CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical name: Tebuconazole (ISO)

1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol

EC Number: 403-640-2

CAS Number: 107534-96-3

Index Number: 603-197-00-7

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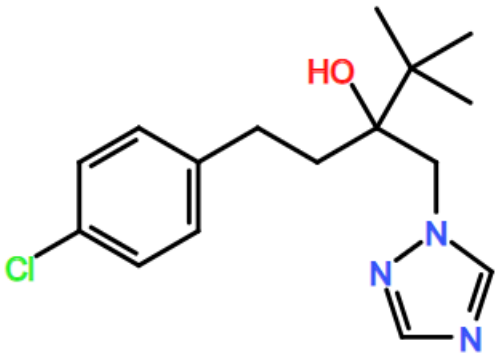
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol
Other names (usual name, trade name, abbreviation)	(±)-α-[2-(4-chlorophenyl)ethyl]-α-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol
ISO common name (if available and appropriate)	Tebuconazole
EC number (if available and appropriate)	403-640-2
EC name (if available and appropriate)	-
CAS number (if available)	107534-96-3
Other identity code (if available)	CIPAC: 494 Anex VI Index number: 603-197-00-7
Molecular formula	$C_{16}H_{22}ClN_3O$
Structural formula	
SMILES notation (if available)	CC(C)(C)C(O)(CCC1=CC=C(Cl)C=C1)CN1C=NC=N1
Molecular weight or molecular weight range	307,8 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	The method of manufacture is non enantioselective, therefore a racemic mixture of the ® and (S) enantiomers are expected. Racemic (50:50; R:S) Tebuconazole was used thoroughly the evaluation and no further consideration of the isomeric composition was made.
Description of the manufacturing process and identity of the source (for UVCB substances only)	Confidential/Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 950 g/kg

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol or (±)-α-[2-(4-chlorophenyl)ethyl]-α-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol Tebuconazole (ISO)	Minimum 95 % w/w	Acute Tox. 4; H302 Repr. 2; H361d Aquatic Acute 1; H400 and Aquatic Chronic 1; H410	Acute Tox. 4; H302 Repr. 2; H361d Aquatic Acute 1; H400 and Aquatic Chronic 1; H410

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Not relevant				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Not relevant					

Table 5: Test substances (non-confidential information) (this table is optional)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
Tebuconazole technical substance	Range between 95.6 % - 98.6 %	-	-	-
Tebuconazole pure substance	Range between 99.1 % - 100 %	-	-	-

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6: For substance with an existing entry in Annex VI of CLP

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	603-197-00-7	tebuconazole (ISO); 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol	403-640-2	107534-96-3	Repr. 2 Acute Tox. 4 Aquatic Acute 1 Aquatic Chronic 1	H361d*** H302 H400 H410	GHS08 GHS07 GHS09 Wng	H361d*** H302 H410		 M = 1 M = 10	
Dossier submitter's proposal	603-197-00-7	tebuconazole (ISO); 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol	403-640-2	107534-96-3	**Add** STOT RE 2 **Modify** Repr. 1B **Retain** Acute Tox. 4	**Add** H373 (eyes) **Modify** H360FD **Retain** H302	**Modify** Dgr **Retain** GHS08 GHS07	**Add** H373 (eyes) **Modify** H360FD **Retain** H302		**Add** oral: ATE = 1700 mg/kg bw	
Resulting entry in Annex VI if adopted by RAC and agreed by Commission	603-197-00-7	tebuconazole (ISO); 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol	403-640-2	107534-96-3	Repr. 1B Acute Tox. 4 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H360FD H302 H373 (eyes) H400 H410	GHS08 GHS07 GHS09 Dgr	H360FD H302 H373 (eyes) H410		oral: ATE = 1700 mg/kg bw M = 1 M = 10	

Table 7: Reason for not proposing harmonised classification and status under consultation

Hazard class	Reason for no classification	Within the scope of consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	Acute Tox. 4, ATE 1700 mg/kg bw	Yes
Acute toxicity via dermal route	data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	data conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	data conclusive but not sufficient for classification	Yes
Skin sensitisation	data conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	data conclusive but not sufficient for classification	Yes
Carcinogenicity	data conclusive but not sufficient for classification	Yes
Reproductive toxicity	Repr. 1B, H360FD	Yes
Specific target organ toxicity-single exposure	data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	STOT RE 2 (eyes)	Yes
Aspiration hazard	data conclusive but not sufficient for classification	Yes
Hazardous to the aquatic environment	hazard class not assessed in this dossier	No
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The current (first) harmonised classification and labelling of Tebuconazole was adopted by ECHA's Committee for Risk Assessment (RAC) on 5 June 2013. Tebuconazole was included in Annex VI of Regulation (EC) No 1272/2008 with ATP07 of the Regulation.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level as substance is regulated under Regulation EC 1107/2009 and Regulation (EU) No 528/2012.

5 IDENTIFIED USES

Tebuconazole is a renewal active substance which acts as a fungicide to control multiple fungal diseases in crops under Regulation EC 1107/2009 and as a wood preservative in product type 8, 7 and 10 under Regulation EU 528/2012.

Tebuconazole belongs to the group of demethylation inhibitor compounds (DMI-fungicides, Class I), in the chemical sub-group of triazoles.

6 DATA SOURCES

Draft Renewal Assessment Report (dRAR) prepared according to Regulation EC 1107/2009. The dRAR was initially prepared by UK-RMS. In March 2019 the assessment was taken over by DK-RMS.

Public literature obtained from a literature review performed in line with the EFSA guidance (2011) on the submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009.

7 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference

Method	Results	Remarks	Reference
ADME study - single dose study U. S. EPA Pesticide Assessment Guidelines, Subdivision F, 85-1, which correspond to OECD 417 GLP Rats, Wistar (BOR:WISW), males and females Oral/gavage Dose Levels: 2 and 20 mg/kg bw Tebuconazole (99.5 %) (specific activity: 84.4 µCi/mg) Batch no: APF 13028500 Vehicle: 0.5% aqueous tragacanth gel solution	Absorption: almost complete absorption of tebuconazole after oral administration. Excretion: a large part of the elimination of tebuconazole was via the bile (91 % within 48 h; 50 % of the total biliary excretion was eliminated after 2.5 h and 90 % after 7 h, indicating a significant first pass effect).	Acceptable study, However, the substance used is not the fully representative for the technical tebuconazole used in the toxicological studies Phenyl-labelled tebuconazole	Vol 2 B.6.1.1.1/01

Method	Results	Remarks	Reference
Whole-body autoradiographic distribution U. S. EPA Pesticide Assessment Guidelines, Subdivision F, 85-1, EPA 540/9-82-025, November 1982: "Rat Metabolism Study" (biokietic part) - correspond to OECD 417 GLP Male Wistar (BOR:WISW), rats Oral/gavage Dose Level : 20 mg/kg bw Tebuconazole (99.5 %) (specific activity: 84.4 µCi/mg) Batch no: APF 13028500 Vehicle: 0.5% aqueous tragacanth gel solution	Distribution: the study showed an even distribution of tebuconazole. 1 hour after administration radioactivity was detectable in all body tissues with the exception of the compact bone substance.	Acceptable study Phenyl-labelled tebuconazole	Vol 3 B.6.1.1.2/01

Method	Results	Remarks	Reference
Metabolism study U. S. EPA Pesticide Assessment Guidelines, Subdivision F, 85-1, which correspond to OECD 417 GLP Male and female Wistar (BOR:WISW), rats Single dose of 20 mg/kg bw; in some groups pretreatment with 2 mg/kg bw of nonradioactive test substance Tebuconazole (99.5 %) (specific activity: phenyl-UL-^{14}C-HWG 1608: 84.4µCi/mg, triazol-3,5-^{14}C-HWG 1608: 56.5 µCi/mg) Batch no: not stated Vehicle: 0.5% aqueous tragacanth solution	Metabolism: Tebuconazole was efficiently metabolised as hardly any unchanged parent compound was found in the excreta 72 h after administration.	Acceptable study Phenyl-labelled and triazolelabelled tebuconazole	Vol 3 B.6.1.1.3/01

Method	Results	Remarks	Reference
In-vitro metabolism study No guideline available GLP Male and female CD1 mice, liver S9 fractions 1 and 10 µM Incubation: 1 and 2 hours Triazole-labelled tebuconazole (Purity > 98 (HPLC)) Batch : KML 9879 Vehicle: phosphate buffer K_2HPO_4 (50mM + 1mM EDTA, pH 7.4)	Slight or no biotransformation of tebuconazole after incubation with mouse liver S9 fraction.	Acceptable	Vol 3 B.6.1.2/01

Method	Results	Remarks	Reference
In-vitro metabolism study No guideline available GLP Male and female Wistar rats and humans liver S9 fractions 1 and 10 µM Incubation: 1 and 2 hours Triazole-labelled tebuconazole (Purity > 98 (HPLC)) Batch : KML 9879 Vehicle: phosphate buffer K_2HPO_4 (50mM + 1mM EDTA, pH 7.4)	High or moderate biotransformation of tebuconazole after incubation with rat and human liver S9 fraction. No human unique metabolites were detected.	Acceptable	Vol 3 B.6.1.2/01

Method	Results	Remarks	Reference
In-vitro metabolism study No guideline available Non-GLP Mouse (MH, 2 strains: NMRI and CD1), dog (DH, Beagle), rat (RH, Wistar) and human (HH, Caucasian) 1, 5, 10 and 20 µM Incubation: 0, 1, 2, or 4 hrs Dog, human, rat, mouse; hepatocytes Triazole-labelled tebuconazole (Purity > 99 (HPLC)) Batch : KML 9879 Vehicle: Williams E medium buffered with Carbogen® (5% CO2; 95% O2) (pH 7.4)	Intense biotransformation of tebuconazole after 2 h incubations at 1 and 5 µM. The principal metabolic reactions were hydroxylation, oxidation, and conjugation with glucuronic acid as the preferred molecule for the conjugation. No unique human metabolites were detected.	Acceptable	Vol 3 B.6.1.2/02

Method	Results	Remarks	Reference
Protein binding study No guideline No GLP Diluted Plasma from: Rat (female) Wistar (RccHan:WIST), Dog (female) Beagle, Rabbit (female) Himalayan, Mouse (female) NMRI, Human (female) Caucasian 1,000 µg/L to 100,000 µg/L Phenyl-labelled tebuconazole (Purity > 99 (HPLC)) Batch : KML 12016 Vehicle: PBS buffer	The binding of tebuconazole to plasma proteins was moderate in all species investigated without evidence for a concentration dependency. Mean unbound fractions: 3.24% in mouse, 5.09% in man, 5.57% in rabbit, 5.74% in dog and 5.82% in rat. No relevant species differences	Acceptable	Vol 3 B.6.1.2/03
Publication: Metabolites of tebuconazole in human (vineyard workers) urine. Unknown exposure Human (Vine yard workers) Tebuconazole (Purity and batch not stated)	TEB-OH and TEB-COOH were identified, both as free molecules and as glucuronide conjugates, as the main metabolites of TEB.	Reliable with restrictions	Vol 3 B.6.1.1.4/01

7.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

The absorption of tebuconazole from the gastro-intestinal tract of the rat is rapid and complete based on urinary (7.4 %) and biliary (90.9 %) excretion by the cholecystotomized animals within 48 hours. A figure of > 98 % (100 % for the purpose of AOEL derivation) of the oral dose was therefore obtained for the degree of oral absorption. Peak relative concentration in the blood plasma was found from 20 to 100 minutes after administration (B.6.1.1.1/01). Absorption of tebuconazole was slower at the high dose and repeated pre-treatment with the non-labelled test substance had no impact on absorption. Sex-dependent differences were apparent.

The distribution in the body was studied in a whole-body autoradiographic study (B.6.1.1.2/01). One hour after oral administration tebuconazole was rapidly and evenly distributed into organs and tissues, except for compact bone substance. Highest levels were found in the liver. Tebuconazole did not accumulate in any organ or tissue after oral administration: radiolabelled residues in tissues and organs were low at termination, but generally higher in male rats compared with females.

The metabolism study in rats revealed that tebuconazole is efficiently metabolised as hardly any unchanged parent compound (0.5 - 2.2 % administered dose) is found in the excreta 72 h after administration. Ten compounds, excluding the parent compound, were identified in urine and faeces. M03 (tebuconazole-1-hydroxy) and M06 (tebuconazole-carboxylic acid) were the main metabolites in all test groups with a slight tendency towards higher amounts in females. Both metabolites were predominantly found in faeces with up to 30 and 33% of the administered dose for M03 and M06, respectively. The same metabolites (TEB-OH and TEB-COOH) were identified, both as free molecules and as glucuronide conjugates, as the main metabolites of tebuconazole in the urine of vineyard workers exposed to tebuconazole (B.6.1.1.4/01).

Distinct sex differences were seen in the metabolic pattern of tebuconazole which mainly involves oxidations as phase 1- reactions, resulting in hydroxy, carboxy, triol and ketoacid metabolites and the phase 2 - conjugates were glucuronides and sulphates. Furthermore, the break-down product M26 (1,2,4-triazole) amounted to 5.4 % in the urine of the male and 1.6 % in that of the female rat (B.6.1.1.3/01). 1,2,4-triazole is a toxicologically relevant metabolite due to the classification as Repr 2 and a proposed upclassification as Repr 1B (RAC 2019). Neither the dose level nor the repeated pre-treatment showed a significant influence on the metabolic pattern in any of the dose groups.

The only metabolite above 10% in rat urine was M06 in females (11.5% in repeated low dose). However, in males it was less than 10% and would not be considered a major rat metabolite.

A comparative *in-vitro* metabolism study using mouse, rat and human liver S9 fractions showed metabolism of tebuconazole with the highest transformation in rat liver homogenates. One major metabolite was formed similarly in all test systems. Comparison of the metabolic profiles showed that no unique human metabolite had been formed.

In a further comparative *in vitro* metabolism study, rat and human cells showed the highest metabolic transformation of tebuconazole and the most similar metabolic pattern compared to the other species. While the principal metabolic reactions (hydroxylation, oxidation, and conjugation) were similar in all hepatocytes, the kind of oxidation and conjugation was most similar again in rat and human hepatocytes. Glucuronidation was the most important detoxification pathway in both species. In contrast, incubation with mouse hepatocytes led to different oxidised metabolites and less conjugation. These in vitro studies therefore indicate that the metabolism of tebuconazole by human hepatocytes is broadly comparable to that by rat hepatocytes.

