Committee for Risk Assessment

RAC

Annex 1

Background document

to the 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

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

4 December 2015

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: TRIADIMENOL

EC Number:	259-537-6
CAS Number:	55219-65-3
Index Number:	Not allocated
Contact details for dossier submitter:	UK CLP Competent Authority, Chemicals Regulation Directorate, Health and Safety Executive, United Kingdom.

Version number: **2** **Date:** **September 2014**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Triadimenol***
EC number:	***259-537-6***
CAS number:	***55219-65-3***
Annex VI Index number:	***Not allocated***
Degree of purity:	***The minimum purity is 97 %. Comprising of A-isomer (threo or RS+SR) and B-isomer (erythro or RR+SS), which are generally in the ratio 80:20, with tolerances of 78-88% A to 12-22% B***
Impurities:	***The manufacturer has requested that the impurities remain confidential. According to the present specification of industrially-produced triadimenol, all impurities are individually present at ≤ 1 %.***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Not listed
Current proposal for consideration by RAC	Acute Tox 4; H302 Repr Cat 2; H361f Aquatic Chronic 2; H411
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox 4; H302 Repr Cat 2; H361f Aquatic Chronic 2; H411

1.3 Proposed harmonised classification and labelling

Table 3: Proposed classification

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
2.15.	Organic peroxides	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification

2.16.	Substance and mixtures corrosive to metals	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	**Acute Tox 4; H302**	None	None	Not applicable
	Acute toxicity - dermal	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
	Acute toxicity - inhalation	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	Not classified	Not applicable	Not applicable	No data
3.4.	Skin sensitisation	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
3.7.	Reproductive toxicity	**Repr Cat 2; H361f**	**None**	**None**	Not applicable
3.8.	Specific target organ toxicity –single exposure	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	**Aquatic chronic 2; H411**	None	None	Not applicable
5.1.	Hazardous to the ozone layer	Not classified	Not applicable	Not applicable	Conclusive but not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Pictograms: CHS07, GHS08, GHS09
Signal word: Warning
Hazard statements: H302, H361f, H411
Precautionary statements: Not required as PS are not included in Annex VI.

Proposed notes assigned to an entry: None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Triadimenol has not previously been reviewed for harmonised classification and labelling and does not have an existing entry in Annex VI of CLP. However, a CLH proposal was submitted by the UK in December 2010. This was published for consultation in July 2011, but was subsequently withdrawn in July 2012 to allow for the inclusion of additional information. In particular, subsequent to submission of the triadimenol DAR, the Notifier provided two additional tests on potential endocrine-mediated or disruptive effects of the substance on fish. These were a fish screening assay (FSA) on fathead minnow (*Pimephales promelas*) by Teigeler, M. (2007) and a fish sexual development test (FSDT) also on fathead minnow by Bomke C. (2010).

2.2 Short summary of the scientific justification for the CLH proposal

Triadimenol is a triazole systemic fungicide that is used as a seed treatment and a foliar spray. In 2008, it was approved for Annex I listing as a 3A review substance under Council Directive 91/414/EEC, with the UK as Rapporteur Member State. In accordance with Article 36 (2) of the CLP Regulation, triadimenol should now be considered for harmonised classification and labelling. Therefore, this proposal considers all human health and environmental end points. This CLH dossier presents a classification and labelling proposal based mainly on the information presented in the assessment of triadimenol under Directive 91/414/EEC, although some of the acute toxicity studies were submitted after that assessment.

Classification with Acute Tox 4; H302 is proposed based on the lowest observed LD_{50} value of 720 mg/kg in rats.

Triadimenol reduced fertility in a multi-generation study in rats (showing an increase in severity of the effects through the generations such that all dose groups were affected by the final generation), with supportive evidence provided by a two-generation study in rats that used lower doses. The reduced fertility index in these studies is not regarded as being a non-specific secondary consequence of parental toxicity. As such, classification with Repr 2; H361f is proposed.

The substance is not classed as readily biodegradable or rapidly degradable. The ecotoxicity test results suggest the substance exhibits acute aquatic toxicity between 10-100 mg/l. This is consistent across all three trophic levels tested. The long-term aquatic data suggest toxicity in the range 0.1-1 mg/l. Fish are the most sensitive taxa, based on a study on *Pimephales promelas* (with 35-day mean-measured growth NOEC of 0.17 mg/l).Using the chronic ecotoxicity criteria, triadimenol fulfils the criteria for aquatic environmental hazard chronic category 2.

At the time of writing, no REACH registration dossiers had been submitted for this substance.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Not currently listed on Annex I of the CLP Regulation.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

The majority of notifiers to the Classification and Labelling Inventory classify triadimenol with Acute Tox 4; H302 and Aquatic Chronic 3; H412. However, a number of notifiers additionally apply Acute Tox 3; H332, whilst a small number have included no classification in the notified information.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

In 2008, Triadimenol was approved for Annex I listing as a 3A review substance under Council Directive 91/414/EEC, with the UK as Rapporteur Member State. In accordance with Article 36 (2) of the CLP Regulation, triadimenol should now be considered for harmonised classification and labelling. Therefore, this proposal considers all human health and environmental end points.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

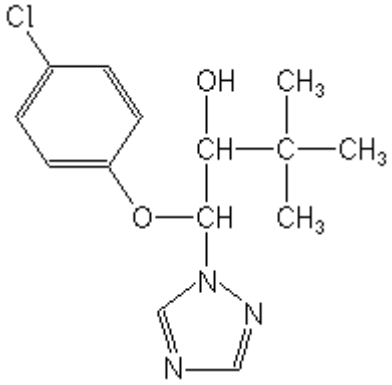
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	259-537-6
EC name:	α-tert-butyl-β-(4-chlorophenoxy)-1H-1,2,4-triazole-1-ethanol
CAS number (EC inventory):	55219-65-3
CAS number:	55219-65-3
CAS name:	1H-1,2,4-triazole-1-ethanol, .beta.-(4-chlorophenoxy)-.alpha.-(1,1-dimethylethyl)-
IUPAC name:	1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol
CLP Annex VI Index number:	Not listed
Molecular formula:	C_{14} H_{18} Cl N_3 O_2
Molecular weight range:	295.8 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Triadimenol	≥ 97 %	97 to 100 %	

Current Annex VI entry: Not listed

Triadimenol is a mixture of two diastereomers, isomer A (threo-configuration, *RS*- and *SR*-form) and isomer B (erythro-configuration, *SS*- and *RR*-form). Each isomer is in itself a racemic mixture of two optical isomer forms. The ratio of the two isomers in the currently manufactured material is ≈ 80 % isomer A: ≈ 20 % isomer B (hereafter referred to as 80:20 material). Further details on the composition are provided in the technical dossier.

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
All impurities are confidential		Process impurities are individually present in the range of 0 to 1 %	

Current Annex VI entry: Some of the impurities are listed on Annex VI. These impurities were thoroughly evaluated and do not impact on the classification proposed in this dossier.

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

Current Annex VI entry: Not applicable

1.2.1 Composition of test material

Triadimenol is a mixture of two diastereomers, isomer A (threo-configuration, *RS*- and *SR*-form) and isomer B (erythro-configuration, *SS*- and *RR*-form). Each isomer is in itself a racemic mixture of two optical isomer forms. The ratio of the two isomers in the currently manufactured material is ≈ 80 % isomer A: ≈ 20 % isomer B (hereafter referred to as 80:20 material). Further details on the composition are provided in the technical dossier.

During the development of triadimenol in the 1970s, material was produced on a laboratory scale with an approximate A:B ratio of 60:40. In the 1980s and beyond, the 80:20 material was produced. Information on the test material's specification is presented as far as possible, but was not always stated in the study reports.

Test materials with varying purities were used in the toxicology studies. The current minimum purity is 97 %, which is similar to or more pure than the material used in the majority of studies. During the evaluation under Directive 91/414/EEC, it was concluded that the data provided a worst-case scenario with respect to impurities. It was also concluded that the profile of impurities present in the older material was almost identical to those in the current specification.

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance at 20°C and 101,3 kPa	Pure: colourless crystals As manufactured: white to grey powder		Visual inspection
Melting point	Isomer A: 138.2ºC Isomer B: 133.5ºC	Dörr, 1979	Measured, OECD 102 DTA
Boiling point	Mixture of *SR* and *SS* or *RR*: 155 to 270ºC	Klusacek & Krasemann, 1986	Measured, OECD 113
Relative density	Isomer A: 1.237 at 22ºC Isomer B: 1.299 at 22ºC	Weber, 1987a; Weber, 1987b	Measured, OECD 109
Vapour pressure	Isomer A (*SR*): $6x10^{-7}$ Pa at 20ºC; $1x10^{-6}$ at 25ºC (extrapolated) Isomer B (*SS* and *RR*): $4x10^{-7}$ Pa at 20ºC; $9x10^{-7}$ at 25ºC (extrapolated)	Weber & Krohn, 1996	Measured, OECD 104
Surface tension	Mixture of *SR* and *SS* or *RR: 54.4* mN/m at 20ºC and 130 mg/L	Krohn, 1999	Measured, EEC method A 5
Water solubility	Isomer A: 0.049 g/L at 20ºC Isomer B: 0.095 g/L at 20ºC As it is a very weak base that can only be completely protonated in non-aqueous systems in the presence of very strong acids, water solubility in the acidic and alkaline pH range was not determined	Leimkuehler, 1980	Measured, OECD 105 (Flask)
Partition coefficient n-octanol/water (log value)	Isomer A: 3.08 at 25ºC Isomer B: 3.28 at 25ºC The effect of pH was not investigated	Krohn, 1984	Measured, OECD 107 (Shake flask)
Flash point	Not applicable (melting point above 40ºC)		
Flammability	Not highly flammable and does not liberate gases in hazardous amounts	Eberz, 1999	Measured, EEC A 10 and 12
Explosive properties	Not explosive	Eberz, 1999	Measured, EEC A 14
Self-ignition temperature	Does not spontaneously combust	Eberz, 1999	Measured, EC A.16
Oxidising properties	No oxidising properties	Eberz, 1999	Measured, EEC A 17
Granulometry			

Stability in organic solvents and identity of relevant degradation products			
Dissociation constant	No dissociation occurred		Measured, OECD 112
Viscosity			

2 MANUFACTURE AND USES

2.1 Manufacture

Triadimenol is manufactured outside of the EU.

2.2 Identified uses

Triadimenol is used as a fungicidal seed and foliar spray treatment in agricultural applications within the EU.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Refer to table 9			

3.1 Physico-chemical properties

3.1.1 Conclusions on classification and labelling

In a standard study (Eberz, 1999), triadimenol was found not to exhibit any explosive properties and no classification for explosivity is proposed.

In standard studies (Eberz, 1999) triadimenol was found to be non-flammable, it did not exhibit any pyrophoric properties and did not liberate any flammable gases in contact with water. No classification for flammability is proposed.

In a standard study (Eberz, 1999), triadimenol was found not to exhibit any oxidising properties and no classification is proposed.

.**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.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

The following summary is derived from the assessment made for the review under Directive 91/414/EEC.

4.1.1 Non-human information

The toxicokinetics and metabolism of triadimenol have been investigated in the rat. The active substance was rapidly and extensively (almost 100%) absorbed following oral administration of low (1 mg/kg/d) and high (100 mg/kg/d) doses. Peak concentrations were achieved in most organs and tissues within one hour, with the highest peak concentrations occurring in fat, the urinary bladder and the liver. Excretion was also extensive and was mainly via the faeces in males (> 90 % of the administered dose in the bile) and via both faeces and bile in females. At 24 hours after administration of single or repeated doses, total excretion was 80-90% in males and females; as excretion was not complete by 24 hours, accumulation of triadimenol may occur. By 120 hours, excretion had reached almost 100%. The half-life of elimination from plasma was between 6.4 and 9 hours after single or repeated (for 14 days) administrations. Retention in tissues was very limited, with residues in the body (excluding the gastrointestinal tract) being up to 0.06% of the administered dose after single low (1 mg/kg/d, determined in males and females) or high (100 mg/kg/d in males) doses and after a repeated low-dose administration (males only; residues determined at 120 hours). After a single dose, the highest residues (determined in male rats) were found in fat, the bladder and the liver; amounts of radioactivity were below the limit of detection by 72 hours after administration of the dose. The results indicated that enterohepatic recirculation occurred. Triadimenol was rapidly metabolised, predominantly by hydroxylation to triadimenol-hydroxy then further oxidation to triadimenol-carboxy. Oxidation of the secondary hydroxy group to form metabolites related to triadimefon was indicated at low levels (< 2 %), but triadimefon itself was not identified. Differences between the metabolites formed from different dose levels and the sexes were minor.

4.1.2 Human information

No available information.

4.1.3 Summary and discussion of toxicokinetics

See section 4.1.1.

4.2 Acute toxicity

Acute toxicity has been investigated by the oral, inhalation and dermal routes in rats and mice. There is also limited information on intra-peritoneal and sub-cutaneous administration in these species.

Table 11: Summary table of relevant acute toxicity studies*

Method	LD_{50}	Remarks	Reference
Oral Rats/Wistar, 3 females/group, repeated (total of 6 animals tested) 2000 mg/kg in 2% Cremophor EL 14-day observation period 80:20 mixture, purity 97.2% OECD 423 (acute toxic class), GLP	> 2000 mg/kg	Animals were fasted for 16-24 hours prior to administration of the test substance. In the first test at 2000 mg/kg, no animals died. In the repeat of the test with this dose, one animal died on day 3. Clinical signs, which appeared between 2 and 11 days after dosing, included piloerection, increased motility, uncoordinated gait, spasmodic state, aggression, hunched posture, laboured breathing. Gross necropsy did not show any treatment-related findings.	Schüngel, 2005c
Oral Rats/Wistar, 15/sex/group 250-1500 mg/kg in water/Cremophor EL 14-day observation period 80:20 mixture, purity 92.7% Not guideline or GLP	720 mg/kg (fasted animals) 1068 mg/kg (unfasted animals)	Two investigations were performed: in one, rats were fed normally; in the other, they were fasted for 16 hours prior to dosing. Deaths occurred from day 1 in fasted and unfasted animals. Clinical symptoms were consistent with effects on the CNS and included lethargy, piloerection, laboured breathing. Severe behavioural disturbances (aggression, self-mutilation) were noted. Gross necropsy of animals that died showed signs of irritation of mucous membranes of the gastrointestinal tract, which were present to a lesser degree in unfasted animals.	Mihail & Thyssen, 1980
Inhalation Rats/Wistar, 10/sex/group 1 hour exposure: 0.31, 0.67, 1.56 mg/L 4 hours' exposure: 0.088, 0.31, 0.95 mg/L Nose-only exposure 14-day observation period 80:20 mixture, purity 92.7% Not guideline or GLP	> 0.95 mg/L	Test atmosphere concentration was determined by analysis. Particle size analysis was not performed. The highest concentrations tested in each of the 1-hour and 4-hour exposures were stated to be the maximum attainable. There were no deaths, clinical signs, effects on body weight or abnormal gross necropsy findings following a single 1-hour exposure. Following a single 4-hour exposure, one female in the 0.31 mg/L group died on day 14, without having shown any previous symptoms. The study authors attributed this death to acute pneumonic bacterial disease. There were no other deaths or treatment-related gross necropsy findings.	Mihail & Thyssen, 1980
Dermal Rats/Wistar, 5/sex/group 2000 mg/kg applied under a semi-occlusive dressing for 24 hours 14-day observation period 80:20 mixture, purity 97.2% OECD 402, GLP	> 2000 mg/kg (males and females)	There were no deaths, clinical signs, effects on body weight or gross pathology.	Schüngel, 2005b
Dermal	> 5000 mg/kg	There were no deaths, clinical signs of toxicity or abnormal gross necropsy findings.	Mihail & Thyssen,

Rats/Wistar, 5/sex/group 2500 & 5000 mg/kg applied for 24 hours (dressing unspecified) 14-day observation period 80:20 mixture, purity 92.7% Not guideline or GLP			1980

**The studies included in this table are regarded as the key studies for classification purposes. Additional studies can be found in the pesticide evaluation report conducted under Directive 91/414/EEC; however, in the opinion of the dossier submitter, these do not impact the classification decision.*

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Two acute oral toxicity studies have been conducted on triadimenol. LD_{50} values of 720 (fasted rats), 1068 (unfasted rats) and > 2000 mg/kg (fasted rats) were obtained.

4.2.1.2 Acute toxicity: inhalation

One acute inhalation toxicity study has been conducted, in which the maximum attainable concentration was used. An LC_{50} of > 0.95 mg/L was obtained.

4.2.1.3 Acute toxicity: dermal

LD_{50} values of > 2000 and > 5000 mg/kg were obtained in two acute dermal studies.

4.2.1.4 Acute toxicity: other routes

Not relevant for classification.

4.2.2 Human information

No information available.

4.2.3 Comparison with criteria

A range of LD50 values, from 720 mg/kg to > 2000 mg/kg, were obtained from acute oral toxicity studies. The value of 720 mg/kg falls within the range for classification with Acute Tox 4: H302 (i.e. >300 mg/kg but ≤ 2000 mg/kg). There is no obvious explanation why the studies gave such different LD50 values. There were no major differences in the experimental animals used, the administered formulation was comparable, and there was not a marked difference in the purity/impurity profiles of the tested materials. As there are no data to indicate that the Schüngel, 2005c data should be given preference over the older LD_{50} values, it is proposed to classify for acute oral toxicity.

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

The LD_{50} values obtained from two acute dermal studies were above the values for classification (≤ 2000 mg/kg).

4.2.4 Conclusions on classification and labelling

Acute Tox 4; H302

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. **Additional key elements** Additional information was provided by the DS on the study by Mihail and Thyssen (1980) (indicated in the RCOM by the DS to be Thyssen and Kimmerle, 1980). This information included deaths at each dose as well as the deviations from the draft OECD TG 401: • Body weights were not reported • The reporting of the results was limited.

Table: Deaths at each dose

Fasted rats Dose (mg/kg)	**Males Deaths/total number**	**Females Death/total number**
250	0/15	0/15
500	3/15	3/15
600	-	3/15
700	8/15	9/15
1000	13/15	13/15
1500	15/15	14/15
Unfasted rats Dose (mg/kg)		
500	0/15	1/15
750	1/15	0/15
850	-	4/15
1000	6/15	8/15
1000*	13/15	
1250		12/15
1500		15/15

*Mistake in the study report; the top dose in males is unclear.

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).

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

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

The information gained from the acute toxicity studies (section 4.2 – table) did not indicate that triadimenol resulted in toxicity to specific organs (other than the CNS) after a single exposure.

In addition to these acute toxicity studies, information on specific target organ toxicity – single exposure is available from two acute neurotoxicity studies.

Table 12. Summary of acute neurotoxicity studies

Method	Results and remarks	Reference
Oral (gavage) Mice/CFW1/males and rats/WISW/males, 3 to 10 animals/group Doses between 3 & 60 mg/kg in polyethylene glycol 400 Test material presumed to be 80:20 mixture; purity 98% Not guideline, not GLP	Pilot study (not a comprehensive evaluation of neurotoxicity) in which six pharmacological tests were conducted to establish if triadimenol had a stimulating effect on the CNS. The findings were that triadimenol: potentiated hexobarbital 'sleeping time' (anaesthesia) of mice; had a statistically significant stimulating effect on spontaneous motility of mice, which was less potent than with caffeine; resulted in effects consistent with stimulation of the CNS in mice (less potent than caffeine); had a stimulating effect on motor activity, rearing, licking/sniffing and grooming in rats; increased motor activity in mice (less potent than caffeine); transiently antagonised the ptosis and inhibition of spontaneous motility that was induced by pre-treatment with reserpine in mice (less potent than caffeine).	Polacek, 1983
Oral (gavage) Rats/Long Evans, 8-12 males/group 50, 100, 200, 400 mg/kg Presumed to be 80:20 mixture, purity not stated Guideline and GLP status not reported	To determine if the hyperactivity in rats associated with triadimefon administration was characteristic of other triazole compounds, 14 triazoles and structurally-related substances were tested, including triadimenol. Triadimenol induced hyperactivity in all dose groups.	Crofton, 1996

From the acute toxicity studies (section 4.2 - table), 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 rats.

In a pilot study that comprised a number of tests, triadimenol at doses of 3 mg/kg and above demonstrated a stimulating effect on the CNS, which was less potent than that of caffeine in the tests in which there was a comparison. The study authors surmised that the potentiation of hexobarbital anaesthesia was more likely to be a peripheral effect on the liver, such as an inhibition of barbiturate metabolism, than an effect on the CNS.

According to Crofton, 1996), it has been proposed that the hyperactivity induced by triazoles, of which triadimenol is a member, is related to altered monoamine metabolism (decreased synaptosomal dopamine reuptake); triadimenol decreases dopamine uptake in *in vitro* brain synaptosomal preparations. Crofton concluded that the crucial site in structure-activity relationship considerations of these CNS effects of triazoles is the ether oxygen, which is present in triadimenol.

4.3.2 Comparison with criteria

Triadimenol resulted in effects on the CNS in acute neurotoxicity studies. There is no indication that, at higher doses of the substance, these effects would lead to death of the animals (in an acute toxic class method test, the cut-off was 2500 mg/kg), and so a classification for STOT-SE should be considered.

STOT-SE is divided into three categories. Categories 1 and 2 are assigned on the basis of significant or severe toxicity and their criteria include guidance cut-off values.

STOT-SE 3 is reserved for transient target organ effects, which are limited to respiratory tract irritation and narcotic effects. Although triadimenol had a transient effect on the CNS, this was stimulant as opposed to narcotic, and so STOT-SE 3 would not be appropriate.

In consideration of categories 1 and 2, triadimenol induced functional disturbance that was not associated with morphological changes: there was no neuropathy or other adverse findings at histopathology. The effects were possibly mediated through a pharmacological effect rather than damage to the CNS; the transient nature of the effects would support this hypothesis. An additional consideration is triadimenol's potency in the neurotoxicity tests, which was less than that of caffeine. For these reasons, it is decided not to propose a classification for STOT-SE.

4.3.3 Conclusions on classification and labelling

Not classified: Conclusive but not sufficient for classification

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.

4.4 Irritation

4.4.1 Skin irritation

The potential of triadimenol to cause skin irritation has been tested in rabbits and humans.

Table 13: Summary table of relevant skin irritation studies*

Method	Results	Remarks	Reference
Rabbit/albino, 3 females 80:20 mixture, purity 97.2% OECD 404, GLP	Draize scores were 0 for all animals at all time points.	Not irritant	Schüngel, 2005a
Rabbit/New Zealand White, 3 females 80:20 mixture, purity 95.8% OECD 404, GLP	The Draize scores were 0 in all animals at all time points up to and including 14 days.	Not irritant	Krötlinger, 1993
Rabbits, New Zealand White, 6 animals 24 hour exposure on intact and abraded skin 80:20 mixture, purity 92.7% Not guideline or GLP	*Intact skin:* 1/6 animals had grade 1 erythema at 24 and 72 hours, and grade 1 oedema at 24 hours. All other animals scored 0. *Abraded skin*: 4/6 animals had grade 1 erythema, 1/6 animals had oedema (grade and time points of these observations not recorded).	Not irritant	Mihail & Thyssen, 1980
Rabbits/albino/6 males 80:20 mixture, purity 97.5% Similar to OECD 404, not GLP	Draize scores were 0 for all animals at all time points.	Not irritant	Nagashima, 1982a

**The studies included in this table are regarded as the key studies for classification purposes. Additional studies can be found in the pesticide evaluation report conducted under Directive 91/414/EEC; however, in the opinion of the dossier submitter, these do not impact the classification decision.*

4.4.1.1 Non-human information

Triadimenol did not cause skin irritation in two well-conducted studies in rabbits. Supportive evidence was provided by additional, non-standard, studies in rabbits.

4.4.1.2 Human information

No information.

4.4.1.3 Comparison with criteria

Several studies in rabbits gave no indication that triadimenol causes skin irritation. Triadimenol did not meet the criteria for classification as a skin irritant under the CLP Regulation.

4.4.1.4 Conclusions on classification and labelling

Not classified: Conclusive but not sufficient for classification

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**.

