Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Triadimenol (ISO); α-tert-butyl-β-(4-chlorophenoxy)-1*H*-1,2,4-triazole-1-ethanol

EC Number: 259-537-6
CAS Number: 55219-65-3

CLH-O-0000001412-86-93/F

Adopted

4 December 2015

4 December 2015

CLH-O-0000001412-86-93/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 harmonized classification and labelling (CLH) of:

Chemicals name: **Triadimenol (ISO); α-tert-butyl-β-(4-chlorophenoxy)-1*H*-1,2,4-triazole-1-ethanol**

EC number: **259-537-6**

CAS number: **55219-65-3**

The proposal was submitted by the **United Kingdom** and received by RAC on **25 September 2014.**

In this opinion, all classifications are given in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonized System (GHS).

PROCESS FOR ADOPTION OF THE OPINION

The **United Kingdom** 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 **14 October 2014**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **28 November 2014**.

ADOPTION OF THE OPINION OF THE RAC

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

Co-rapporteur, appointed by RAC: **Michael Neumann**

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 harmonized classification and labelling was reached on **4 December 2015** and was adopted by **consensus**.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	**No current Annex VI entry**										
Dossier submitters proposal	xxx-xxx-xx-x	triadimenol (ISO); α-tert-butyl-β-(4-chlorophenoxy)-1H-1,2,4-triazole-1-ethanol	259-537-6	55219-65-3	Repr. 2 Acute Tox. 4 Aquatic Chronic 2	H361f H302 H411	GHS08 GHS07 GHS09 Wng	H361f H302 H411			
RAC opinion	xxx-xxx-xx-x	triadimenol (ISO); α-tert-butyl-β-(4-chlorophenoxy)-1H-1,2,4-triazole-1-ethanol	259-537-6	55219-65-3	Repr. 1B Lact. Acute Tox. 4 Aquatic Chronic 2	H360 H362 H302 H411	GHS08 GHS07 GHS09 Dgr	H360 H362 H302 H411			
Resulting Annex VI entry if agreed by COM	xxx-xxx-xx-x	triadimenol (ISO); α-tert-butyl-β-(4-chlorophenoxy)-1H-1,2,4-triazole-1-ethanol	259-537-6	55219-65-3	Repr. 1B Lact. Acute Tox. 4 Aquatic Chronic 2	H360 H362 H302 H411	GHS08 GHS07 GHS09 Dgr	H360 H362 H302 H411			

GROUNDS FOR ADOPTION OF THE OPINION

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

No classification was proposed for physical hazards.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC agrees with the proposal of the dossier submitter (DS) not to classify triadimenol for physical hazard.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity had been investigated in two oral studies (one study according to OECD Test Guideline (TG) 423 and GLP and another study that was not guideline or GLP compliant), one inhalation study (the study was not performed according to guideline or GLP protocol) and two dermal studies (one study according to OECD TG 402 and GLP and another study not according to OECD TG or GLP) in rats.

The LD_{50} values of 720 mg/kg bw and > 2000 mg/kg bw, were obtained from the non-guideline and OECD TG 423 acute oral toxicity studies, respectively. The value of 720 mg/kg bw fell within the range for classification with Acute Tox. 4; H302 (i.e. >300 mg/kg bw but ≤ 2000 mg/kg bw). The DS could not explain why the studies gave such different LD_{50} values. There were no major differences in the experimental animals used, the administered test substance formulations were comparable, and the purity/impurity profiles of the tested substances were not markedly different. As there were no data to indicate that the higher LD_{50} value obtained in the guideline study should be given preference over the LD_{50} value obtained in the older non-guideline study, the DS proposed to classify for acute oral toxicity.

In the acute inhalation study, the maximum attainable concentration of 0.95 mg/L triadimenol did not result in any deaths or clinical signs of toxicity and no classification was proposed for acute inhalation toxicity.

The LD_{50} values obtained from two acute dermal studies were above the range of values warranting classification (≤ 2000 mg/kg bw) and no classification was proposed for acute dermal toxicity by the DS.

Comments received during public consultation

Four MSCAs supported the DS proposal to classify triadimenol as Acute Tox. 4; H302. One MSCA asked for more information regarding the non-guideline acute oral toxicity study (Mihail and Thyssen, 1980) especially regarding the mortality at different dose-levels.

Assessment and comparison with the classification criteria

Acute toxicity: oral

In the acute oral toxicity study in rats by Mihail and Thyssen (1980) using triadimenol with a purity of 92.7%, the lowest LD_{50} value in fasted animals was 720 mg/kg bw and in unfasted animals 1068 mg/kg bw. This study was not performed according to OECD TG or GLP. However, there was no OECD TG for acute oral toxicity available in 1980. The first conventional OECD TG 401 for acute oral toxicity was adopted in 1981 and the second alternative, OECD TG 423 was adopted in March 1996. OECD GLP criteria were established in 1992. The study was described as "acceptable" in the DAR of Triadimenol.

In the second oral acute toxicity study in rats by Schüngel (2005c) using triadimenol with a purity of 97.2% the LD_{50} value in fasted animals was above 2000 mg/kg bw. In the repeated test with the same dose, one animal died on day three. This study was performed according to OECD TG 423 and GLP.

In the most reliable study, Schüngel (2005c), the LD_{50} value was above the limit for classification (2000 mg/kg bw) for Acute Tox. 4; H302 (triadimenol purity 97.2%). However, in the study by Mihail and Thyssen (1980), the lowest LD_{50} value in fasted animals was 720 mg/kg bw and in unfasted animals 1068 mg/kg bw (triadimenol purity 92.7%).

The DS stated that all impurities were thoroughly evaluated and did not impact the classification proposed in the CLH report.

There were no data to indicate that the LD_{50} value obtained in Schüngel (2005c) should be given preference over the LD_{50} values obtained in Mihail and Thyssen (1980).

In agreement with the DS proposal RAC therefore concludes that classification of triadimenol as **Acute Tox. 4; H302** according to CLP (Category 4: 300 < ATE ≤ 2000 mg/kg bw) is warranted.

Acute toxicity: inhalation
One acute inhalation toxicity study was included in the CLH report with a maximum attainable concentration of 0.95 mg/L of triadimenol. The study was not performed according to OECD TG or GLP. No clinical signs or death were reported in this study.

In agreement with the DS proposal, RAC concludes that **no classification for triadimenol for acute inhalation toxicity** is warranted.

Acute toxicity: dermal

The CLH report contained two acute dermal toxicity studies. One study was performed according to OECD TG and GLP with one dose of triadimenol (2000 mg/kg bw). The second study, with triadimenol doses of 2500 and 5000 mg/kg bw, was not performed according to OECD TG or GLP. No deaths were reported in either study.

In agreement with the DS proposal, RAC concludes that **no classification for triadimenol for acute dermal toxicity** is appropriate, since the LD_{50} values obtained from two acute dermal toxicity studies were above the range of values warranting classification for acute dermal toxicity (2000 mg/kg bw).

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

Summary of the Dossier Submitter's proposal

In the acute toxicity studies, there were some signs that were indicative of triadimenol having an effect on the CNS (increased motility, behavioural changes, drowsiness and lethargy). Additional information was obtained from acute neurotoxicity investigations in mice and rats. In a pilot study that comprised a number of tests, treatment of mice with triadimenol was reported to potentiate

hexobarbital-induced anaesthesia, to have a statistically significant stimulating effect on spontaneous motility, to result in effects consistent with stimulation of the CNS, to increase motor activity and to transiently antagonise the ptosis and inhibition of spontaneous motility that was induced by pre-treatment with reserpine (Polacek, 1983). In the same study, triadimenol was also reported to have a stimulating effect on motor activity, rearing, licking/sniffing and grooming in rats. According to the DS, triadimenol in this pilot study at doses of 3 mg/kg bw and above demonstrated a stimulating effect on the CNS, which was stated to be less potent than that of caffeine. The study authors had surmised that the potentiation of hexobarbital anaesthesia was more likely due to inhibition of barbiturate metabolism than an effect on the CNS.

In the acute neurotoxicity study in rats (Crofton, 1996), triadimenol induced hyperactivity in all dose groups (50-400 mg/kg bw). According to the DS, the study authors had proposed that the hyperactivity induced by triazoles, including triadimenol, was related to an altered monoamine metabolism (decreased synaptosomal dopamine reuptake).

The DS concluded that although triadimenol had a transient effect on the CNS, it acted as a stimulant as opposed to a narcotic, and therefore classification as STOT SE 3 was not considered to be appropriate. The DS did not propose classification for STOT SE category 1 or 2, because triadimenol induced a functional disturbance that was not associated with morphological changes, because the effects were possibly mediated via a pharmacological mechanism rather than by damage to the CNS, and because the potency of triadimenol was lower than that of caffeine.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

No human data on relevant to classification for STOT SE was reported. The experimental animal studies included acute toxicity studies and two acute neurotoxicity studies; one in mice and one in rats. Triadimenol induced stimulating effects on the CNS in the acute neurotoxicity studies. These were manifested as a statistically significant stimulating effect on spontaneous motility, hyperactivity, stimulating effect on motor activity, rearing, licking/sniffing and grooming. The effects on spontaneous motility and motor activity were stated to be less potent than those of caffeine. However, no information was included by the DS regarding the amount of caffeine intake or on the quantitative results, so the extent to which the findings were adverse could not be assessed by RAC. Triadimenol also potentiated hexobarbital "sleeping time".