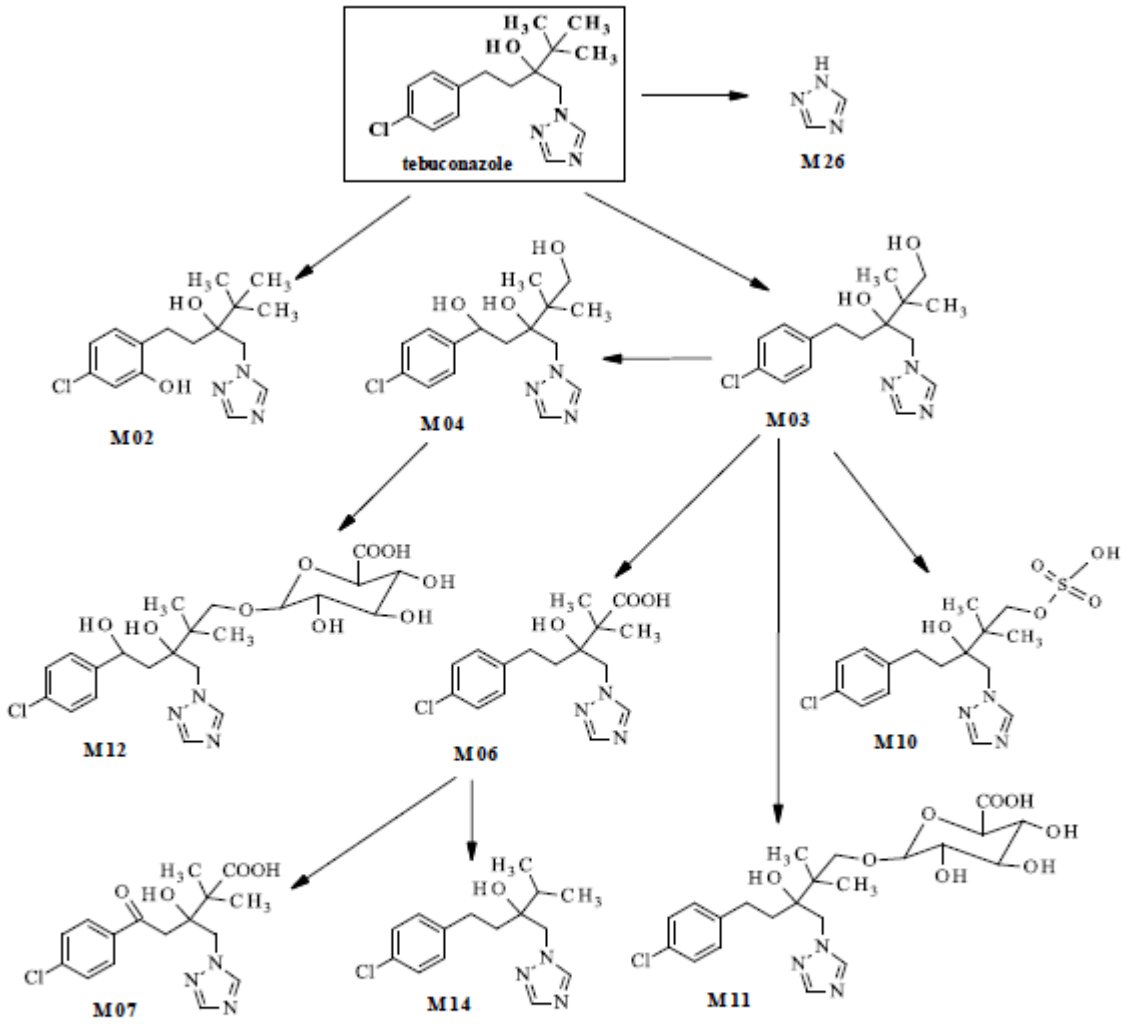
The protein binding of [phenyl-UL-^{14}C] tebuconazole was investigated in-vitro in diluted plasma of mouse, rat, rabbit, dog and human. The unbound fraction of tebuconazole in plasma was about 5 % in all species

indicating that the systemically available concentration of unbound tebuconazole in toxicological studies in-vivo is likely to be much lower than indicated by the given dose.

The excretion of the radioactivity was a fast and complete process which mainly took place via faeces as 62 – 82 % of the administered dose was eliminated by the route, whereas elimination in urine amounted to about 15 – 33 % (B.6.1.1.1/01) in intact animals. For cannulated animals 92.2 % of the administered dose were eliminated by the biliary and faecal route and 7.4 % via urine (B.6.1.1.1/01). Sex-dependent differences were apparent including generally slower clearance of tebuconazole in male rats at all doses. Biliary and faecal elimination was greater in males than in females with correspondingly lower excretion via urine. The amount excreted was not related to the administered dose. The results indicate that enterohepatic recirculation occurs in intact animals. Less than 1 % of the administered dose was recovered in the tissues two to three days after administration, with the liver containing most of the tissue residues. Male animals in all groups had higher residue levels than females. Only a very small amount of radioactivity (0.032 %) was detected in the exhaled air within 3 days of oral administration of 20 mg/kg bw.

The proposed metabolic partway of tebuconazole is shown the figure below:

Figure 5.1.1-1 Proposed metabolic pathway of tebuconazole in the rat



M02: HWG 1608-o-hydroxy
M03: HWG 1608-1-hydroxy
M04: HWG 1608-1,5-dihydroxy
M06: HWG 1608-carboxylic acid
M07: HWG 1608-5-ketocarboxylic acid
M10: HWG 1608-1-hydroxysulfate
M11: HWG 1608-1-OH-glucuronide
M12: HWG 1608-1,5-di-OH-glucuronide
M14: HWG 1608-desmethyl
M26: 1,2,4-triazole

8 EVALUATION OF HEALTH HAZARDS

Acute toxicity

8.1 Acute toxicity - oral route

Table 9: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 401 Acceptable	Rat, fasted Wistar rats /Bor:WISW (SPF-Cpb) M/F 5 rats/sex/group	(HWG 1608) Tebuconazole	1000, 2500, 4500 and 5000 mg/kg bw (male) 1000, 2500, 3150, 3550 and 5000 mg/kg bw (female) Single dose administration	M: LD_{50}: > 5000 mg/kg bw F: LD_{50}: 3933 mg/kg bw	Vol 3 B.6.2.1.1/03
OECD 401 Acceptable	Rat, non-fasted Wistar rats /Bor:WISW (SPF-Cpb) M/F 5 rats/sex/group	(HWG 1608) Tebuconazole	500, 1000, 3550, 3750, 4000, 5000 mg/kg bw (male) 500, 1000, 2500, 3550, 4250, 4500 mg/kg bw (female) Single dose administration	M: LD_{50}: 4264 mg/kg bw F: LD_{50}: 3352 mg/kg bw	Vol 3 B.6.2.1.1/03
Based on OECD 401 (1981) Acceptable	Rat, fasted Wistar rats/ Bor:WISW (SPF-Cpb) 5 male rats	(HWG 1608) Tebuconazole	5000 mg/kg bw Single dose administration	LD_{50}: > 5000 mg/kg bw	Vol 3 B.6.2.1.1/02
JMAFF (59 Nohsan no. 3850); comparable to OECD 401 (1987) Acceptable	Rat, fasted Sprague-Dawley rat (Crj:CD) M/F 5 rats/sex/group	(HWG 1608 technical) Tebuconazole	Males: 1600, 2300, 3000, 3900 and 5000 mg/kg body weight. Females: 730, 950, 1230, 1600, 2300, 3000, 3900 and 5000 mg/kg body weight. Single dose administration	M: LD_{50}: 4000 mg/kg bw F: LD_{50}: **1700** mg/kg bw	Vol 3 B.6.2.1/01
OECD 401 Acceptable – supplementary	Mouse, fasted NMRI mice M/F 5 mice/sex/group	(HWG 1608) Tebuconazole	100, 500, 1000, 1800, 2500, 3150 and 3550 mg/kg bw (male) 500, 1000, 1800, 2500, 3550 and 5000 mg/kg bw (female) Single dose administration	M: LD_{50}: **1615** mg/kg bw F: LD_{50}: 3023 mg/kg bw	Vol 3 B.6.2.1.2/02
JMAFF (59 Nohsan no. 3850); comparable to	Mouse, fasted ICR (Crj:CD-1) mice	(HWG 1608 technical) Tebuconazole	Males: 1600, 2300, 3000, 3900 and 5000 mg/kg body	M: LD_{50}: 2800 mg/kg bw	Vol 3 B.6.2.1.2/01

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 401 (1987) Acceptable – supplementary	M/F 5 mice/sex/group		weight. Females: 3000, 3900 and 5000 mg/kg body weight. Single dose administration	F: LD_{50}: 5200 mg/kg bw	
OECD 401 Acceptable – supplementary	Rabbit, fasted Albino rabbits (HC:NZW) M/F 3 rabbits /sex / dose	(HWG 1608) Tebuconazole	500 and 1000 mg/kg bw (male and female) Single dose administration	M: LD_{50}: >1000 mg/kg bw F: LD_{50}: >1000 mg/kg bw	Vol 3 B.6.2.1.3/01

Table 10: Summary table of human data on acute oral toxicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 11: Summary table of other studies relevant for acute oral toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

8.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Tebuconazole is of low to moderate acute oral toxicity in the rodent species rat and mouse.

Clear sex differences were observed with female rats being most sensitive, while it was opposite with mice. Administration of high oral doses to rats induced sedation, spastic gait, abnormal breathing, locomotor incoordination and emaciation, the symptoms beginning within 5 hours at the dose 950 mg/kg bw for females and within 20 minutes at the dose 1600 mg/kg bw for males. Tebuconazole was of low oral toxicity to fasted male and female rabbits after administration at the dose 1000 mg/kg bw. It was not possible from the acute oral toxicity studies to rank the sensitivity to tebuconazole in the three species tested.

8.1.2 Comparison with the CLP criteria

The results of these studies show that the acute oral LD50 of tebuconazole is 1700 mg/kg bw in rats. Therefore, Tebuconazole fulfils the criteria for acute oral toxicity as category 4, H302 (LD50: 300 < Category 4 ≤ 2000 mg/kg bw). This is consistent with the harmonised classification of tebuconazole.

8.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the LD50 in rats, classification for acute oral toxicity, category 4, H302, is required under CLP Regulation (EC) No. 1272/2008 with an ATE of 1700 mg/kg bw.

8.2 Acute toxicity - dermal route

Table 12: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 402 Acceptable	Rat Wistar rats / Bor:WISW (SPF-Cpb) M/F 5 rats/sex/group	(HWG 1608) Tebuconazole	5000 mg/kg bw 24 hours (occlusive dressing method)	M: LD_{50}: > 5000 mg/kg bw F: LD_{50}: > 5000 mg/kg bw	Vol 3 B.6.2.2/02
JMAFF (59 Nohsan no. 3850); comparable to OECD 402 (1987) Acceptable	Rat Sprague-Dawley rats (Crj: CD, SPF) M/F 5 per sex/group	(HWG 1608 Technical) Tebuconazole	2000 mg/kg bw 24 hours (semi-occlusive conditions)	M: LD_{50}: > 2000 mg/kg bw F: LD_{50}: > 2000 mg/kg bw	Vol 3 B.6.2.2/01
OECD Guideline 402 Acceptable	Rat CD /CrI : CD(SD) rats M/F 5 rats/sex/dose	Tebuconazole TC	2060 mg/kg bw 24 hours (semi-occlusive dressing method)	LD_{50} > 2060 mg/kg bw	Vol 3 B.6.2.2/03

Table 13: Summary table of human data on acute dermal toxicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 14: Summary table of other studies relevant for acute dermal toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

8.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

Tebuconazole is of low acute dermal toxicity. In the limit tests with doses of 2000 or 5000 mg/kg bw no mortality, clinical signs or local effects were observed.

8.2.2 Comparison with the CLP criteria

These three studies in rats show that tebuconazole is of low acute dermal toxicity with LD50 greater than 2000 mg/kg bw. Thus, tebuconazole does not fulfil any criteria for acute dermal classification, as the LD50 is beyond the range of values for category 4 ($1000 < LD50 \leq 2000$ mg/kg bw). This is consistent with the harmonised classification of tebuconazole.

8.2.3 Conclusion on classification and labelling for acute dermal toxicity

Based on the LD50 in rats no classification is required according to Regulation (EC) No. 1272/2008.

8.3 Acute toxicity - inhalation route

Table 15: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
OECD 403 Acceptable Deviation: MMAD>4 µm BW not measured on day 1 and 3	Rat Wistar rats / Bor:WISW (SPF-Cpb) M/F 5-10 rats/sex/group	(HWG 1608-technical grade, Purity 97.1%) Tebuconazole particle size was approx. 50% ≤ 5 µm	Nominal concentration: 100, 250, 2500, 5000 mg/m³ Analytical concentration: 16, 49, 387, 818 mg/m³ 1 x 4 hours (aerosol)	M: LC_{50}: > 0.818 mg/L F: LC_{50}: > 0.818 mg/L (max. attainable concentration)	
Range-finding OECD 403 Acceptable Deviation: MMAD>4 µm BW not measured on day 1 and 3 More than 3 rats/sex/dose used in range finding study	Rat Wistar rats / Bor:WISW (SPF-Cpb) M/F 5-10 rats/sex/group	(HWG 1608-technical grade, Purity 97.1%) Tebuconazole MMAD = 7.1 µm ± GSD (2.0µm), 31% ≤ 5 µm (100 mg/m³) MMAD = 5.0 µm ± GSD (1.8 µm), 51% ≤ 5 µm (300 mg/m³) MMAD = 4.6 µm ± GSD (1.8 µm), 55% ≤ 5 µm (1000 mg/m³)	Nominal concentration: 0, 100, 300, 1000 mg/m³ Analytical concentration: 0, 24, 60, 240 mg/m³ 5 x 6 hours (aerosol)	M: LC_{50}: > 0.24 mg/L F: LC_{50}: > 0.24 mg/L	Vol 3 B.6.2.3/02
OECD 403 (1981) Acceptable Deviations from OECD TG 403 (2009): The MMAD (dust) > recommended 1-4 µm BW not measured on day 1	Rat Wistar rats / Bor:WISW (SPF-Cpb) M/F 5 rats/sex/group	(HWG 1608-technical grade, purity 96.2%) Tebuconazole MMAD = 1.4 µm ± GSD (1.4 µm), 100% ≤ 5 µm (Aerosol), MMAD = 12.8 µm ± GSD (1.9 µm), 8% ≤ 5 µm (Dust)	Nominal concentration 4000 mg/m³ (aerosol) Analytical concentration: 371 mg/m³ (aerosol), 5093 mg/m³ (dust) 1 x 4 hours (dust, aerosol)	M/F LC_{50}: > 5.093 mg/L (dust) LC_{50}: > 0.371 mg/L (aerosol) (max. attainable concentration)	Vol 3 B.6.2.3.1/01
OECD 403 (1981) Acceptable Deviations:	Rat Hsd Cpb:WU M/F 5 rats/sex/group	Tebuconazole white powder, Purity 97.1% MMAD = 2.76 µm ± GSD (1.84 µm), 55% ≤ 3 µm (2118 mg/m³, target	2118 mg/m³ 1 x 4 hours (solid aerosol)	M: LC_{50}: > 2.118 mg/L F: LC_{50}: > 2.118 mg/L (max. attainable concentration)	Vol 3 B.6.2.3/03

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
None		concentration 5000 mg/m³)			
OECD Guideline 403. Acceptable Deviations: BW not measured Day 1+3 MMAD>4 µm	Rat CD /CrI : CD(SD) rats M/F 5 rats/sex/dose	Tebuconazole Purity 97.1-97.2% MMAD = 16.324 µm ± GSD (2.38 µm (5000 mg/m³)	5.00 mg/L air (limit test) 1 x 4 hours (aerosol)	M: LC_{50}: > 5.0 mg/L F: LC_{50}: > 5.0 mg/L	Vol 3 B.6.2.3/04

Table 16: Summary table of human data on acute inhalation toxicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 17: Summary table of other studies relevant for acute inhalation toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

8.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

In the inhalation studies with rats from the original DAR (2006) no deaths occurred and LC50 > 371 mg/m3 (aerosol) and > 5093 mg/m3 (dust) was determined by nose/head-only exposure under dynamic conditions. No clinical symptoms were observed at these maximum attainable concentrations.

