was ossified during development of the offspring and an earlier ossification was considered to have no impact on survival or quality of life. Other variations in the high dose group included increased incidences of variations of the midline anterior cranial bones, rudimentary floating 13th rib, and reduced/incomplete ossification of metacarpals and phalanges (also seen at 50 mg/kg bw/d). RAC considered that the malformations and variations reported on litter parameters were only reported in the presence of marked maternal toxicity including maternal death and do not support a classification for developmental toxicity. The increased incidence of precocious ossification of acromion process starting at doses without maternal toxicity was by RAC not considered to be of significant severity to warrant classification for developmental toxicity.

***Table**: Incidences of skeletal findings in the rabbit developmental toxicity study modified from table 3.10.1.6-6 from Annex I to the CLH report)*

Dose (mg/kg bw/d)	**0**	**5**	**25**	**50**	**75**
No. litter	20	16	18	16	13
No. foetuses	124	118	121	103	63
Skeletal malformations					
Frontal bone fusion and major cranial anomalies foetal incidence no./% Litter incidence HCD[1]: 0.0 – 1.5	0/0 0	0/0 0	0/0 0	1/1 1	1/1.6 1
Two or more caudal vertebra fused and/or reduced, short kinky tail foetal incidence no./% Litter incidence HCD[1]: 0.0 – 0.8	0/0 0	0/0 0	0/0 0	0/0 0	2/2.2 2
Skeletal variations					
Elongation of acromion process foetal incidence no./% Litter incidence HCD[1]: 0 - 1.1%	3/1.7 1	0/0 0	6/3.4 4	8/5.3 5	10/10.9* 4

* $p \leq 0.05$

1 HCD from laboratory collected in 18 studies 5 years near the time of the present study

Comparison with the criteria

Category 1A: Based on evidence from human data. No human epidemiological data was available. Classification as Repr. 1A is not justified.

Category 1B/2: Based mainly on evidence from animal study where the data shall provide clear evidence/some evidence of an adverse effect on development in the absence of other toxic effects or if occurring together with other toxic effects the adverse effect on development is considered not to be a secondary non-specific consequence of other toxic effects. Clear maternal toxicity was reported in the developmental toxicity studies in rat and rabbit including death in maternal rabbits in the presence of developmental toxicity. RAC therefore considered that the effects were not relevant for classification for developmental toxicity.

In conclusion, RAC agreed with the DS that **classification for developmental toxicity is not justified.**

Adverse effects on or via lactation

A lactation effect was not supported by the data. Perinatal toxicity due to maternal toxicity was much more likely since observations on the pups during necropsy ("no milk in stomach") in the two-generation toxicity study indicated nursing deficits of the female rats.

A classification for effects on or via lactation can be assigned on:

a) human evidence indicating a hazard to babies during the lactation period; and/or
b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or
c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

A lactation effect was not supported by the data. Perinatal toxicity due to maternal toxicity was most likely since observations on the pups during necropsy with "no milk in stomach" indicated nursing deficits of the female rats.

RAC agreed with the DS that **classification for effects on or via lactation is not justified.**

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Triticonazole currently has the following classification for the environment in Annex VI of the CLP Regulation: Aquatic Chronic 2 (H411). It was included in the Annex VI by ATP01. The DS's proposal for consideration by RAC was to change the environmental hazard classification to Aquatic Acute 1 (M=1) and Aquatic Chronic 1 (M=1).

Degradation

Regarding abiotic degradation, based on two available tests on hydrolysis performed in accordance with OECD TG 111 and GLP, triticonazole is hydrolytically stable at pH 4-9 and at 25°C. In three tests on direct photochemical degradation in aqueous solutions following OECD

TG 316 and GLP, triticonazole was observed to reversibly convert into its Z-isomer (RPA 406203) and equilibrium between the isomers was established within 1 to 2 days. No other metabolites were observed > 5% AR. The DT_{50} values for the dissipation of triticonazole were 7.4 and 32.7 days once equilibrium had been reached. The DT_{50} value for the dissipation of Z-isomer was 27.6 days.

Regarding biodegradation in aquatic environments, the dossier includes a biodegradation screening study following OECD TG 301B and GLP in which triticonazole at initial concentrations of 10 and 20 mg/L was incubated in test flasks containing activated sludge. No degradation of triticonazole was observed after 28 days.