4.4.2 Eye irritation

Triadimenol's potential to cause eye irritation has been assessed in rabbits.

Table 14: Summary table of relevant eye irritation studies*

Method	Results	Remarks	Reference
Rabbits/albino/3 females 80:20 mixture, 97.2% OECD 405, GLP	All scores were 0 apart from redness of the conjunctiva, which gave mean scores over 24-72 hours of 0.3, 0.7 and 0.3 in the three animals, respectively. This effect was reversible within 3 days.	Slightly irritant	Schüngel, 2005d
Rabbits/New Zealand White, 3 females 80:20 mixture, purity 95.8% OECD guideline 405, GLP	Only effects on the conjunctiva were observed. The mean scores for conjunctival redness were 0.3, 0 and 0.3, with full resolution by 48 hours. Grade 1 chemosis occurred in 3/3 animals at 1 hour but had fully resolved by 24 hours (mean score 0 for each animal).	Slightly irritant	Krötlinger, 1993
Rabbits/New Zealand White, 8 animals 80:20 mixture, purity 92.7% Not guideline or GLP	*5 minutes' exposure* All scores were 0 apart from grade 1 conjunctival redness in 5/5 animals from 5 minutes; fully resolved in all animals by 48 hours. *24 hours' exposure* All scores were 0 apart from grade 1 conjunctival redness in 3/3 animals; fully resolved by 24 hours.	Slightly irritant	Mihail & Thyssen, 1980
Rabbits/albino, 9 males 80:20 mixture, purity 97.5% Not guideline or GLP	*Washed eyes* Grade 1 conjunctival effects in 3/3 animals at 1 to 4 hours only. *Unwashed eyes* Grade 1 corneal effects in 4/6 animals, reversible by 72 hours. Grade 1 or 2 conjunctival redness or chemosis in 6/6 animals, reversible by 96 hours.	Slightly irritant	Nagashima, 1982b

**The studies included in this table are regarded as the key studies for classification purposes. An additional study can be found in the pesticide evaluation report conducted under Directive 91/414/EEC; however, in the opinion of the dossier submitter, these do not impact the classification decision.*

4.4.2.1 Non-human information

Several studies in rabbits have consistently shown that triadimenol has only a mild irritant effect on the eyes.

4.4.2.2 Human information

No available information.

4.4.2.3 Comparison with criteria

The main effect observed was conjunctival redness, with occasional conjunctival chemosis. Since all effects were fully reversible before 21 days, Category 1 (irreversible eye effects) according to CLP is not appropriate. The grades of conjunctival effects (mean scores of ≤ 0.7 in the guideline-compliant studies, individual scores of generally 1 in the non-guideline compliant studies) did not meet the criteria for classification as Category 2 (irritating to eyes) in the CLP Regulation (mean

scores of ≥ 2 in at least 2 of 3 animals for conjunctival redness or chemosis). Conclusions on classification and labelling

Not classified: Conclusive but not sufficient for classification

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.

4.4.3 Respiratory tract irritation

4.4.4 Respiratory tract irritation

No evidence of respiratory tract irritation was found in one acute inhalation study in rats (section 4.2.1).

4.4.4.1 Conclusions on classification and labelling

Not classified: Data lacking

4.5 Corrosivity

Triadimenol was not corrosive when tested for skin and eye irritation

4.5.1 Conclusions on classification and labelling

Not classified: Conclusive but not sufficient for classification

4.6 Sensitisation

4.6.1 Skin sensitisation

The potential for triadimenol to induce skin sensitisation has been investigated in two guinea pig studies.

Table 15: Summary table of relevant skin sensitisation studies

Method	Doses	Results	Reference
Guinea pig/SPF, females, 20 in test group, 10 in negative control group Buehler test 80:20 mixture, purity 97.2% OECD 406 (1992), GLP	*Induction* 62.5% *Challenge* 62.5% Formulated in polyethylene glycol	0/20 animals were sensitized. The positive control substance, alpha hexyl cinnamic aldehyde, gave a positive response in 60% of the animals. Conclusion: non-sensitising	Vohr, 2005
Guinea pig/Pirbright white, 10/sex/group Maximisation test 80:20 mixture, purity 92.7% Similar to OECD 406 (1981), not GLP	*Induction* Intradermal: 2.5% Topical: 25% *Challenge* 25% Formulated in Cremophor EL	0/20 positive at 24 hours 1/20 animals gave a response (score 0.5) at 48 hours. Conclusion: non-sensitising There did not appear to be a positive control group.	Flucke, 1981

4.6.1.1 Non-human information

Triadimenol did not induce skin sensitisation reactions in any animals in a Buehler assay when it was tested at 62.5%. Appropriate responses were obtained in the positive and negative groups.

In a guinea pig maximisation test, triadimenol was negative for skin sensitisation. However, limitations of the study included the lack of: a positive control group; a justification for the concentrations used; and information on irritation (although the animals were pre-treated with 10% sodium lauryl sulphate).

4.6.1.2 Human information

No information available.

4.6.1.3 Summary and discussion of skin sensitisation

Triadimenol did not induce skin sensitisation in a well conducted Buehler study. Supportive evidence was obtained from a guinea pig maximisation test.

4.6.1.4 Comparison with criteria

The criteria for classification as a skin sensitiser (positive in ≥ 15% of animals in a Buehler/non-adjuvant assay, positive in ≥ 30% of animals in a guinea pig maximisation test/adjuvant assay) were not met.

4.6.1.5 Conclusions on classification and labelling

Not classified: Conclusive but not sufficient for classification

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).

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

4.6.2 Respiratory sensitisation

There is no available information on the potential of triadimenol to induce respiratory sensitisation.

4.7 Repeated dose toxicity

The repeated-dose toxicity of triadimenol has been investigated in rats, mice, dogs and rabbits by the oral, inhalation and dermal routes.

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Rat

Table 16.1: Summary table of relevant repeated dose toxicity studies in the rat (oral)

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Oral (gavage) for 28 days Rats/Wistar, 20/sex/group 24 hours after the final dose, half the animals in each group were sacrificed. The remaining animals were observed for a further 28 days. 60:40 material, purity 98.5% Not guideline or GLP Thyssen & Kaliner, 1976	0, 5, 15, 45 mg/kg/d An extra group of 10/sex received 1.5 mg/kg/d, but there were limited investigations in this group	Terminal observations and samples for haematology and clinical chemistry were taken 24 hours, or, for the recovery animals, 28 days after the final treatment. There were no deaths, clinical signs or effects on body weight. Haematology, clinical chemistry and urinalysis values were similar between groups. Gross necropsy did not reveal any treatment-related findings, nor did a test to detect the presence of blood in faeces. Thyroid weights were slightly increased in high-dose males - absolute: 0%, 10%, 10%, 20%**; relative: 0%, 0%, 0%, 25%** in the 0, 5, 15, 45 mg/kg/d groups. In females, triadimenol had a statistically significant effect on ovary weights in all treatment groups, with increases of absolute: 0%, 14%**, 14%*, 20%**; relative: 0%, 18%**, 14%*, 18%** in the 0, 5, 15, 45 mg/kg/d groups. The increased weights of the thyroid in males and ovary in females were not associated with histopathological findings. Because of this, the small differences in absolute terms and the lack of a dose-response relationship in effects on ovary weights, the study authors considered that these organ weights were within the normal range. Histopathology of other organs and tissues did not reveal any treatment-related effects. Following the 4-week recovery period, there were no differences between exposed and control animals. The NOAEL was concluded to be 45 mg/kg/d.
Oral (gavage) for 28 days Rats/Wistar, 20/sex/group 80:20 (purity 98.3%) and 60:40 (purity 84.7%) materials tested Half the animals in each group were sacrificed at the end of the treatment period. The remaining animals were observed for a further 28 days. Not guideline or GLP Mihail & Vogel,	80:20 material: 0, 15, 45, 100 mg/kg/d 60:40 material: 0, 45, 100 mg/kg/d	There were no deaths. The only clinical sign noted was a slight increase in mobility that persisted for up to two hours after dosing and that occurred from day 3 onwards in those animals that received 45 or 100 mg/kg/d of either test substance. *Results obtained immediately after dosing period* Body weights, haematology and urinalysis parameters were unaffected by triadimenol. Slight reductions in various clinical chemistry parameters (AST, bilirubin in males; glucose in females) were stated by the study authors to be within the normal range of physiological variation, apart from those for creatinine, which were reduced in males by 19%** at 100 mg/kg/d and up to 19%* at 45 & 100 mg/kg/d 60:40 material. In females, creatinine was reduced by 10%*, 15%*, 27%** at 15, 45, 100 mg/kg/d; and by 19%** and 29%** at 45 & 100 mg/kg/d 60:40 material. Microsomal enzyme activities were higher (more marked in males) in liver samples after exposure to either test material ≥ 45 mg/kg/d. The following increases in activity were noted: P450 activity by up to 78%** in males and females; aminopyrine-N-DEM by up to 92%** in males and 58%** in females; O-DEM by up to 40%** in males and 34%** in females. There were marginal increases in liver weights at 100 mg/kg/d of both test materials. In males, the increases were 0% / 5% (absolute/relative), whilst in females they were up to 13%** / 11%**. Thyroid weights were not increased and differences in ovary weights were

1981		minimal: 0%, 6%, 5%, 13% (absolute) and 0%, 5%, 5%, 11% (relative) at 0, 15, 45, 100 mg/kg/d of 80:20 mixture; 8% & 16% (absolute) and 11% & 14% (relative) at 45 & 100 mg/kg/d 60:40 material. There were no other effects on organ weights. There were no treatment-related gross necropsy or histopathological findings. *Results obtained after 4-week recovery period* There were no clinical signs and no effects of treatment on body weight, haematology or urinalysis after the recovery period. There were slight reductions in urea (males and females), bilirubin (females only) and creatinine (up to 14% in males at 100 mg/kg/d; and up to 20% in females with 100 mg/kg/d 60:40 material). Microsomal enzyme induction in liver samples was similar between the groups, indicating that the enzyme induction was reversible. There were no notable gross necropsy findings (histopathology was not performed) and no differences in organ weights between the groups. The NOEL was 15 mg/kg/d.
Oral (dietary) for 90 days Rats/Wistar, 15/sex/group (30/sex control group) 60:40 material, purity 98% Not guideline or GLP Loeser & Kaliner, 1977	0, 150, 600, 2400 ppm Equivalent to Males: 0, 12, 49, 203 mg/kg/d Females: 0, 17, 71, 287 mg/kg/d	One female in the 600 ppm group died on day 83. The cause of death was myocardial necrosis and nephritis and was not thought to be treatment-related. There were no clinical signs of toxicity and food consumption was unaffected, although body weight gains were statistically significantly reduced in the high-dose groups of each sex. Haematology investigations were conducted at one month and three months. At the former, the only adverse finding was a slightly decreased haematocrit in high dose females. At the latter time, there were decreases in mean corpuscular haemoglobin (MCH) (by 7%**) and mean corpuscular volume (MCV) (by 13%**) in high-dose males, and decreased haematocrit (by 4%*) in high-dose females. The relative proportion of eosinophils was also reduced in high-dose females (by 86%*). No changes in clinical chemistry were noted at one and three months. There were no adverse findings at gross necropsy. Liver weights were increased by 1%, 1%, 7%* in males; and 0%, 3%, 17%** (absolute values) in females at 150, 600, 2400 ppm. Other organ weight effects were also recorded in high-dose females: absolute ovary weights were increased by 5%, -3%, 23%**; absolute kidney weights were increased by 8%, 2%, 10%* at 150, 600, 2400 ppm. There were no treatment-related histopathological findings in any organs or tissues. The NOAEL was 600 ppm, equivalent to 49-71 mg/kg/d.
Oral (dietary) for 90 days Rats/Sprague Dawley, 20/sex/group 80:20 material, purity 94% Not guideline or GLP Nishimura, 1983	0, 120, 600, 3000 ppm Equivalent to Males: 0, 8, 40, 209 mg/kg/d Females: 0, 9, 46, 221 mg/kg/d	There were no treatment-related deaths. In the high-dose groups, food consumption was reduced by up to 25%, and overall body weight gain was 11% lower in males and 24% lower in females. At 3000 ppm, decreased haemoglobin (by 4%**) and haematocrit values (by 4%**) in males and females were indicative of mild anaemia. Platelet counts were also lower in high-dose males (by 13%**). A lower percentage of reticulocytes (up to 25% reduction at 600 ppm) was also noted in males, although not in a dose-related pattern. Clinical chemistry changes associated with effects on the liver through lipid metabolism were identified at the high-dose level: there were increases in total cholesterol (by 39%**), phospholipid (by 18%*) and total protein (by 3%*) and decreases in albumin (by 7%**) and the albumin/globulin ratio (by 14%**) in females; decreased triglycerides (by up to 32%**) and free fatty acids (by up to 18%**) occurred in males and females. At gross necropsy, treatment-related effects on the liver were observed with a

dose-response relationship: enlarged liver in 1, 0, 2, 4 males and 0, 0, 1, 7 females (group sizes were 20); and accentuated lobular pattern in 0, 0, 2, 5 females at 0, 120, 600, 3000 ppm.

Absolute and relative liver weights were significantly increased by: absolute values of 0%, 0%, 10%*, 17%** in males and 0%, -1%, 9%*, 35%** in females; relative values of 0%, 2%, 7%**, 28%** in males and 0%, 3%, 13%**, 58%** in females at 0, 120, 600, 3000 ppm.

At histopathology of the liver, triadimenol was associated with an increased incidence and severity of fatty change and increased incidence of eosinophilic degeneration of hepatocytes, as recorded below.

	Males				**Females**			
mg/kg/d	**0**	**8**	**40**	**209**	**0**	**9**	**46**	**221**
Number of livers	18	19	18	19	20	20	20	20
Fatty change	1	3	5	15	2	4	9	18
Slight	1	3	4	4	2	4	9	5
Mild			1	9				5
Moderate				1				8
Severe				1				
Eosin. degen.	0	0	0	13	0	0	0	9
Slight				12				9
Mild				1				

The fatty changes were found mainly in the central to mid-zonal region in males and the mid-zonal to peripheral regions in females. Additionally, at 3000 ppm, one male had ground glass appearance and two had nuclear alteration (anisokaryosis).

There were no treatment-related adverse histopathological findings in other organs or tissues.

The NOAEL was 120 ppm (8-9 mg/kg/d).

*N.B. The values for NOAEL/NOEL are provided for information only; they have been agreed by a PRAPeR Expert Meeting. * Statistically significant at $p \leq 0.05$. ** Statistically significant at $p \leq 0.01$*

In several rat studies, triadimenol targeted the liver, kidneys, ovaries and thyroid. There were no deaths below the guidance value for classification of $\leq$ 100 mg/kg/d (90-day study in rats). No serious effects occurred below these values.

In a 28-day study, the only liver effects were microsomal enzyme induction (from 45 mg/kg/d) and increases in liver weight (at 100 mg/kg/d), neither of which persisted during a 4-week recovery period; these changes were indicative of adaptive rather than adverse changes. In one of the 90-day studies, liver weights were increased from 40 mg/kg/d. Clinical chemistry changes associated with effects on the liver through lipid metabolism only occurred at 209/221 mg/kg/d, as was the case for the gross necropsy findings (enlargement, accentuated lobular pattern). Upon histopathology, dose-related increases in the incidence and severity of fatty change (from 8/9 mg/kg/d) and eosinophilic degeneration of hepatocytes (at 209/221 mg/kg/d) were observed.

Apart from the liver, the only organs that were targeted in more than one study were the kidneys and the ovaries. Increased kidney weights were reported in one of the 90-day studies, but only

above the guidance value (at 287 mg/kg/d). The effect on the ovaries was somewhat more consistent, in that increased organ weights were recorded after exposures for 28 days (both studies: from 5 mg/kg/d, but without a dose-response relationship with unspecified isomer composition; and from 15 mg/kg/d with the 80:20 mixture) and 90 days (at 287 mg/kg/d). This effect was reversible during a four-week recovery period that followed 28 days' administration. None of these changes in organ weight was associated with adverse gross or histopathology findings.

In one of the 90-day studies there was an indication of mild anaemia in the high-dose group (209/221 mg/kg/d).

Mouse

Table 16.2: Summary table of relevant repeated dose toxicity studies in the mouse (oral)

<table>
<tr><th>Method</th><th>Dose levels</th><th>Observations and remarks
(effects of major toxicological significance)</th></tr>
<tr><td>Oral (dietary) for 13 weeks
Mice/CD-1, 10/sex/group
Presumed to be 80:20 material, purity 97.4%
OECD 408, GLP
Schladt & Sander, 1998</td><td>0, 160, 500, 1500, 4500 ppm
Equivalent to
Males: 0, 25, 77, 235, 872 mg/kg/d
Females: 0, 31, 94, 297, 797 mg/kg/d</td><td>
A limited histopathology was performed, since the study was initiated to find suitable dose ranges for a chronic study.

One male of the high-dose group died on day 4, but the cause of death was not established. There were no other deaths. Piloerection (5/10 males) and squatting position (7/10 males) occurred in the high-dose group, but otherwise there were no clinical signs of toxicity.

Food consumption was increased by 13% in high-dose males but at the end of the treatment period these animals had body weights that were 9%** (1500 ppm) and 13%** (4500 ppm) lower than controls; body weights were 9%** lower than controls in females of the 4500 ppm group.

The haematology investigations showed that the haematocrit was decreased by 7%* and the mean corpuscular haemoglobin increased by 8%** in high-dose females. In males, decreased leucocyte counts were recorded in the 1500 and 4500 ppm groups, with reductions of 21%* and 27%, respectively.

The clinical chemistry investigations on plasma revealed increases in enzymes that were indicative of effects on the liver. In both sexes, AST and ALT were increased from 500 ppm (AST up to 340%** increase, ALT up to 684%** increase at 4500 ppm). Glutamate dehydrogenase was increased in males from 160 ppm (but within the historical control range at this dose) and in females from 500 ppm (both over 1000%** increase at 4500 ppm). ALP was increased by 40%* in males at 4500 ppm. Decreases in total protein, albumin, cholesterol and bilirubin were evident in both sexes at various dose levels from 500 ppm.

Additionally, homogenized samples of liver were analysed for enzyme activity, with increases in the activities of aminopyrine-N-demethylase and cytochrome P-450 from 500 ppm. Increased triglyceride concentrations also showed a dose-response relationship from 500 ppm.

Gross necropsy of the high-dose male that died revealed black areas in the glandular stomach. There were no treatment-related gross necropsy findings in the other animals.

Triadimenol administration had effects on liver and adrenal weights. In males, absolute liver weights were increased by 37%** and relative weights by 59%** at 4500 ppm. In females, absolute liver weights were increased by 34%** and relative weights 51%** at 4500 ppm. Adrenal weights were reduced in the high-dose groups only in males (absolute weight reduced by 34%, relative by 22%) and females (absolute weight by 47%**, relative by 36%*).

The effects on organ weights of the liver and adrenals were associated with various histopathological findings in these organs. The incidences of the liver findings are presented below.

<table>
<tr><th></th><th colspan="5">Males</th><th colspan="5">Females</th></tr>
<tr><th>mg/kg/d</th><th>0</th><th>25</th><th>77</th><th>235</th><th>872</th><th>0</th><th>31</th><th>94</th><th>297</th><th>797</th></tr>
<tr><td>No. livers</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td><td>10</td></tr>
<tr><td>Hypertrophy</td><td>0</td><td>0</td><td>3</td><td>9</td><td>9</td><td>0</td><td>0</td><td>0</td><td>3</td><td>9</td></tr>
<tr><td>Cytoplasmic vacuolation</td><td>0</td><td>0</td><td>0</td><td>3</td><td>9</td><td>0</td><td>2</td><td>1</td><td>4</td><td>8</td></tr>
</table>
</td></tr>
</table>

Single cell necrosis	0	0	0	1	8	0	0	0	3	9
Fat storage	0	1	2	8	9	1	4	3	8	10
Slight		1	2	4	2	1	4	3	6	7
Mild				4	6				2	3
Moderate					6					
Severe										

The liver hypertrophy mainly affected centrilobular areas, although the whole lobule was often involved in high-dose animals. The hypertrophic cells usually showed a dense and homogeneously oesinophilic cytoplasm with apparently reduced glycogen storage. The observed increases in cytoplasmic vacuolation were associated with increased incidences of fat storage. The severity as well as the incidence of single cell necrosis showed a dose-response relationship: at 1500 ppm, the effect was graded as minimal in 1/10 males and 3/10 females, whereas at 4500 ppm it was graded as minimal in 3/10 males and 5/10 females, and as slight in 5/10 males and 4/10 females. One of the high-dose males also had focal hepatocellular necrosis (graded as minimal).

Histopathology of the adrenals revealed that there were no vacuoles in the X-zone of the adrenal cortex in any of the high-dose females, with incidences of 5/8, 8/10, 7/9, 5/10, 0/9 at 0, 160, 500, 1500, 4500 ppm. The study authors considered the toxicological significance of this finding to be uncertain. There were no treatment-related findings in other organs or tissues, but investigations were limited to liver, spleen, adrenal glands, thyroid glands, femur, sternum, kidneys and testes/epididymides, plus organs/tissues with macroscopic findings.

The NOAEL was 160 ppm (25-31 mg/kg/d).

N.B. The values for NOAEL are provided for information only; they have been agreed by a PRAPeR Expert Meeting.
** Statistically significant at $p \leq 0.05$. ** Statistically significant at $p \leq 0.01$*

The liver and adrenals were the target organs, as evidenced by changes in organ weights, liver enzymes and histopathology. Although effects on liver microsomal enzymes and glutamate dehydrogenase were recorded at 160 ppm (25/31 mg/kg/d), these were within the historical control ranges of the laboratory and so were not considered to be treatment-related. Additionally, there were no other indications of liver toxicity at this dose level. However, the effects at the next dose level (77/94 mg/kg/d) were regarded as treatment-related. Although liver enzyme activity was increased from this dose, adaptive changes of the liver, as evidenced by increased organ weight, were only apparent from 297 mg/kg/d. Findings at histopathology that were indicative of hepatotoxicity (necrosis) mainly occurred from 235/297 mg/kg/d, although increased fat storage was reported in all dose groups.

A further histopathological finding in female mice was the absence of vacuoles in the X-zone of the adrenal cortex at 797 mg/kg/d. The study authors noted that this finding had been recorded in a number of previous studies on substances with various chemical structures: it was not considered to be a rare occurrence. They explained that the X-zone of the adrenal cortex was a transient feature in young mice, and in females the regression of this zone was characterised by the development of large vacuoles.