A new acute inhalation study was provided for the purpose of renewal, and an additional new study was provided. These new acute inhalation toxicity studies confirm that tebuconazole is of low acute inhalation toxicity, with an inhalation 4hr-LC50 in rats greater than 2.118 mg/L (max. attainable concentration with MMAD<4 µm, Bayer TF), and greater than 5.0 mg/L (MMAD>4 µm).

8.3.2 Comparison with the CLP criteria

Overall, these 4 studies in rats show that tebuconazole is of low acute inhalation toxicity (4hr-LC50 > 5.0 mg/L aerosol). Tebuconazole does not fulfil criteria for acute inhalation classification, as the LC50 is beyond the range of values for category 4 (1.0 < LC50 ≤ 5.0 mg/L). This is consistent with the harmonised classification of tebuconazole.

8.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on the LC50 in rats, classification for acute inhalation toxicity is not required under Regulation (EC) No. 1272/2008.

8.4 Skin corrosion/irritation

Table 18: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results - Observations and time point of onset[2] - Mean scores/animal - Reversibility	Reference
OECD 404, (1981) Acceptable	Rabbit New Zealand White rabbit, HC:NZW 3 adult rabbits (male and female)	(HWG 1608-technical grade) Tebuconazole	500 mg 4 hours	- Skin sites were evaluated before and 60 minutes, 24, 48, 72 hours and 7 days after patch removal. - No skin irritation was observed at any of the time points investigated No reported erythema or oedema (mean score were 0.00 at 24, 48 and 72 hours) Non-irritating	Vol 3 B.6.2.4/01
US-EPA-FIFRA, Section 158.135, Guideline 81-5 (1984) Acceptable	Rabbit New Zealand White rabbit, 6 young rabbits (3 male and 3 female)	(Folicur technical grade) Tebuconazole	500 mg 4 hours	- The skin sites were evaluated before and 60 minutes, 24, 48, 72 hours after patch removal - No reported erythema or oedema (mean score were 0.00 at 24, 48 and 72 hours) Non-irritating	Vol 3 B.6.2.4/02
OECD Guideline 404. Acceptable	Rabbit 3 male Himalayan rabbits	Tebuconazole,	500 mg 4 hours	- The skin sites were evaluated before and 60 minutes, 24, 48, 72 hours after patch removal. - No skin irritation was observed (average erythema and oedema formation was 0,0) Non-irritating	Vol 3 B.6.2.4/03

Table 19: Summary table of human data on skin corrosion/irritation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 20: Summary table of other studies relevant for skin corrosion/irritation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

8.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

The potential for tebuconazole to induce skin irritation was investigated in two studies in rabbits. The available studies were evaluated in the original DAR (2006), peer reviewed at EU level (EFSA

Conclusion, 2008), and considered acceptable. A new skin irritation study was provided for the purpose of renewal. Overall, these three studies show that tebuconazole is not a skin irritant.

8.4.2 Comparison with the CLP criteria

These three studies show that tebuconazole is not a skin irritant and hence classification according to Regulation (EC) No. 1272/2008 is not required. This is consistent with the harmonised classification of tebuconazole.

8.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Classification for skin irritation according to Regulation (EC) No. 1272/2008 is not required.

8.5 Serious eye damage/eye irritation

Table 21: Summary table of animal studies on serious eye damage/eye irritation

<table>
<tr><th>Method, guideline, deviations[1] if any</th><th>Species, strain, sex, no/group</th><th>Test substance</th><th>Dose levels duration of exposure</th><th>Results
- Observations and time point of onset[2]
- Mean scores/animal
- Reversibility</th><th>Reference</th></tr>
<tr><td>OECD 405 (1981)

Acceptable</td><td>Rabbit
New Zealand White rabbit, HC:NZW
male
3 adult rabbits</td><td>(HWG 1608) Tebuconazole</td><td>100 µl (weight 50 mg) of test compound

Single dose instillation</td><td>- The eyes were examined ophthalmoscopically before the administration and 1, 24, 48, 72 hours and 7 days after the administration.
- There were no signs of corneal opacities or lesions involving the iris in any animal during the study. Reddening of conjunctiva was observed in one animal (average score 0.3; reversible at 48 hours). No chemosis was observed.
<table>
<tr><th>Males
Animal</th><th>Effects</th><th>Mean scores (24, 48 and 72 h)</th><th>Reversible day</th></tr>
<tr><td>1</td><td>Corneal opacity</td><td>0</td><td>n/a</td></tr>
<tr><td></td><td>Iritis</td><td>0</td><td>n/a</td></tr>
<tr><td></td><td>Redness conjunctivae</td><td>0.33</td><td>2</td></tr>
<tr><td></td><td>Chemosis conjunctivae</td><td>0</td><td>1</td></tr>
<tr><td></td><td>Discharge</td><td>0</td><td>1</td></tr>
<tr><td>2</td><td>Corneal opacity</td><td>0</td><td>n/a</td></tr>
<tr><td></td><td>Iritis</td><td>0</td><td>n/a</td></tr>
<tr><td></td><td>Redness conjunctivae</td><td>0</td><td>1</td></tr>
<tr><td></td><td>Chemosis conjunctivae</td><td>0</td><td>1</td></tr>
<tr><td></td><td>Discharge</td><td>0</td><td>n/a</td></tr>
<tr><td>3</td><td>Corneal opacity</td><td>0</td><td>n/a</td></tr>
<tr><td></td><td>Iritis</td><td>0</td><td>n/a</td></tr>
</table>
</td><td>Vol 3 B.6.2.5.1/02</td></tr>
</table>

<table>
<tr>
<td></td>
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<td></td>
<td>
<table>
<tr><td></td><td>Redness conjunctivae</td><td>0</td><td>1</td></tr>
<tr><td></td><td>Chemosis conjunctivae</td><td>0</td><td>1</td></tr>
<tr><td></td><td>Discharge</td><td>0</td><td>n/a</td></tr>
</table>

Non-irritating
</td>
<td></td>
</tr>
<tr>
<td>US-EPA-FIFRA, Section 158.135, 81-4 (1984)

Acceptable</td>
<td>Rabbit
New Zealand White rabbit
6 young rabbits (3 male and 3 female)</td>
<td>(Folicur-technical grade) Tebuconazole</td>
<td>0.1 g

Single dose instillation</td>
<td>- The eyes were examined ophthalmoscopically before the administration and 1, 24, 48, 72, hours after the administration. Additional examinations occurred on days 7, 8, 14, and 21, in order to characterize the time-course and reversibility of lesions.

- There were no signs of corneal opacities or lesions involving the iris in any animal during the study.

All six rabbits developed redness (grade 1), chemosis (grade 1-2) and discharge (grade 2-3) of the conjunctiva 1-24 h after dosing. Chemosis and discharge had resolved in five animals by 72 h after dosing and in all animals by day 7. Redness had resolved in five rabbits by 72 hours after dosing and in the one remaining animal by day 8.

The average scores, which is based on individual scoring values were: Cornea (24h-72h: 0.00), Iris (24h-72h: 0.00), Redness (24h-72h: 0.78), Chemosis (24h-72h: 0.50).
<table>
<tr><th>Females
Animal</th><th>Effects</th><th>Mean scores (24, 48 and 72 h)</th><th>Reversible day</th><th>Males
Animal</th><th>Mean scores (24, 48 and 72 h)</th><th>Reversible day</th></tr>
<tr><td rowspan="5">1</td><td>Corneal opacity</td><td>0</td><td>n/a</td><td rowspan="5">1</td><td>0</td><td>-</td></tr>
<tr><td>Iritis</td><td>0</td><td>n/a</td><td>0</td><td>-</td></tr>
<tr><td>Redness conjunctivae</td><td>1</td><td>8</td><td>0.67</td><td>3</td></tr>
<tr><td>Chemosis conjunctivae</td><td>1.33</td><td>4-7</td><td>0.33</td><td>2</td></tr>
<tr><td>Discharge</td><td>1.33</td><td>3</td><td>0</td><td>1</td></tr>
<tr><td rowspan="5">2</td><td>Corneal opacity</td><td>0</td><td>n/a</td><td rowspan="5">2</td><td>0</td><td>-</td></tr>
<tr><td>Iritis</td><td>0</td><td>n/a</td><td>0</td><td>-</td></tr>
<tr><td>Redness conjunctivae</td><td>0.67</td><td>3</td><td>0.67</td><td>3</td></tr>
<tr><td>Chemosis conjunctivae</td><td>0.33</td><td>2</td><td>0.33</td><td>2</td></tr>
<tr><td>Discharge</td><td>1</td><td>2</td><td>0</td><td>1</td></tr>
<tr><td rowspan="5">3</td><td>Corneal opacity</td><td>0</td><td>n/a</td><td rowspan="5">3</td><td>0</td><td>-</td></tr>
<tr><td>Iritis</td><td>0</td><td>n/a</td><td>0</td><td>-</td></tr>
<tr><td>Redness conjunctivae</td><td>1</td><td>4-7</td><td>0.67</td><td>3</td></tr>
<tr><td>Chemosis conjuncti</td><td>0.33</td><td>2</td><td>0.33</td><td>2</td></tr>
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<table>
<tr><td></td><td>vae</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td>Discharge</td><td>0</td><td>1</td><td></td><td>1</td><td>2</td></tr>
</table>
Mildly irritating
</td>
<td></td>
</tr>
<tr>
<td>OECD Guideline 405

Acceptable</td>
<td>Rabbit
Male Himalayan rabbits
3/dose</td>
<td>Tebuconazole</td>
<td>100 mg

Single dose instillation</td>
<td>- The eyes were examined ophthalmoscopically before the administration and 1, 24, 48, 72, hours and 4 days after the administration.
- Corneal opacity (grade 1) was observed in one animal 24 to 72 hours after instillation: the fluorescein test performed 24 hours after instillation revealed corneal staining in this animal. Conjunctival redness (grade 1) was observed in two animals (24 and 48 hours in animal no. 3, and 24 until 72 hours in animal no. 1) after instillation. Chemosis (grade 1) was observed in one animal 24 hours after instillation. The iris was not affected.
<table>
<tr><th>Males
Animal</th><th>Effects</th><th>Mean scores (24, 48 and 72 h)</th><th>Reversible day</th></tr>
<tr><td>1</td><td>Corneal opacity</td><td>1</td><td>4</td></tr>
<tr><td></td><td>Iritis</td><td>0</td><td>n/a</td></tr>
<tr><td></td><td>Redness conjunctivae</td><td>1</td><td>4</td></tr>
<tr><td></td><td>Chemosis conjunctivae</td><td>0</td><td>n/a</td></tr>
<tr><td>2</td><td>Corneal opacity</td><td>0</td><td>n/a</td></tr>
<tr><td></td><td>Iritis</td><td>0</td><td>n/a</td></tr>
<tr><td></td><td>Redness conjunctivae</td><td>0</td><td>n/a</td></tr>
<tr><td></td><td>Chemosis conjunctivae</td><td>0</td><td>n/a</td></tr>
<tr><td>3</td><td>Corneal opacity</td><td>0</td><td>n/a</td></tr>
<tr><td></td><td>Iritis</td><td>0</td><td>n/a</td></tr>
<tr><td></td><td>Redness conjunctivae</td><td>0.67</td><td>2</td></tr>
<tr><td></td><td>Chemosis conjunctivae</td><td>0.33</td><td>1</td></tr>
</table>
Slightly-irritating
</td>
<td>Vol 3 B.6.2.5.1/ 03</td>
</tr>
</table>

Table 22: Summary table of human data on serious eye damage/eye irritation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 23: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

8.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

No irritation of cornea or iris was observed in the eye irritation studies, only very mild redness and swelling of conjunctiva, both fully reversible within 72 hours.

A new eye irritation study has been provided for the purpose of renewal. This new study confirms that tebuconazole is only slightly irritating to the eye (but requiring no classification).

8.5.2 Comparison with the CLP criteria

Overall, these three studies in rabbits show that tebuconazole is only slightly irritating to eye but, the criteria for category 2 are not fulfilled, thus no classification is required according to Regulation (EC) No. 1272/2008. This is consistent with the current harmonised classification of tebuconazole.

8.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Classification for eye irritation according to Regulation (EC) No. 1272/2008 is not required.

8.6 Respiratory sensitisation

Table 24: Summary table of animal studies on respiratory sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Results	Reference
No data					

Table 25: Summary table of human data on respiratory sensitisation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 26: Summary table of other studies relevant for respiratory sensitisation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

8.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

There is no relevant in vitro, in vivo or human data available for classification for this hazard class.

8.6.2 Comparison with the CLP criteria

Not applicable.

8.6.3 Conclusion on classification and labelling for respiratory sensitisation

Not applicable.