A surface water simulation study conducted according to OECD TG 309 is also available. In this study, phenyl- and triazole-labelled triticonazole was stable (92.4-99.9% of AR after 59 days) at both low (0.008-0.009 mg/L) and high (0.084-0.093 mg/L) test concentrations. The amount of evolved CO_2 after 60 days reached 1-3% AR and the amounts of other metabolites (the most abundant ones being different isomers of the substance) were below 4.3% AR. Due to the negligible degradation observed, it was not possible to estimate a DT_{50} value.

Phenyl labelled triticonazole also showed slow degradation in the available water-sediment simulation study (OECD TG 308). Only 1.3-1.7% CO_2 evolved after 105 days of incubation, and the three unknown radioactive fractions found in the water and the sediment phases did not exceed levels of 2.5% AR. NER accounted for maximum 25% AR at study end. The $DegT_{50}$ of triticonazole in the total system ranged from 225 to 399 days.

Several soil simulation studies with triticonazole are also included in the dossier. All studies are performed according to or broadly following the OECD TG 307. The estimated DT_{50} values of triticonazole for the different tested soils at standard conditions of 20°C and pF 2 varied from 109 to 565 days (geometric mean 246 days) and at 10 °C from 176 to >1000 days. The main transformation products were the mono-hydroxylated RPA 406341 (Trans-diol) (max. 20.2% AR at 22 °C) and RPA 404766 (Cis-diol) (max. 13.9% AR at 10 °C). Several other metabolites were observed above 5% AR (and some > 10% AR) but they could not be unambiguously identified. Under aerobic conditions mineralisation to CO_2 was limited (0.1 - 8.1% AR after around 120 days) and the level of non-extractable residues (NER) reached 4.5 - 27.3% AR after around 120 days. Degradation of triticonazole under anaerobic conditions was negligible and only led to minor metabolites as well as to the formation of non-extractable residues (max. 25.2% AR).

Information from 8 soil field dissipation studies on triticonazole performed in Europe is also included in the dossier. A geometric mean of the estimated $DegT_{50}$ values of 78.7 days is calculated although it is noted that there is some uncertainty in the values due to the applied test conditions.

The dossier includes also information from laboratory soil simulation and soil field dissipation studies with the metabolites RPA 406341 (Trans-diol) and RPA 404766 (Cis-diol). Regarding the laboratory studies, the estimated DT_{50} values of RPA 406341 and RPA 404766 for the different tested soils were in the range of 56-450 days (geometric mean 163 days) and 24-175 days (geometric mean 75.3 days), respectively, at standard conditions of 20°C and pF 2, and in the range of 309-393 days and 140-296 days, respectively, at 10°C or at reduced soil moisture. The field dissipation study with RPA 406431 resulted in estimated time-step normalized (20 °C and pF 2) field DegT50 values of 33-56 days.

Based on the available information, the DS concluded that the substance is not rapidly degradable for classification purposes.

Bioaccumulation

The log K_{ow} of triticonazole is 3.29 at 20°C based on a HPLC study (OECD TG 117).

A bioconcentration in fish study performed according to US EPA guideline 165-4 is included in the dossier. Bluegill sunfish (*Lepomis machrochirus*) was exposed to [^{14}C]-triticinozale at nominal concentration of 89 µg/L during 28 days in a flow-through system, followed by a 14 days depuration period. A whole fish kinetic BCF of 72.55, a depuration rate constant k2 of 0.8 ml/g/day and a depuration half-life of 0.86 days based on total radioactivity are reported. Based on chemical analysis performed during the uptake phase, the principal radioactive components were triticonazole with the isomeric forms RPA 404886 and RPA 405826, RPA 406972, glucuronide conjugates of RPA 404886 and RPA 405862 and glucuronide conjugates of RPA 406972 and RPA 406341. Triticonazole was found to comprise less than 5% of the total radioactive residue in fish after 28 days of exposure.

According to the DS, the study contains some uncertainties, as the bioconcentration factor seems to first decrease and then increase again. Furthermore, some information is missing in the study report (lipid content of fish, TOC, testing of a second concentration). However, the DS concluded that the results of the study can be used to indicate a low potential of the substance to bioaccumulate in fish, which is also supported by the log K_{ow} below 4.