Dog

Table 16.3: Summary table of relevant repeated dose toxicity studies in the dog (oral)

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Oral (dietary) for 13 weeks Dogs/Beagle, 4/sex/group 60:40 material, purity 98.5% Similar to OECD 409 (1981), not GLP Hoffmann & Kaliner, 1977	0, 150, 600, 2400 ppm Equivalent to 0, 3.75, 15, 60 mg/kg/d for males and females combined	Up to 24 hours elapsed between the final feeding and the terminal procedures (blood sample collection and necropsy). There were no deaths or clinical signs of toxicity. Food consumption was similar overall, but delayed food intake was observed in high-dose animals. There were no statistically significant changes in body weights. Haematology and urinalysis parameters were unaffected by treatment at weeks 7 and 13. Clinical chemistry investigations indicated possible liver effects. Alkaline phosphatase activity was slightly increased in all treated groups at week 13, but without a dose-response relationship. The level of ALT activity was slightly higher at 15 and 60 mg/kg/d in week 7 but not at termination. Plasma cholesterol levels were also slightly increased in these dose groups in both sexes in week 7 and in males in week 13. Activities of cytochrome P-450 and aminopyrine-N-demethylase in liver tissue were increased in high-dose males and females, but only those of N-demethylase had statistical significance (increased by 78%**). Gross necropsy was normal in all groups. The relative liver weight was increased in high-dose females (by 11%*), and the absolute and relative kidney weights were increased in high-dose males (absolute by 16%* and relative by 20%). There were no effects in other organs. Histopathology did not reveal any adverse findings; in particular, findings in the liver and kidney were normal. The NOAEL was 15 mg/kg/d.
Oral (dietary) for 6 months Dogs/beagle, 6/sex/group 80:20 material, purity 98% Not guideline or GLP Hoffmann, 1984	0, 10, 30, 100 ppm Equivalent to 0, 0.25, 0.75. 2.5 mg/kg/d	This study was a follow-up to a two-year chronic study in order to determine a clear NOAEL. As a result, histopathology was not performed. Up to 24 hours elapsed between the final feeding and the terminal procedures (blood sample collection and necropsy). Triadimenol exposure had no effects on any of the investigated parameters. In particular, body weights were unaffected. There were also no changes in alkaline phosphatase, aminopyrine-N-demethylase and cytochrome P-450 activities. Organ weights were unchanged and there were no notable gross necropsy findings. The NOAEL was 100 ppm (2.5 mg/kg/d).
Oral (dietary) for two years Dogs/beagle, 4/sex/group 60:40 material, purity 94.9% Not guideline or GLP Hoffmann & Vogel, 1984	0, 150, 600, 2400 ppm Equivalent to 0, 3.75, 15, 60 mg/kg/d	Up to 24 hours elapsed between the final feeding and the terminal procedures (blood sample collection and necropsy). There were no treatment-related deaths, clinical signs of toxicity or effects on reflexes, body temperature, pulse rate or ophthalmoscopy. One high-dose female was killed for humane reasons in week 22 and was found to have a 'deteriorating disease of the knee joints'. This was not considered to be treatment related. Food and water consumptions were not affected by triadimenol. Body weight gains were lower than controls in males and females in all treatment groups. It was reported that the body weight gains in the control animals were extremely high, being outside or only just within the mean plus two standard deviations of historical control data. The body weight gains of all treated groups were within the normal range. Haematology and urinalysis parameters were normal in all dose groups. Some effects on clinical chemistry were recorded. Alkaline phosphatase levels

		were consistently higher than controls in the high-dose groups from week 40 (up to 300% increase). At week 104, aminopyrine-N-demethylase activity was higher than controls in males and females from 15 mg/kg/d (up to 269%); and the activity of cytochrome P450 was increased in males at 60 mg/kg/d (by 71% compared with controls) and from 15 mg/kg/d in females (by up to 93%). There were no treatment-related effects on organ weights or gross necropsy and histopathology findings. The NOEL was 15 mg/kg/d.

N.B. The values for NOAEL/NOEL are provided for information only; they have been agreed by a PRAPeR Expert Meeting.
** Statistically significant at $p \leq 0.05$. ** Statistically significant at $p \leq 0.01$*

In a 90-day dog study, slightly increased alkaline phosphatase activity and cholesterol levels were indicative of a moderate increase in liver activity. Consistent with this were increased liver weights, but these effects were not associated with adverse gross necropsy or histopathology changes, and so are considered to be adaptive rather than adverse. Kidney weights were increased in high-dose males only but, again, occurred in the absence of gross or histopathological changes.

In studies of longer duration but with lower doses, the only clear adverse effect was a change in some clinical chemistry parameters in a two-year study, which was suggestive of increased liver activity. This was not associated with changes in organ weights, gross necropsy or histopathology.

4.7.1.2 Repeated dose toxicity: inhalation

Table 16.4: Summary table of relevant repeated dose toxicity studies (inhalation)

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Inhalation for one week (five exposures) Rats/Wistar, 10/sex/group Nose-only exposure 14-day observation period 60:40 mixture, purity not stated Not guideline or GLP Thyssen & Kimmerle, 1976	0.064 and 0.32 mg/L for 4 hours per day	Test atmosphere concentration was determined by analysis. Particle size analysis was not performed. It was not indicated if the test atmosphere concentration was the maximum attainable. The methods and result were poorly reported. However, five 4-hour exposures did not result in any deaths, clinical signs or signs of irritation on the visible mucosa.
Inhalation for 3 weeks (5 days/week, total 15 exposures) Rats/Wistar, 10/sex/group Nose-only exposure Not guideline or GLP Isomer composition and purity not stated Kimmerle, 1976	0, 0.03, 0.068, 0.23 mg/L in aerosol form for 6 hours per day Test atmosphere analyses and particle size analyses were not fully reported	There were no deaths or clinical signs of toxicity in any group. Body weights, haematology and clinical chemistry determinations were unaffected by triadimenol. Urinalysis parameters were reported as being unchanged, but no data were presented. No treatment-related effects were noted on organ weights or gross necropsy and histopathology findings (samples for laboratory analysis were taken and animals were sacrificed 24 hours after the final test substance administration). The NOAEL was 0.23 mg/L.

N.B. The values for NOAEL are provided for information only; they have been agreed by a PRAPeR Expert Meeting.

Two repeated dose inhalation studies, in the rat, are available, in which triadimenol did not give rise to deaths or clinical signs. It also had no effects on the measured parameters, which included gross examination, histopathology and weight determination of the liver, kidneys, ovaries and adrenals.

4.7.1.3 Repeated dose toxicity: dermal

Table 16.5: Summary table of relevant repeated dose toxicity studies (dermal)

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Dermal for 3 weeks (5 days/week, total 15 exposures) Rabbits/New Zealand White, 6/sex/group 80:20 material, purity 98% Not guideline or GLP Heimann & Schilde, 1984	0, 50, 250 mg/kg/d in Cremophor EL/distilled water Applied to clipped skin and left uncovered for 6 hours (animals were immobilized), then washed off. The skin of 3 animals/sex was abraded 24 hours before 1st application so that oedema and slight erythema occurred.	There were no deaths or clinical signs of toxicity during the exposure period in any group. Body weights (measured weekly) were unaffected by treatment, as were haematology, clinical chemistry and urinalysis parameters at the end of the treatment period. Liver enzyme activity values at termination of the study were slightly higher than controls in the treatment groups, but a dose-response relationship was not evident and the values were within the laboratory's normal range for rabbits. There were no notable gross necropsy findings (animals sacrificed 24 to 48 hours after the final treatment). Likewise, relative and absolute organ weights and histopathology findings were unaffected. The NOAEL was 250 mg/kg/d.

N.B. The values for NOAEL are provided for information only; they have been agreed by a PRAPeR Expert Meeting.

A single dermal repeated-dose study is available in rabbits, in which two doses of triadimenol were tested. No clinical signs or skin irritation were noted, and there were no effects on the measured parameters, including those of the liver, kidneys, ovaries and adrenals.

4.7.1.4 Repeated dose toxicity: other routes

No information available.

4.7.1.5 Human information

No information available

4.7.1.6 Other relevant information

The chronic/carcinogenicity studies in rats and mice are presented in section 4.10. The non-tumour findings of note are summarised here.

In rats, increased liver weights were apparent at 106 mg/kg/d, which were not associated with any other adverse findings.

In mice, increased liver nodules were recorded in males at 60 mg/kg/d but not at the higher dose (340 mg/kg/d). Liver weights increased from 60 mg/kg/d and were associated with hypertrophy, with the severity of this effect being increased at 340 mg/kg/d. Fatty change in females was also reported at 472 mg/kg/d, and single cell necrosis from 60 mg/kg/d in males. In a second mouse study, increased liver weights, enlarged/swollen livers and nodular formations occurred at 300 mg/kg/d. Histopathology changes in the liver were only noted at this dose level.

4.7.1.7 Summary and discussion of repeated dose toxicity

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 were indicative of adaptive rather than toxic responses, consisting of increased organ weights (in some cases associated with hypertrophy) and liver enzyme activities. 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) in 90-day and longer-duration studies were consistent with bioaccumulation. In 90-day studies, the only toxic effect that occurred below the guidance values was fatty change (from 8/9 mg/kg/d in rats) / increased fat storage (from 25/31 mg/kg/d in mice). In both cases, the changes at these doses were slight. After chronic administration for up to two years, there was no liver toxicity in dogs or rats, although single cell necrosis (slight to minimal, severity not increased compared with the controls) was reported in male mice in one study from 60 mg/kg/d and fatty change in female mice at 472 mg/kg/d.

In various oral studies, triadimenol also increased the weights of the kidneys, ovaries, thyroid and adrenals. Since there was no evidence of organ dysfunction, these organ weight changes do not justify classification, although they may indicate an endocrine-disrupting potential for triadimenol.

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

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

STOT-RE is assigned on the basis of a substance demonstrating evidence of significant or severe toxicity, generally at or below the guidance value of 100 mg/kg/d (for a classification in category 2) obtained in a 90-day rat study. ‘Significant’ toxicity is taken to mean changes that clearly indicate functional disturbance or morphological changes that are toxicologically relevant. ‘Severe’ toxicity is considered to be more profound or serious and indicates changes that are of a considerably adverse nature with a significant impact on health.

In several repeated-dose oral studies of durations from 28 days to two years, the only effects that could be regarded as significant or severe and that were reported at doses below the guidance value were fatty change of the liver (in 90-day rat and mouse studies) and hepatocellular necrosis (in an 18-month mouse study). When the guidance value is adjusted from a 90-day study (100 mg/kg/d) to one of 12-months’ duration, a value of 25 mg/kg/d is obtained, which is clearly below the dose (60 mg/kg/d) at which hepatocellular necrosis occurred. Additionally, the necrosis was graded as slight to minimal in all groups, with no increase in severity from controls even at doses of up to 340/472 mg/kg/d. Therefore, this effect will not be considered further in deciding upon a classification for repeated-dose toxicity.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

A classification of STOT-RE is indicated when toxic effects that may include the following descriptions occur at or below 100 mg/kg/d.

a) Morbidity or death resulting from repeated or long-term exposure

There were no treatment-related deaths or cases of moribund animals below the guidance value.

b) Significant functional changes in the central or peripheral nervous systems or other organ systems

There were no such changes in any organ systems.

c) Any consistent and significant adverse change in clinical biochemistry, haematology or urinalysis parameters

Although there were changes in some clinical chemistry parameters, particularly liver enzyme induction, at dose levels relevant for classification these were indicative of increased liver activity as a result of an adaptive change and were reversible. Such adaptive responses constitute a normal biochemical or physiological response and do not indicate classification.

d) Significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination

There were no such effects at doses below the guidance values.

e) Multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity

There were no such effects.

f) Morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g. severe fatty change in the liver)

Hepatic fatty change/increased fat storage was reported from 8/9 mg/kg/d, but at these dose levels it was graded as slight. Moderate and/or severe fatty change only occurred at doses well above the guidance value.

g) Evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration

There were no such effects.

Additionally, there were no generalised changes that involved several organ systems or significant/severe changes in the general health status of the animals.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Not classified: Conclusive but not sufficient for classification

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	1	3	5	15	2	4	9	18
• Slight	1	3	4	4	2	4	9	5
• Mild			1	9				5
• Moderate				1				8
• Severe				1				

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		1	2	8	9	1	4	3	8	10
• Slight	0	1	2	4	2	1	4	3	6	7
• Mild				4	6				2	3
• Moderate					6					
• Severe										

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**.

4.9 Germ cell mutagenicity (Mutagenicity)

The genotoxic potential of triadimenol has been investigated in several *in vitro* and *in vivo* studies.

Table 17: Summary table of relevant *in vitro* and *in vivo* mutagenicity studies

Method	Concentrations tested	Results	Reference
IN VITRO			
Bacterial reverse mutation (Ames) *S. typhimurium* TA98, 100, 1535, 1537 60:40 material, purity 93.7% Not guideline or GLP	With S9: 4 to 2500 µg/plate Without S9: 2500 µg/plate	**Negative** ± metabolic activation	Herbold, 1979a
Bacterial reverse mutation (Ames) and DNA repair test (rec$^-$ assay) Ames: *S. typhimurium* TA98, 100, 1535, 1537, 1538, *E. coli* WP2 *uvrA* rec$^-$ assay: *Bacillus subtilis* rec$^+$ strain H17 and rec$^-$ strain M45 Isomer composition not stated, purity 97.5% Not guideline, GLP	Ames test With and without S9: 7 test concentrations from 5 to 5000 µg/plate rec$^-$ assay Eight concentration from 50 to 10 000 µg/disk	Ames test. **Negative** ± metabolic activation rec$^-$ assay **Negative**	Tanahashi & Moriya, 1982
Bacterial reverse mutation (Ames) and DNA repair test (rec$^-$ assay) Ames: *S. typhimurium* TA98, 100, 1535, 1537, 1538, *E. coli* B/r try $^-$hcr rec$^-$ assay: *Bacillus subtilis* rec$^+$ strain NIG17 and rec$^-$ strain NIG45 80:20 material, purity 97.5% Not guideline, GLP	Ames test With and without S9: 5 to 5000 µg/plate rec$^-$ assay single concentration of 200 µg/disk	Ames test. **Negative** ± metabolic activation rec assay **Negative**	Nagane *et al.*, 1982
In vitro mammalian cell gene mutation test Mouse lymphoma cell line (L5178Y) 80:20 material, purity 97.5% Not guideline, GLP	With and without S9: 3.9 to 125 µg/ml in the 1st assay and 25 to 150 µg/ml in the 2nd assay	**Negative** ± metabolic activation	Cifone, 1982
In vitro unscheduled DNA synthesis assay Primary rat hepatocytes were isolated from male Fischer 344 rats. 80:20 material, purity 97.5% Not guideline, GLP	Ten concentrations ranging from 0.25 to 250 µg/ml	**Negative**	Myhr, 1982
E. coli Pol A$_1^-$ test DNA deficient strain: *E. coli* p 3478 (pol A$_1^-$). DNA proficient strain: *E. coli* W3110 (pol A$^+$) 80:20 material, purity 97.5% Not guideline or GLP	Five concentrations in the range 62.5 to 1000 µg/plate	**Negative**	Herbold, 1981
In vitro sister chromatid exchange test Chinese hamster ovary cells Isomer composition not stated, but presumed to be 80:20. Purity 93%	Concentrations between 0.1 and 1000 µg/ml to test for cytotoxicity. With S9: 100 to 225 µg/ml	**Negative** ± metabolic activation	Putman, 1987

US EPA guideline, GLP	Without S9: 38 to 300 µg/ml		
IN VIVO			
In vivo micronucleus test (oral) Mice, NMRI, 5/sex/group 60:40 material, purity 93.7% Not guideline or GLP	Two doses of either 175 mg/kg or 350 mg/kg administered by oral gavage, 24 hours apart. Femoral bone marrow was prepared 6 hours after the second application.	**Negative**	Herbold, 1978b
In vivo micronucleus test (oral) Mice, NMRI, 5/sex/group 80:20 material, purity 96.5% Not guideline or GLP	Two doses of either 350 mg/kg or 500 mg/kg administered by oral gavage, 24 hours apart. Femoral bone marrow was prepared 6 hours after the second application.	**Negative**	Herbold, 1979b
Germ cell effects (rodent dominant lethal assay) Mice/NMRI, 50 males 60:40 material, purity 93.7% Not guideline or GLP	Single gavage dose of 500 mg/kg.	**Negative**	Herbold, 1978a

4.9.1 Summary and discussion of mutagenicity

Triadimenol was negative in a series of *in vitro* genotoxicity assays. Two *in vivo* micronucleus assays have been conducted in which triadimenol was also negative, although these assays included only one sampling time (of six hours, compared with the current OECD 474 guideline-recommended sampling times of 18-24 hours and 36-48 hours). However, the negative results in these two assays are supported by the result of a dominant lethal assay.

No information from humans or other relevant information is available

4.9.2 Comparison with criteria

There was no indication that triadimenol has a mutagenic effect on somatic or germ cells in several *in vitro* and *in vivo* assays. The criteria for classification for mutagenicity were not met.

4.9.3 Conclusions on classification and labelling

Not classified: Conclusive but not sufficient for classification

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**.

4.10 Carcinogenicity

The chronic toxicity and carcinogenic potential of triadimenol have been investigated in rats and mice.

Table 18: Summary table of relevant combined chronic/carcinogenicity studies

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Oral (dietary) 2 years Rats/Wistar, 60/sex 60:40 material, purity 94.9% Not guideline or GLP Krötlinger *et al*., 1982	0, 125, 500, 2000 ppm Equivalent to: males: 0, 5, 19, 77 mg/kg/d females: 0, 6, 25, 106 mg/kg/d	Treatment did not affect survival rates, which were ≥ 70% in all groups. There were no treatment-related clinical signs, and food consumption was similar between all groups. *Non-tumour findings* Body weights gains were lower (by up to 19%) and overall body weights were reduced (by up to 13%) throughout the study in high-dose males and females. Slightly reduced erythrocyte counts were recorded in high-dose females at 3 and 6 months but not at later time points, and slightly lower haemoglobin at 6 months but not at the other time points. Slightly reduced erythrocyte counts were recorded in high-dose males at 6 months only. All the values were reported to be within the normal physiological range. Various clinical chemistry findings were reported. AST and ALT were higher from 500 ppm; in the high-dose groups, they were consistently increased at most time points, with males affected to a greater extent (up to 135%** increase for AST, 142% for ALT). GLDH was increased only in high-dose males (by 300%**). Plasma urea was slightly increased from 500 ppm in both sexes (by up to 11%*), but without a consistent pattern. Creatinine levels were consistently lower in high-dose males (by up to 36%**). Glucose levels were higher in all treated groups at 104 weeks, with a dose-response relationship in females but not males. All the differences were stated to be within the normal physiological range. Cholesterol levels and urinalysis were unaffected by triadimenol. Gross necropsy of all animals did not identify any treatment-related effects. Liver weights were higher in high-dose females (absolute increase of 6%*, relative of 21%**). There were no other treatment-related effects on organ weights. The incidence of females with mucosal retention cysts in the stomach was increased in all treatment groups: 13%, 28%, 15%, 33% at 0, 125, 500, 2000 ppm, respectively. The severity was also increased: graded as minimal: 13%, 23%, 13%, 27%; graded as moderate: 0%, 5%, 1.7%, 7% at 0, 125, 500, 2000 ppm. There were no other non-neoplastic histopathological findings. *Tumour findings* The pattern of neoplastic findings did not indicate any relationship to treatment. The numbers of rats with tumours was similar between all the groups, the number of rats with benign or malignant tumours was not increased by treatment and the time to the first tumour was not shortened by treatment. The NOEL was 125 ppm (5-6 mg/kg/d).
Oral (dietary) 18 months Mice/CD-1, 50/sex/group 80:20 mixture, purity 96.8-97.6% OECD 451 (1981), GLP Schladt, 1998	0, 80, 400, 2000 ppm Equivalent to Males: 0, 11, 60, 340 mg/kg/d Females: 0, 17, 91, 472 mg/kg/d	Clinical chemistry investigations were not performed. The survival rate was unaffected by treatment and was 68-90% at 18 months. There were no clinical signs of toxicity. Food consumption was slightly increased in the high-dose males, by 5%, but was otherwise similar between the groups. *Non-tumour findings* Body weights of both sexes at the high dose were significantly lower than controls from week 2 and throughout the remainder of the study, with reductions of up to 21%** in males and 16%** in females. There were no consistent, toxicologically important findings from the haematology investigations. At gross necropsy, nodules were identified in the liver, but the incidence was only increased in the 60 mg/kg/d males: 6/50, 6/50, 14/50, 5/50 at 0, 11, 60, 340 mg/kg/d, respectively. The incidences of liver nodules in the females were: 1/50,

4/50, 3/50, 1/50 at 0, 17, 91, 472 mg/kg/d.

Liver weights were increased in males: absolute by 7%, 10%, 27% and relative by 7%, 13%**, 48%** at 11, 60 and 340 mg/kg/d. They were also increased in females: absolute by -2%, 5%, 15%** and relative by 1%, 6%, 27%** at 17, 91 and 472 mg/kg/d. There were no other treatment-related organ weight changes.

A number of treatment-related histopathological liver findings were recorded in males from 60 mg/kg/d and in females from 472 mg/kg/d. These are summarised in the table below. The increased incidence of diffuse/centrilobular hepatocellular hypertrophy was accompanied by a dose-related increase in the severity in the males only. A dose-related increase in severity was not noted for the other non-neoplastic histopathological findings. Hepatocellular vacuolation (observed in females only) was characterised by large intracytoplasmic vacuoles containing eosinophilic fluid. The accumulated pigment that was observed was yellow/brown in colour.

Liver tumours

There was an increased incidence of neoplastic findings in the liver of males of the 400 ppm group; however, there was no dose-related response. The neoplastic and non-neoplastic liver histopathology findings are summarised in the table below.

	Males				**Females**			
mg/kg/d	**0**	**11**	**60**	**340**	**0**	**17**	**91**	**472**
Number of livers	50	50	50	49	50	50	48	50
Hypertrophy (total)	5	8	34**	49**	2[t]	2	2	45**
Grade 1	0	1	0	0	0	0	0	0
Grade 2	1	7	31	7	1	1	1	23
Grade 3	4	0	3	33	1	1	1	22
Grade 4	0	0	0	9	0	0	0	0
Fatty change	8	12	7	5	5[t]	2	7	23**
Cellular vacuolation	0	0	0	0	0[t]	2	2	12
Inflammatory infiltration	31[t]	32	35	45**	36	41	32	43**
Pigment accumulation	7[t]	7	10	36**	29[t]	33	25	40**
Single cell necrosis	6[t]	9	20**	42**	8[t]	5	8	25**
Basophilic foci of cellular alteration	0[t]	3*	2	5**	0	1	0	2
Adenoma	7	5	10	5	1	1	2	0
Adenocarcinoma	0	3*	4*	2	0	0	0	0
Haemangioma	0	0	1	0	0	1	1	0
Haemangiosarcoma	0	1	1	1	0	2	0	0

Historical control data from the RITA database[1] are available for this strain of mouse. Four studies of two-year duration (performed at the same laboratory as the present study) gave a range of 2-19.4% for hepatocellular adenoma and 6-17.6% for hepatocellular carcinoma. Nine studies of 18-19 month duration performed in various laboratories (with start dates of -6 to +2 years from the present study) gave a range in males of 0-13.6% (mean 5.8%) for adenoma and 4-22% (mean 11.9%)

		for carcinoma. The combined historical control range for hepatocellular adenoma/carcinoma was 8-32%, whereas the combined incidence in the present study was 14%, 16%, 28%, 14% at 0, 11, 60, 340 mg/kg/d. *Ovarian tumours* The incidence of ovarian luteoma in females was: 0/49[t] (0%), 0/50 (0%), 0/48 (0%), 2/50 (4%). Historical control data are available in the RITA database[1] from studies conducted in CD-1 mice between -6 and +3 years of the date of the present study. There were no reports of ovarian luteoma in historical control data from seven studies of 18-month duration. For 13 studies of two-year duration, the historical control range was 0-10% (mean 1.7%). Of these studies, six of them were without any incidences of ovarian luteoma. In the remainder, the mean was 3.5%, with 1, 2 or 5 animals being affected in each study. Taking all the historical control data together, this tumour occurred in 13 out of 1112 females. There were no other tumour findings of note. The total number of tumours, the incidence of benign and malignant tumours and the time of their appearance were not changed by triadimenol. The NOAEL for all effects was 80 ppm (11 mg/kg/d in males). The NOAEL for carcinogenicity was 2000 ppm (340-472 mg/kg/d).
Oral (dietary) 2 years Mice/CF_1/W 74, 50/sex/group 60:40 material, purity 94.9% Not guideline or GLP Bomhard & Loeser, 1982	0, 125, 500, 2000 ppm Equivalent to 0, 19, 75, 300 mg/kg/d for males and females	This study had the following deficiencies: there was not a histopathological investigation of a full range of organs and tissues; thorough investigation of abnormalities identified at the 24-month differential blood count could not be completed because of fading of the slides and the possibility that artefacts had been introduced during preparation; up to 50% of thyroids were not available for histopathological examination. Survival was unaffected by treatment and was 68-80% at 18 months, and 22-38% at 24 months. There were no clinical signs of toxicity and food consumption was similar between all groups. *Non-tumour findings* There were treatment-related effects on body weights and body weight gains at different time points from 19 mg/kg/d; these parameters were lower than controls for high-dose males throughout the study (up to 15% reduction in body weight, 40% reduction in body weight gain) and for high-dose females for the majority of the study (up to 11% reduction in body weight, 37% reduction in body weight gain). Body weight gains of mid-dose females were reduced by 16% over the duration of the study. There were no obvious haematology findings at 12 and 24 months, although blood smears (on 10/sex/group) at 24 months revealed some unusual findings. Howell-Jolly bodies were observed in all females (including controls), along with polychromatophilia in a single 19 mg/kg/d female. Howell-Jolly bodies and polychromatophilia were recorded in 9/10 high-dose and one 75 mg/kg/d males. Howell-Jolly bodies and polychromatophilia with basophilic stippling were observed in separate, single males at 19 mg/kg/d. Further investigation was prevented because of technical problems. Changes in clinical chemistry parameters at 12 and 24 months were suggestive of adverse liver effects. AST was markedly increased in high-dose animals (by up to 364%**), as was ALT (by up to > 800%**). Cholesterol was reduced in high-dose groups of both sexes at 12 months but only in the males at 24 months (up to 31%** reduction at 300 mg/kg/d). Alkaline phosphatase was increased by up to 250%** in both high-dose groups. At 12 months, total protein was slightly lower in the high-dose groups, but there were no differences from the controls at 24 months. Urea was slightly increased in females of the dosed groups at 24 months, but without a clear dose-response relationship. The only findings of note at gross necropsy were an increased incidence of enlarged or swollen livers and/or nodular formations in the liver in high-dose animals. Liver weights were markedly increased in these animals: in males, the

absolute and relative weight increases were 50%** and 66%**, respectively, whilst those in females were 73%** and 71%**, all at 300 mg/kg/d.