8.7 Skin sensitisation

Table 27: Summary table of animal studies on skin sensitisation

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
OECD 406 (1992) Maximization Test Acceptable	Guinea pig Hsd Poc:DH (SPF-bred) Female 35 guinea pigs (20 in test group, 10 in control group, 5 in range finding)	(HWG 1608) Tebuconazole	Intradermal induction (0.1 mL/animal, 5 % suspension) Topical induction (0.5 mL/patch, 50%) Topical challenge (0.5 mL/patch, 40%)	No skin effects in the test substance group and in the control group. Not sensitizing	Vol 3 B.6.2.6.1/01
According to scientific standard; comparable to OECD 406 (1981) Maximization Test Acceptable	Guinea pig Pirbright white W58 (breeder Winkelmann, Borchen) 40 male guinea pigs (20 in test group, 20 in control group)	(HWG 1608) Tebuconazole	Intradermal induction (0.1 mL/animal, 1% suspension) Topical induction (25%) Topical challenge (25%)	Evaluation revealed the same number of positive skin reactions in the test compound group and control group on compound and control flanks (positively reacting animals in test compound group: 8 compound and 8 control and in control group: 3 compound and 3 control). Not sensitizing	Vol 3 B.6.2.6.1/02
OECD Guideline 406. Maximization Test Acceptable	Guinea pig Male and female Dunkin Hartley Guinea pigs 8 males preliminary study, 10 female test group, 5 females control group.	Tebuconazole	Intradermal induction (0.1 mL/animal, 10% suspension) Topical induction (50%) Topical challenge (10%)	Intradermal induction with 10 % suspension of tebuconazole TC in sesame oil revealed a discrete or patchy erythema, or a moderate and confluent erythema in all 10 animals 24 h after start of exposure and a discrete or patchy erythema in 4 animals 48 h after start of exposure. The skin was coated with SDS on the day prior to the topical induction (50% suspension Tebuconazole) to induce skin irritation. The challenge with 10% suspension revealed no skin reactions in any animal. Not sensitizing	Vol 3 B.6.2.6.1/03
OECD 406 (1981) Buehler Test	Guinea pig DHPW (SPF-bred) 36 male	(HWG 1608-technical) Tebuconazole	First to third induction (0.5 mL/patch, 25%, 6 hours) First challenge	There were no skin reactions in the induction or the challenge. Because of the conclusive results of the 1st challenge, a 2nd challenge was not performed.	Vol 3 B.6.2.6.2/01

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Acceptable	guinea pigs (12 in test group, 12 in first control group (1st challenge), 12 in second control group (2nd challenge))		(0.5 mL/patch, 25%, 6 hours)	Not sensitizing	
OECD 406 (1981) Buehler Test Acceptable	Guinea pig Hartley albino (Sasco, Madison, WI) 30 male guinea pigs (15 in tebuconazole test group, 5 in tebuconazole control group), (5 in DNCB test group, 5 in DNCB control group)	(Folicur-technical grade) Tebuconazole	First to third induction (0.4 g, 6 hours) First challenge (0.4 g, 6 hours)	Tebuconazole did not produce any erythema at the dose site of test- or non-induced control groups after the challenge dose (average dermal score were 0/0 in incidence/severity). The DNCB test group showed an average dermal score of 1.0/0.9 after third induction and 1.0/1.3 after challenge. No evidence of irritation at the dose site of the non-induced control group. Not sensitizing	Vol 3 B.6.2.6.2/02

Table 28: Summary table of human data on skin sensitisation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 29: Summary table of other studies relevant for skin sensitisation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

8.7.1 Short summary and overall relevance of the provided information on skin sensitisation

Four studies investigating the skin sensitisation potential of tebuconazole were conducted: two guinea pig maximisation tests, and two Buehler patch tests. In the sensitisation studies no skin sensitisation was observed in the guinea pigs tested by the Buehler patch tests or by the more sensitive Magnusson-Kligman maximisation test.

A new skin sensitisation study (guinea pig maximisation test) was provided for the purpose of renewal. Overall, these 5 studies (3 GPMTs and 2 Buehler tests) show that tebuconazole is not a skin sensitiser.

8.7.2 Comparison with the CLP criteria

These 5 studies (3 GPMTs and 2 Buehler tests) show that tebuconazole is not a skin sensitiser and no classification according to Regulation (EC) No. 1272/2008 is required. This is consistent with the harmonised classification of tebuconazole.

8.7.3 Conclusion on classification and labelling for skin sensitisation

Classification for skin sensitisation according to Regulation (EC) No. 1272/2008 is not required.

8.8 Germ cell mutagenicity

Table 30: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations[1] if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Rosenkranz and Leifer, Chemical Mutagens, Principles and Methods for their detection, Vol. 6, 109 (1980) Acceptable – Supplementary, as a non-standard test	(HWG 1608) Tebuconazole (Purity 97.1%)	Pol test *E. coli* (K12)p 3478; W 3110 (+/- S-9 mix) 625-10000 µg/plate	Non-mutagenic in the Pol test at concentrations up to and including 10000 µg/plate, with or without metabolic activation. Negative	Vol 3 B.6.4.1.1/01
comparable to OECD 471 (1983) Acceptable in a WoE approach with more modern Ames studies.	(HWG 1608) Tebuconazole (Purity 97.0%)	*Salmonella*/microsome test (TA1535, TA100, TA1537, TA98 ; +/– S-9 mix) 20-12500 µg/plate	Non-mutagenic in the *Salmonella*/microsome assay, with and without metabolic activation when tested at concentrations from 20 to 12500 µg/plate. Negative	Vol 3 B.6.4.1.2/03
OECD 471 (1983) Acceptable in a WoE approach. The limitations of this study are compensated by the availability of more modern Ames studies.	(HWG 1608) Tebuconazole (Purity 96.6%)	*Salmonella*/microsome test (TA98, TA100, TA1535, TA1537, TA1538 ; +/– S-9 mix) 37.5-2400 µg/plate 39.5-450 µg/plate	Not mutagenic up to and including 450 µg/plate with or without metabolic activation in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 or TA1538. Negative	Vol 3 B.6.4.1.2/01
OECD 471 Acceptable	Tebuconazole TGAI (Purity 98.8%)	Reverse mutation assay *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 ; *E. coli* WP2 uvrA	Tebuconazole was not mutagenic up to cytotoxic/limit concentrations in the presence and absence of metabolic activation.	Vol 3 B.6.4.1.2/02

Method, guideline, deviations[1] if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
		+/– S-9 1.00 - 1000 µg/plate (TA 98, TA 100, TA 1535, TA 1537) ; 31.6 - 5000 µg/plate (*E.coli* WP2 uvrA)	Negative	
OECD 471 (1997) Acceptable	(HWG 1608) Tebuconazole (Purity 98.0%)	Reverse mutation assay *S. typhimurium* (TA98, TA100, TA1535, TA1537) *E. coli* (WP2/uvrA) +/– S-9 15.625-500 µg/plate (+/- S-9 mix) 31.25-1000 µg/plate (- S-9 mix) 156.25-5000 µg/plate (+ S-9 mix)	Not found to induce base-pair or frameshift mutations in bacteria in the reverse mutation assay with or without metabolic activation. Negative	Vol 3 B.6.4.1.2/04
OECD 471 (1997) Acceptable	Tebuconazole (Purity 95.7%)	Reverse mutation assay *S. typhimurium* (TA98, TA100, TA102, TA1535, TA1537) +/– S-9 plate incorporation test: 3-5000 µg/plate (+/- S-9 mix) pre-incubation test: 3-5000 µg/plate (+/- S-9 mix)	Not mutagenic up to cytotoxic/limit concentrations in the presence and absence of metabolic activation. Negative	Vol 3 B.6.4.1.2/05
OECD Guideline 471 Acceptable	Tebuconazole (Purity 98.4 %)	Reverse Mutation Assay *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100 and *Escherichia coli* strain WP2 uvrA +/– S-9 Experiment I: 1.5 - 5000 µg/plate Experiment II: 1.5 - 5000 µg/plate	Non-mutagenic under the conditions of the test. Negative	Vol 3 B.6.4.1.2/06
OECD Guideline 471 Acceptable	Tebuconazole (Purity 97.21%)	Reverse mutation test *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 ; *E. coli* WP2 uvrA Experiment I: 5 - 5000	Non-mutagenic under the conditions of the test. Negative	Vol 3 B.6.4.1.2/07

Method, guideline, deviations[1] if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
		µg/plate Experiment II: 50 - 5000 µg/plate		
OECD 473 (1983) Acceptable in a WoE approach. Despite the identified limitations, a modern in vitro micronucleus study is available.	(HWG 1608) Tebuconazole (Purity 96.5%)	Cytogenetic *in vitro* (human lymphocytes) 3-30 µg/mL (- S-9 mix) 30-300 µg/mL (+ S-9 mix)	No clastogenic effect on human lymphocytes in concentrations up to 30 µg/ml without S-9 mix and up to 300 µg/ml with S-9 mix. Negative	Vol 3 B.6.4.1.3/01
According Hsie et al., Mutation Res. 86, 193-214 (1981) This is an old study and deviations from the OECD-Guideline 476 (2016) occurred; however, a new fully guideline compliant study (Sokolowski, A.; 2017) is available Acceptable in a WoE approach. Despite the identified limitations, a second modern assay has been submitted.	(HWG 1608) Tebuconazole (Purity 96.6%)	CHO/HGPRT 80.0-100.0 µg/mL (- S-9 mix) 12.5-200.0 µg/mL (+ S-9 mix)	Not considered mutagenic in the CHO-HPRT assay in concentrations up to 100 µg/ml without S-9 mix or up to 150 µg/ml with S-9 mix. Negative	Vol 3 B.6.4.1.5/01
OECD 476 (2016) Acceptable	Tebuconazole (Purity 95.7%)	CHO/HGPRT Pre-test for cytotoxicity: 0 - 2000 µg/mL (±S9) Experiment I: 0 - 250 µg/mL (±S9) Experiment II: 0 - 120 µg/mL (-S9) Experiment III: 0 - 120 µg/mL (+S9)	No significant and reproducible test substance induced increases in mutant frequencies were observed with and without metabolic activation up to cytotoxic concentrations. Based on these results, tebuconazole is considered to be non-mutagenic in the V79/HPRT Forward Mutation Assay, both with and without metabolic activation. Negative	Vol 3 B.6.4.1.5/02
OECD 479 (1986) Acceptable – as supplementary, as non-standard	(HWG 1608) Tebuconazole (Purity 96.5%)	SCE/CHO 4-30 µg/mL (- S-9 mix) 15-120 µg/mL (+ S-9 mix)	Tebuconazole was negative in the Sister Chromatid Exchange assay in concentrations up to 30 µg/mL in the absence of S-9 mix and up to 120 µg/mL in the presence of metabolic	Vol 3 B.6.4.1.6/01

Method, guideline, deviations[1] if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
test			activation Negative	
OECD 482 (1986) Acceptable – supplementary as non-standard test	(HWG 1608 technical) Tebuconazole (Purity 96.5%)	Primary rat hepatocyte UDS 0.504-25.2 µg/mL	Not mutagenic in the primary rat hepatocyte unscheduled DNA synthesis assay in the dose range 0.504 -25.2 µg/mL. Negative	Vol 3 B.6.4.1.7/01
JMAFF 59 Nosan No. 4200 (1985) Acceptable – supplementary as non-standard test	(HWG 1608) Tebuconazole (Purity 98.0%)	Rec-assay (B. subtilis H17, M45) 0.313-20 µg/plate (+/- S-9 mix)	No DNA-damaging effects, with and without metabolic activation, in this rec-assay test in spores of two strains of B. subtilis in the dose range 0.313 to 20 µg/plate. Negative	Vol 3 B.6.4.1.8/01
OECD guideline 487 Acceptable	Tebuconazole	Micronucleus test in human lymphocytes *in vitro* Experiment I, exposure period 4 hours, with and without S9 mix, concentrations 13 - 2000 µg/mL. Experiment IIB 4 hour exposure period with S9 mix, concentrations 81.7 – 250 µg/mL Experiments IIA and IIB, exposure period 20 hours without S9 mix, concentrations 6.5-250 µg/mL (IIA) and 17.7-94.0 µg/mL (IIB) respectively.	Tebuconazole did not induce micronuclei as determined by the *in vitro* micronucleus test in human lymphocytes and is considered to be non-mutagenic in this *in vitro* micronucleus test, when tested up to cytotoxic or precipitating concentrations (21.9-122 µg/mL (- S9 mix) 39.8-122 µg/mL (+ S9 mix) Negative	Vol 3 B.6.4.1.4/01

Table 31: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations[1] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
OECD 474 (1983) Acceptable – supplementary, as a new, modern study is available. GLP	(HWG 1608) Tebuconazole (Purity 95.3%)	Micronucleus test (male and female NMRI mice) 5/sex/dose group Oral, gavage 200-2000 mg/kg bw	Not mutagenic in the somatic *in vivo* mouse micronucleus test when administered in single oral doses (200 to 2000 mg/kg bw) to male and female NMRI mice but was found to inhibit erythropoiesis. Negative (PE/NE ratio altered)	Vol 3 B.6.4.2.1/01

Method, guideline, deviations[1] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
OECD 474 (1996) Acceptable - supplementary, as a new, modern study is available. GLP	Tebuconazole technical (Purity 99.5%)	Micronucleus test (male and female Crl:CD-1 (ICR) BR mice) 5/sex/dose group Oral, gavage 187.5-750 mg/kg/day (total doses: 375-1500 mg/kg)	Tebuconazole does not induce damage to the chromosomes or the mitotic apparatus of mice bone marrow cells after two oral administrations, with a 24-hour interval, at the dose-levels of 187.5, 375 or 750 mg/kg/day. Negative (PE/NE ratio altered)	Vol 3 B.6.4.2.1/02
OECD 474 (1997) Acceptable - supplementary, as a new, modern study is available GLP	Tebuconazole (Purity 98.2%)	Micronucleus test (male and female NMRI mice) 5/sex/dose group Oral, gavage 500-2000 mg/kg	Tebuconazole tested up to the highest reasonable dose level of 2000 mg/kg b.w. by oral administration showed no mutagenic properties in the mouse bone marrow micronucleus study at the two tested sampling times of 24 hours and 48 hours. Negative (PE/NE ratio altered)	Vol 3 B.6.4.2.1/03
OECD guideline 474 Acceptable	Tebuconazole technical (Purity 97.8%)	<u>Mouse *in vivo* micronucleus test</u> (male and female CD1 mice) Oral, gavage Preliminary tests: 2 mice/sex /dose Main tests: 5 mice/sex /dose* * two additional animals per sex were used in the 1st main test high dose group (2000 mg/kg /day) First preliminary test: 1750, 2000 mg/kg /day Second preliminary test: 750, 875 mg/kg /day First main test: 1000, 1750, 2000 mg/kg /day Second main test: 187.5, 375, 750 mg/kg /day	Tebuconazole technical did not show any evidence of causing an increase in the induction of micronucleated polychromatic erythrocytes, in male and female CD1 mice when administered orally by gavage in this *in vivo* test procedure. However, bone marrow cell toxicity was observed at all dose levels tested Negative (PE/NE ratio altered)	Vol 3 B.6.4.2.1/04
OECD guideline 478 Acceptable – supplementary	(HWG 1608) Tebuconazole (Purity 93.5%)	<u>Dominant lethal test</u> (male NMRI mice) 50 males/dose group. 600 Females remained untreated	The dominant lethal test on the male NMRI mouse of a single oral dose of 2000 mg tebuconazole/kg bw did not indicate a mutagenic effect of the substance.	Vol 3 B.6.4.3.1/01

Method, guideline, deviations[1] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
as non-standard test		2000 mg/kg bw	Negative	

Table 32: Summary table of human data relevant for germ cell mutagenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

8.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

Tebuconazole was negative in an extensive number of in vitro and in vivo studies to investigate its genotoxic potential.
Tebuconazole was negative when tested up to cytotoxic concentrations in numerous Ames tests and in multiple HPRT-locus mammalian cell mutation assays in CHO and V79 cells. A supplementary in vitro rat liver UDS assay was also negative. Tebuconazole did not induce chromosome aberrations in human lymphocytes, or significant increases in the frequency of sister chromatid exchanges in CHO cells, in the presence and absence of metabolic activation up to cytotoxic concentrations. Also, it was clearly not clastogenic or aneugenic to human lymphocytes in a new, guideline compliant in vitro micronucleus assay conducted up to cytotoxic concentrations. Overall, there was no evidence of genotoxicity across these in vitro studies.