The DS also concluded that the bioaccumulation potentials of the major metabolites in water and sediment studies (RPA 404766, RPA 406341, RPA 407922 and RPA 406302) are also assumed to be low, due to their log K_{ow} values clearly below 4.

Aquatic toxicity

Valid acute and chronic toxicity studies are available for fish, aquatic invertebrates and algae. All studies were performed in accordance to GLP.

Acute toxicity

Four different fish species (*Oncorhynchus mykiss, Lepomis macrochirus, Cyprinodon variegatus* and *Cyprinus carpio*) were tested in 7 acute studies performed according to guidelines OECD TG 203, E.P.A./FIFRA Guideline 72-1, FIFRA Guideline 72-3 or OPPTS 850.1075. The 96h LC_{50} values determined in the studies ranged from > 3.6 mg/L for *Oncorhynchus mykiss* to > 18 mg/L for *Cyprinus carpio*. The results are based on mean measured concentrations or nominal concentrations (provided that the measured concentrations were 80-120% of the nominal concentrations). In most of the studies, no mortality was observed and in the ones where mortality was observed it was so low that no LC50 value could be determined. Therefore, all the available LC_{50} values are limit values.

Regarding acute toxicity to aquatic invertebrates, the dossier included an 48h static study with *Daphnia magna* following OECD TG 202 and a 96h flow-through study with the marine species *Americamysis bahia* following U.S. EPA/FIFRA Guideline 72-3. The studies resulted in EC_{50} values of 7.85 mg/L (based on nominal concentrations, mean measured concentrations 92-106% of the nominals) and 1.9 mg/L (based on mean measured concentrations), respectively.

Two algal toxicity studies with triticonazole are available. In a study following OECD TG 201, only 5.6% inhibition in the growth rate of *Pseudokirchneriella subcapitata* was observed at the highest test concentration, and hence, a 72h E_rC_{50} of >10 mg/L is reported based on nominal concentration (measured concentrations were between 96 and 120% of the nominals). In a FIFRA Guideline 122-2/123-2 test with the marine algae *Skeletonema costatum,* 72h and 96h E_rC_{50} values of 0.46 and 0.53 mg/L, respectively, were determined based on mean measured concentration. These are the lowest acute toxicity values used by the DS for triggering the acute hazard classification.

In addition, a study following the U.S. EPA/FIFRA Guideline 72-3 with the oyster *Crassostrea virginica* is included in the dossier and a 96h EC_{50} of 8.9 mg/L based on shell growth is reported.

Chronic toxicity

Five chronic toxicity studies on fish are included in the dossier. Two of them are fish full life cycle studies with *Pimephales promelas* following guidelines (U.S.) EPA-FIFRA 72-5 and OPPTS 850.1500 and one of them also the OECD (2008) Detailed Review Paper on Fish Life-cycle Tests. The studies were performed under flow-through conditions during 192 and 270 days. No substance related effects were observed in survival and reproduction in either of the studies at the tested concentrations (up to 0.0462 and 0.0937 mg/L). A NOEC value of 0.0114 mg/L (based on measured concentrations) is reported for growth and the chronic classification is based on this value.

Two fish early life stage studies performed according to FIFRA Guideline 72-4 and OPPTS Draft Guideline 850.1400 are also available for *Pimephales promelas*. In these flow-through studies 30d-NOEC values of 0.021-0.024 mg/L for length and 0.021-<0.024 mg/L for weight are reported based on nominal concentrations (mean measured concentrations were in the range 92-120% of the nominal concentrations).

Furthermore, an early life stage test (OPPTS Draft Guideline 850.1400) with *Cyprinodon variegatus* is also included in the dossier. No effects in survival or growth were observed at the tested concentrations (up to 0.12 mg/L) during the 28-day study.

Chronic toxicity to *Daphnia magna* was studied in two studies following OPPTS Draft Guideline 850.1300 and under semi-static conditions. In the CLH dossier, for one of the studies a 21d-NOEC of 0.19 mg/L for growth is reported and no effects were observed in survival and reproduction at the tested concentrations (up to 3 mg/L). For the other daphnia study, a 21d-NOEC of 0.11 mg/L is reported for reproduction and no effects were observed in the other endpoints at the tested concentrations (up to 3.5 mg/L). The results are based on mean measured concentrations.