Histopathological findings were observed in the liver and the thyroids. The non-neoplastic findings are recorded together with the neoplastic findings in the table below. In the liver, hyperplasia in high-dose males and hyperplastic nodules in high-dose females were increased, but the incidence of hypertrophy was not increased. In high-dose males, there was an increased incidence of cystic thyroids. This lesion was described as being characterised by the presence of cystic changes in the follicles or in some cases by coalescence of one or two follicles that had also undergone cystic changes. The thyroids were frequently not available for examination, either because they were missing or had undergone autolysis. There were no adverse ovarian findings.

Tumour findings

The incidence of hepatocellular adenomas was increased in females in a dose-response relationship (see table below). In females, hepatocellular adenomas were observed in animals that died or were killed between 613 and 735 days at 75 mg/kg/d, and between days 501 (1 animal) and 735 at 300 mg/kg/d. In males, hepatocellular adenomas were observed between 573 and 734 days in controls and between days 576 and 734 at 300 mg/kg/d. The historical control range for this tumour in CF1 mice was 0 to 25% in males and 0 to 12% in females (8 studies conducted in the same laboratory from 2 years before to 2 years after the triadimenol study). In a study conducted concurrently with the triadimenol study, hepatocellular adenomas occurred in 25% of the male controls and 12% of the female controls.

The incidence of hepatocellular adenocarcinomas was not increased by triadimenol. There was no increase in the incidence of thyroid or ovarian tumours.

Incidences of neoplastic and non-neoplastic liver and thyroid findings

	Males				**Females**			
mg/kg/d	**0**	**19**	**75**	**300**	**0**	**19**	**75**	**300**
Number of livers	50	50	50	50	50	50	50	50
Hypertrophy	0	0	1	1	1	2	1	1
Hyperplasia (focal)	0	0	0	3	0	0	0	0
Hyperplastic nodules	8	6	9	10	2	1	1	7
Hepatocytic hyperpigmentation	4	0	0	9	5	0	0	5
Necrosis	2	0	0	1	0	0	1	0
Adenoma	5	4	5	8	0	0	4	6*
Adenocarcinoma	3	0	0	1	1	0	1	0
Cystadenoma	1	0	0	0	0	0	0	0
Angioadenoma	0	0	0	0	0	0	1	0
Number of thyroids	28	24	34	39	36	36	31	35
Cysts	2	2	2	10	3	3	1	3
Adenoma	0	1	0	0	0	0	0	0

Overall, there was no treatment-related increase in the total incidence of neoplastic findings.
The NOAEL was 19 mg/kg/d.

*N.B. The values for NOAEL/NOEL are provided for information only; they have been agreed by a PRAPeR Expert Meeting. * Statistically significant at $p \leq 0.05$. ** Statistically significant at $p \leq 0.01$. [t] Statistically significant ($p \leq 0.05$)in a trend test*

[1]Registry of Industrial Toxicology Animal Data. This is a database of historical control data from animal carcinogenicity and chronic studies collected from European and American companies and maintained by the Fraunhofer Institute of Toxicology and Experimental Medicine.

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Rat

Reductions in body weights and body weight gains in treated rats indicated that sufficiently high dose levels had been administered to assess triadimenol's carcinogenic potential. The non-neoplastic findings were consistent with those observed in the short-term repeated dose toxicity studies (section 4.7), in that the liver was the target organ (increased weights with some slight changes in clinical chemistry parameters, but in the absence of any gross or histopathology findings). The incidence and severity of mucosal retention cysts in the stomach was increased in females.

In this study there were no indications that triadimenol was carcinogenic.

Mouse

(i). Non-tumour findings

Reductions in body weights in treated mice indicated that sufficiently high dose levels had been administered to assess triadimenol's carcinogenic potential. Changes in clinical chemistry parameters that were suggestive of adverse liver effects were associated with increased liver weights and gross/histopathological liver findings: nodules, hepatocellular hypertrophy, hyperplasia, single cell necrosis (graded as slight and/or minimal in all groups, including the controls) and vacuolation. An increased incidence of cystic thyroids was reported in the second study but not in the first. Ovary weights were unaffected by treatment.

(ii). Malignant and benign liver tumours

At gross necropsy, the incidence of nodules in the liver was increased in the mid-dose male group. This was associated with an increased incidence of neoplastic lesions (hepatocellular adenocarcinoma) in this group, whereas this tumour type did not occur in any females. In males, the incidences of hepatocellular adenoma were 14%, 10%, 20%, 10% at 0, 11, 60, 340 mg/kg/d, whilst the incidences of hepatocellular carcinoma were 0%, 6%, 8%, 4%. Thus, there was not a dose-response relationship. At 60 mg/kg/d, the incidence of adenoma was outside the historical control range for studies of 18-19 months' duration (maximum 13.6%), although the incidence of carcinoma was not (historical control maximum 22%). Since the triadimenol study was of 18 months' duration, the historical control data for 18-19 month studies are more relevant than those of two years' duration. The incidence for combined adenoma/adenocarcinoma was within the historical control data for combined tumours; the study authors argued that it is somewhat arbitrary to distinguish the two tumour types histopathologically and so it is reasonable to combine adenoma/carcinoma. Survival in the high-dose group (90%) was higher than that of the mid-dose group (80%), and the timing of unscheduled deaths was similar between the two groups. Therefore, the lack of increased tumours in the high-dose males is not explained by a reduced opportunity to develop liver tumours compared with the mid-dose male group. The toxicologically significant

histopathology findings that were recorded in mid-dose males were recorded in higher incidences in the high-dose males, indicating that triadimenol's toxic effect on the liver was dose-related. Considering all the evidence, the conclusion is that the increased incidence of liver tumours in the mid-dose group was not related to triadimenol exposure.

Supportive information was provided by a second mouse study. There was no increase in the incidence of hepatocellular carcinoma in any treatment group. The incidence of hepatocellular adenoma was increased in females in a dose-response relationship (0%, 0%, 8%, 12% at 0, 19, 75, 300 mg/kg/d), but the incidence in the high-dose group was the same as that in the control female mice of a concurrently-run study (same strain of mouse and the same laboratory). An interim sacrifice was not included in the study design, so it is not possible to determine if exposure to triadimenol shortened the time to the first appearance of these tumours, although tumours were observed after similar numbers of days in control (between 573 and 734 days) and high-dose males (between 576 and 734 days) that died during and at the end of the study. Likewise, the times at which the tumours were observed were similar between females of the 75 and 300 mg/kg/d groups. Therefore, these tumours are considered to be incidental to triadimenol administration. The histopathological changes in the thyroid were not associated with thyroid tumours.

(iii.) Benign ovarian tumours

Ovarian luteomas occurred in 2/50 (4%) females of the high-dose group (472 mg/kg/d) in one study. This incidence was higher than that from historical control data derived from several studies of 18-months' duration (0%) but within the historical control range derived from studies of 24-months' duration (0-10%). The study pathologist noted that this tumour type is rare, but that when it occurs, there may be multiple spontaneous occurrences within a study (only approximately one half of the control studies accounted for all the incidences of tumours). Additionally, the two lesions reported in the triadimenol study were re-examined, leading to the pathologist's conclusion that neither was completely in concordance with the IARC classification for ovarian luteoma (Mohr *et al*., 2001): one lesion was possibly a 'sex cord stromal tumour (mixed)'; the second was a borderline neoplastic lesion and was possibly a sex cord stromal hyperplasia.

4.10.1.2 Carcinogenicity: inhalation

No information available.

4.10.1.3 Carcinogenicity: dermal

No information available.

4.10.2 Human information

No information available.

4.10.3 Other relevant information

Triadimenol was negative in a series of *in vitro* and *in vivo* assays to detect its genotoxic potential (section 4.9).

4.10.4 Summary and discussion of carcinogenicity

The available information on the carcinogenic potential of triadimenol is provided by three oral studies in which there were increased incidences in liver and ovarian tumours in mice. From the rat study there was no indication that triadimenol had a carcinogenic potential.

In a mouse study, there were increased incidences of benign and malignant liver tumours only in the mid-dose male group. When the two tumour types were combined, the incidence was within the contemporary historical control data. This, together with the absence of a dose-response relationship, suggests that they were incidental to triadimenol exposure. Additional support for the tumours being spontaneous rather than treatment-related was provided by the survival and histopathology data, which indicated that high-dose males had at least an equal opportunity to develop tumours and exhibited greater hepatocellular toxicity than the mid-dose males. Benign liver tumours also occurred in a second mouse study, in a dose-response relationship in females only. The observed incidences were within the normal range for this mouse strain. There were no reports of benign or malignant liver tumours in the rat study.

A low incidence (2/50, 4%) of ovarian tumours was reported in one mouse study at doses of 472 mg/kg/d; tumours of this type were not found in the second mouse study or in rats. The tumours were not associated with effects on ovary weights or histopathology, but were outside the historical control range for studies of the same duration. 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.

4.10.5 Comparison with criteria

Two tumour types in one species (the mouse) occurred in two studies 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.

There is no evidence that triadimenol induces tumours in humans, so category 1A is not appropriate.

Triadimenol is non-genotoxic, which lowers the level of concern for classification. Although increased incidences of liver tumours were observed in one sex in each study (with no dose-response in the male mice), the increased incidences in both studies were within the historical control ranges for those strains of mice; therefore, a causal relationship between triadimenol administration and liver tumour induction was not established and a classification for carcinogenicity is not proposed based on the liver findings. Classification in Category 1B is based on ‘sufficient’ evidence of carcinogenicity: benign/malignant neoplasms in a) two or more species of animals; or b) two or more independent studies in one species conducted at different times or in different laboratories or under different protocols. Since neither of these criteria was met, classification in this category is not appropriate .

It therefore remains to decide if the ovarian tumours best meet the criteria for Category 2 or no classification. Classification in Category 2 is based on ‘limited’ evidence of carcinogenicity where the data suggest a carcinogenic effect but are limited for making a definitive evaluation. Two cases of benign ovarian luteoma occurred in female mice exposed to 472 mg/kg/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 (reports of multiple

spontaneous occurrences within a study) and the pathologist's indeterminate diagnosis of the lesions as luteomas, the overall conclusion is that the data do not suggest a carcinogenic effect and thus classification is not warranted.

4.10.6 Conclusions on classification and labelling

Not classified: Conclusive but not sufficient for classification

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**.

4.11 Toxicity for reproduction

The reproductive toxicity of triadimenol has been investigated in two-/multi-generation and developmental toxicity studies.

4.11.1 Effects on fertility

Two multi-generation studies have been conducted in rats.

Table 19: Summary table of relevant multi-generation studies

<table>
<tr><th>Method</th><th>Dose levels</th><th>Observations and remarks
(effects of major toxicological significance)</th></tr>
<tr><td>Oral (dietary)
Multi-generation
Rats/Long Evans, 10 male, 20 female/group
60:40 material, purity assumed to be 94.9%
Not guideline or GLP
Loeser & Eiben, 1982</td><td>0, 125, 500, 2000 ppm
Equivalent to 0, 15, 60, 240 mg/kg/d in males and females
Administered during 70-day pre-mating, mating at age 100 days, gestation and lactation periods</td><td>The study had a number of deficiencies: food consumption was not measured, so test compound intakes were estimates; fertility of individual males was not determined since females were mated with more than one male; reproductive tissues were not examined histologically; sperm parameters were not examined; mating performance of females was not determined by vaginal smear; pregnancy status was not confirmed (females that did not produce young were assumed to be pregnant if they had clearly gained weight during mating and lost over 30 g three weeks after mating); gross necropsy was not performed on all generations.
Parental toxicity
There were no treatment-related deaths or clinical signs of toxicity in any generation.
Triadimenol had adverse effects on parental body weights, with a worsening of the effects through the generations. Body weight gains of the high-dose F_0 parents were reduced up to the time of the first mating and continued to be reduced in high-dose females up to the second mating. The body weights of the high-dose F_{1B} animals were reduced at weaning and remained lower up until the time of the first mating; the body weights of the mid-dose F_{1B} females were also slightly reduced up to the first mating. The body weights between weaning and the first mating of the F_{2B} generation were similarly affected by exposure to triadimenol, with males of the mid- and high-dose groups being affected. In this generation, females in all the dose groups were affected. Maternal body weights at the time of the first mating are shown below, in the table under developmental toxicity.
Fertility effects
Triadimenol administration consistently reduced fertility in all three generations in a dose-related pattern (apart from the second mating of F_1 animals). The fertility data are presented in the table below.
<table>
<tr><th>mg/kg/d</th><th>0</th><th>15</th><th>60</th><th>240</th></tr>
<tr><td></td><td colspan="4">F_0: first mating (to produce F_{1A})</td></tr>
<tr><td>No. pregnant/no. per group</td><td>17/20</td><td>16/20</td><td>14/19</td><td>4/20</td></tr>
<tr><td>Pregnancy rate (%)</td><td>85</td><td>80</td><td>73.7</td><td>20**</td></tr>
<tr><td></td><td colspan="4">F_0: second mating (to produce F_{1B})</td></tr>
<tr><td>No. pregnant/no. per groups</td><td>16/18</td><td>16/20</td><td>14/19</td><td>13/19</td></tr>
<tr><td>Pregnancy rate (%)</td><td>88.9</td><td>80</td><td>73.7</td><td>68.4</td></tr>
<tr><td></td><td colspan="4">F_1: first mating (to produce F_{2A})</td></tr>
<tr><td>No. pregnant/no. per group</td><td>20/20</td><td>20/20</td><td>14/20</td><td>4/8</td></tr>
<tr><td>Pregnancy rate (%)</td><td>100</td><td>100</td><td>70*</td><td>50**</td></tr>
<tr><td></td><td colspan="4">F_1: second mating (to produce F_{2B})</td></tr>
<tr><td>No. pregnant/no. per group</td><td>16/19</td><td>17/20</td><td>6/20</td><td>4/7</td></tr>
<tr><td>Pregnancy rate (%)</td><td>84.2</td><td>85</td><td>30**</td><td>57.1</td></tr>
</table>
</td></tr>
</table>

			F_2: first mating (to produce F_{3A})		
		No. pregnant/no. per group	17/20	20/20	7/14	5/15
		Pregnancy rate (%)	85	100	50	33.3**
			F_2: second mating (to produce F_{3B})		
		No. pregnant/no. per group	18/20	15/20	10/14	7/14
		Pregnancy rate (%)	90	75	71.4	50*

Developmental toxicity

Developmental landmarks were not assessed, although examination of pups for grossly visible malformations after birth or during lactation did not reveal any malformed pups; however, this observation is of limited value, since dams may eat obviously malformed pups soon after birth.

Triadimenol administration was associated with reduced litter size at birth, reductions in the pup viability indices and reduction in pup weight, although these effects generally occurred together with reductions in maternal body weight. Changes in maternal body weight at the first mating, total litter size at birth, pup viability data and changes in pup weights at birth and after 28 days for all the litters are presented below.

mg/kg/d	**0**	**15**	**60**	**240**
	F_0: first mating (to produce F_{1A})			
Maternal body weight	0%	+1%	-5%	-19%**
Litter size at birth	11.9	11.6	10.6	4.2%*
Pup weight at birth	0%	0%	0%	0%
5d viability index (%)	90.1	85.4	79.9*	41.2**
28d viability index (%)	90.1	79.3*	93.3	14.3**
Pup weight at 28 days	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 weight at birth	0%	0%	0%	-13%
5d viability index (%)	90.8	82.0*	77.2*	81.6
28d viability index (%)	95.6	90.2	91.0	52.6**
Change in pup weight at 28 days	0%	0%	0%	-23%**
	F_1: first mating (to produce F_{2A})			
Maternal body weight	0%	+2%	-8%**	-15%**
Litter size at birth	12.1	11.2	10.1	10.0
Pup weight at birth	0%	0%	0%	0%
5d viability index (%)	87.2	85.3	76.6*	62.5**
28d viability index (%)	98.9	93.9*	70.3**	63.6**
Pup weight at 28 days	0%	0%	0%	-33%**
	F_1: second mating (to produce F_{2B})			

		<table><tr><td>Litter size at birth</td><td>12.7</td><td>10.5</td><td>6.2*</td><td>9.7</td></tr><tr><td>Pup weight at birth</td><td>0%</td><td>0%</td><td>0%</td><td>0%</td></tr><tr><td>5d viability index (%)</td><td>88.7</td><td>81.6</td><td>64.9**</td><td>84.6</td></tr><tr><td>28d viability index (%)</td><td>97.3</td><td>91.3</td><td>95.8</td><td>73.3**</td></tr><tr><td>Pup weight at 28 days</td><td>0%</td><td>0%</td><td>0%</td><td>-21%*</td></tr><tr><td></td><td colspan="4">**F_2**: first mating (to produce F_{3A})</td></tr><tr><td>Maternal body weight</td><td>0%</td><td>-7%*</td><td>-9%**</td><td>-24%**</td></tr><tr><td>Litter size at birth</td><td>11.9</td><td>9.5</td><td>5.7*</td><td>6.8*</td></tr><tr><td>Pup weight at birth</td><td>0%</td><td>-11%</td><td>-20%**</td><td>-20%*</td></tr><tr><td>5d viability index (%)</td><td>89.7</td><td>66.5**</td><td>2.5**</td><td>11.8**</td></tr><tr><td>28d viability index (%)</td><td>70.7</td><td>53.0</td><td>0</td><td>0</td></tr><tr><td>Pup weight at 28 days</td><td>0%</td><td>0%</td><td>-</td><td>-</td></tr><tr><td></td><td colspan="4">**F_2**: second mating (to produce F_{3B})</td></tr><tr><td>Litter size at birth</td><td>10.3</td><td>8.5</td><td>11.0</td><td>3.9**</td></tr><tr><td>Pup weight at birth</td><td>0%</td><td>0%</td><td>0%</td><td>-16%*</td></tr><tr><td>5d viability index (%)</td><td>81.6</td><td>74.0</td><td>96.4**</td><td>29.6**</td></tr><tr><td>28d viability index (%)</td><td>93.9</td><td>93.7</td><td>88.8</td><td>100.0</td></tr><tr><td>Pup weight at 28 days</td><td>0%</td><td>-14%</td><td>-26%*</td><td>-29%</td></tr></table> Sex ratios in pups of F_1 parents were M/F 52/48, 48/52, 57/43, 76.5/23.5 at the first mating and 50/50, 58.7/41.3, 55.7/44.3, 51/49 at the second mating at 0, 15, 60, 240 mg/kg/d. The distortion of the sex ratio at 240 mg/kg/d in the first mating may have been a sampling error resulting from the small group size. The sex ratios of all the litters in the subsequent generations were unaffected by triadimenol, and so the finding at the first mating of F0 animals was unlikely to be treatment related. Gross necropsy was limited to animals that died prematurely and F_{2B} parents / F_{3B} offspring (1/sex/dam/group at 4 weeks old). No treatment-related gross necropsy findings were recorded. Liver, kidney, testicle or ovary weights were examined in the F_{2B} parents. Liver and kidney weights were unaffected relative to body weight, but, even accounting for the lower body weights of these animals compared with controls, testes weights were elevated at 60 and 240 mg/kg/d (relative values of 124% and 142% of the controls at 60 and 240 mg/kg/d), whilst ovary weights were elevated at 240 mg/kg/d (relative value of 123% compared with the controls). Histopathological investigations were not performed. A NOAEL was not identified.
Oral (dietary) Rats/Wistar/ 10 male, 20 female/group 80:20 mixture, purity 97.5% Reference made to OECD 416 (1983), not	0, 20, 100, 500 ppm Equivalent to F_0: males 0, 1.7, 9, 42; females 0, 2.2, 11, 57 mg/kg/d F_1: males 0, 1.2, 6, 29; females 0, 1.8,	Histopathology was not performed on animals that failed to induce pregnancy or failed to become pregnant. Assessment of male fertility was limited, because each female was mated with multiple males. Also, sperm parameters and developmental landmarks were not assessed. Vaginal smears to detect the presence of sperm were taken from F_{1B} females (but not from F_0 animals) to confirm that mating had occurred. *Parental toxicity* There were no treatment-related deaths or clinical signs of toxicity. The body weights of F_0 parent animals were not affected by triadimenol. However, the high-dose F_{1B} animals had lower body weights at weaning (by up

GLP

Loeser & Eiben, 1984

9, 39 mg/kg/d

Administered to F_0 through 100-day pre-mating period, mating, gestation and lactation.

to 6%) that remained lower to the start of mating, although body weight gains in the pre-mating period were comparable with controls. The body weights of these animals were significantly lower during the period between the first and second mating in both sexes, and remained lower for the remainder of the study (up to 9% reduction).

Fertility effects

Fertility of the F_0 parents was unaffected by dosing with triadimenol. There was a slight effect on the fertility of the F_1 parents of the high-dose group as a result of a lower rate of insemination, although the effects were inconsistent. The study report stated that three high-dose females that failed to become pregnant in either of the first two matings produced normal-sized litters in a third mating, although no further details were provided. The fertility data are presented in the table below.

mg/kg/d (female)	**0**	**2.2**	**11**	**57**
	F_0: first mating (to produce F_{1A})			
No. pregnant/no. per group	19/20	19/19	20/20	20/20
Fertility index	95	100	100	100
	F_0: second mating (to produce F_{1B})			
No. pregnant/no. per group	19/20	19/19	18/20	18/20
Fertility index	95	100	90	90
mg/kg/d (female)	**0**	**1.8**	**9**	**39**
	F_1: first mating (to produce F_{2A})			
No. pregnant/no. per group	17/19	16/20	14/20	14/20
Insemination index[1]	95	95	90	70
Fertility index[2]	90	80	70	70
	F_1: second mating (to produce F_{2B})			
No. pregnant/no. per group	15/18	15/20	16/20	15/20
Insemination index	100	95	85	80
Fertility index	83	75	80	75

Developmental toxicity

There was no effect on maintenance of pregnancy or duration of gestation. Total litter sizes at birth, changes in pup weights and viabilities at 5 and 28 days for the two generations are shown in the table below (there were no statistically significant differences).

mg/kg/d (females)	**0**	**2.2**	**11**	**57**

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

[2] = number of pregnant females / number of females in the group x 100

		F_0: first mating (to produce F_{1A})			
		Litter size at birth	9.3	10.5	11.2	9.9
		Pup weight at birth	0%	-2%	-5%	-5%
		5d viability index (%)	94.4	97.0	91.1	92.9
		28d viability index (%)	98.1	97.2	96.7	94.6
		Pup weight at 28 days	0%	-4%	-6%*	-5%
		F_0: second mating (to produce F_{1B})			
		Litter size at birth	9.8	10.3	9.9	9.3
		Pup weight at birth	0%	-2%	0%	-9%**
		5d viability index (%)	96.8	97.4	100	94.0
		28d viability index (%)	93.9	98.9	97.5	87.5
		Pup weight at 28 days	0%	-4%	-1%	-11%
		mg/kg/d (females)	**0**	**1.8**	**9**	**39**
		F_1: first mating (to produce F_{2A})			
		Litter size at birth	10.5	11.0	11.0	10.6
		Pup weight at birth	0%	+2%	+6%	+2%
		5d viability index (%)	78.7	93.7	96.8	96.0
		28d viability index (%)	79.8	89.3	92.7	88.5
		Pup weight at 28 days	0%	-12%*	+11%	-18%**
		F_1: second mating (to produce F_{2B})			
		Litter size at birth	11.5	10.1	10.8	10.3
		Pup weight at birth	0%	+4%	0%	+2%
		5d viability index (%)	90.7	97.4	92.5	89.0
		28d viability index (%)	87.7	96.9	81.6	90.4
		Pup weight at 28 days	0%	+1%	-7%	-3%
		There was no effect of treatment on sex ratios. Apart from animals that died prematurely, gross necropsy and histopathology were restricted to the F_{1B} parents and F_{2B} offspring. No treatment-related gross necropsy or histopathology findings were recorded. The NOAEL for parental toxicity and reproductive effects was 100 ppm. Gross necropsy of decedents, F_{1B} parents and F_{2B} offspring (1/sex/dam/group) did not show any treatment-related findings. In the high-dose F_{1B} parents, absolute and relative ovary weights were increased by 9% and 14%*, respectively, whilst in males of this group, relative testes weights were increased by 12%*. There were no corresponding histopathological findings in these organs. The NOAEL for parental toxicity and reproductive effects was 11 mg/kg/d.				