Four in vivo mouse bone-marrow micronucleus assays, all via oral administration, were available. In all of these, no increase in the incidence of micronuclei was induced. The assays were compliant with the contemporary OECD guideline 474. Bone-marrow toxicity was demonstrated in these assays (reduced PCE/NCE ratio). A dominant lethal test in mice with a single oral dose of 2000 mg /kg bw did not indicate a mutagenic effect of the substance. Overall, there was no evidence of genotoxicity across these in vivo studies.

According to Regulation (EU) no 283/2013, photo-mutagenicity testing is not required for substances with a UV/VIS molar extinction/absorption coefficient less than 1000 L x mol-1 x cm-1. There is no relevant absorption in the range 290 - 700 nm and the ultraviolet/visible molar extinction/absorption coefficient of the active substance is less than 10 L x mol-1 x cm-1 (see chemistry evaluation section B.2.4). Photo-mutagenicity testing is therefore not required for tebuconazole.

8.8.2 Comparison with the CLP criteria

The genotoxic potential of tebuconazole has been investigated in a series of in vitro and in vivo studies. A summary of the available genotoxicity studies is presented in the table above. With the exception of new studies (dated 2008 onwards), these were all evaluated in the original DAR (2006).

The results of these available in vitro and in vivo tests are all negative. Thus the criteria for Category 1A or B classification is not fulfilled. Also classification in Category 2 is not justified based on the available in vitro tests or in vivo mammalian somatic cell chromosomal aberration or genotoxicity tests. According to CLP, a classification for germ cell mutagenicity category 2 is based on positive somatic cell mutagenicity tests in vivo, in mammals, or other positive in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays, or

positive in vitro mammalian mutagenicity assays for substances which also show chemical structure activity relationship to known germ cell mutagens.

Based on these results, the aforementioned classification criteria are not met. Classification criterion for substances which show chemical structure activity relationship to known germ cell mutagens is also not met since tebuconazole does not show a chemical structure activity relationship to known germ cell mutagens and the dominant lethal test resulted negative.

Based on these data it is concluded that no classification for germ cell mutagenicity is warranted for tebuconazole according to Regulation (EC) No 1272/2008.

8.8.3 Conclusion on classification and labelling for germ cell mutagenicity

No classification for germ cell mutagenicity is warranted for tebuconazole according to Regulation (EC) No 1272/2008.

8.9 Carcinogenicity

Table 33: Summary table of animal studies on carcinogenicity

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Chronic/Carcinogenicity, OECD 453 (1981) GLP Deviations to the OECD guideline 453 (2009): the deviations are minimal and can be compensated by results of other studies and thus do not prohibit an evaluation of the results which are important for this type of study, therefore, the study is regarded as acceptable **Rat** Male and female Wistar Bor:WISW (SPF-Cpb) 50/sex/dose	HWG 1608 (Syn. Tebuconazole) Mixed batch no.: Fl. no.: 132; Purity: >95% Oral (diet) study, 2 years 0, 100, 300, 1000 ppm (M/F: 0/0, 5.3/7.4, 15.9/22.8, 55.0/86.3 mg/kg bw/day)	**NOAEL Carcinogenicity**: 1000 ppm for M & F (M/F: 55/86.3 mg/kg bw/day) **NOAEL Systemic toxicity**: 300 ppm for F (equal to 23 mg/kg bw/day) and 1000 ppm for M (equal to 55 mg/kg bw/day) **LOAEL for carcinogenicity**: No carcinogenic effects observed. C-cell adenomas and carcinomas of the thyroid were increased in all treated males (not dose-related, not statistically significant). Hence, not considered treatment-related. In all treated females, a lower frequency of endometrial adenocarcinoma was found. These incidences were small and not dose-related and therefore, considered not treatment-related. **LOAEL for systemic toxicity**: 1000 ppm (86.3 mg/kg bw/day) At 1000 ppm in females: ↓body weight gain -14.5 % week 0-27, -13 % week 0-52 and -2 % week 0-104. Histopathological findings of the adrenal (↓ harmorrhagic degeneration, Control: 23/50 vs 4/50 at 1000 ppm), spleen ↑ haemosiderin accumulation; control: 2/50 vs 19/50 at 1000 ppm in spleen) and liver (pigment deposit in the Kupffer star cells (2/49 in control vs 7/50 at 1000 ppm). Spleen weight significantly ↑; abs (17.2 %) and rel (mg/100g) (15.4 %) at 12 months. Adrenal weight	Vol 3 B.6.5.1/01

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
		significantly ↓; abs (26.9 %) + rel (22.6 %)at 24 months No significant systemic toxicity in males up to the top dose.	
Chronic/Carcinogenicity, OECD 453 (1981) GLP Deviations to the current OECD guideline 453 (2009): the deviations are minimal and can be compensated by results of other studies and thus do not prohibit an evaluation of the results which are important for this type of study type, therefore, the study is regarded as acceptable **Mice** Male and female Bor:NMRI (SPF-Han) 50/sex/dose	HWG 1608 (Syn. Tebuconazole) Mixed batch no.: Fl. no.: 132; Purity: approx. 95% Oral diet study, 21 months, interrim at 12 months 0, 20, 60, 180 ppm (M/F: 0/0, 5.9/9.0, 18.2/26.1, 53.1/80.5 mg/kg bw/day)	**NOAEL Carcinogenicity**: 180 ppm for M & F (M/F: 53/81 mg/kg bw/day). **NOAEL Systemic toxicity**: 20 ppm for M & F (M/F: 6 /9 mg/kg bw/day). **LOAEL for carcinogenicity**: none No treatment-related neoplastic effects in treated animals. The number, type, location and distribution of the neoplasms found throughout the study groups do not provide any indications of a carcinogenic action for the test substance. **LOAEL for systemic toxicity**: 60 ppm (M/F: 18/26 mg/kg bw/day) At 180 ppm: temporarily ↓ erythrocyte counts (M at 21 months (-6.7%) and F at 12 months (-5.1%)), for males ↑ erythrocyte counts at 12 months (5.2%). ↓ hemoglobin (-6.3%) and hematocrit values (-4.3%) 12 months only (F). ↑ in bilirubin levels (F) (+37 % at 12 months, +63.6% at 21 months). ↓ cholesterole in males (-22.5 at 12 months and -24.1 % at 21 months , only statistically significant at 12 months). In females ↓ cholesterole (-36.8% only at 12 months not at 21 months). Slight fatty degeneration /periportal vacuolation of the liver: Interrim: 4/10 in males, 6/10 females vs 0/10 in controls. Terminal: 9/50 vs 0/50 in control in males, 8/50 vs 2/50 in control in females, respectively. Centrilobular vacuolation in the liver in females at interrim (4/10 vs 0/10 in control). Centrilobular vacuolation in the liver in males at 21 months (17/50 vs 5/50 in control). In females 9/50 vs 7/50 in control. At ≥ 60 ppm: slight fatty degeneration / vacuolation of the liver mostly centrilobular vacuolation in the liver in	Vol 3 B.6.5.2/01

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
		males at terminal autopsy (10/50 vs 5/50 in control), and in females at interrim autopsy 2/10 vs 0/10 in control. , ↑ in bilirubin levels (F) (+22.2% at 12 months not significant and + 54.5% at 21 months).	
Chronic/Carcinogenicity, OECD 453 (1981) GLP Deviations to the current OECD guideline 453 (2009): the deviations are minimal and can be compensated by results of other studies and thus do not prohibit an evaluation of the results, therefore, the study is regarded as acceptable **Mice** Male and female NMRI mice, SPF-bred 50/sex/dose	HWG 1608 (Syn. Tebuconazole) Batch no. 816896061 Purity: 96.2 % Oral (diet) study, 21 months 0, 500, 1500 ppm (M/F: 0/0, 85/103, 279/357 mg/kg bw/day) In a first mouse oncogenicity study with doses up to 180 ppm, no significant effects except fatty degeneration of the liver were seen, so this study was repeated with higher concentrations.	**NOAEL Carcinogenicity**: 500 ppm, (M/F: 85/103 mg/kg bw/day) **NOAEL Systemic toxicity**: None, as effects were seen from the lowest dose tested **LOAEL for carcinogenicity**: 1500 ppm, (M/F: 279/357 mg/kg bw/day) At 1500 ppm: ↑ incidence of liver tumours in both sexes. MTD has been exceeded at this dose level. Furthermore, mechanistic data indicate that the MoA is via the CAR/PXR and not relevant for humans. No carcinogenic effects in other organs. **LOAEL for systemic toxicity**: 500 ppm (M/F: 85/103 mg/kg bw/day) At 500 ppm : pronounced liver toxicity (enlarged liver (M,F:2/50,5/50 vs 1/50,0/50 in control), ↑ liver weight at terminal kill males abs 17.1% and rel (mg/100g) 21.7%. , ↑ single cell and focal necroses, inflammation, bile duct hyperplasia and steatoses) and changes in some clinical-chemistry indicative of liver damage e.g. ↑ ALAT, ASAT, alkaline phosphatase and changes in haematological parameters (↓ haematocrit, MCH, MCHC, ↑ thrombocyte count). Please see tables 53-55 below for more details on effect size of histopathology and clinical chemistry/haematology. At 1500 ppm: pronounced liver toxicity (enlarged liver (M,F:35/50,32/50 vs 1/50,0/50 in control) ↑ abs and rel (mg/100g) liver weight in males and females (>100% compared with control at 52 week autopsy, > 200 % 91 weeks), ↑ abs and rel (mg/100g) adrenal weight in females (>20 %) statistically significant only at terninal autopsy. ↑ single cell and focal necroses, inflammation, bile duct hyperplasia and steatoses) and changes in some clinical-chemistry indicative of liver damage e.g. ↑ ALAT, ASAT, alkaline phosphatase and changes in	Vol 3 B.6.5.2/02

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
		haematological parameters (↓ haematocrit, MCH, MCHC, ↑ thrombocyte coundt). Please see tables 53-55 for more details on effect size of histopathology and clinical chemistry/haematology.	

Table 34: Summary table of human data on carcinogenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 35: Summary table of other studies relevant for carcinogenicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
28-day liver mechanistic study in the male and female mice by dietary administration **(liver enzyme activity and gene transcript investigation)** Non-GLP Acceptable as a mechanistic study	Tebuconazole (Purity 97.5%) Batch: K689052	Mouse, NMRI, 20/sex/dose. Diet: 0, 25, 500 and 1500 ppm equivalent to 4, 72 and 201 mg/kg bw/day in males and 5, 87 and 273 mg/kg bw/day in female in the 7-day study and to 4, 77 and 231 mg/kg bw/day in males and 5, 90 and 276 mg/kg bw/day in females in the 28-day study Additional group: Phenobarbital 80 mg/kg bw/d, oral gavage	In conclusion, these results suggest that tebuconazole is not a peroxisome proliferator since neither an induction of LAH – Lauric Acid Hydroxylase (at 28-day exposure) nor an increase in Acoxl gene transcript (at the two periods of exposure) were observed after tebuconazole treatment in both sexes. In contrast, tebuconazole induced BROD and PROD activities as well as an increase in Cyp 2b and Cyp 3a gene transcripts (males > females) to a lesser extent than phenobarbital, indicating that CAR/PXR receptors were indeed activated by tebuconazole	Vol 3 B.6.5.3/01
28-day liver mechanistic study in the male and female mice by dietary administration (**liver histopathology and cell proliferation investigations**) GLP Acceptable as a mechanistic study	Tebuconazole (Purity 97.5%) Batch: K689052	Mouse, NMRI, 20/sex/dose. Diet: 0, 25, 500 and 1500 ppm equivalent to 4, 72 and 201 mg/kg bw/day in males and 5, 87 and 273 mg/kg bw/day in female in the 7-day study and to 4, 77 and 231 mg/kg bw/day in males and 5, 90 and 276 mg/kg bw/day in females in the 28-day study	In conclusion, 1500 ppm of tebuconazole induced marked liver cytotoxicity and cell proliferation throughout the study, for both the 7- and 28-day exposure periods. At 500 ppm liver cytotoxicity (hepatocellular single cell necrosis) was noted in both sexes for both the 7- and 28-day exposure periods, as well as a slight cell proliferation in females only for the 7-Day period. No effect on the liver was observed at the lowest dose level investigated (25 ppm) for the parameters examined.	Vol 3 B.6.5.3/02
DNA-synthesis induction in	Tebuconazole (Purity	Exposure to 5 concentrations (0,	Overall, treatment of isolated male C57BL/6 WT mouse	Vol 3 B.6.5.3/03