The dossier included also a flow-through study with *Americamysis bahia* performed according to FIFRA Guideline 72-4 and OPPTS Draft Guideline 850.1300. The 28d-NOEC values determined for survival, reproduction, length and weight were 0.16, 0.041, >0.32 and 0.16 mg/L, respectively, based on mean measured concentrations.

The chronic toxicity values determined in the available algal studies were a 72h NOE_rC of 1.0 mg/L for *Pseudokirchneriella subcapitata* and 72h- and 96h-NOEC values of 0.066 and 0.031 mg/L, respectively, for *Skeletonema costatum*.

In addition, the dossier included a chronic toxicity study following the BBA Guideline (1995) with the sediment-dwelling midge *Chironomus riparius*, although its reliability is considered borderline by the DS. No significant effects were observed in the emergence or development rate at the tested concentrations during the 26 day exposure period. Therefore, a NOEC of 0.0777 mg/L is reported based on the initial measured concentration of the test substance in the overlying water.

Comments received during public consultation

Four Member State Competent Authorities expressed their support to the proposed environmental classification and M-factors during the public consultation.

One company submitted comments on the available fish bioaccumulation study and Chironomid studies, as well as two editorial comments regarding the correct test species and NOEC of two aquatic toxicity studies, which the DS confirmed in their responses. Regarding the bioaccumulation study, the company presented its opinion that the bioaccumulation study can be taken into account for concluding that triticonazole has a low potential for bioaccumulation

and that a new study is not required (although this is more related to the pesticide renewal process). Some further information was provided in order to clarify the study uncertainties identified by the DS (related to the BCF values, fish lipid content, TOC level and lack of second test concentrations). The DS responded by indicating that they do not agree that it can be demonstrated with certainty that steady state was reached after 28 days and that lipid correction with historical data may be valid would need more information to be done in this case. The DS indicated that the approach proposed regarding TOC as reasonable.

The comments on the reliability of the studies on sediment dwelling species were concerned with the requesting of further studies. The company was of the opinion that the studies were valid and no further data was required, although the DS considered that the available Chironomid study was not reliable and that further data should be requested. This data request is in the context of the pesticide renewal process and is outside the scope of the CLP Regulation.

Assessment and comparison with the classification criteria

Degradation

Based on the available hydrolysis study, triticonazole is hydrolytically stable at pH 4-9. In aqueous photolysis studies, the substance was observed to reversibly transform to its Z-isomer and dissipation DT_{50} values in the range of 7.4 and 32.7 days (once equilibrium had been reached) were determined for the substance.

In the available ready biodegradation screening test, no degradation of triticonazole was observed after 28 days. In a surface water simulation study, the substance showed negligible degradation during 60-d study period, and hence, no DT_{50} value could be determined. In the available water-sediment simulation studies, the $DegT_{50}$ values of triticonazole in the total systems ranged from 225 to 399 days. Slow degradation (DT_{50} values ranging from above 100 days to above 1000 days) was also observed in the available soil simulation studies.

In conclusion, RAC agreed with the DS's proposal that triticonazole is not rapidly degradable for classification purposes.

Bioaccumulation

In the available bioconcentration in fish study performed according to US EPA guideline 165-4, a whole fish kinetic BCF of 72.55 based on total radioactive residues is determined for *Lepomis machrochirus*. The BCF values calculated directly from the concentration of total radioactivity in water and fish during the uptake phase ranged from 28 to 94, the highest value of 94 observed after 21 days and a BCF of 65 determined on the last day of the uptake phase. RAC notes that none of the BCF values are lipid-normalised (lipid content not measured in the study) and the kinetic BCF is not growth-corrected either. Since there is no information on the lipid content of the test fish, it is not possible to know whether the lipid-normalisation would lead to higher or lower BCF values. The growth-correction normally increases the BCFk. On the other hand, according to the information in the Annex to the CLH report, triticonazole was found to comprise less than 5% of the total radioactive residue in fish after 28 days exposure. Therefore, the reported BCF values determined based on total radioactivity are likely to overestimate the bioaccumulation of the parent substance triticonazole.