STOT SE 1 and 2:

Triadimenol induced functional disturbances (transient stimulant effects on the CNS), that were not associated with morphological changes since histopathology indicated no neuropathy or other adverse effects.

In conclusion, in agreement with the DS proposal and based on the available data RAC concludes that classification of triadimenol for STOT SE 1 or 2 is not warranted.

STOT SE 3 (transient target organ effects; narcotic effects):

The data indicated that triadimenol had a transient stimulant effect on the CNS. However, the extent to which the stimulant effect was adverse was not assessed and this effect was opposite to narcotic effects. Therefore a classification as STOT SE 3 was not considered justified by RAC. Triadimenol also potentiated hexobarbital-induced "sleeping time", but this effect was more likely due to inhibition of barbiturate metabolism than an effect on the CNS and therefore it also did not support classification as STOT SE 3.

RAC concludes in agreement with the DS proposal that the available data do not justify classification of triadimenol as STOT SE 3.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

Four rabbit studies on skin corrosion/irritation were provided by the DS; two OECD TG 404 studies according to GLP, one non-GLP study similar to OECD TG 404, and one study that was not performed according to either OECD TG or GLP.

According to the DS, these studies in rabbits gave no indication that triadimenol caused skin corrosion/irritation and therefore did not meet the criteria for classification for skin corrosion/irritation under the CLP Regulation.

Comments received during public consultation

No comments received during public consultation.

Assessment and comparison with the classification criteria

In the absence of any signs of skin corrosion/irritation in two well conducted studies in rabbits performed according to OECD TG 404 and GLP and with supportive evidence from two additional non-standard studies in rabbit, triadimenol does not fulfil the criteria for skin corrosion/irritation under CLP either in terms of severity of scores or in terms of irreversibility.

In agreement with the DS proposal, RAC concludes that **classification for triadimenol for skin corrosion/irritation is not warranted**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier submitter's proposal

Four rabbit studies investigating serious eye damage/irritation were provided by the DS; two OECD TG 405 studies according to GLP, and two studies that were not performed according to OECD TG or GLP.

The main effect observed was conjunctival redness, with occasional conjunctival chemosis. Since all these effects were fully reversible within 21 days, and since the grades of conjunctival effects did not meet the criteria for classification as category 2 in the CLP Regulation, the DS did not propose any classification for serious eye damage/eye irritation.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

The main effect reported in OECD TG compliant studies was conjunctival redness with occasional conjunctival chemosis. However, the effects were fully reversible in 21 days and category 1 (irreversible effects on the eye) according to CLP is not considered appropriate by RAC. The grades of conjunctival effects (mean score of ≤ 0.7 in the OECD TG studies, and individual scores of 1 in most animals in the non–guideline studies) do not meet the criteria for a classification as category 2 (irritation to eyes) according to CLP (mean scores of ≥ 2 at least in 2 of 3 animals for conjunctival redness or chemosis).

In agreement with the DS proposal RAC concludes that **no classification for triadimenol for severe eye damage/eye irritation** is warranted.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

There were two guinea pig studies on skin sensitisation: an OECD TG 406 (GLP) compliant Buehler assay and a (non-GLP) Guinea Pig Maximisation test (GPMT) similar to this guideline.

Triadimenol did not induce skin sensitisation in any animals in the Buehler assay when it was tested at 62.5% induction and challenge doses, and appropriate responses were obtained in the positive and negative control groups. Triadimenol was also negative for skin sensitisation in the supporting GPMT, which however had limitations including the lack of a positive control group, no justification for the concentrations used (2.5% intradermal dose, 25% topical and challenge doses) and no information on irritation responses.

The DS did not propose classification for skin sensitisation.

Comments received during public consultation

One MSCA questioned the presence of the triadimenol metabolite triadimefon in the tests performed to assess the skin sensitisation potential of triadimenol since triadimefon has a harmonised classification as Skin Sens. 1 according to CLP. The MSCA also questioned the negative results from the Buehler test and the GPMT performed with triadimenol, and suggested to label triadimenol with EUH 208 if triadimenol is not classified as a skin sensitiser due to the possible presence of the metabolite/impurity triadimefon.

Assessment and comparison with the classification criteria

The skin sensitising potential of triadimenol was assessed in a Buehler test and a GPMT, both with a purity of triadimenol of 97.2%. Triadimenol did not induce skin sensitisation in the Buehler test performed according to OECD TG 406 and GLP (induction and challenge dose of 62.5%). This result was supported by the absence of skin sensitisation (only 1/20 animals giving a positive response at 24 h) in the GPMT. According to the CLP criteria, classification as a skin sensitiser is warranted if at > 20% topical induction dose ≥ 15% of animals give a positive response in a Buehler test/non-adjuvant assay and/or if at > 1% intradermal induction dose ≥ 30% of animals give a positive response in a GPMT/adjuvant assay. As none of the animals were positive in the Buehler test and less than 30% were positive in the Guinea Pig Maximisation test, the classification criteria were not met.

During PC information was received that tradimenol may contain the impurity triadimefon at concentrations of ≤ 1%. Triadimefon has a harmonised classification as Skin Sens. 1. How to handle the presence of impurities in substances are described in CLP Regulation Article 2(7) and Article 11.

Taking these CLP Regulation articles into account, triadimenol should be considered classified as Skin Sens. 1 when the impurity triadimefon reaches 1.0%, i.e. the generic concentration limits (GCL). However, information was received from Industry that in the new specification the maximum content of triadimefon in triadimenol is set at 0.9% or 9 g/kg.

The presence of the impurity triadimefon in triadimenol used in the GPMT and the Buehler test and a possible impact on the results should be considered. The results from the Guinea Pig Maximisation test and from the Buehler test were negative and the classification criteria for skin sensitisation were not fulfilled as described above. However, according to the OECD TG 406 the epidermal induction dose should be maximised until it produces *mild to moderate* or *mild* skin irritation, respectively. This is considered difficult to achieve for triadimenol due to the possible formation in situ of the metabolite triadimefon, which has been classified as Skin Sens. 1. Therefore negative test results for substances containing sensitising impurities, or for mixtures in general containing sensitisers, should be interpreted with great care (see section 3.4.3 of the CLP Guidance).

In agreement with the DS proposal and based on the available data, **RAC concludes not to classify triadimenol for skin sensitisation**.

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

Summary of the Dossier submitter's proposal

The CLH report included two 28-day oral gavage studies and two 90-day oral dietary studies in rats (all non GLP or guideline), a 13-week oral dietary study in mice (OECD TG 408) and in dogs (similar to OECD TG 409), a 6-month and 2-year oral dietary study in dogs (non GLP or guideline), 1- and 3-week inhalation studies in rats (non GLP or guideline) and a 3-week dermal study in rabbits (non GLP or guideline). In addition, results of chronic/carcinogenicity studies in rats and mice were taken into consideration by the DS when assessing the potential classification for STOT RE.

According to the DS, after oral administration, the main target organ in rats, mice and dogs was the liver. At lower doses and with shorter durations of exposure, the liver effects, increased weights associated in some cases with hypertrophy and increased liver enzyme activities, were indicative of adaptive rather than toxic responses. The effects were reversible after administration of triadimenol for 28 days. A progressive worsening of the liver effects (gross and histopathology findings that were evidence of toxicity) was observed in 90-day and longer-duration studies and that was considered by the DS to be consistent with triadimenol bioaccumulation. In 90-day studies, the only toxic effect that occurred below the guidance values was fatty change (from 8/9 mg/kg bw/d in rats) / increased fat storage (from 25/31 mg/kg bw/d in mice). In both cases, the changes were slight at these doses. After chronic administration for up to two years, there was no liver toxicity in dogs or rats, although slight to minimal single cell necrosis was reported in male mice in one study from 60 mg/kg bw/d and fatty change in female mice at 472 mg/kg bw/d. In various oral studies, triadimenol also increased the weights of the kidneys, ovaries, thyroid and adrenals. According to the DS these organ weight changes did not justify classification since there was no evidence of organ dysfunction. However, the DS pointed out that they may have indicated an endocrine-disrupting potential for triadimenol.

Inhalation and dermal administration of triadimenol did not result in any adverse effects.

The DS did not propose classification for STOT RE because there were no significant adverse effects below the guidance values. Although changes were observed in some clinical chemistry parameters, particularly in liver enzyme induction at dose levels relevant for classification, these reversible effects were indicative of increased liver activity as a result of an adaptive change. According to the DS, such adaptive responses constituted a normal biochemical or physiological response and did not warrant classification. Slight hepatic fatty change/increased fat storage was reported below the guidance value for classification but that did not warrant classification according to the DS.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

The main target organ in rats, mice and dogs following exposure to triadimenol is the liver. In rats, a dose-related increase in the severity of fatty change from 8/9 mg/kg bw/d in male and female rats was reported in a 90-day study and increased fat storage from 25/31 mg/kg bw/d in a 13-week study in mice (see tables below). However, the changes at these dose levels were slight.