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
cultured male C57BL/6 mouse hepatocytes **(the potential to stimulate hepatocellular proliferation in mice)** GLP Acceptable as a mechanistic study	98.2%) Batch 95893115	1, 3, 10 and 30 µM) for 96 hours. Positive control: phenobarbital and epidermal growth factor Solvent control: DMSO	hepatocyte cultures with tebuconazole resulted in concentration-dependent increases in replicative DNA synthesis as determined by the S-phase labelling index, which peaked at 3 µM. Treatment with the positive control items PB and EGF gave the expected set of responses, indicating the suitability of the test system	
DNA-synthesis induction in cultured male CarKO/PxrKO mouse hepatocytes **(the potential to stimulate hepatocellular proliferation in mice when CAR/PXR not present)** GLP Acceptable as a mechanistic study	Tebuconazole	Exposure to 5 concentrations (0, 1, 3, 10 and 30 µM) for 96 hours Positive control: phenobarbital and epidermal growth factor Solvent control: DMSO	There was no increase in replicative DNA synthesis following treatment of isolated male CarKO/PxrKO mouse hepatocyte cultures with either tebuconazole or PB, as determined by S-phase labelling index. Treatment with the positive control item EGF gave the expected response, indicating the suitability of the test system.	Vol 3 B.6.5.3/04
DNA-synthesis induction in cultured male human hepatocytes from three individual donors **(the potential to stimulate hepatocellular proliferation in humans)** GLP Acceptable as a mechanistic study	Tebuconazole (Purity 98.2%) Batch: 95893115	Human hepatocyte cultures from 3 individual donors. Exposure to 5 concentrations (0, 3, 10, 30 and 50 µM) for 96 hours Positive control: phenobarbital and epidermal growth factor Solvent control: DMSO	There was no increase in replicative DNA synthesis following treatment of male primary human hepatocyte cultures from three individual donors with either tebuconazole or PB, as determined by the S-phase labelling index. Treatment with the positive control item EGF gave the expected set of responses, indicating the suitability of the test system.	Vol 3 B.6.5.3/05
Dose-response involvement of constitutive androstane	Tebuconazole (Purity 97.3%)	Dose: 0, 375, 750 or 1500 ppm equivalent to 0, 74, 104, 209 mg/kg bw/day in WT mice and 0, 92, 150, 203 mg/kg bw/day in	The results of this study indicate that while CAR activation is fully responsible for the liver hypertrophy,	Vol 3 B.6.5.3/06

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
receptor in mouse liver hypertrophy induced by triazole fungicides Non-GLP Acceptable as a mechanistic study	Batch: not specified	CARKO mice 5 animals/ group Positive control: phenobarbital	CYP2B induction and hepatocyte proliferation caused by PB in the mouse, CAR activation is not solely responsible for the equivalent liver effects caused by Teb in the mouse. The data suggest that for Teb non-CAR routes, in particular PXR activation, are also important in causing liver hypertrophy, CYP2B induction and hepatocyte proliferation in the mouse	
Involvement of constitutive androstane receptor in liver hypertrophy and liver tumour development induced by triazole fungicide Non-GLP Acceptable as a mechanistic study s	Tebuconazole (Purity 97.3%) Batch: not specified	Administration ot tebuconazole to groups of 25 males at 0, or 1500 ppm (equivalent to 0, 226-336 mg/kg bw/day)	The results of this study suggest that for Teb non-CAR routes, in particular PXR activation, are also important in causing liver hypertrophy, CYP2B induction and hepatocyte proliferation in the mouse. However, CAR activation seems essential in Teb-induced mouse liver tumour development.	Vol 3 B.6.5.3/07
CYP1A1 induction and CYP3A4 inhibition by the fungicide imazalil in the human intestinal Caco-2 cells-Comparison with other conazole pesticides Non-GLP Acceptable as a mechanistic study	Tebuconazole (Purity not specified) Batch not specified		Tebuconazole induced the CYP1A1 activity, but to a much lesser extent than imazalil.	Vol 3 B.6.5.3/08
Azole Fungicides Disturb Intracellular Ca2+ in an Additive Manner in Dopaminergic PC12 Cells Reliable with restrictions	Tebuconazole (purity not specified) Batch not specified	Effects on cell viability were tested using a combined alamar Blue/CFDA-AM assay and on oxidative stress using a H2-DCFDA fluorescent assay Rat dopaminergic pheochromocytoma cells (PC12 cells) were treated *in vitro* with 100 µM tebuconazole for 24	Tebuconazole, concentration-dependently inhibit depolarization-evoked calcium influx. Tebuconazole induce a (near) complete inhibition indicative of a nonspecific inhibition of VGCCs. Exposure to tebuconazole, does not increase ROS production therefore tebuconazole did not inducean	Vol 3 B.6.7.1.3/01

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		hours. Following treatment, the production of ROS and changes in the levels of intracellular Ca2+ were investigated.	effect on oxidative stress	

8.9.1 Short summary and overall relevance of the provided information on carcinogenicity

In a carcinogenicity study in rats, no increases in liver tumour incidences were noted. Two mouse oncogenicity studies were conducted with tebuconazole. In the initial study (B.6.5.2/01), no clear treatment-related oncogenic effects were observed following treatment of male and female mice up to a dose of 180 ppm (53/81 mg/kg bw/day in M/F) for up to 21 months. In a follow-up oncogenicity study in mice (B.6.5.2/02) with dietary doses of 0, 500 or 1500 ppm over a period of 21 months an increased incidence of liver adenomas and carcinomas in both sexes at the highest dose of 1500 ppm (280/357 mg/kg bw/day in M/F) was observed.

Rat
Administration of tebuconazole produced a range of non-neoplastic treatment-related effects at the highest dose and only in females. At the top dose (1000 ppm – estimated to be 55 mg/kg bw/day in M and 86.3 mg/kg bw/day in F) the following effects were seen in females: a treatment-related decrease in body weight, adrenal weight associated with a reduction in individuals with haemorrhagic degeneration of the cortex, increased spleen weight with associated haemosiderin accumulation and pigment deposits in the Kupffer star cells in the liver. Based on these findings, a NOAEL of 300 ppm for females, equivalent to 23 mg/kg bw/day, was determined for systemic effects. Findings in this 2-year study were similar to those in repeat-dose toxicity studies conducted for 28- and 90-days.

There was a slightly higher number of neoplasms in the males at a dose of 1000 ppm, due to a higher benign tumour count.

Table 36 Neoplastic histopathology results of the combined chronic toxicity/carcinogenicity study in rats (main groups):

Dose [ppm]	Males				Females			
	0	100	300	1000	0	100	300	1000
Number of animals examined	50	50	50	50	50	50	50	50
Mortality (%)	18	14	16	6	20	24	20	16
Body weight at termination [g]	396	404	413	387	253	264	259	248
Number of animals examined	50	50	50	50	50	50	50	50
Overall tumour incidence:	25	30	28	29	33	23	35	24
No. of animals with neoplasms	18	19	21	26	26	27	30	20
No. of animals with benign neoplasms	13	12	16	21	19	20	23	17
No. of animals with malignant neoplasms	6	9	5	6	11	10	7	4
No. of animals with multiple neoplasms	5	5	1	5	8	6	3	1

The incidence of C-cell adenomas and carcinomas of the thyroid was increased in all treated male rats

compared to controls (see below).

Table 37 Thyroid and uterus tumour incidences:

Dose [ppm]	Males					Females				
	0	**100**	**300**	**1000**	**HCD[b]**	**0**	**100**	**300**	**1000**	**HCD[b]**
Thyroid tumours										
Follicle adenoma (b)[a]	0/50	1/50	0/50	3/50	--	0/49	0/50	1/50	2/50	--
%	*0*	*2*	*0*	*6*	*0 – 5.2*	*0*	*0*	*2*	*4*	*0 - 4.0*
Follicle carcinoma	*0/49*	*0/49*	*0/50*	*0/50*		*0/50*	*0/50*	*0/50*	*0/50*	
%	*0*	*0*	*0*	*0*		*0*	*0*	*0*	*0*	
C-cell hyperplasia	1/50	3/50	7/50	6/50	--	1/49	2/50	3/50	0/50	--
%	*2*	*6*	*14*	*12*	--	*2*	*4*	*6*	*0*	--
C-cell adenoma (b)[a]	0/50	1/49	3/50	2/50	--	1/49	0/50	1/50	1/50	--
%	*0*	*2*	*6*	*4*	*0 – 17.0*	*2*	*0*	*2*	*2*	*0 –14.3*
C-cell carcinoma (b)[a]	0/50	1/49	0/50	1/50	--	0/49	0/50	0/50	0/50	--
%	*0*	*2*	*0*	*2*	*0 - 16.0*	*0*	*0*	*0*	*0*	*0 – 4.3*
Uterus tumours										
Adenoma (b) [a]						0/50	0/50	1/50	0/50	--
Adenocarcinoma (m) [a]						2/50	1/50	0/50	1/50	
%						*4*	*2*	*0*	*2*	*0 - 14.4[c]*
Carcinoma; atypical (m) [a]						0/50	3/50	2/50	1/50	--
%						*0*	*6*	*4*	*2*	
Adenocarcinoma total [a]						1/50	4/50	2/50	2/50	
%						*4*	*8*	*4*	*4*	

[a] number of animals affected/total number of animals
[b] HCD from 25 studies (1981 – 1987), Wistar (BOR:WISW(SPF-CPB)), same breeder:
[c] Combined historical control data from 11 studies conducted at Bayer (1973 – 1976), Wistar (BOR:WISW(SPF-CPB)), same breeder, outside of the five year range of the study
(b) = benign neoplasms; (m) = malignant neoplasms

Relevant HCD on thyroid tumors were available that consists of data from 25 studies (1981 – 1987) conducted at the same laboratory, same strain and same supplier as the tebuconazole study, strain Wistar (BOR:WISW(SPF-CPB)), same breeder.

For C-cell thyroid tumours, there was no clear dose-response relationship, the histopathology data revealed no evidence of progression from adenoma to carcinoma and the incidences of C-cell thyroid tumours observed in the study were within the historical control data (HCD) provided.

Overall, therefore, the increased incidence of C-cell tumours of the thyroid were considered unrelated to treatment. Follicular adenoma was slightly above the HCD range of relevant HCD in high dose males and just within HCD range in high dose females but no progression to carcinomas were observed.

In female rats, a lower frequency of endometrial adenocarcinoma was found in comparison with controls. These incidences were small and not dose-related. Overall, there were no treatment-related tumours of the uterus or of any other organ

No carcinogenic effect was seen up to the top dose of 1000 ppm, equivalent to 55 mg/kg bw/day for males and 86 mg/kg bw/day for females respectively.

Mice
The long-term toxicity and carcinogenic potential of tebuconazole was assessed in two studies conducted in the mouse over 21 months.

First study in mice:
The first study tested doses of 20 – 180 ppm (6 – 81 mg/kg bw/day). Adverse and treatment-related effects on the liver, including fatty degeneration/vacuolation in both sexes, and an increase in bilirubin in females, were seen at a dose of 60 ppm and above (18 – 26 mg/kg bw/day).

Increases in absolute and relative liver weights (statistically significant for males only), decreases in cholesterol in both sexes and reductions in erythrocyte counts in males were seen at the top dose of 180 ppm. Based on these findings, a NOAEL of 20 ppm for males and females, equivalent to 6 and 9 mg /kg bw/day in males and females respectively, was identified as no effects were seen at this dose level. The number of hepatocellular adenoma in males in the 60 ppm and 180 ppm dose groups was slightly higher compared with the control and low dose group (20 ppm). The increase was not statistically significant.

Table 38 Incidence of hepatocellular tumours

Liver									
		Males				Females			
Dose (ppm)		0	20	60	180	0	20	60	180
Number examined	[N]	50	49	50	49	49	49	50	50
Hepatocellular tumour singular (b)	[N]	2	2	4	6	1	0	0	0
Hepatocellular tumour multiple (b)	[N]	0	0	1	0	0	0	0	0
Hepatocellular adenoma singular or multiple	[N]	2 (4 %)	2 (4 %)	5 (10 %)	6 (12%)	1	0	0	0
Hepatocellular carcinoma	[N]	1	0	0	1	0	0	0	1
Haemangioma (b)	[N]	1	2	0	0	1	0	0	0
Hemangiosarcoma (m)	[N]	0	0	0	1	0	0	0	0

The incidence of hepatocellular adenoma in males was 4 % / 4 % / 10 % / 12 % at 0, 20, 60 and 180 ppm respectively. In the male high dose group (180 ppm) and in the control group there was also one animal each with a carcinoma.

HCD for benign and malignant liver tumours were provided; these ranged from 1/46 (2 %) to 9/50 (18 %) (Table 51). These HCD were obtained within 5 years of the study date, using the same species, strain and breeder as the study. However, no information on the laboratory was provided and more importantly, no distinction between malignant and benign, singular and multiple tumour incidences was presented; therefore, these HCD should not be compared directly with the increased incidences of liver adenoma seen in males in this study. HCD (2 % - 16 %) for liver adenoma in males in the same strain of mice and relevant period (1984 - 1996) from the Registry of Industrial Toxicology Animal-data (RITA) database have also been provided (Table 52); although these data show that the increased incidences (10/12 %) of liver adenoma seen in males in this study were within the range of the RITA HCD, they were not obtained from the same laboratory where the study was conducted and should therefore normally only be considered as supplemental information. The RITA HCD data does however confirm the same trend of liver adenoma formation in

untreated males of same species and strain obtained from the same breeder. Considering this trend of liver tumor formation in males of this strain and the fact that the higher incidence in the 180 ppm is not statistically significant, it can be concluded that the effect is not treatment-related.

Table 39 Incidence of hepatocellular tumours in historical studies

Study Number	**1**	**2**	**3**	**4**	**5**	**6**
Liver						
Number examined	50	50	50	45	46	48
Hepatocellular tumours (b+m) [N]	7	3	9	5	1	6
[%]	14	6	18	11	2	12

(b) = benign neoplasms; (m) = malignant neoplasms

Details of HCD presented:

Years conducted – 1981 – 1988 (Note: within 5 years of the study date 1984 – 1986)

Laboratory – information not provided for the HCD (Note: Laboratory in the study was Bayer)

Species – Mouse (Note: same as used in the study)

Strain – NMRI (Note: same as used in the study)

Breeder – Winkelmann, Borchen (Germany) (Note: same as used in the study)

Table 40 Hepatocellular tumours in mice, historical RITA data

Mice strain	**Studies (N)**	**Dates of study**	**Duration (months)**	**Number of animals**	**Adenoma, hepatocellular**		**Carcinoma, hepatocellular**	
					with lesion	**in %**	**with lesion**	**in %**
Male								
NMRI	6	1984 – 1996	19 – 24	348	27	2.0 – 16.0 mean 7.8	21	0.0 – 20.0 mean 6.0
All*	113	1984 - 2013	6 - 25	5167	391	0.0 – 22.0 mean 7.6	459	0.0 – 22.0 mean 8.9
Female								
NMRI	6	1984 –	19 – 24	210	3	0.0 – 2.0	4	0.0 – 4.0

		1996				mean 1.4		mean 1.9
All*	113	1984 - 2013	6 - 25	5112	74	0.0 – 13.3 mean 1.4	57	0.0 – 12.2 mean 1.1

*the studies conducted over 6 months were in RasH2 and p53 which had very low incidences in liver tumours. Thus, considering only classical carcinogenicity studies conducted over 19 – 25 months, mean incidences are higher.

Second study in mice:

The second study tested higher doses of 500 and 1500 ppm (85 – 357 mg/kg bw/day) as the question of whether a maximum tolerated dose (MTD) had been reached in the first study was raised. Severe liver effects, which included enlargement of the liver, increased liver weights , single cell and focal necrosis, inflammation, bile duct hyperplasia and steatosis, accompanied by clinical-chemistry and haematological changes, were evident at both doses. Given the marked liver toxicity observed, particularly at the top dose of 1500 ppm, the RMS considers the MTD to have been exceeded in this study. Liver weights were increased >100% compared with control at 52 week autopsy and > 200% compared with control at terminal autopsy, respectively and marked effects on liver histopathology were observed. See tables below for details on histopathology.