According to the DS, the results of the study have some uncertainties as the bioconcentration factor seems to first decrease and then increase again, and because some information is missing (lipid content of fish, TOC, testing of a second concentration). 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. However, RAC notes 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, as stated above, RAC agreed that this leads to some uncertainty regarding the BCF values. 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. RAC agreed with the DS that there is some uncertainty in the BCF value as only one concentration has been tested.

RAC also notes that according to the OECD TG 305, the concentration of the test substance should be selected to be below its chronic effect level. There is no chronic data for *Lepomis machrochirus* but the concentration of triticonazole used in the BCF study (89 µg/L) is above the NOEC values of 0.011-0.047 mg/L determined for *Pimephales promelas* based on growth. For another species (*Cyprinodon variegatus*), no effects were observed in the available FELS test at concentrations up to 0.12 mg/L. No information on the growth in the exposure and control groups of the available BCF study are given in the CLH report, and hence, it is not possible to assess whether the substance may have had adverse effects in the growth of the exposed fish. However, as the test concentration was not well above the available NOEC values for fish, any possible effect can be expected to be relatively weak.

Considering that the available BCFs based on total radioactivity are low (below 100) and overestimate the BCF of the parent substance, and that rapid elimination of the substance was observed (depuration half-life < 1 day), RAC concluded that even though some uncertainty remains in the available BCF values, the BCF of triticonazole is expected to be below 500. The measured log K_{ow} of triticonazole is 3.29, which is also below the cut-off value of 4 indicated in the CLP Regulation for bioaccumulation potential.

RAC agreed with the DS's proposal that the substance has low bioaccumulation potential for classification purposes.

Acute toxicity

Valid acute toxicity data is available for all three trophic levels. The available LC_{50} and EC_{50} values for fish and aquatic invertebrates are above 1 mg/L and therefore do not lead to acute classification.

The lowest acute values, 72h and 96h E_rC_{50} values of 0.46 mg/L and 0.53 mg/L, were determined for the alga *Skeletonema costatum* in a study performed according to FIFRA Guideline 122-2/123-2. The study duration was 120h, which is longer than the 96h duration defined in the FIFRA guideline. According to the Annex to the CLH report the section-by-section growth rates of the controls varied by more than 60%, which is above the 30% variation allowed in OECD TG 201. The DS notes that the algal species used is not recommended in OECD TG 201, and hence, the validity criteria indicated in that guideline are not applicable for this species. RAC notes that constant exponential growth of the controls is important and based on the data in the Annex to the CLH report, it seems that during the last test day, the growth rate decreased. However, up to 96h test duration the growth seems exponential and constant, and therefore, RAC considered that the results up to that day can be considered valid. It should be noted that no acute studies are available for *Pimephales promelas*, which is the most sensitive species in the chronic studies on fish. However, based on the acute to chronic ratio, the estimated acute value for *P. promelas* would be in the same order of magnitude as the lowest acute values determined for algae. Therefore, this is not expected to have effect on the proposed classification and labelling. In conclusion, the E_rC_{50} values for *Skeletonema costatum* are below the classification threshold of 1 mg/L for Aquatic Acute 1 and in the range of $0.1 < L(E)C_{50} \leq 1$ mg/L leading to an acute M-factor of 1.

Chronic toxicity

Valid chronic data is available for all three trophic levels. The lowest chronic value is the NOEC of 0.0114 mg/L determined for the fish *Pimephales promelas*. This is below the classification threshold of ≤ 0.1 mg/L for Aquatic Chronic 1 for not rapidly degradable substances and justifies a chronic M-factor of 1 (0.01 < NOEC ≤ 0.1 mg/L). The lowest chronic values for aquatic invertebrates and algae support this classification as they are in the same order of magnitude (28d-NOEC of 0.041 mg/L for *Americamysis bahia* and 96h-NOEC of 0.031 mg/L for *Skeletonema costatum*).

Conclusion on Classification

Based on the above assessment, RAC agreed with the DS's proposal that triticonazole should be **classified as Aquatic Acute 1 (H400) with an acute M-factor of 1 and Aquatic Chronic 1 (H410) with a chronic M-factor of 1.**

Additional references

Reuter *et al*., 2018, Rabbit-specific gastrointestinal toxicity in prenatal developmental toxicity studies and its regulatory impact. Poster presented at Annual conference of the European Teratology Society in 2018 (unpublished).

ANNEXES:

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

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).