N.B. The values for NOAEL are provided for information only; they have been agreed by a PRAPeR Expert Meeting.
** Statistically significant at $p \leq 0.05$. ** Statistically significant at $p \leq 0.01$*

4.11.1.1 Non-human information

A multi-generation study has been conducted in rats. Triadimenol was administered to F_0 males and females for a 70-day pre-mating period before being mated twice. The F_{1B} offspring were retained and subsequently mated twice, from which the F_{2B} offspring were mated twice to produce F_{3A} and F_{3B} pups. All matings within each generation were on the basis of one male to two females, with each female being kept together with three different males over a 20-21 day period. Standardisation of litter sizes to 10 pups took place on post natal day 5.

The pregnancy rate was consistently reduced at 60 and 240 mg/kg/d in both matings of the three generations, although it should be noted that in some generations the 240 mg/kg/d groups contained only small numbers of females for investigation, largely owing to the reduced pregnancy rate. As only limited investigations were performed (mating performance/pregnancy status of females was not confirmed; multiple males were housed with each female; no sperm investigations or histopathology of reproductive tissues), it was not possible to elucidate if the reduced number of pregnancies was the result of impaired mating or interference with another reproductive parameter.

In a two-generation study in rats, each generation was mated twice, with the first litter being sacrificed at weaning and the second generation being retained for mating. All matings were on the basis of one male to two females, with the male being replaced weekly over a three week period. Standardisation of litter sizes at a maximum of 10 pups took place on day 5. A dose-related reduction in the fertility index was recorded in the F_1 generation matings. The effects were less pronounced than those seen in the multi-generation study, but were consistent with the lower doses administered. At the high dose, the reduction in the fertility index was associated with a reduction in the insemination index, indicating that the effect on fertility may have been at least partially mediated through an interference with mating. The appearance and behaviour of all the treated rats did not differ from those of the controls, indicating that the reduced insemination indices were not a result of incapacitation.

4.11.1.2 Human information

No information available.

4.11.2 Developmental toxicity

The developmental toxicity of triadimenol has been investigated in several studies in rats and rabbits.

Table 20: Summary table of relevant developmental toxicity studies

Method	Dose levels	Observations and remarks (effects of major toxicological significance)
Oral (gavage) Rats/Long Evans, 20-22 females/group 60:40 material, purity 93.7% Similar to OECD 414, not GLP Machemer, 1977	0, 10, 30, 100 mg/kg/d in 0.5% Cremophor EL on gestation days 6 to 15 (dams sacrificed on gestation day 20).	*Maternal toxicity* There were no deaths or clinical signs of toxicity in any group. There were 20 pregnant animals in each group (only data from pregnant animals were included). Body weights were reported as mean values for weight gain over the treatment and gestation periods rather than as actual body weights at different time points. Body weight gains during the treatment period were 100%, 101%, 95%, 76%** at 0, 10, 30, 100 mg/kg/d. Weight gains over the whole gestation period were slightly lower at 100 mg/kg/d only (96% of controls). Gross necropsy of dams was not performed. *Developmental toxicity* Litter data was presented only as mean values for each litter. There were no total litter losses or effects of treatment on the number of viable fetuses at day 20, sex ratio, number of resorptions or total number of foetuses. Mean foetal and placental weights of the high-dose group were higher than those of controls, and in this group there were fewer 'stunted' (weight <3 g) foetuses. There were no external or internal malformations at 100 mg/kg/d. A number of foetuses at lower dose levels exhibited malformations of the head (hypoplasia of telencephalon, anophthalmia or microphthalmia), but these were also noted in a control foetus (foetal incidence 1/215, 5/200, 13/215, 0/218 at 0, 10, 30, 100 mg/kg/d). They also tended to be grouped in a small number of litters (litter incidence 1/20, 1/20, 3/20, 0/20 at 0, 10, 30, 100 mg/kg/d). Individual skeletal alterations were not reported (numerical incidence data only). The number of fetuses that showed retarded skeletal development was not increased by triadimenol. The NOAEL for developmental effects was 100 mg/kg/d. The NOAEL for maternal toxicity was 30 mg/kg/d.
Oral (gavage) Rats/Wistar, 25 females/group 80:20 material, purity 97% OECD 414, GLP Becker *et al*., 1987a	0, 30, 60, 120 mg/kg/d in 0.5% Cremophor EL on gestation days 6 to 15 Dams were sacrificed on day 21	Statistical analysis of the findings was limited. *Maternal toxicity* There were no deaths, clinical signs of toxicity or abnormal gross necropsy findings in adult animals. Maternal body weight gains were reduced in the 60 and 120 mg/kg/d groups in the early part of the treatment period (up to day 11), in a dose-response relationship (see table below). Body weight gains over the remainder of the study were comparable to controls. Food consumption was similarly lower in the 60 and 120 mg/kg/d groups up to day 11, but thereafter equalled or exceeded the intakes of the controls. *Developmental toxicity* An increase in the rate of post-implantation loss (embryonic and foetal resorptions were increased) resulted in a reduction in the number of live foetuses at 120 mg/kg/d. Sex ratio and foetal weight were unaffected. Placental weight was not investigated. External abnormalities were recorded in a single foetus at 30 mg/kg/d and visceral abnormalities in a single foetus in each of the control and 30 mg/kg/d groups. Skeletal anomalies occurred with a higher combined incidence in dosed groups, although without a dose-response relationship (3.4%, 6.6%, 11.5%, 6.7% of foetuses were affected at 0, 30, 60, 120 mg/kg/d). There was also no dose-

<table>
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<td>response relationship in the individual skeletal anomalies. These frequencies of anomalies were described by the study authors as being within the normal range for this strain of rats (historical control data for overall incidence of skeletal anomalies were not provided, but data for individual findings were available).

Individual numerical data for skeletal ossification were not reported (only mean values were provided), but triadimenol did not appear to have affected the extent of delayed or absent ossification.

There was a dose-related increase in the incidence of supernumerary ribs (in most cases bilateral); these were small in size, being less than half the size of full ribs.

The main findings relevant to developmental toxicity are recorded below.
<table>
<tr><th>mg/kg/d</th><th>0</th><th>30</th><th>60</th><th>120</th></tr>
<tr><td>Maternal body weight gain (day 6-11)</td><td>100%</td><td>100%</td><td>84%</td><td>58%</td></tr>
<tr><td>Live foetuses: per group</td><td>289</td><td>304</td><td>266</td><td>255</td></tr>
<tr><td>per dam</td><td>12.0</td><td>12.2</td><td>11.6</td><td>11.1</td></tr>
<tr><td>Embryonic resorptions:</td><td></td><td></td><td></td><td></td></tr>
<tr><td>per group</td><td>18</td><td>17</td><td>19</td><td>32</td></tr>
<tr><td>% of implantations</td><td>5.9</td><td>5.3</td><td>6.6</td><td>11.1</td></tr>
<tr><td>per dam: mean</td><td>0.8</td><td>0.7</td><td>0.8</td><td>1.4</td></tr>
<tr><td>Foetal resorptions:</td><td></td><td></td><td></td><td></td></tr>
<tr><td>per group</td><td>0</td><td>1</td><td>2</td><td>2</td></tr>
<tr><td>% of implantations</td><td>0</td><td>0.3</td><td>0.7</td><td>0.7</td></tr>
<tr><td>per dam: mean</td><td>0</td><td>0</td><td>0.1</td><td>0.1</td></tr>
<tr><td>total post-implantation loss:</td><td></td><td></td><td></td><td></td></tr>
<tr><td>per group</td><td>18</td><td>18</td><td>21</td><td>34</td></tr>
<tr><td>% of implantations</td><td>5.9</td><td>5.6</td><td>7.3</td><td>11.8</td></tr>
<tr><td>per dam: mean</td><td>0.8</td><td>0.7</td><td>0.9</td><td>1.5</td></tr>
<tr><td>Supernumerary ribs: no. skeletons</td><td>146</td><td>152</td><td>139</td><td>135</td></tr>
<tr><td>14th rib (left): incidence</td><td>11.6%</td><td>16.4%</td><td>38.8%</td><td>51.1%</td></tr>
<tr><td>14th rib (right): incidence</td><td>13.0%</td><td>19.1%</td><td>41.0%</td><td>51.1%</td></tr>
</table>

The NOAEL for developmental toxicity was 30 mg/kg/d. The NOAEL for maternal toxicity was 30 mg/kg/d.</td>
</tr>
<tr>
<td>Oral (gavage)

Rats/Long Evans, 25 females/group

80:290 material, purity 95.2%

Not guideline or GLP</td>
<td>0, 10, 30 mg/kg/d in 0.5% Cremophor EL

on days 6 to 15 of gestation

Dams were sacrificed on day 20</td>
<td>Maternal toxicity

There were no treatment-related deaths or clinical signs in dams. Body weight gains of the high-dose group were slightly lower than those of controls over the treatment period (reduced by 14%) and over the whole gestation period (by 9%).

Gross necropsy was not performed on dams.

Developmental toxicity

The number of females with live litters and the number of foetuses per litter was unaffected by treatment. There was also no effect on post-implantation losses. A</td>
</tr>
</table>

<table>
<tr><td>Renhof, 1984</td><td></td><td>single foetus of < 3g was identified, in the high-dose group. Mean foetal weight was similar between groups but mean placental weight was increased (106% and 111%* of controls at 10 and 30 mg/kg/d, respectively).

Skeletal findings were reported as numerical incidences only, and individual data were not reported. The number of foetuses with minor skeletal deviations and malformations was not increased by triadimenol.

The NOAEL for developmental effects was 30 mg/kg/d. The NOAEL for maternal toxicity was 10 mg/kg/d.</td></tr>
<tr><td>Oral (gavage)

Rats/Crl;CD, 5 females/group

Assumed to be 80:20 mixture, purity was 95%

OECD 414 (1981), GLP

Clemens et al., 1990</td><td>0, 25, 60, 95, 130, 165 mg/kg/d in 0.5% carboxy-methyl-cellulose / 0.4% Tween 80 NF

on days 6 to 15 of gestation.

Dams were sacrificed on day 20.</td><td>Range-finding study.

Maternal toxicity

Reduced body weight gains were recorded during the treatment period at all dose groups: day 0-20 body weight gains were 100%, 96%, 82%, 76%, 73%, 81% at 0, 25, 60, 95, 130, 165 mg/kg/d.

Developmental toxicity

Post-implantation loss was increased from 130 mg/kg/d: 11%, 4%, 5.5%, 4.7%, 28.4%, 30.8% at 0, 25, 60, 95, 130, 165 mg/kg/d.

There was no clear effect on foetal weights.

1/4 litters of the 165 mg/kg/d group contained 1/15 normal foetuses and 14/15 malformed foetuses (14 with protruding tongue, 5 additionally with cleft palate). A skeletal examination was not performed.</td></tr>
<tr><td>Oral (gavage)

Rats/Crl;CD, 28 females/group

Assumed to be 80:20 mixture, purity was 95%

OECD 414, GLP

Clemens et al., 1990</td><td>0, 5, 15, 25, 60 mg/kg/d in 0.5% carboxy-methyl-cellulose / 0.4% Tween 80 NF

on days 6 to 15 of gestation.

Dams were sacrificed on day 20.</td><td>Maternal toxicity

There were no deaths or clinical signs of toxicity.

Lower food consumption from 15 mg/kg/d during the treatment period was associated with reduced body weight gains: final corrected body weight gains were 100%, 97%, 84%**, 91%, 78%** at 0, 5, 15, 25, 60 mg/kg/d. This effect was evident from the start of treatment, but body weight gains after the end of treatment were comparable between groups.

There were no treatment-related gross necropsy findings in dams, and serum enzyme activities (AST, ALT, alkaline phosphatase) were similar between groups. Absolute and relative liver weights were unaffected by triadimenol.

Developmental toxicity

The number of dams with live litters was similar (≥ 22) between groups. There were no instances of total litter loss or abortion, and the number of corpora lutea and implantations was similar between the groups. There was no evidence of post-implantation loss. The total number of foetuses, mean litter size, mean foetal weight and sex ratio were likewise unaffected by treatment. Median placental weight was increased at 60 mg/kg/d (0.63g compared with 0.52g in the controls); this was outside the historical control range of 0.50 – 0.55 g.

The incidence and pattern of external, visceral and skeletal malformations was not changed by treatment. Likewise, skeletal ossification was unaffected.

There was a dose-related increase in the incidence of extra ribs (no information is provided on the size of the extra ribs). The data are summarised in the table below.
<table>
<tr><th>mg/kg/d</th><th>0</th><th>5</th><th>15</th><th>25</th><th>60</th></tr>
<tr><td>Number of foetuses</td><td>191</td><td>157</td><td>174</td><td>168</td><td>198</td></tr>
<tr><td>Number of litters</td><td>28</td><td>22</td><td>25</td><td>25</td><td>28</td></tr>
<tr><td>Extra lumbar ribs
foetuses (litters)</td><td>1 (1)</td><td>6 (6)</td><td>6 (6)</td><td>13 (9)</td><td>42 (20)</td></tr>
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<tr><td></td><td></td><td>
<table>
<tr><td>foetal % (litter %)</td><td>0.5 (3.6)</td><td>3.8 (27.3)</td><td>3.4 (24)</td><td>7.7** (36)</td><td>21.2** (74.4)</td></tr>
<tr><td>Cervical ribs</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>foetuses (litters)</td><td>4 (4)</td><td>2 (2)</td><td>3 (3)</td><td>6 (3)</td><td>13 (9)</td></tr>
<tr><td>foetal % (litter %)</td><td>2.1 (14.3)</td><td>1.3 (9.1)</td><td>1.7 (12)</td><td>3.6 (12)</td><td>6.6 (32.1)</td></tr>
<tr><td>Extra ribs (combined)</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>foetuses (litters)</td><td>5 (5)</td><td>8 (8)</td><td>9 (8)</td><td>19** (10)</td><td>55 (24)**</td></tr>
<tr><td>foetal % (litter %)</td><td>2.6 (17.9)</td><td>5.1 (36.4)</td><td>5.2 (32.0)</td><td>11.3** (40)</td><td>27.8 (85.7)**</td></tr>
</table>

Historical control data were provided from studies conducted in the same laboratory with the same strain of rat over the 8 years previous to the triadimenol study: foetal incidence = 0.5 – 15.7 %; litter incidence = 3.6 – 66.7%. When only the data from studies (n = 8) conducted up to 3 years before the current study were considered, the foetal incidence was 0.5 – 6.6% and the litter incidence was 3.6 – 38.5%. No data from studies performed concurrently with or after the present study were available.

The NOAEL for developmental toxicity was 15 mg/kg/d. The NOAEL for maternal toxicity was 5 mg/kg/d.
</td></tr>
<tr><td>Oral (gavage)

Rabbits/Chin-chilla, 16 females/group

Assumed to be 80:20 mixture, purity was 97%

OECD 414 (1981), GLP

Becker et al., 1987b</td><td>0, 8, 40, 200 mg/kg/d

in 0.5% Cremophor EL

on days 6 to 18 of gestation

Dams were sacrificed on day 28.</td><td>
Maternal toxicity

There were no treatment-related deaths or gross necropsy findings amongst dams. Clinical signs were recorded at 200 mg/kg/d and included: excited appearance; hair loss; skin injuries attributed to excessive scratching and gnawing; abrupt motions of the head and licking of the forepaws and abdomen.

In the 200 mg/kg/d group, food consumption was markedly reduced during the treatment period, followed by increased food consumption during the post-treatment period. These animals lost weight during days 6 to 9 and showed very low body weight gain over the treatment period (17g gain, compared with 309g gain in the controls). Body weight gain over the treatment period was also reduced at 40 mg/kg/d (272g gain).

Developmental toxicity

The number of dams with live litters was at least 14 in each group.

Post-implantation loss, largely as a result of embryonic resorptions, was increased at 200 mg/kg/d (foetal resorptions were mostly unaffected). The historical control data for post-implantation loss from 21 studies conducted between 1985 and 1987 were: per group = 1 - 14; % of implantations = 0.7 – 10.4; mean per dam = 0.1 – 0.9. The values for embryonic resorptions from the same studies were: per group = 0 - 9; % of implantations = 0 – 6.7; mean per dam = 0 – 0.6.

As a result of the resorptions, the number of live foetuses was lower in this group, as was foetal weight. Placental weight was not determined. Sex ratios were unaffected.

The pattern and incidence of skeletal malformations was similar between the groups. The incidence of skeletal anomalies, mostly in the form of abnormal or incomplete ossification, was increased at 200 mg/kg/d.

The treatment-related developmental findings are presented in the table below.
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<table>
<tr><th>mg/kg/d</th><th>0</th><th>8</th><th>40</th><th>200</th></tr>
<tr><td>Live foetuses: per group</td><td>128</td><td>103</td><td>112</td><td>95</td></tr>
<tr><td>per dam</td><td>8.0</td><td>6.9</td><td>7.5</td><td>6.3</td></tr>
<tr><td>Embryonic resorptions</td><td></td><td></td><td></td><td></td></tr>
<tr><td>per group</td><td>1</td><td>7</td><td>3</td><td>11</td></tr>
<tr><td>% of implantations</td><td>0.8</td><td>6.4*</td><td>2.5</td><td>10.1**</td></tr>
<tr><td>mean per dam</td><td>0.1</td><td>0.5</td><td>0.2</td><td>0.7</td></tr>
<tr><td>no. dams affected</td><td>1</td><td>3</td><td>3</td><td>6</td></tr>
<tr><td>Total post-implantation loss</td><td></td><td></td><td></td><td></td></tr>
<tr><td>per group</td><td>5</td><td>9</td><td>8</td><td>14</td></tr>
<tr><td>% of implantations</td><td>3.8</td><td>8.0</td><td>6.7</td><td>12.8*</td></tr>
<tr><td>mean per dam</td><td>0.3</td><td>0.6</td><td>0.5</td><td>0.9</td></tr>
<tr><td>no. dams affected</td><td>5</td><td>5</td><td>5</td><td>7</td></tr>
<tr><td>Weights of live foetuses (g)</td><td>35.2</td><td>35.6</td><td>34.8</td><td>33.3*</td></tr>
<tr><td>Skeletal anomalies</td><td></td><td></td><td></td><td></td></tr>
<tr><td>foetal incidence</td><td>0%</td><td>2.9%</td><td>1.8%</td><td>7.4%**</td></tr>
<tr><td>litter incidence</td><td>0%</td><td>20%</td><td>13.3%</td><td>42.9%**</td></tr>
</table>

The NOAEL for developmental effects was 40 mg/kg/d. The NOAEL for maternal toxicity was 8 mg/kg/d.
</td>
</tr>
<tr>
<td>Oral (gavage)

Rabbits/New Zealand White, 20 females/group

Assumed to be 80:20 mixture, purity 96%

OECD 414 (1981), GLP

Clemens et al., 1992</td>
<td>0, 5, 25, 125 mg/kg/d in 0.5% carboxymethyl cellulose / 0.4% Tween 80

on days 6 to 18 of gestation.

Dams were sacrificed on day 29.</td>
<td>
Maternal toxicity

There were no deaths or clinical signs of toxicity.

Dams of the 125 mg/kg/d group lost weight over the treatment period (associated with reduced food consumption) and overall weight gains (days 0-29) of this group were significantly reduced compared with the controls (29%* lower). Food consumption and weight gains of the other dose groups were unaffected.

There were no abnormal findings at gross necropsy of dams, and absolute and relative liver weights were similar between groups.

Developmental toxicity

The number of dams with live litters was similar between groups (at least 16). Litter size at 25 and 125 mg/kg/d was lower than controls, but this was attributed to lower numbers of corpora lutea combined with higher pre-implantation losses. The study authors noted that the numbers of corpora lutea and implantations in the controls were at the upper end of the historical control range (from 6 studies conducted within the previous 12 months) whilst the numbers from the 25 and 125 mg/kg/d groups were at the lower end.

Post-implantation loss was not increased by treatment and there were no changes in the sex ratios. Mean foetal weights were increased at 25 and 125 mg/kg/d. Mean placental weight was increased only at 25 mg/kg/d.

There were no adverse effects on external, visceral and skeletal abnormalities. An increased extent of skeletal ossification at 25 mg/kg/d reflected the significantly increased foetal weights in the group.

The NOAEL for developmental effects was 125 mg/kg/d. The NOAEL for maternal toxicity was 25 mg/kg/d.
</td>
</tr>
</table>

N.B. The values for NOAEL are provided for information only; they have been agreed by a PRAPeR Expert Meeting.
** Statistically significant at $p \leq 0.05$. ** Statistically significant at $p \leq 0.01$*

4.11.2.1 Non-human information

Information on litter size at birth, pup body weights, viability and growth after triadimenol administration to dams was obtained from a multi-generation and a two-generation study in rats (section 4.11.1). The multi-generation study (Loeser & Eiben, 1982) showed a pattern of increasing severity of effects on viability and growth through the generations, with both the 5-day (number of pups before litter reduction at day 5 / number live born x 100) and the 28-day (number surviving to day 28 / number after litter reduction on day 5 x 100) viability indices being severely reduced in all dose groups (15, 60 and 240 mg/kg/d) at the first mating of the F_2 animals. At this mating 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 not repeated at the second F_2 mating, and effects in all the generations at 15 mg/kg/d, and possibly also at 60 mg/kg/d, were likely to have been chance findings. In the high-dose group, all the findings were likely to be a consequence of the fairly severe maternal toxicity (as observed in the maternal body weights being decreased by up to 24%), which also increased through the generations.

In a two-generation study (Loeser & Eiben, 1984), there were some reductions in litter size at birth and pup viability that were more apparent at the second mating, but they were small, inconsistent and there was usually no dose-response relationship. A direct comparison of substance intake between the multi-generation and the two-generation study was not possible, since intakes in the first study were estimates based on guidance rather than measured values. However, from the estimated values, the intakes of triadimenol were less in the second study (the high-dose group in the two-generation study was less than the mid-dose group of the multi-generation study).

The number of post-implantation losses was increased in rats from 120 mg/kg/d and in rabbits at 200 mg/kg/d. The increased embryonic and foetal resorptions in rats (Becker *et al*., 1987a; Clemens *et al*., 1990) occurred in a dose-response relationship and were associated with maternal toxicity (reduced body weight gains). In rabbits (Becker *et al*., 1987b), 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) noted in these animals, which was more marked during the early part of gestation.