Table 41 Clinical chemistry and haematology measured in mice treated with tebuconazole in their diet through 21 months

		Males			**Females**		
Dose [ppm]		**0**	**500**	**1500**	**0**	**500**	**1500**
ALAT [U/L]	wk 51	38.0	53.2* (40.0%)	236.3** (522 %)	31.7	51.7* (63.1%)	272.5** (760%)
	wk 90	74.9	123.1** (64.4%)	480.8** (541%)	39.2	64.9* (65.6%)	419.4** (970%)
ASAT [U/L]	wk 51	31.9	37.5 (17.6%)	121.3** (280%)	38.3	47.2* (23.2%)	144.0** (276%)
	wk 90	46.1	60.7 (31.7%)	251.8** (446%)	36.9	59.0** (59.9%)	303.8** (723%)
Alkaline phosphatase [U/L]	wk 51	74	117** (58.1%)	181** (145%)	174	212 (21.8%)	292 (67.8%)
	wk 90	126	156 (23.8%)	531** (321%)	182	328 (80.2%)	517** (184%)
Cholesterol [mmol/L]	wk 51	3.71	1.99** (-46.4%)	1.66** (-55.3%)	2.84	1.48** (-47.9%)	1.92*
	wk 90	3.88	1.57** (-59.5%)	4.55 (17.3%)	3.76	2.25** (-40.2%)	3.59
Bilirubin [µmol/L]	wk 51	1.8	1.3** (-27.8%)	1.3** (-27.8%)	2.2	2.1 (-4.5%)	1.9 (-13.6%)
	wk 90	2.1	1.6** (-23.8%)	5.0 (138%)	2.7	2.1*(-22.2%)	4.9 (81.5%)
Inorg. phosphate [mmol/L]	wk 51	1.90	1.73*	2.14*	1.63	1.69 (3.7%)	1.94** (19%)
	wk 90	1.60	1.75**	2.06**	1.62	1.64 (1.2%)	1.93** (19.1%)
Leucocyte count [10^9/L]	wk 51	5.2	5.6 (7.7%)	10.2** (96.2%)	3.9	3.7 (-5.1%)	10.7** (174%)

Dose [ppm]		Males			Females		
		0	500	1500	0	500	1500
	wk 90/91	6.6	5.0* (-24.2%)	9.8* (48.5%)	7.6	4.3 (-43.4%)	9.5 (25.0%)
Erythrocyte [10^{12}/L]	wk 51	8.90	9.11	8.51	8.40	8.99 (7.0%)	7.49 (-10.8%)
	wk 90/91	9.13	8.25	7.95** (-12.9%)	8.36	8.63 (3.2%)	7.51(-10.2%)
Haemoglobin [g/L]	wk 51	142	144	129** (-9.2%)	139	148**	132
	wk 90/91	143	150	117** (-18.2%)	132	131	125
Haematocrit [L/L]	wk 51	0.435	0.414	0.373** (-14.3%)	0.422	0.432 (2.4%)	0.381* (-9.7%)
	wk 90/91	0.427	0.375** (-12.2%)	0.375** (-12.2%)	0.407	0.401 (-1.5%)	0.380 (-6.6%)
MCHC [g/L erythrocytes]	wk 51	327	348** (6.4%)	347** (6.1%)	331	344 (3.9%)	347* (4.8%)
	wk 90/91	334	402** (20.4%)	313** (-6.3%)	324	328 (1.2%)	328 (1.2%)
MCH [pg]	wk 51	16.0	15.8	15.3 (-4,4%)	16.6	16.6	17.7 (6.6%)
	wk 90/91	15.7	18.3** (16.6%)	14.6* (-7.0%)	15.8	15.3	16.7 (5.7%)
Thrombocyte count [10^9/L]	wk 51	1311	1323	1650** (25.9%)	1024	1274* (24.4)	1284 (25.4%)
	wk 90/91	1678	1668	2223* (32.5%)	904	1374* (52.0%)	1771** (95.9%)
Clotting time (Hepatoquick) [sec]	wk 51	21.1	20.4	20.1* (-4.7%)	20.7	19.7 (-4.8%)	19.2 (-7.2%)
	wk 90/91	19.0	18.9	16.8** (-11.6%)	19.3	18.8 (-2.6%)	16.3** (-15.5%)

* significantly different from control $p \leq 0.05$

** significantly different from control $p \leq 0.01$

wk week

ASAT: Aspartate aminotransferase ALAT: Alanine aminotransferase

MCHC: Mean corpuscular haemoglobin concentration MCH: Mean corpuscular haemoglobin

Table 42 Non-neoplastic findings – Interim phase

Organ	Dose [ppm]					
	0	500	1500	0	500	1500
	Males			Females		

Organ		Dose [ppm]					
		0	**500**	**1500**	**0**	**500**	**1500**
Liver							
Examined	[N]	10	9	10	10	10	10
Focal inflammation with associated hepatocytic generation	[N]	1	5	1	0	**5***	2
Necrosis of individual hepatocytes	[N]	0	**5***	**8*****	0	2	**9*****
Focal necrosis	[N]	0	0	0	0	1	2
Focal hyperplasia of hepatocytes	[N]	0	0	2	0	0	3
Periacinar hepatocytic vacuolation	[N]	0	1	0	0	0	0
Panacinar fine fatty vacuolation	[N]	0	**8*****	**10*****	0	**10*****	**10*****
Centriacinar fatty vacuolation (large)	[N]	1	1	1	0	**9*****	**6***
Periacinar hepatocytic hypertrophy	[N]	0	0	0	0	0	1
Chronic inflammatory cells within the portal area	[N]	1	3	2	1	4	**8****
Bile duct hyperplasia	[N]	0	1	**8*****	0	2	**6***
Periportal fibrosis	[N]	0	1	**5***	0	0	2
Extramedullary haemopoiesis	[N]	0	1	1	0	3	**5***
Eosinophilic focus/foci of hepatocellular alteration	[N]	0	0	0	0	0	3
Pigment laden Kupffer cells	[N]	0	**4***	**8*****	0	0	**8*****
Fat stain: Panacinar fat	[N]	1	**6***	**8****	6	9	5
Fat stain: Pericinar fat	[N]	2	3	2	0	0	**5***
Focal mineralization	[N]	0	0	1	0	0	0

Table 43 Non-neoplastic findings – Terminal phase

Organ		Dose [ppm]					
		0	**500**	**1500**	**0**	**500**	**1500**
		Males			**Females**		
Liver							
Examined	[N]	47	48	48	47	45	46
Focal inflammation with associated hepatocytic generation	[N]	1	1	0	3	2	0
Necrosis of individual hepatocytes	[N]	3	**11***	2	0	2	1
Focal necrosis	[N]	1	1	1	1	3	5
Focal hyperplasia of hepatocytes	[N]	6	2	**23*****	1	0	**12*****
Kupffer cell hyperplasia	[N]	0	3	3	0	0	1
Panacinar hepatocytic fatty vacuolation	[N]	0	5	0	0	0	0
Panacinar fine fatty vacuolation	[N]	0	**14*****	**25*****	1	4	**19*****

Organ		Dose [ppm]					
		0	**500**	**1500**	**0**	**500**	**1500**
Centriacinar fatty vacuolation (large)	[N]	1	1	0	3	**13****	4
Periacinar hepatocytic hypertrophy	[N]	0	0	2	0	0	**13*****
Chronic inflammatory cells within the portal area	[N]	2	2	7	5	1	2
Bile duct hyperplasia	[N]	0	3	5	0	0	1
Oval cells proliferation	[N]	0	0	**23*****	0	0	**17*****
Extramedullary haemopoiesis	[N]	0	2	**7***	5	1	12
Clear cell focus/foci	[N]	0	0	2	0	0	4
Eosinophilic focus/foci of hepatocellular alteration	[N]	0	2	3	0	0	**7****
Biliary cyst(s)	[N]	0	0	2	0	0	0

Based on these findings, a LOAEL of 500 ppm equivalent to 85 and 103 mg /kg bw/day in males and females respectively, was identified for systemic effects.

Liver tumours were significantly increased in both sexes at 1500 ppm (the top dose), a dose at which marked liver toxicity occurred(please see table below). Increases in tumours were markedly above the range of spontaneous incidences observed in this mouse strain.

Table 44 Animals with neoplastic findings in the liver – Terminal phase

Liver							
		males			females		
Doses (ppm)		0	500	1500	0	500	1500
Examined		47	48	48	47	45	46
Hepatocellular Adenoma (b)	[N]	3 (6%)	2 (4%)	**17*** (35%)**	0 (0%)	0 (0%)	2 (4%)
Hepatocellular Carcinoma (m)	[N]	0 (0%)	0 (0%)	**10*** (21%)**	1 (2%)	0 (0%)	**12*** (26%)**
Hemangiosarcoma	[N]	0	0	0	0	1	0

A NOAEL for carcinogenicity in the mouse of 500 ppm, equivalent to 85 and 103 mg/kg bw/day for males and females respectively, was therefore identified.

Proposed mode of action (MoA) and human relevance assessment
The weight of evidence indicates a hypothesized mode of action (MoA) via the constitutive androstane receptor and/or pregnane X receptor (CAR/PXR) activation leading to the observed liver tumours. The key events involved are summarized in the following table:

Table 45 Listing of key events and associative events for a CAR/PXR-mediated liver tumour MOA

Events	Description
Key events (KE)	

KE 1	Activation of CAR/PXR nuclear receptor
KE 2	Altered gene expression secondary to CAR activation
KE 3	Increased hepatocellular proliferation
KE 4	Increased clonal expansion, leading to altered foci
KE 5	Increased incidence of hepatocellular tumours
Associative events (AE)	
AE 1	Increased Cyp2b, Cyp3a enzyme activity and/or protein
AE 2	Hepatocellular hypertrophy
AE 3	Increased liver weight

(From Elcombe et al, Crit Rev Toxicol. 2014;44(1): 64-82)

The mechanistic and regulatory studies described above have demonstrated these key events for tebuconazole. Thus, tebuconazole induced BROD and PROD activities as well as an increase in Cyp2b and Cyp3a gene transcripts in the available *in vivo* studies (KE 1, 2 and AE 1) which indicates that CAR/PXR receptors were activated by tebuconazole with subsequently altered gene expression. These key events together with increased hepatocellular proliferation (KE 3) were demonstrated *in vivo* (B.6.5.3/02).

This was also supported in two publications (B.6.5.3/06 and B.6.5.3/07) about triazole fungicides, including tebuconazole, investigating effects on the liver in wildtype (WT) and CAR knockout (CARKO) mice. Overall, the studies confirmed an involvement of the CAR and PXR receptors in the development of the tumours by showing fewer key events in CARKO mice treated with tebuconazole compared to WT mice treated with tebuconazole; also fewer altered hepatocellular foci occurred in CARKO than in WT mice treated with tebuconazole. The other key and associative events, like hepatocellular hypertrophy (AE 2), increased liver weight (AE 3) and eventually liver tumours (KE 5) were seen in the standard toxicology studies.

In addition to the in vivo mechanistic studies, in vitro studies were conducted with wild-type (WT) mice (B.6.5.3/03), CAR/PXR-knockout (CarKO/PxrKO) mice (B.6.5.3/04) and human (B.6.5.3/05) hepatocyte cultures exposed to tebuconazole. These studies confirmed the involvement of CAR/PXR in the postulated MoA since they demonstrated that hepatocyte proliferation was induced in WT mouse hepatocytes but not in CarKO/PxrKO mouse hepatocytes. Human hepatocytes did not show proliferation which clearly confirms that human hepatocytes are not sensitive to this MoA as the key event of hepatocyte proliferation does not occur in humans. On this basis, the postulated MoA is not relevant to humans.

Based on the available studies, other potential MoAs can also be excluded. A genotoxic MoA can be excluded based on the results of the genotoxicity studies which did not indicate a genotoxic potential for tebuconazole. Furthermore, neither an induction of LAH -Lauric Acid Hydroxylase (at 28-day exposure) nor an increase in Acoxl gene transcript (at 7- and 28-day exposure), two markers of peroxisome proliferation, was observed after treatment with tebuconazole, which suggests that tebuconazole is not a peroxisome proliferator. Also an effect on apoptosis is not likely based on the absence of clear effects on the apoptosis gene transcripts Bax and Bcl-X1. The negative Cyp1a1 results also indicate that there is no involvement of the AhR receptor.

Furthermore, severe liver cytotoxicity alone as a MoA for the liver tumours can be excluded, since in the toxicity studies with tebuconazole in mice at the highest dose tested, no signs of severe cytotoxicity in the liver, like inflammatory signs, broad hepatic necrosis, hepatocellular death, fibrosis, cirrhosis or severely increased transaminase activities were observed.

Conclusion
The lowest long-term systemic toxicity NOAEL, identified in the mouse, was 20 ppm (6/9 mg/kg bw/d in M/F), based on effects at 60 ppm (18/26 mg/kg bw/d in M/F). Tebuconazole was carcinogenic in the mouse, causing liver tumours in both sexes at 1500 ppm (280/357 mg/kg bw/d in M/F). A NOAEL for carcinogenicity of 500 ppm in males and females (85/103 mg/kg bw/d in M/F) was identified as no tumours were seen at this dose level.

Overall, it can be concluded that the available evidence shows that the liver tumours seen in mice at the high dose of 1500 ppm (280/357 mg/kg bw/day in M/F) are most likely to arise through activation of CAR/PXR receptors, with consequent altered gene expression, hypertrophy, and hepatocyte proliferation, leading to altered foci and eventually tumours. The available evidence also shows that hepatocyte proliferation does not occur in human hepatocyte cultures exposed to tebuconazole, which clearly confirms that human hepatocytes are not sensitive to this MoA. On this basis, the postulated MoA and resulting liver tumours are not relevant to humans. Hence, it is concluded that classification for carcinogenicity is not warranted.