Table: Liver effects (90 days oral diet study in rats):

	Males	**Females**

Dose (mg/kg bw/d)	0	8	40	209	0	9	46	221
Numbers of animals	18	19	18	19	20	20	20	20
Fatty changes • Slight • Mild • Moderate • Severe	1 1	3 3	5 4 1	15 4 9 1 1	2 2	4 4	9 9	18 5 5 8

Table: Liver effects (13-week oral diet study in mice):

	Males					**Females**				
Dose (mg/kg bw/d)	0	25	77	235	872	0	31	94	297	797
Number of animals	10	10	10	10	10	10	10	10	10	10
Fatty storage • Slight • Mild • Moderate • Severe	0	1 1	2 2	8 4 4	9 2 6 6	1 1	4 4	3 3	8 6 2	10 7 3

In chronic studies for up to two years, no liver toxicity was reported in rats. However, in a 18-month chronic study in mice, single cell necrosis (slight to minimal severity) was statistically significantly increased from 60 mg/kg bw/d in males and in females at 472 mg/kg bw/d as well as fatty change in females at 472 mg/kg bw/d. However, these effects occurred above the Guidance Value for a classification as STOT RE 2 for 18 months (guidance values adjusted for duration of the study according to Haber's rule: 1.875 to 18.75 mg/kg bw/d).

In a 13-week study in dogs a statistically significant increased liver weight was observed in females at 60 mg/kg bw/d. However, no adverse gross necropsy or histopathological changes were reported. In a 6-month and 2-year study in dogs, with exposure to lower concentrations of triadimenol compared to the 13-week study, no changes in liver weight or histopathology were reported.

Besides effects on the liver, an increase in the relative ovary weight was reported in two 28-day studies in rats from 5 and 15 mg/kg bw/d, respectively, and in a 90-day study at 287 mg/kg bw/d. However, the change in ovary weight was reversible and not associated with histopathological changes.

In conclusion, effects reported in the liver at doses relevant for a classification were as follows:

- Rats: dose-related increase in the severity of fatty change starting from 8/9 mg/kg bw/d in male/female rats in a 90-day study (Guidance value for STOT RE 2: 10-100 mg/kg bw/d)

- Mice: increased fat storage from 25/31 mg/kg bw/d in male/females in a 13-week study (Guidance value for STOT RE 2: 10-100 mg/kg bw/d)

Such effects could be considered to support classification for specific target organ toxicity following repeat exposure, as they are specifically referred to in the CLP Regulation (Annex I 3.9.2.7) as follows "*morphological or diffuse changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in the liver)".*

However, in agreement with the DS, RAC considers that the dose-related fatty changes/ fat storage effects in the liver reported in 90-day studies in rats and mice are not sufficiently severe to justify classification as STOT RE category 2, therefore RAC concludes that **no classification for STOT RE is warranted**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

The genotoxic potential of triadimenol had been investigated in several *in vitro* and *in vivo* studies. There was no indication that triadimenol had a mutagenic effect on somatic or germ cells in any of the assays. The DS proposed no classification for germ cell mutagenicity as the classification criteria for mutagenicity were not met.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

Negative results were obtained in all the available mutagenicity tests performed with triadimenol including *in vitro* and *in vivo* test on somatic and germ cells. The criteria for classification for mutagenicity according to CLP are therefore not met.

In agreement with the DS proposal, RAC concludes that **no classification for triadimenol is warranted for germ cell mutagenicity**.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

The DS assessed a 2-year oral study in rats and mice (non GLP or guideline), and an 18-month oral study (OECD TG 451) in mice.

According to DS, there was no indication that triadimenol had carcinogenic potential in rats. In the 18-month mouse study (OECD TG 451), increased incidences of benign and malignant liver tumours were observed only in the mid-dose group in males. When the two tumour types were combined, the incidence was within the contemporary historical control data (HCD). As no dose-response relationship was observed, the DS concluded that the liver tumours were incidental findings. Benign liver tumours also occurred in the 2-year mouse study, having a dose-response relationship only in females. The observed incidences were within the normal range for this mouse strain.

A low incidence (2/50, 4%) of ovarian tumours was reported in the 18-month mouse study at 472 mg/kg bw/d but not in the 2-year study. The tumours were not associated with effects on ovary weights or histopathology, but ovarian luteoma in HCD from seven studies of 18-month were not reported. Ovarian luteoma is recognised to be a rare tumour, although clustered occurrences in the historical control data suggest that when it does arise, there may be multiple spontaneous occurrences within a study. On re-evaluation of the two lesions, the pathologist was of the opinion that neither was a clear-cut luteoma, as per IARC criteria for lesion classification.

The DS concluded that there were two tumour types in one species (mouse) in the absence of severe toxicity but with reductions in body weights of 11 to 21% (reductions in body weight gain of up to 40%). In neither mouse study was the total number of tumours increased by exposure to triadimenol. Triadimenol is non-genotoxic, which lowered the level of concern according to the DS. Although increased incidences of liver tumours were observed in one sex in each mouse study (with no dose-response relationship in the males), the increased incidences in both studies were within the historical control ranges and therefore a causal relationship between triadimenol administration and liver tumour induction was not established and a classification for carcinogenicity based on the liver findings was not proposed by the DS.

Two cases of benign ovarian luteoma occurred in female mice exposed to 472 mg/kg bw/d triadimenol. This was a dose at which body weight gain was reduced by > 10%, indicating that the maximum tolerated dose was exceeded. Given the uncertainties over the significance of the finding and the pathologist's indeterminate diagnosis of the lesions as luteomas, the overall conclusion of the DS was that the data did not suggest a carcinogenic effect and thus classification was not proposed.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

No evidence of carcinogenicity was reported in a 2-year dietary carcinogenicity study in rats.
In mice, ovarian tumours were reported in a 18-month study, and in the same study liver adenomas and adenocarcinomas were reported in male mice. In female mice, liver adenomas were reported in a 2-year carcinogenicity study. The incidences are shown in the tables below.

Table: Ovarian tumours in the 18-month study in mice

Dose (mg/kg bw/d)	**0**	**17**	**91**	**472**	**HCD**
Ovarian luteoma	0/49 (0%)	0/50 (0%)	0/48 (0%)	2/50 (4%)	0* 0-10%, mean 1.7%** 1.12%***

* HCD between minus 6 years and plus 3 years of present study (7 studies)
** HCD from 13 studies of 2-years duration (6 studies with 0% luteoma, the mean of 7 studies was 3.5% with 1, 2 or 5 animals affected in each study)
*** HCD of all the HCD

Table: Liver adenomas/adenocarcinomas in the 18-month study in mice

	Males				**Females**			
Number of animals	50	50	50	49	50	50	48	50
Dose (mg/kg bw/d)	0	11	60	340	0	17	91	247
Adenoma	7	5 (10%)	10 (20%)	5 (10%)	1	1	2	0
Adenocarcinoma	0	3* (6%)	4* (8%)	2 (4%)	0	0	0	0
Combined adenoma and carcinoma	14%	16%	28%	14%				

Statistically significant at *p≤0.05, **p≤0.01
HCD: 2-year studies; 2-19.4% adenoma and 6-17% carcinoma (4 studies, same laboratory).
HCD: 18-19 month studies between minus 6 years and plus 2 years of present study 0-13.6% (mean 5.8%) adenoma and 4-22% (mean 11.9%) for carcinoma (9 studies from various laboratories). The combined HCD for adenoma/carcinoma was 8-32%.

Table: Liver tumours in the 2-year study in mice

	Males				**Females**			
Number of animals	50	50	50	50	50	50	50	50
Dose (mg/kg bw/d)	0	19	75	300	0	19	75	300
Adenoma	5	4	5	8	0	0	4	6* (12%)
Adenocarcinoma	3	0	0	1	1	0	1	0

Statistically significant at *p≤0.05, **p≤0.01
HCD: 0-25% in males and 0-12% in females (8 studies between minus 2 years and plus 2 years of present study). In a study performed concurrently with the triadimenol study adenomas occurred in 25% of male controls and 12% of female controls.

Two tumour types in one species, mice, occurred in two different carcinogenicity studies in the absence of severe toxicity.

Carc. Cat. 1A:
There is no evidence that triadimenol induces tumours in humans, so RAC considers that classification as Carc. Cat. 1A is not appropriate.

Carc. Cat. 1B:
Triadimenol is not genotoxic. Increased incidences of liver tumours were reported in male mice without a dose-response relationship in a 18-month carcinogenicity study and in female mice with a dose-response relationship in a 2-year carcinogenicity study. The increased incidence of liver tumours was within the HCD in both studies and in both sexes. However, the induction of liver adenomas in the 2-year study in female mice was at the upper limit of the HCD.

Ovarian tumours were reported in the 18-month study in mice in the presence of reduced body weight gain of up to 40%. The ovarian tumour was reported to be a rare tumour type and outside the combined HCD (18 and 24 month studies) and the HCD relevant for the 18-month study. However, the two luteomas were re-examined, and the pathologist's conclusion was that neither of the luteomas was completely in concordance with the IARC criteria for ovarian luteoma. One luteoma was possibly a "sex cord stromal tumour (mixed)" and the second was a borderline neoplastic lesion which could possibly also be a sex cord stromal hyperplasia.

The liver tumours in female mice were not reported in the same study as the ovarian tumours.