In one rat developmental study (Machemer, 1977), a number of foetuses in the lower dose groups (10 and 30 mg/kg/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/d) group, even in the presence of maternal toxicity.

In several other developmental studies, there were dose-related increases in the incidences of supernumerary ribs (two studies, occurred from 5 mg/kg/d) and malformed foetuses (one study, at 165 mg/kg/d) in rats. In one study, the extra ribs were small (Becker *et al*., 1987a). In a second study (Clemens *et al*., 1990), the combined incidence of extra ribs exceeded the most relevant historical control data, with the increased incidence of extra lumbar ribs being particularly marked. There was no information on the size of the extra ribs, so there is uncertainty over the severity of the effect. In a range-finding study in rats (Clemens *et al*., 1990), 14/15 pups from one litter of the high-dose group showed malformations. 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 remains unclear. An increased incidence of extra ribs was not recorded in the rabbit studies. The skeletal anomalies in the form of abnormal or

incomplete ossification that occurred in one study (Becker *et al*., 1987b) were likely to have been a manifestation of developmental delay, attributed to the maternal toxicity and are, additionally, common in rabbits.

Exposure to triadimenol resulted in increases in placental weight in two rat studies (Clemens *et al*., 1990; Renhof, 1984) and one rabbit study (Clemens *et al*., 1992). The study authors stated that this is an effect that is commonly seen with azole-containing substances. The finding is of uncertain significance, but a hormonal effect cannot be excluded.

4.11.2.2 Human information

No information available.

4.11.3 Other relevant information

In a dominant lethal assay (section 4.9), a single dose of 500 mg/kg/d had no effect on male fertility.

4.11.4 Summary and discussion of reproductive toxicity

4.11.4.1 Fertility

The reproductive toxicity of triadimenol has been investigated in oral studies in rats and rabbits.

Both multi-generation studies showed an increase in the severity of the fertility effects through the generations, so that all dose groups were affected by the final generation. The toxicokinetic investigations (section 4.1) 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 occurs.

In these two studies, parental toxicity (as exhibited by decreased body weight gains which also worsened through the generations) was evident but there were no overt clinical symptoms. The effects on fertility were more marked when higher doses (60 & 240 mg/kg/d) were administered, but were still evident at the F_1 first and second matings when lower doses were used (from 1.8 mg/kg/d), and in the F_{3B} generation in the low-dose (15 mg/kg/d) group, when parental toxicity was slight or absent. The insemination index was also reduced in the one study where this parameter was investigated. The reduced fertility index in these studies is not regarded as being a non-specific secondary consequence of parental toxicity. No specific investigations have determined if the possible effect on fertility is mediated through males or females, although male fertility was not affected by a single dose of triadimenol in a dominant lethal assay.

4.11.4.2 Developmental toxicity

When triadimenol was administered just during gestation, post-implantation losses only occurred at higher doses (from 120 mg/kg/d in rats and 200 mg/kg/d in rabbits) and in association with maternal toxicity. Continuous administration of triadimenol in a multi-generation study resulted in statistically significantly reduced total litter sizes from 60 mg/kg/d; it is not known if it was pre- or post-implantation losses that were most affected. However, this effect on litter sizes was associated with quite severe maternal toxicity. Differences in pup viability and body weight were also likely to be due to maternal toxicity (at the higher doses) or chance (at the lower doses), and are not sufficiently convincing to justify classification.

The increased incidences of supernumerary ribs occurred in two rat studies in dose-response relationships, but were only statistically significantly increased or clearly above the control value when maternal toxicity was evident. Uncertainty surrounds the developmental/teratogenic significance of supernumerary ribs, in particular their post-natal reversibility or otherwise. The presence of supernumerary ribs that are small in size may be considered to be less significant with respect to teratogenic potential than ribs that are more than half the size of a full rib, which are considered to be more likely to persist post-natally. Generally, findings of this nature are not used as evidence for classification. Supernumerary ribs did not occur in rabbits when triadimenol was administered in relatively high doses that resulted in quite severe maternal toxicity.

In one rat study, one litter of the high-dose group contained a large number of foetuses with malformations, including cleft palate. However, the fact that they occurred only in one litter seems to provide less evidence of a specific developmental effect than if they had been distributed amongst several litters; one explanation may be that there was a genetic link to one of the parents.

4.11.5 Comparison with criteria

The developmental toxicity observed was generally associated with maternal toxicity and did not provide evidence of a specific effect. Likewise, reduced post-natal survival and weight gain occurred together with maternal toxicity and/or reduced pup birth weight. There was therefore insufficient evidence to propose a classification for developmental toxicity or effects on or via lactation. However, triadimenol reduced fertility in a multi-generation study in rats, with supportive evidence provided by a two-generation study in rats that used lower doses; this effect did not appear to be a secondary effect of non-specific toxicity. Classification for these effects is therefore indicated.

There is no human data available, so category 1A is not appropriate.

In deciding if category 1B or 2 is the more appropriate, several factors are taken into consideration; these include the specificity of the effect and any association with parental toxicity; mechanistic information that indicates the effects may not be relevant for humans; the strength of the evidence; deficiencies in the study that make the quality of the evidence less convincing.

There was no mechanistic information to inform on the relevance of the findings to humans. Thus, they are regarded as being of relevance to humans.

In terms of the multi- and two-generation studies, both of these had deficiencies that reduced their quality and the information available from them. There was no information on whether the effect on fertility was mediated through the males or the females. Given the weaknesses in both studies, together with only one species (rat) having been investigated, the fact that a clear dose-response was not always obtained, with no gross or histopathological evidence of damage to the reproductive organs, Category 2 is considered to be more appropriate for the fertility effects.

4.11.6 Conclusions on classification and labelling

CLP Regulation:	**Repr Cat 2; H361f**

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) Foetal % (litter %)	 1 (1) 0.5 (3.6)	 6 (6) 3.8 (27.3)	 6 (6) 3.4 (24.0)	 13 (9) 7.7** (36)	 42 (20) 21.2** (74.4)
Cervical ribs Foetuses (litters) Foetal % (litter %)	 4 (4) 2.1 (14.3)	 2 (2) 1.3 (9.1)	 3 (3) 1.7 (12)	 6 (3) 3.6 (12.0)	 13 (9) 6.6 (32.1)
Extra ribs Foetuses (litters) Foetal % (litter %)	 5 (5) 2.6 (17.9)	 8 (8) 5.1 (36.4)	 9 (8) 5.2 (32.0)	 19** (10) 11.3** (40)	 55** (24) 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 can not 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.**

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Information on acute neurotoxicity is presented in section 4.3 on STOT-SE. There are no available studies on the repeated-dose neurotoxicity of triadimenol.

4.12.1.2 Immunotoxicity

No information available.

4.12.1.3 Specific investigations: other studies

No information available.

4.12.1.4 Human information

No information available.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Triadimenol exists as two diastereomers, referred to as A and B . Some environmental studies were able to differentiate between the isomers, but others could not. The two environmental simulation studies do have measurements of A:B, and slight differences were noted in the ratios at the end of the tests. The Draft Assessment Report (DAR) produced under Directive 91/414/EEC concluded the differences were not of practical concern for environmental exposure or the risk assessment. The active substance was therefore considered as the sum of the isomers.

5.1 Degradation

Table 21: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Not specified	Hydrolysis T1/2 > one year	Hydrolytically stable	Nicholls & Thornton, 1980
EPA 161-2	Aqueous photolysis T1/2 = 9 days	Not rapidly photodegradable	Brumhard and Sneikus, 2002
Not specified	Water-sediment whole system DT50 > 377 days	Indicates *not readily biodegradable*	Anderson, 1986, revised 2002; Schäfer, 2002

5.1.1 Stability

A hydrolysis study using radio-labelled triadimenol was run at pH 4.5, 7.1 and 9.2 at 20 and 40°C for 32 days. The half-life of triadimenol at 20°C was estimated as above one year at all pHs. Due to the age of the study, this was not to GLP or a specific test guideline. (DAR reference: Nicholls & Thornton, 1980)

Three aqueous photolysis studies are available. The most modern was conducted using radio-labelled triadimenol with high chemical purity (>99% pure) and carried out according to EPA guideline 161-2. The test was run for 12 days and used a xenon lamp filtered to exclude wavelengths below 290 nm. Under sterile experimental conditions of continuous illumination, triadimenol degraded with a mean first order DT50 of 9 days. This was considered by the study authors to be equivalent to an environmental half-life of 48 summer days (June) in Athens, Greece (38.03˚N). It was also found that triadimenol only exhibited very limited adsorption of light in the range 290 – 292 nm. No individual metabolites ≥10% applied radioactivity (AR) were observed to occur. (DAR reference: Brumhard and Sneikus, 2002)

A second study using 96.7% purity triadimenol was considered invalid due to photosensitising impurities in the test solutions. The third study determined a DT50 of 1.7 days under artificial light; however due to its age few details are available for the study, for example the light source. This means that the study is of limited use.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

No estimation of biodegradation was made in the DAR.

5.1.2.2 Screening tests

There is no ready biodegradation study available for this substance, and the notifier proposed that the substance is not readily biodegradable.

5.1.2.3 Simulation tests

An aerobic water/sediment simulation study using radio-labelled triadimenol is available. It was conducted prior to both GLP and a specific test guideline being available. The study was run for 13 weeks at 22°C using two systems, a freshwater sediment (from a drainage ditch of a fruit orchard) and a pond sediment (from a recultivated gravel pit). Samples were taken for analysis in weeks 0, 2.5, 5, 9 and 13.

Mineralisation was relatively low, with 2.8 – 3.8% AR at the end of the test, which supports the assumption of not readily biodegradable. Only two metabolites were identified, triadimefon and M02 (see soil degradation below for structures). The maximum occurrence of triadimefon was 0.7% AR in sediment at 5 weeks after treatment; the maximum occurrence of M02 was 1.7% in water at 13 weeks after treatment. This indicates that little degradation of the parent substance had occurred by the end of the test. The formation of unextracted residues was relatively low, being 0.6 – 2.6% AR at the end of the study.

The study indicated relatively high levels of partitioning of triadimenol to sediment, particularly in the system with higher organic carbon content in sediment. In this system, 52.3% AR was found as triadimenol in sediment at 2.5 weeks after treatment. The fate of the two isomers was similar in both systems. There was a slight change in the isomer ratio during the study but this was not considered to have any practical impact in the DAR. Dissipation from the water phase in both systems could not be calculated with any degree of reliability with first order kinetics. This was explained by water phase kinetics being dominated by partitioning into sediment in both systems. Although there was no calculation of actual degradation rates in water, the results of the sediment/water study were interpreted as indicating that true degradation in this study was likely to be slow, and possibly little different from the slow degradation seen in the aqueous hydrolysis study.

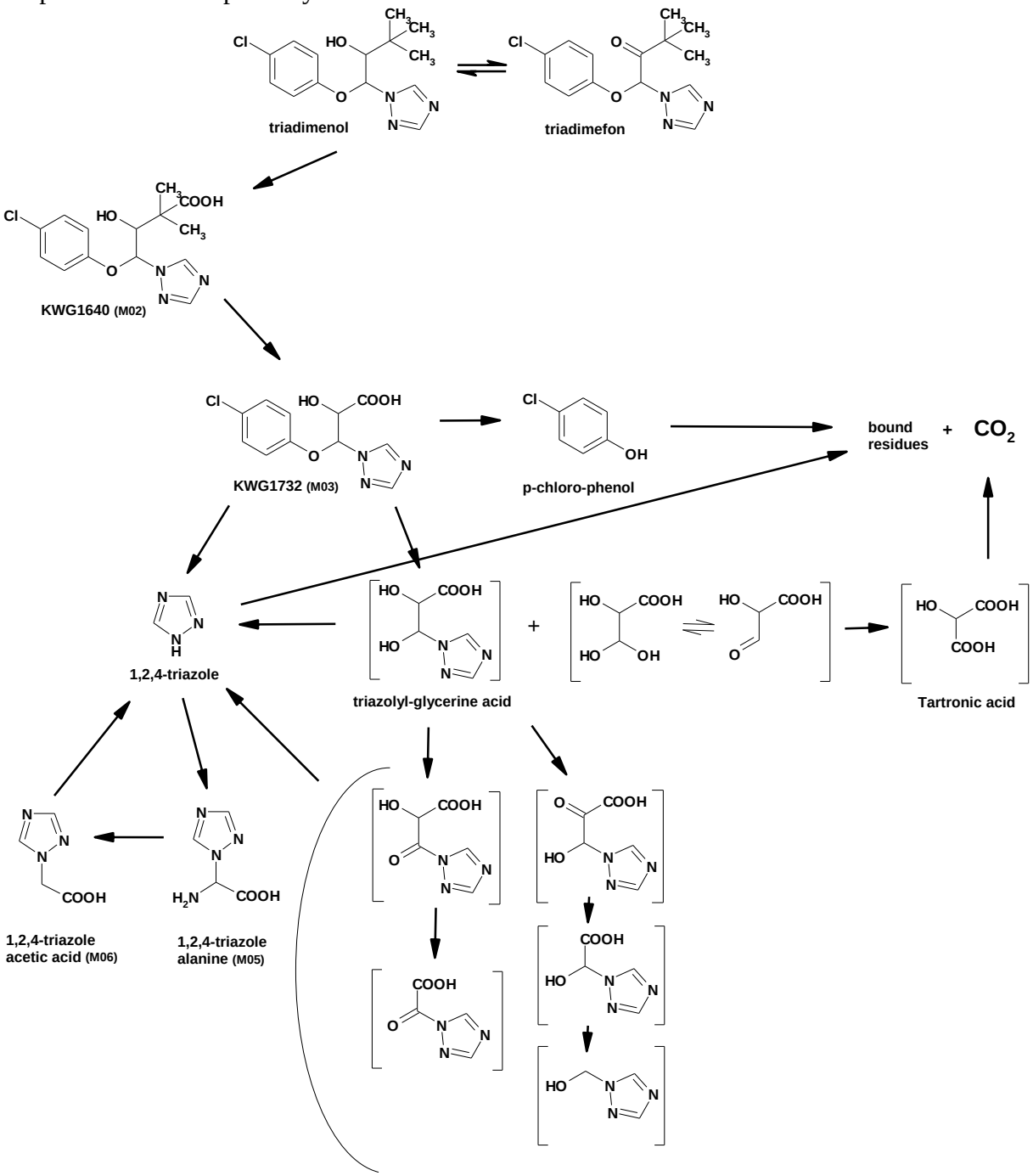
There was some criticism in the DAR of the limited number of samples taken in the study (five) and the location of the radio-labelling in the phenyl ring (the issue being that a third potential metabolite, M04, 1, 2 4-triazole would not be detected). However, given the slow degradation of triadimenol, very low metabolite formation and that identified metabolites all retained the triazole ring, the DAR indicates that it is unlikely that M04 would have been formed in significant quantities in the study. Overall the study was *considered useful for regulatory decision making* in the DAR. (DAR reference: Anderson, 1986, revised 2002)

Later work (DAR reference: Schäfer, 2002) to calculate DT50s at 20°C from the above study determined total system DT50s as 443 and 377 days for the freshwater and pond sediment systems respectively.

Two aerobic soil degradation studies are available. The first study was run according to BBA guidelines IV, 4-1, 1986 and to GLP. It used radio-labelled triadimenol (containing an isomeric ratio A:B of 55:45) applied to four different soils (two silty loams, a sandy loam and a loamy sand). The study was run for 100 days in the dark at 20°C with samples taken on days 0, 3, 7, 14, 28, 49/56, 77 and 100. The ratio of isomers A:B was found to decline gradually in soils with the greatest ability to degrade total triadimenol most quickly (ratio at 100 days: 35:50). In soils with the least ability to degrade total triadimenol, the isomer ratio remained virtually unchanged over time (i.e. 55:45). DT50s for the different soils were calculated as being between 47.3 and 362 days. (DAR reference: Brumhard, 2003). The second study was conducted according to BBA leaflet no. 36 (1973) and used two BBA standard soils 1 and 2. There are only brief details of this non-GLP study. It was run for 180 days at 22°C using cold triadimenol. The calculated DT50s normalised to 20°C were 134 and 349 days. (DAR reference: Voegeler, 1976).

The DAR proposes the metabolic pathway for triadimenol in aerobic soil below.

Proposed metabolic pathway for triadimenol in aerobic soil



Triadimefon = M01; KWG1640 = M02; 1,2,4-triazole = M04; p-chloro-phenol = M07

5.1.3 Summary and discussion of degradation

Triadimenol was found to be stable to hydrolysis. Rapid photolysis was not observed in the laboratory studies. A ready biodegradation study is not available, but no rapid biodegradation was indicated in the water/sediment or the soil degradation studies. Overall there is good evidence that the substance is not rapidly biodegraded. Due to the stability and lack of biodegradation, the environmental classification of the substance should be made using the properties of the parent substance.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

The adsorption of triadimenol was described by three separate studies covering a total of 14 soils. Two of the studies pre-dated requirements for GLP compliance but were considered relatively well reported and the adsorption coefficients derived to be reliable. In spite of the large number of soils tested, the range of pH encompassed did not cover alkaline soils. The notifier has addressed this, considering that the molecule is in a non-ionised state between pH 3-10, and would not be expected to show any particular pH related dependency at environmentally relevant pH. A wide range of other soil properties was considered. There is no obvious trend between pH and Koc in the experimental data.

Koc values were between 108 and 702 ml/g. The arithmetic mean and the median of the values were both 273. (DAR reference: Vogeler, 1978; Puhl and Hurley, 1978, revised 1983; Hein, 2002)

5.2.2 Volatilisation

The DAR states that triadimenol has a vapour pressure of 6×10^{-7} and 4×10^{-7} Pa (diastereomers A and B respectively) at 20˚C and Henry's Law Constant of 3×10^{-6} and 4×10^{-6} Pa.m3/mole (diastereomers A and B respectively). It is therefore not considered volatile. (Webber & Krohn, 1996)

5.2.3 Distribution modelling

Not relevant to classification.

5.3 Aquatic Bioaccumulation

Table 22: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
EPA-FIFRA 72-6	BCF = 21 l/kg		Forbis, 1987

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

The substance has a log K_{ow} between 3.08 and 3.28, which implies a moderate bioaccumulation potential. No other modelled data are available.

5.3.1.2 Measured bioaccumulation data

A fish bioaccumulation study with radio-labelled triadimenol (99% radiochemical purity) was run according to EPA-FIFRA 72-6 and GLP. This used bluegill sunfish (*Lepomis macrochirus*) and was conducted under flow-through conditions. The uptake period was 28 days and the depuration period was 14 days. One test concentration of 0.97 mg a.s./l with dimethyl formamide (DMF) solvent was used. The concentration of DMF was not indicated in the DAR. A control, also using DMF, was run in parallel. The uptake phase was initiated by transferring 125 acclimatised fish into each of the control and test chambers (two chambers per treatment). Fish were sampled on days 1, 3, 7, 14, 21

and 28 of the uptake phase and days 0, 0.17, 1, 3, 7, 10 and 14 of the depuration phases of the study. No treatment-related mortality or adverse effects on the fish were noted and environmental parameters were within acceptable limits throughout the study.

The time to reach 90% of steady-state was 1.4 days. The daily bioconcentration factors of triadimenol ranged from 7.1 to 27 for whole fish, 11 to 44 for visceral portions and 1.9 – 5.4 for edible portions. When moved to clean water, residues of triadimenol depurated with a half-life of 0.42 days. After 14 days in clean water 99% of the mean plateau radioactivity was depurated from whole fish and a whole fish BCF of 21 was estimated. No mention of lipid measurement is made in the DAR. (DAR reference: Forbis, 1987).

Information on degradants: A 42-day flow-through fish bioaccumulation study using *Lepomis macrochirus* is available for the metabolite triadimefon (M01). This determined a whole fish BCF of 64.

5.3.2 Summary and discussion of aquatic bioaccumulation

Measured data suggests that neither triadimenol nor its degradants bioaccumulate significantly. The BCF value for triadimenol of 21 is lower than the trigger values under CLP..

5.4 Aquatic toxicity

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Three 96-h acute fish tests are available. Nominal concentrations were used to calculate the results as measured concentrations were indicated to have been maintained at >80% in all the tests. In addition a prolonged fish toxicity study is also available. The key information from these is summarised in the following table:

Table 23: short-term toxicity to fish

Purity	Species	Test guideline	Endpoint	Toxicity value in mg/l of a.s.	Conditions	Reference
96.3%	Rainbow trout *Oncorhynchus mykiss*	OECD 203	96-h LC50 96-h NOEC	21.3 6.25 (nominal concentrations)	Static	Dorgerloh (1993a)
94.9%	Golden orfe *Leuciscus idus*	n/a	96-h LC50 96-h NOEC	17.4 <15 (nominal concentrations)	Static	Hermann (1979)
96.3%	Golden orfe *Leuciscus idus*	OECD 203	96-h LC50 96-h NOEC	27.3 6.25 (nominal concentrations)	Static	Dorgerloh (1993b)
96.3%	Rainbow trout *Oncorhynchus mykiss*	OECD 204	28-day LC50 28-day NOEC	11.4 3.13 (mean-measured concentration)	Semi-static	Dorgerloh (1993)

The Golden Orfe test (Hermann, 1979) has the lowest 96-h LC50 of 17.4 mg/l. This is an old non-GLP study and was not stated as being run to a specific test guideline. The fish loading appears to exceed the current guideline at some concentrations, and different numbers of animals appear to have been used for different concentrations (between 10 and 30). Nominal concentrations of 0, 15, 16, 17, 17.35, 17.70, 18.4, 19.2 and 20 mg a.i./l were run, with acetone used as an adjuvant (concentration not stated). During the test no effects were observed in the control fish. No mortality was observed in the lowest concentration, although sub-lethal effects were seen. The 96-h LC50 based on nominal concentrations was 17.4 mg a.i./l. The report is relatively concise and some minor information is missing, such as pH and light/dark cycle. However it was stated that oxygen levels were maintained above 7 mg O_2/l. Concentration measurements were made in a separate aquarium that did not contain fish. These indicated that the substance was stable during the test period (>80% between 2 and 96 hours). Comparison with measured concentrations in the other two (GLP compliant) acute fish studies indicated that the test substance was also stable in the presence of fish during the test period (mean-measured concentrations > 80% nominal). Together it seems reasonable to assume that the test substance was stable in the earlier test in the presence of fish, and therefore the reporting of the results as nominal concentrations is acceptable.

Overall while the test has some weaknesses, it is in line with the other fish results, including one conducted on the same species. The purity of triadimenol in the earlier test is slightly lower than the later studies, however again the results are similar for all three tests, so the purity does not appear to have affected the outcome of the tests.

An OECD 204 prolonged fish toxicity study was run for 28 days under semi-static conditions using *Oncorhynchus mykiss*. This used 96.3% purity triadimenol. Fish were hatched at the test facility and held for at least 14 days prior to test commencement. At the beginning of the test mean body weight of the fish was 2.9 g and mean body length was 6.2 cm. Nominal concentrations were 3.13, 6.25, 12.5, 25.0 and 50.0 mg/l. Mean-measured results were LC50 = 11.4mg/l and NOEC = 3.13mg/l. 100% mortality was observed at 25.0 and 50.0mg/l. The study was GLP compliant. (DAR reference: Dorgerloh, 1993)

Tests for degradants: The following endpoints from reliable and GLP-compliant studies are available from the DAR:

Triadimefon (M01): *Oncorhynchus mykiss*: 96-h LC50: 4.08 mg/l; NOEC: 0.71 mg/l

Triadimefon (M01): *Lepomis macrochirus*: 96-h LC50: 10.0 mg/l; NOEC: 3.50 mg/l

1,2,4-triazole (M04): *Oncorhynchus mykiss*: 96-h LC50: 760 mg/l; NOEC: 100 mg/l

5.4.1.2 Long-term toxicity to fish

Table 24: Long-term toxicity to fish

Purity	Species	Test guideline	Endpoint	Toxicity value in mg/l of a.s.	Conditions	Reference
99.2%	Fathead minnow *Pimephales promelas*	OECD 210: Fish early life stage test	35-day NOEC (growth)	0.17 (mean-measured concentration)	Flow-through	Nieden & Lam (2007)
99.2%	Fathead minnow *Pimephales promelas*	Fish screening assay (similar to OECD 230 adopted in 2009)	21-day NOEC, based on plasma vitellogenin (VTG) levels	0.03 (nominal)*	Semi-static (weekly renewal)	Teigeler, M. (2007)
99.9%	Fathead minnow *Pimephales promelas*	Fish sexual development test (similar to OECD 234 adopted in 2011)	129-132 day NOEC,	not determined*	Flow-through	Bomke, C. (2010)

* See following discussion of issues relating to the endpoint from the FSDT and the relevance of this and the FSA endpoint to hazard classification.

Early life stage toxicity of triadimenol to *Pimephales promelas* (Nieden & Lam, 2007)

Subsequent to the submission of the DAR the notifier has provided a FELS test using *Pimephales promelas* to the UK CA. These data are not included in the DAR. This test was conducted using 99.2% purity triadimenol. Newly fertilised eggs were exposed to mean measured concentrations of 0.05, 0.10, 0.17, 0.38, 0.75, 1.69 and 3.23 mg a.i./l dispersed in diluent water using acetone (l00µl/l). Control and solvent controls were also run. Four replicates per level were run, with 35 eggs per replicate, which were thinned to 20 alevin after the hatching phase. The test was conducted at 25°C (± 1°C) under flow-through conditions.

Hatching was completed five days after initiation of the study (designated day 0 post-hatch). There was no significant difference between controls and the other treatments. There was also no difference for alevin survival (measured at day 5), but fry survival was lower for the top two concentrations (measured at day 35). Impacts on growth were also seen for the higher concentrations. The most sensitive end point was growth (weight) with a mean-measured NOEC of 0.17 mg a.i./l. Environmental parameters were within acceptable limits throughout the study.

The study was undertaken according to OECD 210 (without significant deviation) and was GLP compliant. It is considered to be reliable (Klimisch 1) and suitable for use in regulatory hazard classification.

Studies on degradants: The following effects endpoints from reliable and GLP-compliant studies are also available from the DAR:

Triadimefon (M01): 35-day FELS test using *Pimephales promelas*: NOEC (growth – length/weight): 0.17 mg/l.

1, 2, 4-triazole (M04): 28-day fish juvenile growth test using *Oncorhynchus mykiss*: NOErC ≥ 100 mg/l.

Additional testing for endocrine-mediated/disruptive effects in fish

Also subsequent to submission of the triadimenol DAR, the Notifier has provided two additional tests to the UK CA on potential endocrine-mediated or disruptive effects of the substance on fish. These were a fish screening assay (FSA) on fathead minnow (*Pimephales promelas*) by Teigeler, M. (2007) and a fish sexual development test (FSDT) also on fathead minnow by Bomke C. (2010). This follows the possibility of endocrine disruption (ED) being raised during peer review of the DAR under Dir. 91/414/EEC. As ED was not then included as a standard requirement under that Directive, any ED assessment was left as a matter for individual Member States. However, subsequently a need for ED characterisation was highlighted for the triazole group of fungicides, including triadimenol. Most triazole pesticides were then given the following confirmatory data requirement: '*Further information addressing the potential endocrine disrupting properties of [active substance] within two years after the adoption of the OECD test guidelines on endocrine disruption or, alternatively, of Community agreed test guidelines*'.

At the time of writing the Commission has yet to finalise its guidance on ED testing and hazard/risk assessment - the submitted 'confirmatory' ED studies have not therefore been considered at Community level under Dir. 91/414/EEC, or the subsequent pesticides Regulation (EC) No. 1107/2009. There have, however, been developments in ED testing at the OECD and the two submitted studies are based on OECD guidelines which are generally considered to be appropriate

to at least screen for such effects. Whilst there is not yet formal agreement at an EU level on the precise type of testing to be conducted for ED effects and how this should be interpreted, the two submitted studies will be considered against the existing OECD protocols on which they were based. The reliability and relevance of these tests and their endpoints to environmental hazard classification under the CLP Regulation is also discussed.

These ED tests were initially requested by the German regulatory authority (UBA) and they have already conducted an evaluation of them under their national risk assessments for plant production products. These initial evaluations have been translated and submitted to the UK CA and reference is made to them, along with the Notifier's evaluations, in the following summaries:

Fathead minnow, fish screening assay - Triadimenol (Teigeler M. , 2007)

Document No: M-285905-01-1 (report no. EBBTX045)
Guidelines: OECD FSA test protocol (ENV/JM/TG/EDTA (2004) 1 REV 2). There were no substantive differences from the current OECD 230 version adopted in 2009.
GLP: Yes (certified laboratory)

Objective:
A fish screening assay (FSA) was performed to identify a potential impact of the active substance triadimenol on the estrogenic and androgenic pathways of sexually mature fathead minnow during a short-term exposure for 21 days including reproduction.

Material and methods:
Triadimenol Technical, purity: 99.2% a.s., Batch number: 40055060.
The study was performed with male and female adult fathead minnows (*Pimephales promelas*) from an in-house culture. After >2 weeks acclimation, the exposure commenced with sexually dimorphic fish in good spawning condition (<5% mortality over 7 days post 48-h settling in period). Day 0 mean weight & length were: males: 1.7g / 5.2cm; females: 1.0g / 4.4cm; variation was within 20% of mean.
Fish were exposed to the test item for 21 days under static-renewal conditions with weekly renewal at nominal test concentrations of 0.3, 3.0, 30, 300 and 3000 µg a.s./L and a control. The setting of these test concentrations was stated to be based on previously known chronic toxicity. (Note: the guideline suggests using a flow-through design, however the test substance was sufficiently stable in solution to permit static-renewal).
One 260 L glass aquarium per concentration and the control was divided into four compartments each of 60 L with stainless steel nets. Two male and 4 female fish were distributed to each compartment (pseudo-replication).
Ten days before the start of the exposure period, a pre-exposure period was initiated. In this period, the spawning status was recorded and egg numbers were estimated to determine a base-line fecundity of each spawning group.
Fish were fed twice daily *ad libitum* with freshly hatched brine shrimp (*Artemia* sp.) nauplii and ground flake food; any excess was removed. The purified aquarium water had a hardness of 90 mg $CaCO_3$/L; aquaria were kept under 16/8 h light/dark cycle and at 480 lux.

The test item concentrations were chemically analysed (using liquid chromatography tandem mass spectrometry) at the start, before and after each weekly water exchange and at the end of the test period.
The daily biological assessment was based on the following biomarker endpoints: secondary sex characteristics, vitellogenin blood plasma concentration, 11-keto testosterone blood plasma

concentration and gonad histopathology. Fecundity, fertility and growth were also evaluated against controls.

Results and discussion:

Analytical Findings:

The following water quality parameters were determined during the test:
Temperature ranged from 22.7 to 23.5 °C; pH was between 7.1 and 8.5 (Note: guideline variability is 0.5 units) and oxygen saturation was 74% to 94%. Any slight protocol deviations are considered unlikely to have significantly affected the results.
All measured concentrations of triadimenol were between 80% and 111% of nominal concentrations. Thus, effect concentrations were within ±20% and were based on nominal concentrations.

Biological Findings:

Observations regarding abnormal behaviour or symptoms, clinical signs (sublethal effects) or mortality in the tested concentrations resulted in no observable difference compared to the controls during the 21 days of exposure.

The assessment of reproduction did not indicate a statistically significant effect ($p<0.05$) expressed as estimated egg numbers per day and female. Only a slight (non-significant) decrease was observed at 300 and 3000 µg a.s./L. This could hint at an endocrine modulation potential, but as noted under the histopathological results, given the findings in the liver, the reduction in eggs/female/day could also be indicative of generalized systemic toxicity.
No significant effect on the fertilization rate was observed in the assessed egg clutches over the exposure period of 21 days.

No statistically significant effect was detected on fish growth in male and female fish (expressed as pseudo-specific growth rate based on standard length and wet weight, respectively, over 21 days). Only a slight (non-significant) decrease in fish weight was observed in the highest test level at 3000 µg a.s./L in both genders. This slight decrease could be also correlated to the systemic effect of triadimenol considering the histopathological findings in the liver.

Regarding observations of secondary sexual characteristics during the exposure period, no relevant differences were observed in comparison to the control group. Assessment of the specialised male secondary sex characteristic biomarker in fathead minnows at test termination (i.e. the nuptial tubercles) showed the following results:
The number of nuptial tubercles in males was not affected in any of the treatment levels. The nuptial tubercles score values were slightly, but not statistically significantly, reduced at the low and medium test concentrations, but showed no clear concentration-response relationship.
In females all parameters, except the parameter 'vertical bands', showed no difference to the control. The occurrence of such a typically male secondary sex characteristic in a single or a few females can be interpreted in this study as transient, random and not concentration-related observation, which occurred under spawning conditions also in control females in this study, and (according to the authors) frequently in several other experiments.

The biomarker endpoint vitellogenin concentration in blood plasma showed a significant reduction in female fathead minnows at the two highest test concentrations of 300 and 3000 µg a.s./L, respectively. The vitellogenin synthesis in the liver of intact organisms is strictly under endocrine control. The observed decrease of the vitellogenin concentration was explained as an inhibition of the key enzyme aromatase, which catalyses the formation of 17 beta estradiol by aromatisation of

testosterone. However, taking into account the clearly observed degeneration of the liver tissue at 3000 µg a.s./L and the incipient liver changes observed at 300 µg a.s./L, the study authors proposed that reduced VTG levels at these concentrations could also be explained with generalised systemic liver toxicity effects.

Regarding the vitellogenin concentration in male blood plasma, a slight increase was seen across concentration levels but this was only statistically significant at 30 µg a.s./L Considering all concentration levels, no other statistically significant effect was seen and there was no clear concentration-related response.

The biomarker endpoint 11-keto testosterone (relevant male sex steroid in fish) in blood plasma of male fish was slightly, but not statistically significantly decreased at test concentrations of 3.0, 30 and 300 µg a.s./L and showed a possible non-monotonic concentration response curve. The 11-keto testosterone plasma level reached the control level again at higher test concentrations or was slightly increased. This may indicate active endocrine regulation mechanisms (e.g. anti-androgenic versus androgenic), but was considered by the authors not to be relevant for assessing effects at the population level.

The female and male gonads were unaffected by exposure to the test compound up to and including 3000 µg a.s./L regarding the stage of development and the distribution of histopathological findings throughout the groups. From the viewpoint of overall pathology, a concentration of 3000 µg a.s./L lead to systemic toxicity but did not cause any effect on gonad morphology.

In the liver, clear degenerative and inflammatory changes were seen in fish exposed to 3000 µg a.s./L. What were described as 'incipient liver changes' were observed at 300 µg a.s./L. This included single cell necrosis, condensed hepatocellular cytoplasm and a slight increase of fatty vacuolation. However, these were not all clearly concentration-correlated, they were described as minimal or slight effects and were only found in a few of the necropsied fish. Therefore, the concentration of 300 µg a.s./L was proposed as a 'borderline level' for liver toxicity. Since no statistics were done on the histopathology, it is not clear whether the NOEC for liver effects in this study would be 30 or 300 µg a.s./L.

Conclusion of FSA:

A significant reduction of the vitellogenin concentration in female blood plasma at 300 and 3000 µg a.s./L was observed, which might indicate an inhibition of sexual endocrine mechanisms. However, since liver toxicity occurred in the same concentrations (particularly at 3000 µg a.s./L) a general systemic toxicity effect cannot be excluded.

The general systemic toxicity effect could be applied also for the interpretation of the reproduction (fecundity) and the growth (male and female pseudo-specific growth rate based on wet weight) results, where slight but non-statistically significant decreases were recorded at the highest test concentration(s).

The fertilization rate was not affected in any test level in this study. In view of the lack of adverse findings in the gonad tissues of both sexes (up to the highest test level), no adverse effect on the reproductive success were anticipated.

The potential non-monotonic concentration response relationship of the 11-keto testosterone level, which showed a slight decrease at the medium test levels, was interpreted as a slight trend (e.g. anti-

androgenic effect) and thus not relevant, because no corresponding effects on reproduction were seen at this concentration.

Therefore, based on the above findings, the overall concentration (NOEC) where no treatment-related adverse effects were seen in this 21-day fathead minnow study was considered to be 30 µg a.s./L (0.03 mg triadimenol/L).

This fish screening assay is considered to be reliable without restriction (Klimisch 1).

Triadimenol - fish sexual development test (FSDT) with fathead minnow (Bomke C., 2010)

Document No: M-386734-01-1 (report no. EBBTL007)
Guidelines: OECD FSDT draft test protocol (version of February 2009). There were no substantive differences from the current OECD 234 version adopted in 2011, or the existing guideline 210.
GLP: Yes (certified laboratory)

Objective:
The aim of this Fish Sexual Development Test (FSDT) was to determine potential effects of the test item during the early-, juvenile- and sexual maturation life stages of fathead minnow (*Pimephales promelas*), with the main focus on sexual differentiation, gonad development *via* histopathological evaluations and plasma vitellogenin (VTG) concentrations.

Material and methods:
Test item: triadimenol (tech.) – KWG 0519, purity 99.9% (w/w), specified by batch No.: 40085003, article No.: 04900928, specification No.: 102000006060.
Test organism: Fathead minnow (*Pimephales promelas*) from in-house culture, freshly fertilised eggs (840 total) < 24 hours old, were introduced at the start of exposure. The minnows were exposed to the test item concentrations or a dilution water control under continuous flow-through conditions for 129-132 days (123-126 days post-hatch). There were four replicates per test concentration and eight replicates of the control, with 30 eggs per replicate. Post-hatch began on day 6; at day 39, the fish were transferred to larger aquaria, at day 46 the fish numbers across the replicates were adjusted to be similar (range across replicates reduced from 15-26 to 18-21).
Mortality and larval hatching were assessed daily. Behavioural impacts and abnormal physical changes were recorded at least on working days. Hatched larvae were fed with live brine shrimp nauplii three times daily (twice at weekends). Additionally, ground flake food (Tetramin®) was fed on working days once per day. Excess food was removed and all feeding ceased 24-h prior to study termination. Termination of test was over four days (days 129 - 132) due to need to assess fish instantly.

In Phase 1 of the study (days 0-39) there were 11 exchanges of solution/day; in Phase 2 (days 39-132) there were 6 exchanges of solution/day. There was a minor issue with the solution delivery system but it was not deemed to affect the test concentrations or results.
Stock solutions were prepared by either ultrasonic or heating (up to 100 ^{o}C), then intense stirring at room temperature overnight. Measured concentrations appear unaffected by this treatment (see analytical findings), although it is unclear if there was any effect on the stereo-chemistry. The definitive study was conducted at the nominal test concentrations of 0 (control), 5.12, 12.3, 29.5, 70.8 and 170 µg a.s./L. Concentrations of triadimenol were measured in the test media from alternating replicates using HPLC, starting on study day -1 and 0 and thereafter in weekly intervals.

Results and discussion:

Analytical Findings:

The analytical findings corresponded well the expected nominal concentrations of the test item. The mean measured concentrations of triadimenol were 0, 5.50, 13.1, 32.2, 72.0 and 178 µg a.s./L, which ranged from 102 to 109% of nominal during the test period in all test levels. The reported results therefore refer to the nominal concentrations of triadimenol.

Test temperature in controls in Phase 1 was: 23.5 - 26.2 ^{o}C; in Phase 2 it was: 23.1 - 26.1 ^{o}C. In the treatment groups temperature varied from: 23.6 - 26.0°C overall.

Hardness was 43.6 - 49 mg $CaCO_3$/L. pH was 6.7 - 7.5.

Oxygen saturation varied between 87-89% of the air saturation and ranged from 61 - 101% during the whole test period for all test levels.

The aquaria were kept under a 16/8 hour light/dark cycle at 120 - 208 lux (Phase 1) and 215 - 496 lux (Phase 2).

Biological Findings:

Regarding the time to hatch and the egg hatchability on study day 6; the hatching rate on days 6 and 7 indicates a reduction at 70.8 and 170 µg/L, although the effect (approx. 10 %) is not statistically significant ($p<0.05$). However, the control shows a relatively moderate hatching rate (84.6 +/- 9.7 % for 8 replicates) with two negative outliers (70.0 % and 73.3 %). The validity criterion for hatching success in controls from OECD 234 is >80%, so the control variability drops below this level. Overall no statistically significant difference to the pooled control could be observed in all test levels. According to the study authors, the endpoint 'time to hatch' from study day 4 - 7 and 'egg hatchability' on study day 6 resulted in a NOEC ≥ 170 µg a.s./L and a LOEC > 170 µg a.s./L.

Regarding the embryo survival between study day 0 and 6, the larval survival between study day 7 and 45 and the juvenile or adult survival between study day 46 and test termination (study day 129-132); no significant difference compared to the control data for all test levels was determined. Regarding the cumulative survival between study day 0 and test termination (study day 129-132); a slight statistically significant difference was observed at the concentration of 70.8 µg a.s./L. At the higher concentration of 170 µg a.s./L the cumulative survival was not significantly different to the control so there was no clear concentration-response relationship. However, it is observed that survival was also relatively low in the controls (66.3 +/- 3.3 %). The validity criterion for post-hatch survival in controls from OECD 234 is ≥ 70%, so this raises concerns about the reliability of this endpoint and the study. Overall, the endpoint 'survival' (embryo, larval, juvenile, adult and cumulative, respectively) was considered by the study authors to result in a NOEC ≥ 170 µg a.s./L (the higher test concentration) and a LOEC > 170 µg a.s./L.

Morphological and behavioural effects (sublethal findings/symptoms) including secondary sexual characteristics assessed during the exposure and also gross morphological observations at necropsy (test termination) resulted in a NOEC ≥ 170 µg a.s./L and a LOEC > 170 µg a.s./L.

Regarding the growth parameters at test termination (study day 129-132) a statistically significant increase in male wet weight in comparison to the control data was determined at the test level of 70.8 µg a.s./L. At the higher concentration of 170 µg a.s./L the male wet weight was not significantly different to the control, so no clear concentration-response relationship was observed. For the standard length of males and the standard length and wet weight of females, respectively, there was also a slight increase in male length at 70.8 µg a.s./L but no statistically significant effect was seen up to and including 170 µg a.s./L. The endpoint 'growth at test termination', expressed as standard length and wet weight, therefore resulted in a NOEC ≥ 170 µg a.s./L and a LOEC > 170 µg a.s./L for males and females.

Endocrine Biomarkers:
Regarding the mean total number of nuptial tubercles per male; a concentration-related increase from controls was observed at all concentrations leading from 5.12 up to 70.8 µg a.s./L and the difference was statistically significant at this penultimate concentration. However, at the higher concentration of 170 µg a.s./L the number of nuptial tubercles per male was not significantly different to the control and were near control numbers and less than that seen at 5.12 µg/L. Therefore no concentration response relationship was observed. The authors argued that fewer males at 70 µg/L meant higher numbers of nuptial tubercles were likely to be due to there being fewer fish, and in particular fewer males, present. The biomarker 'secondary sexual characteristics', based on the number of nuptial tubercles per male at test termination, therefore resulted in a NOEC ≥ 170 µg a.s./L and a LOEC > 170 µg a.s./L.

Data analysis regarding the male VTG levels resulted in a statistically significant increase of vitellogenin concentration at the test level of 70.8 µg a.s./L only. At the higher concentration of 170 µg a.s./L the male vitellogenin plasma concentration was not significantly different to the control. Therefore, according to the study authors, no concentration-response relationship was observed. An increasing trend was seen at the lower concentrations, but this was not statistically different. The authors argued that the wider control fish data available in the literature for the test suggest the male VTG concentrations in the study are not unusual but possibly just reflect earlier sexual maturation of the male fish. Again it was suggested that this effect results from there being fewer fish at 70 µg/L. In females no statistically significant difference in the vitellogenin plasma concentration compared to the control data was determined. The authors therefore considered that the biomarker 'vitellogenin plasma concentrations' in males and females at test termination, resulted in a NOEC ≥ 170 µg a.s./L and a LOEC > 170 µg a.s./L.

According to the study authors, the determination of gonadal sex revealed no exposure related shift of sex ratio up to and including 170 µg a.s./L. The further detailed histopathological investigations apparently gave no evidence of any influence of the test item on gonads and on gonad maturation in both sexes (males: testes and sperm ducts; females: ovaries and oviducts) up to and including 170 µg a.s./L. The investigation of other organs present (liver, kidneys, intestine) also reported no evidence of any induced findings up to and including 170 µg a.s./L. According to the authors, the biomarkers 'sex ratio' and 'gonad histopathology at test termination' therefore resulted in a NOEC ≥ 170 µg a.s./L and a LOEC > 170 µg a.s./L for males and females.

Validity criteria:
Test conditions of the study met the validity criteria, given by the draft guideline proposal as well as most of those in the later agreed OECD guidelines no. 210 and 234. As mentioned above, the OECD 234 validity criteria for hatching success and post-hatch survival in controls were however not entirely met. It is also noted that that the use of fathead minnow was only partially validated and it is not one of the standard species recommended in OECD 234.

Conclusion of FSDT:
Based on the results of this study it was proposed by the study authors and Notifier that the overall chronic (129-132 day) NOEC for triadimenol to early, juvenile and sexual maturation life stages of fathead minnow is the highest concentration tested, i.e. 170 µg a.s./L. The overall chronic (129-132 days) LOEC would therefore be > 170 µg a.s./L.

There was disagreement from the UBA evaluators on some aspects of the study. They reported

increased liver damage for all treatments, especially at 70.8 µg/L (increased vaculation); increased kidney damage (nephropathy/tubular dilation/proteinaceous casts) at 70.8 µg/L and less underdeveloped seminal tubules at up to and including 12.3 µg/L, with mineralisation increased for all treatments. Of the statistical comparisons, only the liver damage in males was statistically significant at 70.8 µg/L, – no statistical difference was observed at the higher 170 µg/L test concentration and therefore a concentration-response was not observed.

The UBA also point out that in the current version of the OECD guideline, the sex ratio should be evaluated separately for (a) clearly male, (b) clearly female, (c) intersex and (d) undifferentiated fish. Results of a revised statistical evaluation show that the ratio of females at 170 µg/L is significantly reduced compared to the control (ToxRat 2.10, two-page Williams Test with $\alpha = 0.05$). A NOEC of 70.8 µg/L would therefore be derived based on sex ratio. However, it is unclear if the UBA evaluation is based on sex differentiation due to histological sex (examination of gonads at necropsy, as recommended in the OECD 234 guideline) or phaenotypic sex. The Notifier has argued that after physical inspection of fish at test termination, the sex of all individuals (including undifferentiated) could be clearly identified as male or female and they still consider the NOEC to be ≥ 170 µg/L.

The UBA also note that data from the VTG plasma concentrations are asymmetrical and show multi-modal distribution; there are only few samples (n = 4-9) and many outliers. Consideration of the medians shows clear differences between the VTG plasma concentrations of the males (median of all VTG data of a treatment throughout all replicates) with the median at 170 µg/L indicating reduced VTG plasma concentration in males. This is linked to the concentration-related increase (reported above) up to 70.8 µg/L and then the apparent drop in male VTG at 170 µg/L back to control levels.

Overall, a number of less apparent 'effects' appear to occur at the highest test concentration of 170 µg/L than at the lower concentration of 70.8 µg/L. The UBA speculate that this could be due to a regulation which compensates any endocrine effect, or the toxic effects predominating here and preventing a shift to more males. Such a mechanism is unclear as histopathological results do not indicate significant effects on the liver at the highest test concentration. There may, however, be a more general problem with the performance of the study in that on day 39 the fish were transferred into larger aquaria but 9% of the fish could not be found, so that the total number was reduced from 615 to 561 (if this was due to mortality or mis-counting, it is not a specific validity criterion for the test but it could nevertheless affect reliability). Subsequently, on day 46, individual fish were moved between the replicates to achieve a comparative number of fish per replicate and treatment (mostly 20 fish/aquarium), also endangering the independence of the replicates. In particular 8 control replicates show a high variance for the relevant endpoints VTG (vitellogenin) content and sex ratio and make a reliable statistic evaluation more complicated. One reason could be that 7 out of these 8 control replicates were affected by the moving of fish between replicates. The study authors also argue that some of the apparent effects could be due to their being fewer and larger males at 70 µg/L and that wider control fish data available in the literature suggest the effects are not unusual but possibly just reflect earlier sexual maturation of the males resulting from there being fewer fish at 70 µg/L.