Classification in Carc. Cat. 1B is based on sufficient evidence of carcinogenicity where a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. RAC considers that neither of these criteria was met, and classification in Carc. Cat. 1B is not warranted.

Carc. Cat. 2:
Classification in Carc. Cat. 2 is based on limited evidence of carcinogenicity. Limited evidence can be shown by (a) the tumour findings having been seen only in one study or (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies or (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential.

RAC considers that the induction of ovarian luteoma in a 18-month carcinogenicity study in mice above the HCD as well as the dose-dependent statistically significant increase in hepatocellular adenoma in female mice in a 2-year carcinogenicity study reported at the upper boundary of the HCD range indicate that triadimenol may have carcinogenic potential. However, ovarian luteomas were reported in the presence of a 40% reduction in body weight gain and was not reported in the 2-year carcinogenicity study in mice, and upon re-examination, the pathologist's conclusion was that neither of the luteomas was completely in concordance with the IARC classification for ovarian luteoma. As regards the hepatocellular adenomas/adenocarcinomas, a statistically significant increase was only reported in a 2-year carcinogenicity study in female mice, and not in a 18-month carcinogenicity study in male and female mice and the incidences were not above the HCD. Furthermore, no carcinogenicity was reported in a 2-year carcinogenicity study in rats. RAC concludes that the criteria for classification in category were not met.

Conclusion

In agreement with the DS proposal, RAC concludes **not to classify triadimenol for carcinogenicity**.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

Fertility

The DS proposed a harmonised classification and labelling for triadimenol as Repr. Cat. 2; H361f.

A multi-generation study (Loeser and Eiben, 1982; not guideline or GLP compliant) and a two-generation study (Loeser and Eiben, 1984; OECD TG 416 compliant, non GLP) in rats were included in the CLH report. Both studies showed an increase in the severity of the fertility effects through the generations, such that all dose groups were affected by the final generation. The toxicokinetic investigations indicated that excretion reached approximately 80%-90% of an administered single or repeated dose at 24 hours, so it is possible that bioaccumulation of triadimenol occurred. In these two studies, parental toxicity (as exhibited by decreased body weight gains which also increased in severity through the generations) was evident but there were no overt clinical symptoms. The effects on fertility were more marked when higher doses (60 and 240 mg/kg bw/d) were administered, but were still evident at the F1 first and second matings when lower doses were used (from 1.8 mg/kg bw/d), and in the F2B generation in the low-dose (15 mg/kg bw/d) group, when parental toxicity was slight or absent. The insemination index was also reduced in the one study (Loeser & Eiben, 1984) where this parameter was investigated. The reduced fertility index in these studies was not regarded as being a non-specific secondary consequence of parental toxicity. No specific investigations have determined if the possible effect on fertility was mediated through males or females, although male fertility was not affected by a single dose of triadimenol in a dominant lethal assay.

As triadimenol reduced fertility in a multi-generation study in rats (Loeser and Eiben, 1982), with supportive evidence provided by a two-generation study in rats (Loeser and Eiben, 1984) that used lower doses and the effect did not appear to be a secondary non-specific effect of other toxic effects, the DS concluded that classification for these effects should be proposed. There was no human data available, and in the absence of mechanistic information to the contrary, the effects were regarded as being of relevance to humans.

Both multi- and two-generation studies had deficiencies that, according to DS, reduced their quality and the information available. There was no information on whether the effect on fertility was mediated through the males or the females. Overall, category 2 was considered to be an appropriate classification for the fertility effects because (i) the weaknesses in both studies, (ii) only one species (rat) had been investigated, (iii) a clear dose-response was not always obtained and (iv) no gross or histopathological evidence of damage to the reproductive organs was available.

Development

The DS evaluated the multi-generation study (Loeser and Eiben, 1982) and the two-generation study (Loeser and Eiben, 1984) in rats, three GLP-compliant OECD TG 414 studies in rats, two GLP-compliant OECD TG 414 studies in rabbits and two non-guideline studies in rats to assess the developmental toxicity of triadimenol.

The multi-generation study (Loeser and Eiben, 1982) showed a pattern of increasing severity of effects on viability and growth through the generations, with both the 5-day and the 28-day viability indices being severely reduced in all dose groups at the first mating of the F2 animals. There was also a treatment-related reduction in the litter size and pup weight at birth in all the treatment groups. However, these results were less pronounced at the second F2 mating. According to the DS, the effects in all generations at 15 mg/kg bw/d and possibly also at 60 mg/kg bw/d were likely to be chance findings. The DS further concluded that all the findings in the high-dose group were likely to be consequences of the fairly severe maternal toxicity (maternal body weights being decreased by up to 24%), which also increased through the generations. In the two-generation study (Loeser and Eiben, 1984), there were some reductions in litter size at birth and pup viability that were more apparent at the second mating, but according to DS they were small, inconsistent and there was usually no dose-response relationship. In the

developmental studies an increased number of post-implantation losses in rats and rabbits were observed. According to the DS, the increased embryonic and foetal resorptions in rats had a dose-response relationship and were associated with maternal toxicity (reduced body weight gains). In rabbits, the greater extent of embryonic resorptions compared with foetal resorptions in the high-dose group was consistent with the maternal toxicity (clinical signs and weight loss), which was more marked during the early part of gestation. The DS concluded that post-implantation losses, reduced total litter size, differences in pup viability and body weight were likely to be due to maternal toxicity (at the higher doses) or chance (at the lower doses), and were not sufficiently convincing to justify classification.

In one rat developmental study (Machemer, 1977) a number of foetuses in the lower dose groups (10 and 30 mg/kg bw/d) exhibited malformations of the head, which tended to be clustered in a small number of litters, but there were no occurrences in the high-dose (100 mg/kg bw/d) group, even in the presence of maternal toxicity. In several other rat developmental studies, dose-related increases in the incidences of supernumerary ribs (from 5 mg/kg bw/d; Becker *et al.*, 1987a and Clemens *et al.*, 1990) and malformed foetuses (one study, at 165 mg/kg bw/d, Clemens *et al.*, 1990) were reported. In one study, the extra ribs were small (Becker *et al.*, 1987a). In another study, the combined incidence of extra ribs exceeded the most relevant historical control data, with the increased incidence of extra lumbar ribs being particularly marked (Clemens *et al.*, 1990). There was no information on the size of the extra ribs, leaving the DS with uncertainty over the severity of the effect. The DS concluded that uncertainty surrounds the developmental/teratogenic significance of supernumerary ribs, and generally findings of this nature would not be used as evidence for classification.

In a range-finding study in rats (Clemens *et al.*, 1990), 14/15 pups from one litter of the high-dose group showed malformations (14 with protruding tongue, 5 additionally with cleft palate). These were not clearly associated with maternal toxicity, since the body weight gains of the two next lower dose groups were more affected than those of the high-dose group, but without any malformations in the pups. However, since only one litter was affected, the toxicological significance of this finding remained unclear for the DS.
According to the DS, the skeletal anomalies in the form of abnormal or incomplete ossification that occurred in one rabbit study (Becker *et al.*, 1987b) were likely to be manifestations of developmental delay attributed to the maternal toxicity and common in rabbits.

Triadimenol also caused increases in placental weight in two rat studies (Clemens *et al.*, 1990; Renhof, 1984) and in one rabbit study (Clemens *et al.*, 1992). According to the study authors, this effect was commonly seen with azole-containing substances, and the DS concluded that the finding was of uncertain significance, but a hormonal effect could not be excluded.

Overall, the DS concluded that the developmental toxicity observed was generally associated with maternal toxicity and did not provide evidence of a specific effect. There was therefore insufficient evidence to propose a classification for developmental toxicity or effects on or via lactation.

Comments received during public consultation

Four MSCAs supported the classification proposed by the DS as Repr. 2; H361f. One MSCA noted that classification for effects on or via lactation as well as development may be appropriate. Consequently the MSCA proposed the following classification for reproductive toxicity: Repr. 2; H361fd and Lact.; H362.

The MSCA suggestion for classification for lactation was based on the finding that in ruminants the level of triadimenol in milk decreased rather slowly, although the level was significantly lower than in other tissues.

The MSCA comment suggesting classification for development was based on the reported findings of supernumerary ribs. Although they are considered as asymptomatic in rodents, in human cervical ribs are often associated with a pathological conditions known as Thoracic Outlet Syndrome (Solecki *et al.*, 2013). Additionally cervical ribs are anomalies often observed with triazole compounds. The MSCA also noted the occurrence of cleft palates with 5 cleft palate

reported in one litter in a range finding study with 5 animals/dose group. Since this effect is commonly reported for triazoles they are unlikely to be a secondary-non-specific consequence of maternal toxicity.

Assessment and comparison with the classification criteria

Developmental toxicity

Five GLP compliant developmental toxicity studies performed according to or similarly to OECD TG 414 (three in rats and two in rabbits), as well as a multi-generation study, a 2-generation study, a range finding developmental toxicity study and a non-guideline developmental toxicity study in rats, were available for the assessment of developmental toxicity following exposure to triadimenol.

Post-implantation losses

In the study by Becker *et al.* (1987a), a rat developmental toxicity study performed according to GLP and OECD TG 414, increases in post-implantation losses were reported at the top dose of 120 mg/kg bw/d (5.9%, 5.6%, 7.3% and 11.8% at 0, 30, 60 and 120 mg/kg bw/d, respectively) in the presence of decreased maternal body weight gain as compared to controls (100%, 100%, 84% and 58% at 0, 30, 60 and 120 mg/kg bw/d, respectively). An increase in post-implantation losses was also reported in the range-finding rat study by Clemens *et al.* (1990) from 130 mg/kg bw/d (11%, 4%, 5.5%, 4.7%, 28.4% and 30.8% at 0, 25, 60, 95, 130 and 165 mg/kg bw/d, respectively) in the presence of decreased maternal weight gain as compared to controls (100%, 96%, 82%, 76%, 73% and 81% at 0, 25, 60, 95, 130 and 165 mg/kg bw/d, respectively). In the main study by Clemens *et al.* (1990), performed according to GLP and OECD TG 414, no increase in post-implantation-losses was reported. However, the doses were lower, up to 60 mg/kg bw/d. No increases in post-implantation losses were reported in the GLP non-compliant rat study performed similarly to OECD TG 414 (Machemer, 1977) with doses up to 100 mg/kg bw/d in a non-guideline rat study (Renhof, 1984) with doses up to 30 mg/kg bw/d or in the 2-generation rat study with doses up to 57 mg/kg bw/d (Loeser and Eiben, 1984). Also in these studies the tested doses were lower than those associated with increased post-implantation losses. In the multi-generation study in rats (Loeser and Eiben, 1982) with doses up to 240 mg/kg bw/d, a dose-related decrease in pregnancy rates was reported in the presence of decreased maternal body weight gain up to 28% as compared to controls, but due to the experimental design of the study it was not possible to determine if this was related to pre- or post-implantation losses. In rabbits, a statistically significant increase in post-implantation losses was reported in the OECD TG 414 study by Becker *et al.* (1987b) at 200 mg/kg bw/d (3.8%, 8.0%, 6.7% and 12.8% at 0, 8, 40 and 200 mg/kg bw/d, respectively) in the presence of decreased maternal body weight gain as compared to controls (17 g vs 309 g in controls on gestation day (GD)6-9 and 17 g vs 272 g in controls on GD6-18), but no developmental effects were reported in the other OECD TG 414 rabbit study with doses up to 125 mg/kg bw/d.

As a conclusion, in developmental toxicity studies post-implantation losses were reported in rats and rabbits in the presence of maternal toxicity evident as reduced body weight gain. In rats, the increases in post-implantation losses were consistently reported at and above 120 mg/kg bw/d in the two rat studies in which these higher doses were administered. Also in rabbits, the increase in post-implantation losses was only observed at the high dose, but this was statistically significant. The associated reduced maternal body weight gains could have been direct consequences of increased post-implantation losses, being intrauterine rather than maternal effects, as the body weight gains were not reported as corrected body weight gains (*i.e.* as the difference between the initial and terminal body weight minus the gravid uterine weight). However, in a study by Fleeman *et al.* (2005) it has been shown that a reduced body weight gain of 50% and more up to a negative body weight gain did not cause an increased number of post-implantation losses. Therefore, the observed increases in post-implantation losses are not considered to be secondary consequences of maternal toxicity.
RAC concludes that the observed post-implantation losses provide clear evidence of developmental toxicity.

Malformations/variations

An increased incidence of supernumerary ribs (14th left rib; 11.6%, 16.4%, 38.8%, 51.5% and 14th right rib; 13, 19.1, 41, 51.1% at 0, 30, 60 and 120 mg/kg bw/d, respectively) was reported in the OECD TG 414 study by Becker *et al.* (1987a) in the presence of decreased maternal body weight gain as compared to controls (100%, 100%, 84% and 58% at 0, 30, 60 and 120 mg/kg bw/d, respectively). Furthermore, an increased incidence of extra ribs (lumbar and cervical) was reported in the OECD TG 414 developmental toxicity study by Clemens *et al.* (1990) (main study) with the incidences included in the table below. The maternal body weight gain on GD6-16 was decreased at 15 and 60 mg/kg bw/d as compared to controls (100%, 97%, 84%, 91% and 78% at 0, 5, 15, 25 and 60 mg/kg bw/d, respectively), but was similar to controls at all doses on GD20.

Table: Extra ribs in the main study (Clemens *et al.*, 1990)

Dose (mg/kg bw/d)	**0**	**5**	**15**	**25**	**60**
Number of foetuses	191	157	174	168	198
Number of litters	28	22	25	25	28
Extra lumbar ribs					
Foetuses (litters)	1 (1)	6 (6)	6 (6)	13 (9)	42 (20)
Foetal % (litter %)	0.5 (3.6)	3.8 (27.3)	3.4 (24.0)	7.7** (36)	21.2** (74.4)
Cervical ribs					
Foetuses (litters)	4 (4)	2 (2)	3 (3)	6 (3)	13 (9)
Foetal % (litter %)	2.1 (14.3)	1.3 (9.1)	1.7 (12)	3.6 (12.0)	6.6 (32.1)
Extra ribs					
Foetuses (litters)	5 (5)	8 (8)	9 (8)	19** (10)	55** (24)
Foetal % (litter %)	2.6 (17.9)	5.1 (36.4)	5.2 (32.0)	11.3** (40)	27.8** (85.7)

Statistically significant at *p≤0.05, **p≤0.01

HCD for extra ribs was available from the same laboratory with the same strain of rat from 8 years before the study; the foetal incidence varied from 0.5% to 15.7% and the litter incidence from 3.6% to 66.7%. When only data from 3 years before the triadimenol study were included, the HCD for foetal incidence varied from 0.5% to 6.6% and the litter incidence from 3.6% to 38.5%.

According to Solecki *et al.* (2013), in rodents short supernumerary ribs were transient findings that disappeared after birth, while full supernumerary ribs seemed to be permanent structures. Both short and full supernumerary ribs were believed not to adversely affect rodent survival or health, and therefore they were generally classified as variations. In humans, cervical supernumerary ribs were regarded as infrequent variations in Solecki *et al.* (2013), but according to the two-category classification scheme proposed for developmental toxicity studies (Solecki *et al.*, 2001), they could be considered as malformations. Although being asymptomatic in many individuals, human cervical ribs were often associated with a pathologic condition known as Thoracic Outlet Syndrome caused by pressure of the nerves of the branchial plexus and on the subclavian artery (Sanders *et al.*, 2002). In the (main) study by Clemens *et al.* (1990), a dose-related increase in cervical ribs was reported. The increase in the combined incidence of extra ribs (lumbar and cervical) was statistically significant and above the HCD range for foetal and litter incidences, although the increase in cervical ribs per se did not reach statistical significance. However, RAC concludes that the observed increase in cervical ribs in rats may be of some concern for humans due to its association with Thoracic Outlet Syndrome. RAC concludes that the observed extra ribs were unlikely to be secondary non-specific consequences of decreased maternal body weight gain because the maternal body weight did not correlate with the number of extra ribs at the mid doses. In addition, the maternal body weight gains were similar to controls at all doses on GD20.

In the range-finding study by Clemens *et al.* (1990), 1/4 litters in the 165 mg/kg bw/d dose group contained 14/15 malformed foetuses (14 with protruding tongue, 5 additionally with cleft palate). Only one litter was affected, but there were only 4 litters in the high dose group (maternal toxicity consisted of decreased maternal body weight gain (81%) as compared to controls (100%)), and comparable doses were not tested in other available studies on triadimenol. Furthermore, triadimenol is a triazole and cleft palates have been reported following exposure to other triazoles

(Menegola *et al.*, 2005 and Menegola *et al.*, 2009). As cleft palates are also severe findings, RAC concludes that the effect cannot be totally dismissed and that it cannot be excluded that the observed cleft palates in 1/4 litters were not only chance findings.

Developmental landmarks were not assessed in the multi- and two-generation rat studies, and in the study by Machemer (1997) (similar to OECD TG 414) the observed malformations were not dose-related.

In the developmental toxicity study in rabbits by Becker *et al.* (1987b), increases in skeletal anomalies, mostly in the form of abnormal or incomplete ossification, were reported at 200 mg/kg bw/d (0%, 20%, 13.3% and 42.9%** on a litter basis at 0, 8, 40 and 200 mg/kg bw/d, respectively) in the presence of decreased maternal body weight gain as compared to controls (17 g vs 309 g in controls on GD6-9 and 17 g vs 272 g in controls on GD6-18). In the second rabbit study by Clemens *et al.* (1992) no developmental effects were reported up to doses of 125 mg/kg bw/d. In the OECD TG 43 (2008) it is stated that severe decrease in body weight gain in rabbits resulting in body weight loss can result in reduced foetal weight, alterations in ossification and abortion, but not in malformations. In Cappon *et al.* (2005), feed restriction and the subsequent reduction in maternal body weight gain resulted in an increase in abortion, reduced foetal weight, and an increased incidence of foetuses with unossified sternebrae, metatarsals, metacarpals, and caudal vertebrae, but there were no foetal malformations associated with feed restriction. Based on this information, RAC concludes that it is reasonable to assume that the reported abnormal or incomplete ossification in rabbits is produced solely as a secondary non-specific consequence of decreased maternal body weight gain.