The UK CA also notes that although meeting the draft guideline validity criteria, the study did not entirely meet those finalised in OECD 234 for control hatching success and post-hatch survival. Fathead minnow were not actually one of the species fully validated and recommended for use in OECD 234, although, for comparison, it is useful to have the ELS, FSA and FSDT all conducted on the same species. Use of this test species is further justified in a separate expert statement (N. König and C. Bomke, 30/9/2010, 'Statement regarding the fish species (*Pimephales promelas*, fathead minnow) used for the Fish Sexual Development Test (FSDT) with the active substance triadimenol').

However, it's use does lead to increased uncertainty over reliance on this study.

Due to these uncertainties over performance and interpretation, the results from this study are not considered to be entirely reliable or convincing (Klimisch 3). The UK CA proposes that a NOEC cannot currently be determined from this study. It is not, in any case, used in the hazard classification decision below but could be examined further during the pesticide registration process if considered crucial to that process.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

One acute invertebrate study is available, and is indicated to be GLP compliant. This was conducted to OECD 202 using *Daphnia magna* and under static conditions. It used 96.6% purity triadimenol and determined a 48-h EC50 of 51 mg/l and a NOEC of 10 mg/l. Results were based on nominal concentrations. Mean-measured concentrations were only available for the lowest concentration, which suggested that the test solutions were stable. (DAR reference: Heimbach, 1989)

Studies on degradants:

Triadimefon (M01): *Daphnia magna* 48-h EC50: 7.16 mg/l, NOEC: 2.35 mg/l

1, 2, 4-triazole (M04): *Daphnia magna* 48-h EC50: >100 mg/l, NOEC: 100 mg/l

1, 2, 4-triazole (M04): *Daphnia magna* 24-h EC50: 800 mg/l (graphic estimation), NOEC: 320 mg/l

5.4.2.2 Long-term toxicity to aquatic invertebrates

Table 25: long-term toxicity to aquatic invertebrates

Purity	Species	Test guideline	Endpoint	Toxicity value in mg a.i./l	Conditions	Reference
92%	*Daphnia magna*	n/a	21-day NOEC (reproduction) 21-day NOEC (mortality)	0.199 0.145 (mean-measured concentration)	Flow-through	Lamb, 1982
97.3%	*Daphnia magna*	OECD 211	21-day NOEC (reproduction) 21-day NOEC (mortality)	1.25 n.d. (mean-measured concentration)	Semi-static	Dorgerloh & Sommer, 2001
96.9%	*Chironomus riparius*	BBA (1995)	28-day NOEC (development & emergence)	≥ 0.1 (initial concentration)	Static	Heimbach, 1998

Long-term toxicity of triadimenol to *Daphnia magna* (Lamb, 1982)

In a non-GLP study the toxicity of triadimenol (purity: 92%) to *Daphnia magna* was investigated over a period of 21 days in a flow-through system. The study was not conducted to any recognised

protocol, but generally it appears to have been conducted and reported in line with the principles of OECD 211. It used nominal concentrations of 0.050, 0.100, 0.200, 0.400, 0.800 mg a.i./l and a control. Two replicates were run at each level, with 15 animals per replicate. The daphnids were fed twice daily, and environmental parameters were within acceptable limits throughout the study. The mean concentrations of triadimenol in the test chambers ranged from 100 to 145% of the nominal concentrations.

Sub-lethal effects (loss of equilibrium) were observed at 24 and 48 hours in the 0.400 and 0.800 mg a.i./l levels. No sub-lethal effects were observed after 48 hours. There was no mortality at the two lowest concentrations tested but there was 100% mortality at the highest concentration tested. The control group appeared normal throughout the 21 days of exposure. There were no significant differences in the number of young produced in the 0.050 and 0.100 mg a.i./l concentrations compared to the young produced in the control. Even though there was 50% mortality in the 0.200 mg a.i./l level, the daphnia produced approximately the same number of young per surviving adult as the control group. There were no young produced at 0.800 mg a.i./l, and reproduction was reduced by approximately 56% among daphnia at 0.400 mg a.i./l. The mean-measured NOEC for reproduction was 0.199 mg a.i./l, and mean measured NOEC for mortality was 0.145 mg a.i./l.

Long-term toxicity of triadimenol to *Daphnia magna* (Dorgerloh & Sommer, 2001)

A second GLP study was run according to OECD 211 and US EPA 72-4 using 97.3% triadimenol using static renewal conditions (renewal every 48-72 hours). Nominal test concentrations of 0.04, 0.08, 0.16, 0.32, 0.64, 1.28 and 2.56 mg a.i./l were run as well as a control and a solvent (DMF) control. DMF solvent was used at 100 µl/l. There were ten test vessels per concentration with one test animal per vessel. The study was run for 21 days with test solutions renewed every 48 hours during the week and at 72 hours over weekends. The test organisms were fed (daily, except for the first weekend of the study), but the test solutions were not aerated during the study. Light intensity was indicated to be about 1500 lux and water quality was indicated to be within the guideline recommendations. Measured concentrations of the test solutions were between 83% and 116% of nominal. Little difference in the measurements of fresh and expired concentrations was observed.

Statistically significant mortality (>20%) was not observed in any test level and the controls. Similarly there was no statistical difference between the body length of the controls and the animals at any test level, and so the NOEC for the body length of the parent animals was 2.56 mg as/l. For reproduction, statistically significant effects were observed at 2.56 mg a.s./l, and the NOEC for reproduction was 1.28 mg a.s./l.

Long-term toxicity of triadimenol to *Chironomus riparius* (Heimbach, 1998)

A GLP compliant study was conducted in accordance with BBA (1995) method, the effect of technical triadimenol (purity: 96.9%) on the development and emergence of *Chironomus riparius* larvae in a sediment/water system was investigated. This was run using a single test concentration of 0.1 mg a.s./l and a control. 25 animals were used for each replicate and four biological replicates were run for each level. In addition one replicate was run for each level for analytical purposes. Water quality parameters for control and treatment replicates were within levels permitted under the test guideline. Measured concentrations of the test water during the study showed these declined from 100% of nominal at one hour, 80% on day 7 to 57% on day 28. Analysis of the pore water detected at triadimenol at 3.9% on day 7 and 2.9% on day 28. The DAR concluded these findings

suggested a small amount of the test substance adsorbed to the sediment and/or was degraded during the study.

The number of emerged midges was not significantly different to the control at the test concentration of 0.1 mg a.s./l, and neither was the development rate. Therefore the NOEC for both development and emergence was 0.1 mg a.s./l (initial concentration), the highest concentration tested.

Studies on degradants:

Triadimefon (M01): *Daphnia magna* – 21-day non-GLP NOEC (mortality): 0.100 mg/l

Triadimefon (M01): *Daphnia magna* – 21-day NOEC (growth): 0.052 mg/l

5.4.3 Algae and aquatic plants

One algal study is available. This was conducted to OECD 201 using *Pseudokirchneriella subcapitata* and 97.3% purity triadimenol. The 72-h ErC50 was 38 mg/l, and the NOErC was 4.7 mg/l. Results were based on nominal concentrations as measurements indicated levels were maintained at >80% of initial concentrations throughout the test. The study was GLP compliant. (DAR reference: Scheerbaum, 2001)

Studies on degradants:

Triadimefon (M01): *Pseudokirchneriella subcapitata:* 120-h ErC50: 2.01 mg/l, NOErC: 1.20 mg/l

1, 2, 4-triazole (M04): *Pseudokirchneriella subcapitata:* 72-h ErC50: >31 mg/l, NOErC: 3.1 mg/l

5.4.4 Other aquatic organisms

None

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

A ready biodegradation study is not available. The substance is hydrolytically stable and not rapidly photodegraded. Data from long-term degradation studies in water-sediment systems and soil do not indicated rapid primary degradation or mineralisation. On this basis it is concluded the substance should not be classified as readily biodegradable or rapidly degradable. The BCF value of triadimenol is 21, which is lower than the trigger values under CLP.

The ecotoxicity test results suggest the substance exhibits acute aquatic toxicity between 10-100 mg/l. This is consistent across all three trophic levels tested. The results indicate that fish are the most sensitive taxa, with all three acute fish test results lower than the *Daphnia* and algal results. The most sensitive acute endpoint is a 96-h LC50 = 17.4 mg a.i./l (nominal concentration) for *Leuciscus idus*. Whilst the result from this non-GLP test should be treated with caution, it is in agreement with the other two fish test results.

The long-term aquatic data suggest toxicity in the range 0.1-1 mg/l. Fish are the most sensitive taxa, based on the FELS study on *Pimephales promelas* (35-day mean-measured growth NOEC of 0.17 mg/l). The non-GLP 21-day *Daphnia* study also has results in this range (albeit using lower purity

test substance), but the more modern 21-day *Daphnia* study using a purer test substance found a higher NOEC (1.28 mg/l). No effects were observed in the *Chironomid* study, and so a true NOEC cannot be derived from this limit test. The growth NOEC for algae was 4.7 mg/l indicating that aquatic plants are not the most sensitive trophic level. Under the 2nd ATP of the CLP Regulation, using the chronic ecotoxicity criteria, triadimenol fulfils the criteria for aquatic environmental hazard chronic category 2. This is using the 35-day growth NOEC of 0.17 mg/l (mean-measured) from the FELS test using *Pimephales promelas*.

The recently included fish screening assay and fish sexual development test (also on fathead minnow (*Pimephales promelas*)) are of uncertain relevance in relation to aquatic hazard classification and labelling. The FSA (Teigeler, M., 2007) does give a reliable NOEC of 0.03 mg a.s./L, but it is unclear whether this resulted from an impact on sexual endocrine mechanisms or from a more generalised systemic liver toxicity. Data from mammalian toxicology tests (e.g. see Section 4.8) indicate that triadimenol can target the liver, but significant adverse liver pathology was generally seen at relatively high concentrations. It is not clear whether a NOEC for liver effects in the FSA would be 0.03 or 0.3 mg a.s./L, but the main basis for the overall FSA NOEC of 0.03 mg a.s./L was the effect on female plasma VTG levels.

In the FSDT (Bomke C.,2010) there is uncertainty over the nature of any ED effects seen in terms of a clear concentration-dependant cause and effect. There were also concerns regarding the overall validity and reliability of this test and it is the opinion of the UK CA that a clear NOEC has not been established.

Regardless of whether the FSA NOEC of 30 µg a.s./L is based on putative ED (i.e. female VTG) effects or on slight histopathological liver changes, such effects are not considered relevant in relation to aquatic hazard classification. Although current CLP Guidance mentions that the lowest NOEC is generally used, it does not yet specifically address which effects can be used as the basis for chronic classification. A precedent for consideration of such effects was given in a recent RAC assessment of tebuconazole (EC no.: 403-640-2, CAS no.: 107534-96-3). This included information from an ED test with fathead minnow. Although this was an FSDT rather than FSA, it also gave information on potential ED-related effects and histopathological changes in the liver at levels lower than those 'traditionally' used for chronic classification (e.g. growth, survival, reproduction). The RAC opinion was that "*these are too specific effects to be used as a basis for classification*". The RAC, along with the Evaluating MS and Dossier Submitter, agreed that whilst such studies might provide supporting data when based on endpoints for mortality, growth and fertility, ED endpoints were currently not considered for the purposes of aquatic hazard classification in the harmonised system.

The UKCA nevertheless proposes that it would be useful to 'flag up' the use of such endpoints for further public, MS and RAC consultation with the aim of updating CLP guidance on this matter.

For the purposes of this report, it is proposed not to use the information submitted from either the FSDT or the FSA and instead to rely on the 35-day growth NOEC of 0.17 mg/L from the FELS test on *Pimephales promelas* for the chronic classification. Based on this result and in accordance with the chronic ecotoxicity criteria in the 2nd ATP of the CLP Regulation, triadimenol requires an aquatic hazard classification of Chronic Category 2. No M factor is required.

One of the degradants of triadimenol (triadimefon, M01) appears to be more ecotoxic than the parent substance, however the rate of its formation appears to be very slow from the environmental simulation studies. Therefore the following classification should be based on the parent substance:

Aquatic Chronic 2; H411

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 <u>not</u> 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 <u>not</u> 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 <u>not</u> 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.

Additional key elements

The FSDT data (Bomke, 2010) was re-evaluated by one commenting MS and provided to RAC (summarised under heading No 1 below). After the PC it was observed that the original study report was not sufficient for understanding the data and results and therefore RAC requested the data owner to provide clarification and re-analysis of certain parts of the original study

report (summarised under heading No 2 below).

1) RAC's summary of the commenting MSCA's re-evaluation of the FSDT report (Bomke, 2010):

Reason for this test

The test was requested in order to answer the question of whether triadimenol, as a potential aromatase inhibitor, presents as a potent endocrine disruptor, and to derive a NOEC for the endpoints sex ratio, vitellogenin plasma concentration of the males and weight. Contrary to the recommendation of the German Federal Environment Agency (UBA), the test was not carried out with *Danio rerio*, but instead with fathead minnow (*Pimephales promelas*). This species is considered less sensitive to the core endocrine endpoints aromatase inhibition and sex differentiation.

Materials and methods

Potential effects of triadimenol on sexual differentiation and development during the early-, juvenile- and sexual maturation life stages were evaluated in a flow-through test with fathead minnow (*Pimephales promelas*) at nominal test concentrations of 0 (Control), 5.12, 12.3, 29.5, 70.8 and 170 µg/L. Each treatment group included four, and the control eight, test chambers consisting of 30 freshly fertilized eggs. Mortality and larvae hatching were recorded daily. Behavioural impacts and abnormal physical changes were recorded at least on working days. Hatched larvae were fed with live brine shrimp nauplii three times daily except on weekends/holidays when food was added two times daily. Additionally, ground flake food (Tetramin®) was fed on working days once per day. On post-hatch day 33, all surviving individuals of each test level were transferred to corresponding new replicate aquaria with larger dimensions. 9% of the fish could not be found so that the total number was reduced from 615 to 561. In addition, on day 46, individual fish were moved between the replicates to achieve a comparative number of fish per replicate and treatment (mostly 20 fish/aquarium), thus endangering the independence of the replicates. Eggs and hatched larvae of fathead minnow were exposed to the test item over a period of 129-132 days; duration adjusted to the tested species. After test end, all fish were prepared for histological investigation. At the end of the test, the fish were almost at the age of sexual maturity in the controls (low number of nuptial tubercles, eggs); the test duration was therefore sufficient. The nominal concentrations, temperature, oxygen concentration and pH were observed as being satisfactory. The validity criteria stated in the draft OECD TG 234 for the Fish Sexual Development Test (21.12.2010) were fulfilled. The 8 control replicates showed a high variance for the relevant endpoints VTG (vitellogenin) content and sex ratio which makes a reliable statistical evaluation difficult. One reason could be the moving of fish between the replicates which took place in 7 out of the 8 control replicates.

Results

The phenotypically or histologically determined sex ratios ranged between 40% and 60% in all aquaria; the evaluation by the test author only considered the gonad histology to determine the sex but ignored the findings of the phenotypic sex differentiation. This differentiation into the two groups, male and female only, ignoring the undifferentiated/not determinable and the intersex fish, did not show any significant effects. When developing the OECD guideline, this kind of analysis was identified as problematic and in the current version of the OECD guideline (28.07.2011), it is now pointed out that the sex ratio should be evaluated separately for (a) clearly male, (b) clearly female, (c) intersex and (d) undifferentiated fish. Using this definition, the commenting MSCA performed an evaluation of the original data from Annex F3 of the study report, leading to the following results:

Table: Results for treatment groups in % performed by the commenting MSCA:

Triadimenol [µg/L]	0		5.12		12.3		29.5		70.8		170	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Female	54,29	14,19	40,18	9,36	52,96	16,09	54,11	9,07	55,53	16,05	36,48	8,86
Male	41,43	15,69	58,63	9,31	41,78	11,98	42,20	8,31	40,45	11,66	54,82	7,21
Intersex	3,69	4,41	1,19	2,38	2,63	3,04	3,69	4,77	4,02	5,07	4,94	7,07
Undifferentiated	0,60	1,68	0,00	0,00	2,63	3,04	0,00	0,00	0,00	0,00	3,76	2,51

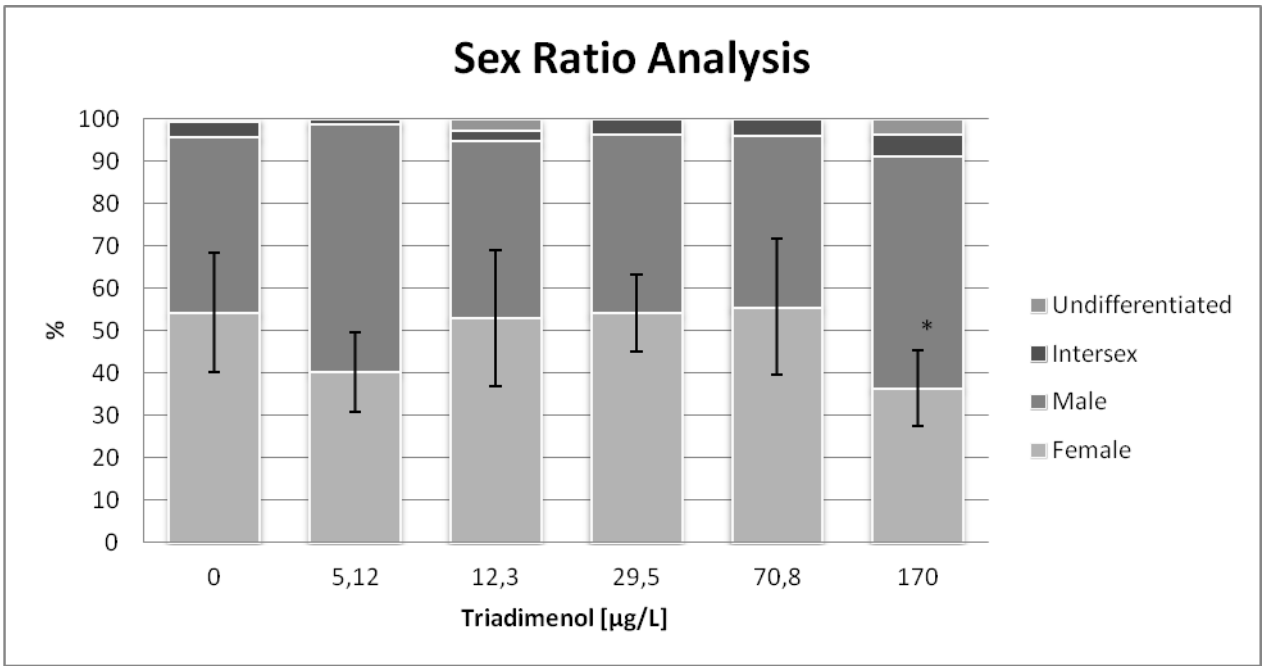


Figure: Results of statistical re-evaluation of the sex ratio performed by the commenting MSCA

The statistical evaluation showed that the ratio of the females at 170 µg/L is significantly reduced compared to the control (ToxRat 2.10, two-page Williams t-Test with α = 0.05); a NOEC of 70.8 µg/L can be derived.

2) RAC's summary of the data owner's clarification of the FSDT report (Bomke, 2010):

Clarification on the biomarker "phenotypic sex (via gonad histology)" mentioned on page 13 of the study report

In the study report from Bomke (2010) it is noted that a biomarker "phenotypic sex (via gonad histology)" was measured. This phrasing is not correct because the phenotypic sex was obtained via the analysis of the external appearance, i.e. from the observation of secondary sexual characteristics. These were listed in column 3 of the appendix F3 of the study report: presence of dorsal nape pad (male characteristic), presence and number of nuptial tubercles (male characteristics), presence of vertical coloured bands on the body (male characteristic) and presence of an ovipositor (female characteristic). However, RAC notes that appendix F3 of the Bomke study report does not list any male characteristic for the majority of the individuals determined to be phenotypic males, leaving still some ambiguity with the phenotypic sex. Furthermore, only very rarely were fish classified as "phenotypic undifferentiated".

The histological sex was defined based on the gonad histology according to standard OECD

procedures, as described in the histology report (Annex J of the Bomke study report). All individuals were either males or females based on gonad histology and no undifferentiated or intersexes were seen.

There were some mismatches between the phenotypic sex and the histological sex. These mismatches correspond to the 4.4% error reported in the study report (page 31) for the endpoint phenotypic sex ratio. This sexing error is a well-known drawback of the endpoint phenotypic sex ratio. In the OECD TG 234 it is therefore recommended that the histological sex ratio be used.

Clarification on the appendix F3 and on the appendix J in Bomke (2010)

The methodology used for the plasma VTG measurements is described in the study report (Bomke, 2010) but it is not documented which individuals specifically were discarded from the randomised samples because they were not suitable for the VTG analysis. Fish with morbidity symptoms (haemorrhages, swollen belly) were sacrificed shortly before test termination. These fish were categorized as dead during exposure. For other individuals, no plasma samples or insufficient plasma quantity could be collected because those fish were undeveloped: the gonads were very small and mature spermatozoa were not observed. In other cases plasma samples were taken but the samples were lost and no plasma analysis was performed. The data owner presented a table that summarised all available raw data for each individual fish in the experiment.

Clarification on the deviations of Bomke (2010) to the current version of the OECD TG 234
This study was carried out when the OECD TG 234 was under development. Thus deviation from the adopted (current) version are expected, and in addition there are some experimental draw backs which may influence the reliability of the results.

- One major difference between the draft and the final OECD guideline 234 lies in the choice of the fish species. The species fathead minnow which was used by Bomke (2010) has been removed from the final OECD TG 234. The choice of the test species for the FSDT with triadimenol was discussed in detail in König and Bomke (2010) and the study authors admit that fathead minnow is considered to be less sensitive to the endocrine endpoints aromatase inhibition and sex differentiation.
- Further, it was concluded that the number of tubercles cannot deliver reliable information in the present study due to the high variability in the developmental stages of the (male) fish.
- During the data re-evaluation, discrepancies between the raw data and the data presented in Table 8 and in Annex F3 of the study report were discovered. A corrigendum to the study report was provided.
- It was underlined that in the histopathology report (Appendix J) the basic evaluation of the findings was not performed on a replicate base: only the total ratios per treatment levels were given. Thus, the statistical procedures recommended in OECD TG 234, which use the replicates as relevant statistical units, could not be applied to reassess the raw staging data. A high variation in the development stages for both testes and oocytes was observed. Consequently, it was stated by the data owner that this variation just underlines that many fish have not reached sexual maturity, which was the case for all test levels including controls. Thus, "it is unlikely that more information from a statistical re-evaluation of these parameters" can be achieved.
- On day 46, fish groups were reduced from 45 to 20 fish per replicate, and were redistributed among replicates to achieve equal conditions in all replicate vessels. Due to the mixing of individuals and to the missing data, it was concluded that it is statistically not reliable to address survival along the whole study duration (0-132 days) in a single statistical test. The informative value of the parameter 'overall survival' can thus be considered as limited.
- While performing the re-analysis of the endpoint hatching rate several typing mistakes in Table 8 of the study report were spotted. However, the correct data were reported in the raw data file.

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**.

Note that under the 2nd ATP of the CLP Regulation, using the chronic ecotoxicity criteria, triadimenol fulfils the criteria for aquatic environmental hazard chronic category 2. This is using the 35-day growth NOEC of 0.17 mg/l (mean-measured) from the FELS test on *Pimephales promelas*. No M factor is required.

6 OTHER INFORMATION

This substance has been reviewed by the United Kingdom Competent Authority under Council Directive 91/414/EEC. The studies evaluated in this dossier were largely taken from the draft assessment report produced under this review programme, although additional information was obtained from studies submitted by the Notifier after the assessment report had been published. A search of the publicly-available literature did not reveal any additional information relevant for inclusion in this report.

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