Decreased litter size at birth and decreased postnatal viability

In the multi-generation study in rats (Loeser and Eiben, 1982), a statistically significant reduction in the litter size and pup body weight at birth, as well as in the pup viability indices at post-natal day (PND) 5 and 28 were reported, and the incidences of these effects as well as the maternal body weights are shown in the table below:

Table : Maternal, litter and pup parameters in the multi-generation study

Dose (mg/kg bw/d)	**0**	**15**	**60**	**240**
F_0: fist mating to produce F_{1A}				
Maternal bw gain (g) (weeks 0-10) (as compared to control)	242 (0%)	245 (+ 1%)	231 (-5%)	197 (-19%**)
Litter size at birth	11.9	11.6	10.6	4.2*
Pup bw weight at birth[1]	0%	0%	0%	0%
5d viability index (%)[2]	90.1	85.4	79.9*	41.2**
28d viability index (%)[3]	90.1	79.3*	93.3	14.3**
28d pup bw weight gain	0%	0%	0%	-28%**
F_0: second mating to produce F_{1B}				
Litter size at birth	11.5	10.7	10.6	3.8*
Pup bw weight at birth[1]	0%	0%	0%	-13
5d viability index (%)[2]	90.8	82*	77.2*	81.6
28d viability index (%)[3]	95.6	90.2	91.0	52.6**
28d pup bw weight gain	0%	0%	0%	-23%**
F_1: fist mating to produce F_{2A}				
Maternal bw gain (g) (weeks 5-16) (as compared to control)	207 (0%)	210 (+1%)	182 (-12%)	189 (-9%)
Litter size at birth	12.1	11.2	10.1	10.0
Pup bw weight at birth[1]	0%	0%	0%	0%
5d viability index (%)[2]	87.2	85.3	76.7*	62.5**
28d viability index (%)[3]	98.9	93.9*	70.3**	63.6**
28d pup bw weight gain	0%	0%	0%	-33%**
F_1: second mating to produce F_{2B}				
Litter size at birth	12.7	10.5	6.2*	9.7

Pup bw weight at birth[1]	0%	0%	0%	0%
5d viability index (%)[2]	88.7	81.6	64.9**	84.6
28d viability index (%)[3]	97.3	91.3	95.8	73.3**
28d pup bw weight gain	0%	0%	0%	-21%*
F_2: fist mating to produce F_{3A}				
Maternal bw gain (g) (weeks 5-15) (as compared to control)	218 (0%)	197 (-9%)	198 (-9%)	156 (-28%)
Litter size at birth	11.9	9.5	5.7*	6.8*
Pup bw weight at birth[1]	0%	-11%	-20%**	-20%*
5d viability index (%)[2]	89.7	66.5**	2.5**	11.8**
28d viability index (%)[3]	70.7	53.0	0	0
28d pup bw weight gain	0%	0%	-	-
F_2: second mating to produce F_{3B}				
Litter size at birth	10.3	8.5	11.0	3.9**
Pup bw weight at birth[1]	0%	0%	0%	-16%*
5d viability index (%)[2]	81.6	74	96.4**	29.6**
28d viability index (%)[3]	93.9	93.7	88.8	100.0
28d pup bw weight gain	0%	-14%	-26%*	-29%

Statistically significant at *p≤0.05, **p≤0.01

[1] Changes in pup weight compared to control animals

[2] (No. of live pups after 5 days / no. of pups born) x 100

[3] (No. of live pups after 28 days / no. of live pups after 5 days, after culling) x 100

In the multi-generation study a significant reduction in litter size at birth was reported. The effect was more pronounced in the high dose animals of the F1 and F3 generations (the mean litter size was reduced by 43-67% as compared to control animals), but it was also reported in both F2 generations. Also the 5 day- and 28-day viability indices were significantly decreased in all generations. The effect was not statistically significant and dose-related in all matings, but it was statistically significant in some of the matings also at the low dose. Reduced maternal body weight gains of 9-28% as compared to controls were observed in the high dose group during a 10-week period preceding the first matings, but no information on the body weights during pregnancies were provided. However, in a study by Carney *et al.* (2004) it was shown that a reduced body weight up to 32% as compared to control animals did not affect the litter size or offspring viability. This information indicates that the decreased litter size and reduction in postnatal survival were not secondary non-specific consequences of maternal toxicity. In addition, in the mid-dose group in the F1 second mating and F2 first mating there were also indications of litter losses in the absence of marked maternal toxicity.

In the 2-generation study (Loeser and Eiben, 1984), no clear effects on litter size, pup weight and pup viability were reported, however, lower doses (up to 57 mg/kg bw/d) were tested in this study.

RAC concludes that the decrease in litter size at birth observed in the multi-generation study may be a consequence of pre- or post-implantation losses (decreased pregnancy rates were reported in this study but the effect cannot be assigned to either impairment of sexual function and fertility or to development as is discussed under the section on fertility). The decreases in 5- and 28-day viability indices also provide evidence of developmental toxicity.

Overall, RAC concludes that the developmental toxicity observed in the form of post-implantation losses, increase in cervical ribs and cleft palates reported following exposure to triadimenol during gestation and in the form of decreased postnatal viability following maternal exposure during the 10-week premating period up to weaning is not considered to be a secondary non-specific consequence of maternal toxicity, and provide altogether clear evidence of developmental toxicity.

Fertility

There is a multi-generation study (Loeser and Eiben, 1982) and a 2-generation reproductive toxicity study (Loeser and Eiben, 1984) in rats available for the assessment of effects on fertility following exposure to triadimenol. The multi-generation study was not performed according to OECD Test Guidelines or GLP. As regards the 2-generation reproductive toxicity study, a reference was made to OECD TG 416. The study was not in accordance with GLP. However, OECD TG 416 was not available in 1982, as the first conventional OECD TG 416 was adopted in 1983. The OECD GLP criteria were established in 1992.

In the multi-generation study dose-related statistically significant decreases in pregnancy rates (no. of pregnant/mated rats) were reported in three generations at 60 and/or 240 mg/kg bw/d as reported in table 9 below. The findings were consistently reported over the generations in a dose-related way except for one out of six matings, in which the response was not dose-related. However, in this mating the number of animals in the high dose group was low (only 7 animals as compared to 14-20 animals in other groups) which may have caused a result that was not statistically significant at this high dose group.

Table: Fertility data from multi-generation study

Dose (mg/kg bw/d)	**0**	**15**	**60**	**240**
F_0: fist mating to produce F_{1A}				
Pregnancy rate[1] (%) No. pregnant/no. per group	85 17/20	80 16/20	73.7 14/19	20** 4/20
F_0: second mating to produce F_{1B}				
Pregnancy rate (%) No. pregnant/no. per group	88.9 16/18	80 16/20	73.7 14/19	68.4 13/19
F_1: fist mating to produce F_{2A}				
Pregnancy rate (%) No. pregnant/no. per group	100 20/20	100 20/20	70* 14/20	50** 4/8
F_1: second mating to produce F_{2B}				
Pregnancy rate (%) No. pregnant/no. per group	84.2 16/19	85 17/20	30** 6/20	57.1 4/7
F_2: fist mating to produce F_{3A}				
Pregnancy rate (%) No. pregnant/no. per group	85 17/20	100 20/20	50 7/14	33.3** 5/15
F_2: second mating to produce F_{3B}				
Pregnancy rate (%) No. pregnant/no. per group	90 18/20	75 15/20	71.4 10/14	50* 7/14

Statistically significant at *p≤0.05, **p≤0.01
[1]Pregnancy rate: no. of pregnant/mated rats.

The weights of the reproductive organs were studied in the animals that died prematurely and in F_{2B} parents. The relative testis weights were increased at 60 and 240 mg/kg bw/d (124 and 142% of controls, respectively) and the ovary weights were increased at 240 mg/kg bw/d (123% of controls). Histopathological investigations were not performed on testis or ovaries. Increases in ovary weight were also reported in the repeated dose toxicity studies. In the 28-day study in rats (Thyssen and Kaliner, 1977) the increase in relative ovary weight was 0, 18**, 14** and 18**% at 0, 5, 15 and 45 mg/kg bw/d. In the 90-day study in rats (Loeser and Kaliner, 1977) the increase in absolute ovary weight was also reported (0, -3 and 23**% as compared to controls at 17, 71 and 287 mg/kg bw/d). No histopathological findings were reported in the ovary in the repeated dose toxicity studies.

Regarding maternal toxicity, there were no treatment-related deaths or clinical signs of toxicity. However, decreases in maternal body weight gain were reported (see Table below).

Table: Changes in body weight gain in the multi-generation study

mg/kg bw/day	**0**	**15**	**60**	**240**
F0 bw gain week 1-10*	242	245 (+1%)	231 (-5%)	197 (-18%)
F1b bw gain week 5-16*	207	210 (+1%)	182 (-12%)	189 (-9%)
F2b bw gain week 5-15*	218	197 (-9%)	198 (-9%)	156 (-28%)

*To start of first mating

Reduced maternal body weight gains of 9-28% as compared to controls were observed in the high dose group during a 10-week period preceding the first matings, but no information on the body weights during pregnancies was provided. In the study by Chapin *et al.* (1993) it was shown that a body weight reduction of 10% and 20% induced by feed restriction did not affect the pregnancy rate. The effects on pregnancy rates were therefore not considered as being secondary non-specific consequences of parental toxicity and according to RAC provided clear evidence of an adverse effect on reproductive toxicity.

Although the study had several deficiencies (e.g. food consumption was not measured, no vaginal smears of females, fertility of individual males was not determined since females were mated with more than one male, reproductive tissue was not examined histologically, sperm parameters were not examined, mating performance of females were not determined in all matings, pregnancy status was not confirmed and gross necropsy was not performed in all generations), RAC concludes that the deficiencies in the multi-generation study did not render the quality of the clear evidence on decreased pregnancy rates less convincing.

In the 2-generation reproductive toxicity study a slight insignificant dose-related decrease in fertility index and/or insemination index was reported in the F1 generation (see the table below).

Table: Fertility data from 2-generation study - female

Dose (mg/kg bw/d)	**0**	**2.2**	**11**	**57**
F_0: fist mating to produce F_{1A}				
Fertility index[1] (%)	95	100	100	100
F_0: second mating to produce F_{1B}				
Fertility index (%)	95	100	90	90
Dose (mg/kg bw/d)	**0**	**1.8**	**9**	**39**
F_1: fist mating to produce F_{2A}				
Fertility index (%)	90	80	70	70
Insemination index[2] (%)	95	95	90	70
F_1: second mating to produce F_{2B}				
Fertility index (%)	83	75	80	75
Insemination index (%)	100	95	85	80

[1](number of pregnant females /number of females in the group) x 100
[2]Determined from vaginal smears to detect the presence of sperm = (number of inseminated females/number of females in the group) x 100

The effects were less pronounced than those seen in the multi-generation study, but they were consistent with the lower doses used in this study. Effects on reproductive organs were studied in F_{1B} parents. These included an increase in relative testis weight at 29 mg/kg bw/day (12% higher as compared to controls) and increased ovary weight at 39 mg/kg bw/d (14% higher as compared to controls, statistically significant at $p \leq 0.05$). There were no histopathological findings in these organs, but histopathological examinations were not performed on animals that failed to induce pregnancy. As regards parental toxicity, there were no deaths or clinical signs. The F_0 body weight gain was not affected by the treatment, and it was 115, 106, 113 and 108 g at 0, 1.8, 9 and 39 mg/kg bw d, respectively, in F_1 dams from week 5 to week 16.

RAC concludes that the 2-generation study testing only lower doses provided weak supporting evidence on reproductive toxicity.

Overall, RAC concludes that the deficiencies in the multi-generation study do not render the quality of the clear evidence on decreased pregnancy rates less convincing. However, the observed decrease in pregnancy rates cannot be assigned to either impairment of sexual function and fertility or to developmental toxicity, because it was not determined in the study whether the effect was caused by impaired sexual function and fertility or by post-implantation losses which is an adverse effect on development. According to the CLP criteria, if reproductive toxic effects cannot be clearly assigned to either impairment of sexual function and fertility or to developmental toxicity, chemicals with these effects shall be classified as reproductive toxicants with a general hazard statement. The CLP criteria (4th ATP) Annex VI section 1.2.3 and Annex VII Note 4 to table 1.1 state that *"Hazard statements H360 and H361 indicate a general concern for effects on fertility and/or development: "May damage/Suspected of damaging fertility or the unborn child". According to the criteria, the general hazard statement can be replaced by the hazard statement indicating the specific effect of concern in accordance with section 1.1.2.1.2 in Annex VI. When the other differentiation is not mentioned, this is due to evidence proving no such effect, inconclusive data or no data and the obligations in Article 4(3) shall apply for that differentiation*" and in the CLP Guidance section 3.7.4.1 (version 4.0, November 2013) it is further described that "*where the effect cannot be specified with respect to fertility or development the general statement must be applied"*.

RAC conclusion on classification and labelling

Repr. 1A:

There is no information available regarding effects on fertility following exposure to humans, so RAC considers that a classification of triadimenol as Repr. 1A is not appropriate.

Repr. 1B:

According to the CLP criteria a classification of a substance in category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on reproductive toxicity 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.

RAC concludes that the developmental toxicity observed in the form of post-implantation losses in rats and rabbits, and the increase in cervical ribs, cleft palates and decreased postnatal viability in rats provide altogether clear evidence of developmental toxicity. The dose-related decrease in pregnancy rates that was observed in all three generations in the multi-generation rat study (with the weak supporting evidence in the form of decreased fertility index in the 2-generation rat study testing only lower doses) and the associated decrease in litter sizes provide clear evidence of reproductive toxicity. RAC concludes that the adverse effects on reproduction were not secondary non-specific consequences of parental toxicity, and that there is no evidence that these effects are not relevant to humans. In addition, the deficiencies in the multi-generation study do not render the quality of the clear evidence on decreased pregnancy rates in rats less convincing. Although no gross or histopathological examinations of the reproductive organs were performed, adverse effects on sexual function and fertility include e.g. alterations in the female and male reproductive system, adverse effects on gamete production and transport, sexual behaviour, fertility **or** pregnancy outcomes. There is clear evidence of an adverse effect on pregnancy rates and further investigations on the cause of that effect (e.g. gross or histopathological examinations of the reproductive organs) are not required for a specific classification. In addition, pregnancy rates and fertility index were studied only in one species, but studies in the second species are not required in the CLP Regulation in order to conclude on a specific classification for reproduction. As the observed decrease in pregnancy rates could not be assigned to either impairment of sexual function and fertility or to developmental toxicity, **RAC concludes that Repr 1B; H360 without 'F' and 'D' should be assigned for triadimenol**.

Lactation:

In the multi-generation study a dose-depended decrease in the 5-day viability index was reported (see the table below). However, from the study report it is difficult to elucidate if the decrease in the 5-day viability index is only related to exposure to triadimenol after birth, or if also exposure during gestation is necessary to reduce the 5-day viability index.

Table: 5 day Viability index from the multigeneration study (Loeser and Eibem, 1992)

Dose (mg/kg bw/d)	**0**	**15**	**60**	**240**
F_0: fist mating to produce F_{1A}				
5d viability index %	90.1	85.4	79.9*	41.2**
F_0: second mating to produce F_{1B}				
5d viability index %	90.8	82*	77.2*	81.6
F_1: fist mating to produce F_{2A}				
5d viability index %	87.2	85.3	76.7*	62.5**
F_1: second mating to produce F_{2B}				
5d viability index %	88.7	81.6	64.9**	84.6
F_2: fist mating to produce F_{3A}				
5d viability index %	89.7	66.5**	2.5**	11.8**
F_2: second mating to produce F_{3B}				
5d viability index %	81.6	74	96.4**	29.6**

Statistically significant at *p≤0.05, **p≤0.01

Maternal toxicity was evident as a decrease in maternal body weight gain (at 240 mg/kg bw/d an 18% decrease in the body weight gain from week 1 to week 10 in the F_0 generation as compared to controls, and a 28% decrease in the body weight gain from week 1 to week 15 in the F_2 generation as compared to controls). No information on maternal body weights during lactation was provided. However, in a study by Carney *et al.* (2004) it was shown that reduced maternal body weight up to 32% did not seem to affect survival during lactation. A decrease in viability index was not reported in the 2-generation study, however, lower doses were used in this study, up to 57 mg/kg bw/d in females.

In the toxicokinetics section it is described that triadimenol excretion was 80-90% at 24 hours after administration of single or repeated doses. This indicates that bioaccumulation may occur. Furthermore, triadimenol was rapidly and extensively absorbed (almost 100%) following oral administration with the highest peak concentration in fat, urinary bladder and liver. There are no measurements of triadimenol in milk available. However, triadimefon (a metabolite of triadimenol) was measured in goat milk at a concentration lower than in liver and kidney. It is anticipated that this will also be the case following exposure to triadimenol due to similar physical/chemical properties of the two substances. Furthermore, due to the bioaccumulation potential of triadimenol and information of absorption into fat, a potential transfer of triadimenol to milk cannot be excluded.

According to the CLP criteria a classification for effects on or via lactation can be assigned on the basis of:

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 effects in the offspring due to transfer in the milk or adverse effects on 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.

Based on the significantly reduced viability index on PND 5 seen in several generations in the multi-generation study together with the information from toxicokinetic studies, it cannot be excluded that due to its properties triadimenol may be transferred to milk. RAC concludes that triadimenol should be classified for effects on or via lactation with H362, as was proposed during public consultation.

Overall, RAC agrees to classify triadimenol as **Repr. 1B; H360 and Lact.; H362.**

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier submitter's proposal

Triadimenol is a triazole systemic fungicide that is used as a seed treatment and a foliar spray treatment. Triadimenol exists as two diastereomers, referred to as A and B. The Draft Assessment Report (DAR) produced under Directive 91/414/EEC concluded that differences in ratios of the two diastereomers in the active substance were not of practical concern for environmental exposure or risk assessment. The active substance was therefore considered as the sum of the isomers. Triadimenol has not previously been reviewed for harmonised classification and labelling and does not have an existing entry in Annex VI of CLP.

Degradation

The dossier submitter considered triadimenol as not rapidly degradable. Triadimenol degrades very slowly in water and sediment (Anderson, 1986, revised 2002; Schäfer, 2002) with calculated DT_{50} values at 20°C for the total W/S-system of 443 and 377 days and in soil with calculated DT_{50} values at 20°C in various soils between 47.3 and 362 days (Brumhard, 2003) and between 134 and 349 days (Voegeler, 1976). A ready biodegradation study was not available. Triadimenol is also stable to hydrolysis (Nicholls & Thornton, 1980).

Aquatic Bioaccumulation

The DS regarded triadimenol as being not bioaccumulative in the aquatic environment. The basis for this was that the log K_{ow} of between 3.08 and 3.28 (Krohn, 1984) and the measured BCF value of 21 (Forbis, 1987) are lower than in the criteria in the CLP Regulation.

Acute Toxicity

The DS proposed to not classify triadimenol as acutely hazardous to the aquatic environment; the short-term ecotoxicity test results showed toxicity between 10 to 100 mg/L across all three trophic levels. The results indicated that fish were the most sensitive taxon, with all three acute fish test results lower than the results in *Daphnia* and algae. The most sensitive acute endpoint was a 96h LC_{50} = 17.4 mg/L (nominal concentration) for *Leuciscus idus*.

Chronic Toxicity

The DS proposed to classify triadimenol as Aquatic Chronic 2; H411. The basis for this proposal was the 35-day growth NOEC of 0.17 mg/L (mean-measured) from the FELS test using *Pimephales promelas*. The results of the non-GLP 21 day *Daphnia* study were also in this range (albeit the test substance used was of lower purity). The NOE_rC for algae was 4.7 mg/L indicating that aquatic plants are not the most sensitive trophic level.

The Fish Screening Assay (FSA) (Teigeler, 2007) provided a reliable NOEC of 30 µg/L based on a statistically significant reduction (of around 38% compared with control) in vitellogenin (VTG) levels in female fish and on slight histopathological liver changes. However, the DS stated that such effects are not considered relevant in relation to aquatic hazard classification.

This argumentation is in line with the recent RAC assessment of tebuconazole where a Fish Sexual Development Test (FSDT) with fathead minnow gave information on effects (degenerative liver toxicity, reduction in yolk accumulation and pancreas effects) at levels lower than those effects 'traditionally' used for chronic classification (e.g. growth, survival, reproduction). RAC, along with the Evaluating MSCA and DS, agreed in the case of tebuconazole that whilst such studies might provide supporting data when based on endpoints for mortality, growth and fertility, such effects

(some of which may be ED-related endpoints) were currently not considered as a sole basis for the purposes of aquatic hazard classification.

The DS assessed the Fish Sexual Development Test (FSDT) (Bomke, 2010) with uncertainty over the nature of any ED effects seen in terms of a clear concentration-dependent cause and effect. There were also concerns regarding the overall validity and reliability of this test and that a clear NOEC had not been established. However, in the CLH report itself the dossier submitter did not evaluate the sex ratio as a prospective endpoint to be considered for the purposes of aquatic hazard classification.

Comments received during public consultation

Comments on the proposed classification related to environmental hazards were received from three MSCAs. While one supports the proposed classification Aquatic Chronic 2 for triadimenol, the two others find it justified to classify it as Aquatic Chronic 1 with an M-factor of 1. This latter proposal was based on the measured NOEC of 0.030 mg/L in the reported FSA (Teigeler, 2007) which was evaluated as a valid and reliable study without restriction. The observed effects in the liver included single cell necrosis, condensed hepatocellular cytoplasm and a slight increase in fatty vacuolation. The commenting MSCAs argued that even if these effects are considered as minimal or slight, they do not represent an ED endpoint and this liver toxicity should be considered for classification purposes.

In addition, one commenting MSCA argued that the FSDT (Bomke, 2010) is a valid study and the assessment "reliable with restrictions" is only related to non-sufficient data for vitellogenin. In addition to the Vitellogenin (VTG) concentration, the secondary sexual characteristics (nuptial tubercles) and the histology of the male genitals are the most sensitive endpoints in the test system and therefore, the commenting MSCA argued, these endpoints are reliable without restrictions. In contrast to the test report itself, a re-evaluation by the commenting MSCA discovered significant effects on the two endpoints. For the endpoint of secondary sexual characteristics, the mean amount of nuptial tubercles per male fish increased depending on concentration and decreased again at the highest concentration. The highest effect occurred at 70.8 µg/L (2.85-fold the control level, statistically significant) giving a sensitive and relevant endpoint.

The commenting MSCA also stressed that for the endpoint sex ratio (ratio of distinct females) the phenotypically and histologically determined sex ratios range between 40% and 60% in all aquaria and the assessment by the study authors did not show significant effects. The commenting MSCA's re-evaluation of the sex ratio was done by applying separate categories for (a) clearly male, (b) clearly female, (c) intersex (both oocytes and spermatogenetic cells in one gonad) and (d) undifferentiated sex as recommended in the current (2011) version of the OECD TG 234 which showed that the ratio of the females at 170 µg/L is significantly reduced compared to the control (ToxRat 2.10, two-page Williams Test with $\alpha = 0.05$). Consequently a NOEC of 70.8 µg/L was derived by the commenting MSCA (see details in additional key elements).

The DS pointed out that at this point it is not clear if the commenting MSCA's re-evaluation of the sex ratio was based on secondary sexual/morphological characteristics. The data holder argued that when sex was determined based on histological examination of the gonads, the sex of all individuals could be clearly identified, and no significant difference was observed. The DS concluded, having considered the methodology and reporting of effects in the FSDT study, as well as the re-evaluation by the MSCA and the data holder's assessment of it, that its results could not be relied upon for classification purposes.
Thus the DS considered the NOEC of 0.17 mg/L from the original report as valid for classification purposes.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS that triadimenol cannot be considered as rapidly degradable.

Aquatic Bioaccumulation

RAC agrees with the DS: triadimenol has low potential for bioaccumulation and therefore does not meet the criteria for bioaccumulation.

Acute Toxicity

RAC agrees with the DS proposal and argumentation that **triadimenol does not meet the criterion for aquatic acute classification**.

Chronic Toxicity

RAC considers the observed effects on reduction in vitellogenin (VTG) level and histopathological changes in the liver from the FSA (Teigeler, 2007) as supporting data for the purposes of aquatic hazard classification. However, without further guidance and clarification on how these endpoints should be used, RAC has not considered them for the purposes of aquatic hazard classification of triadimenol.

RAC considers that changes in the sex ratio of fish provide potential evidence of reprotoxicity and can lead to adverse effects at the population level; consequently this is relevant for aquatic hazard classification. According to OECD TG 234, sex ratio is to be determined via gonad histology and whenever possible via genetic markers (i.e. positive identification of the genetic sex). Optionally, in histological determination, evaluation and staging of oocytes and spermatogenetic cells may also be determined.

RAC understands that the FSDT (Bomke, 2010) was carried out parallel to the development of the corresponding OECD TG 234 and thus the study does not fulfil fully the currently adopted version of the guideline. It was evaluated as reliable only with restrictions and several experimental draw backs have been discovered by the data owner. RAC notes that the tested species fathead minnow (*Pimephales promelas)* is no longer included in OECD TG 234, because it is considered less sensitive to the core endocrine endpoints aromatase inhibition and sex differentiation (König & Bomke, 2010). The data owner confirmed that all fish were either males or females based on gonad histology and no undifferentiated or intersex fish were seen. In contrast, the re-evaluation of the sex ratio submitted by one commenting MSCA used a discrepancy between phenotypic sex and histological sex to identify undifferentiated or intersex fish and to derive a NOEC of 70.8 µg/L. RAC considers this procedure and the NOEC provided by the MSCA as not appropriate for the purpose of aquatic hazard classification.

In the study (Bomke, 2010) there is an unusually high variance in the eight control replicates for the relevant endpoints. This hinders a meaningful statistical evaluation. No reliable explanation for 9% lost fish at day 39 and for a significantly higher mortality in the test concentration of 70.8 µg/L is given. An error in the re-distribution of the fish at day 46 might be an explanation. While a lower density might explain a significantly faster development (larger size and higher weight) of the fish, it remains unclear why this should be responsible for a high VTG level in male fish. The hatchability seems to indicate – although statistically not significant – a delayed development caused by triadimenol. While the data owner only stressed that the statistically significant effects do not result in a clear dose response, the commenting MSCA in light of the assumed endocrine mode of action of triadimenol requested an assessment of the biological significance of (and correlation between) the effects observed in this study. Overall, RAC agrees with the dossier submitter that this study seems not reliable enough for the purpose of aquatic hazard classification.

Based on the 35-day mean, measured growth NOEC of 0.17 mg/L from the FELS test using *Pimephales promelas*, RAC agrees with the proposal and argumentation of the DS, to classify triadimenol as **Aquatic Chronic 2; H411**.

Additional references

Additional references not included in the CLH report

Cappon GD, Fleeman RE, Chapin RE, and Hurtt ME. (2005) Effects of feed restrictions during organogenesis on embryo-fetal development in rabbit. Birth Defects Research (Part B) 74: 442-430.

ANNEXES:

Annex 1 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 rapporteurs' comments (excl. confidential information).