8.9.2 Comparison with the CLP criteria

Table 46 Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Rat, Wistar	**Thyroid Tumours** **Follicule adenoma** 0-100-300-1000 ppm: M: 0/49[0%]-1/49/2%]-0/50[0%]-3/50[6%] F: 0/50[0%]-0/50[0%]-1/50[2%]-2/50[4%]	No	n.a.	No	In both sexes	No	Oral (diet)	Slight incidences, not statistically significant, considered of spontaneous origin
	C-Cell adenoma 0-100-300-1000 ppm: M: 0/49[0%]-1/49[1%]-3/50[3%]-2/50[2%]		No evidence of progression from adenoma to carcinoma		In males			Not dose-related, not statistically significant and lack of evidence of progression from adenoma to carcinoma
	C-cell carcinoma 0-100-300-1000 ppm: M: 0/49[0%]-1/49[2%]-0/50[0%]-1/50[2%]				In males			
	Uterus tumours **Endometrial adenocarcinoma** 0-100-300-1000 ppm: F: 2/50-1/50-0/50-1/50	No	n.a.	Small incidence	Females	No	Oral (diet)	Small incidences, not dose-related, considered not treatment related
Mouse,	**Liver Tumours**	No evidence	No	No	Both	Yes	Oral	MoA not relevant to

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
NMRI	**Hepatocellular adenoma** 1500 ppm: 17/48* (M) 2/46 (F) Incidences in concurrent control animals: 3/47 (M) 0/45 (F) **Hepato-cellular carcinoma** 1500 ppm: 10/48* (M) 12/46* (F) Incidences in concurrent control animals: 0/47 (M) 1/47 (F)	for carcinogenic effects of tebuconazole in other organs			sexes	RMS considers the MTD to has been exceeded	(diet)	human: increase liver tumors due to activation of CAR/PXR receptors, with consequent altered gene expression, hypertrophy, and hepatocyte proliferation, leading to altered foci and eventually tumours

*Significantly different from control $p \leq 0.001$

According to the above mentioned ECHA Guidance a classification for carcinogenicity requires an increased incidence of neoplasms due to exposure to the substance.

In the two-year rat study, the type, location and distribution of the neoplasms did not indicate a carcinogenic potential of tebuconazole (B.6.5.1/01).

In a 21-month mouse study with doses of 0, 20, 60 and 180 ppm, no clear treatment-related carcinogenic effect was seen (B.6.5.2/01).

In a second 21-month mouse study, with doses of 0, 500 and 1500 ppm, the incidences of liver tumours were significantly increased in both sexes at 1500 ppm, a dose at which marked liver toxicity occurred. Increases in tumours were markedly above the range of spontaneous incidences observed in this mouse strain. A NOAEL for carcinogenicity in the mouse of 500 ppm, equivalent to 85 and 103 mg/kg bw/d for males and females respectively, was therefore identified.

However, mechanistic studies demonstrated that tebuconazole caused activation of the CAR and PXR receptor with consequent altered gene expression, hypertrophy, and hepatocyte proliferation, leading to altered foci and eventually tumours. The available evidence has also shown that hepatocyte proliferation does not occur in human hepatocyte cultures exposed to tebuconazole, which clearly confirms that human hepatocytes are not sensitive to a key event in this MoA. No other MoA relevant to humans was identified.

It was further concluded that the liver tumors occurred only at a dose exceeding the maximum tolerated dose (i.e. at the highest dose of 279 mg/kg bw/d).

Based on the MoA of a CAR/PXR-mediated effect together with the high dose beyond the MTD at which liver tumors were seen, the liver tumors in mice are not regarded as relevant to humans.

8.9.3 Conclusion on classification and labelling for carcinogenicity

Classification for carcinogenicity is not warranted.

8.10 Reproductive toxicity

8.10.1 Adverse effects on sexual function and fertility

Table 47: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL in mg/kg bw/day (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL	Reference
Developmental toxicity studies relevant for sexual function and fertility			
Two-generation, dietary GLP OECD test guideline no. 416 (1983) Tebuconazole, batch FL 132 (mixed batches), 95.2 %. Rat Bor: WISW (SPF Cpb) 25 males and 25 females/dose	Tebuconazole 95.2% purity ppm: 0, 100, 300 and 1000 Equivalent to (mg/kg bw/d): 0, 9.1 – 11.1, 27.8 – 33.9 and 94.8 – 111.4 in F and 0, 7.1 – 9.2, 21.6 – 27.1 and 72.3 – 97.2 in M. Exposure from 17 weeks premating, during mating, gestation, lactation of F1 and continued until PND21 of the F2 generation.	**NOAEL:** 300 ppm. Equivalent to: 21.6 – 27.1 mg/kg bw/d (males) and 27.8 – 33.9 mg/kg bw/d (females). **LOAEL:** 1000 ppm. Equivalent to: 72.3 - 97.2 mg/kg bw/d (males) and 94.8 - 111.4 mg/kg bw/d (females). **Effects at the LOAEL** Reproductive: One dam was found moribund, possibly due to dystocia. When sacrificed there were foetuses in both uterine horns, and the placentas in one horn were found to be very thick, beige coloured and hard (**see table W – appendix II**). Systemic toxicity: Decreased food consumption was seen but was not statistically significant (8-11% of control, no change in first generation females). Body weight gains and body weights were slightly retarded (less than 10% and not considered toxicologically relevant). Organ weight decrease (absolute liver (12%) & kidney (7-10%) weight, not relative) secondary to decreased body weights (9%) in F1B parental animals. (**see table W and Z – appendix II**).	Vol 3 B.6.6.1.1/01
Developmental neurotoxicity (DNT), oral dietary GLP Not in accordance with OECD test guideline, but in accordance with US-EPA, OPPTS	Tebuconazole, batch 603-00l 3, 96.0 – 96.9 % ppm: 0, 100, 300, 1000. Exposure from GD6 to lactation	**NOAEL**: 300 ppm Eqivalent to: 22 and 41.3 mg/kg bw/d during gestation and lactation, respectively. **LOAEL**: 1000 ppm. Equivanent to: 65 and 125.4 mg/kg bw/d during gestation and lactation, respectively. **Effects at the LOAEL** Reproductive: prolonged gestation, two maternal deaths/moribund	Vol 3 B.6.6.2.1.2/01

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL in mg/kg bw/day (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL	Reference
870.6300; US-EPA, Pesticide Assessment Guidelines Sprague-Dawley rats (Crl:CD®BR VAF/Plus®) (25 presumed pregnant rats per dosage group)	day 11 Equivalent to (mg/kg bw/d) Gestation days 6-21: 0, 8.8, 22.0 and 65.0; Lactation days 1-12: 0, 16.3, 41.3 and 125.4.	sacrifices (GD 22 or 23) related to dystocia (**see table W**). Systemic: Reduced body weight and feed consumption (6-16%) (**see table W**).	
Developmental neurotoxicity (DNT), perinatal dosing, gavage, dams and pups Not GLP Not in accordance with OECD test guideline Sprague-Dawley rats (strain: Tac: N(SD)fBR) 100 mated female rats, ≥15/dose	Tebuconazole, no information about batch number, 97.4 % 0, 6, 20, 60 mg/kg bw/d, Eposure from GD 14 to PND 7	**NOAEL** : 20 mg/kg bw/d **LOAEL** : 60 mg/kg bw/d **Effects at the LOAEL :** Reproductive: no effect on gestation length and no dystochia was seen Systemic: Maternal weight gain during pregnancy reduced from 87.8 g (control) to 74.0 g (60 mg/kg bw/d group). (For more details **see table W**).	Vol 3 B.6.6.2.1.2/01
Developmental toxicity, oral gavage Not GLP No guideline specified Pregnant female Wistar rats, 20-24/dose group	Tebuconazole 98% Control; 24 dams of which 19 were pregnant and 6 were sacrificed at GD 21 50 mg⁄kg bw/d; 19 pregnant dams of which 7 for GD 21 section 100 mg/kg bw/d: 18 pregnant of which 8 for GD 21.	**NOAEL:** 50 mg/kg bw/d, **LOAEL:** 100 mg/kg bw/d, **Effects at the LOAEL** Reproductive: prolonged gestation seen in both dose groups (only statistically significant at 100 mg/kg bw/d) and two dams in the 100 mg/kg group were unable to give birth due to dystocia (**see table W**). Systemic: reduced maternal weight gain (29-34% at high dose), reduced dam weight (8% reduction at high dose GD 21), no change in dam weight adjusted for litter and uterine weight. .	Vol 3 B.6.6.3/07

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL in mg/kg bw/day (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL	Reference
	Exposure from GD 7 to GD 21 and from PND1-16		
Developmental toxicity, oral gavage Not GLP Pregnant female Wistar rat, 10-22 mated per dose group	Tebuconazole 98.5%, 0, 12.5, 50 mg/kg bw/day Exposure from GD7 to PND16. 15, 8 and 6 dams with viable litters in each exposure group respectively.	**NOAEL** 50 mg/kg bw/d **LOAEL** : ND **Effects at the LOAEL** Reproductive: no change in gestation length or number of implantation scars in the uterus. Sytemic: no statistically significant effects on maternal body weight gain, and no clinical signs of toxicity . Increased liver weight (7%) at PND16 in males from the 50 mg/kg bw/d group, this was not evident in adulthood. (For more details **see table W and Z**).	Vol 3 B.6.6.3/04 Vol 3 B.6.6.3/05 Vol 3 B.6.6.3/06
Prenatal developmental toxicity study in rats			
Developmental toxicity, oral gavage Not GLP Pregnant female Wistar rats, 10 mated per dose group.	Tebuconazole 98%, Control; 10 dams of which 6 were pregnant 50 mg/kg bw/d: 9 dams. Exposure from	**NOAEL**: ND **LOAEL**: 50 mg/kg bw/day **Effects at the LOAEL** Reproductive: decrease in estradiol in dams (58 %). Systemic: no effect on maternal weight gain (For more details **see table W**).	Vol 3 B.6.6.3/08

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL in mg/kg bw/day (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL	Reference
	GD 7 to GD 21, with Caesarean sections performed on GD 21.		
Four other prenatal developmental toxicity studies have been performed in **rats**. They were all performed according to GLP and were complient with OECD TG 414 (1981), (n=25). B.6.6.2.1.1/01 and B.6.6.2.1.1/02) used oral gavage doses up to 120 mg/kg bw/day from GD 6-15, while B.6.6.2.1.3/01 and B.6.6.2.1.3/02 tested dermal doses up to 1000 mg/kg bw/day from GD6-15 in Wistar rats. None of the studies found any adverse effect in preimplantation loss. Gestation length and dystochia could not be examined using this study design. For additional details, please go to section on developmental toxicity and table W and Z in annex III.			
Four prenatal developmental toxicity studies have also been performed in **rabbits**. They were all performed according to GLP and were complient with OECD TG 414 (1981), (n= 15-16). B.6.6.2.2.1/01, B.6.6.2.2.1/02, B.6.6.2.2.1/03 and B.6.6.2.2.1/04 used oral gavage doses up to 100 mg/kg bw/day from GD 6-18/19, in either Himalayan- or Chinchilla rabbits. None of the studies found any adverse effect in preimplantation loss. Gestation length and dystochia could not be examined using this study design. For additional details, please go to section on developmental toxicity and table W and Z in annex III.			
Four prenatal developmental toxicity studies have been performed in **mice.** Three of them used oral gavage doses up to 100 mg/kg bw/day, and they were performed according to GLP and were complient with OECD TG 414 (1981), B.6.6.2.3.1/01), B.6.6.2.3.1/02) and B.6.6.2.3.1/03. Additionally, B.6.6.2.3.2/01tested dermal doses up to 1000 mg/kg bw/day in NMRI mice according to a US EPA teratogenicity study guideline (1984). None of the studies found any adverse effect in preimplantation loss. Gestation length and dystochia could not be examined using this study design. For additional details, please go to section on developmental toxicity and table W and Z in annex III.			
Pubertal toxicity studies relevant for sexual function and fertility			
Pubertal assay, oral gavage. US-EPA OPPTS 890.1500, 890.1450 (2009) Acceptable Rat Crl:CD (SD) Sprague Dawley, 15 male and 15 female	Tebuconazole Batch N.o: K 689052 Purity: 97.5 %. 0, 75 and 150 mg/kg bw/d Females exposed from PND 22 to 42, and males exposed from PND 23 to 53.	**NOAEL** - **LOAEL** 75 mg/kg bw/d **Effects at the LOAEL** 75 mg/kg bw/day: Reproductive: ↓ , dorsolateral prostate (21%) and adrenal (21%) weights; macroscopic effects (atrophic/small seminal vesicle); ↓ testosterone (17%, not statistically significant). Systemic: Mean body weight of females was comparable to controls throughout treatment, whereas male body weight was significantly reduced (6-7%) on selected days of treatment. No statistically significant differences in male or female final body weights. **Adverse fertility effects were seen even more clearly at the highest tested dose of 150 mg/kg bw/day and included the following:** Delay in PPS and VO (see table W), ↓ adrenal (13%)& sexual organ weights (seminal vesicle (23-33% relative and absolute), dorsolateral prostate (21%, absolute), epididymis (13%, absolute) and LABC (24%, absolute)); macroscopic effects (atrophic/small prostate (1 vs 5/15), seminal vesicle (2 vs 7/15), LABC (0 vs 3/15) and testis (1 vs 3/15), not assessed statistically); ↓ testosterone (53%). Systemic: ↓ body weight at treatment start (10% of control), ↓ body	Vol 3 B.6.8.3.1.2/01

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL in mg/kg bw/day (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL	Reference
		weight gains in males and females during treatment (varies from day to day, e.g. 61% lower bw gain from dosing start to day 4 of dosing in males and 71% cumulative bw gain on day 3 of treatment in females), final bw 8%↓(males only, no difference in final female bw). After initial growth impairment, the growth of exposed animals was comparable to that of controls. Evaluation of individual animal data reveal that on the first day of dosing 5 /15 males displayed weight loss (2 to 5 g equal to 5-10% of own bw), which was regained on the next day (up to 12 g weight increase equal to 20% of own bw).	
(Late) pubertal exposure Not GLP, No guideline specified Rat, male, 6/dose	Tebuconazole, 95%, 0, 25, 50, 100 mg/kg bw/d Male rats exposed for 21 days from PND35-56	**NOAEL:** 50 mg/kg bw/d **LOAEL:** 100 mg/kg bw/d **Effects at the LOAEL:** Increased serum testosterone level (~175%) and lowered serum estradiol (~40%). Increased gene and protein expression of Cyp11a1, Hsd11b1, Fshr (~1.5-2 fold). No effects on Lhcgr, Scarb1, Star, Hsd3b1, Cyp17a1, Hsd17b3, Dhh, Amh, Sox9). No change in body weight or epididymis and testis weight. For more details see table W.	Vol 3 B.6.8.3.1.2/02

Table 48: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 49: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
See annex II for the assessment of ED properties for supportive *in vitro* investigations on EAS-modality				

8.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

The main adverse effects of tebuconazole identified by the DK-RMS as relevant for classification for reproductive performance and fertility are 1) dystocia and prolonged gestation, and 2) effects on the reproductive system of pubertally exposed males. Usually, in an evaluation of reproductive performance and fertility it is relevant to also include information from repeated dose studies in adult animals, investigating weight and histopathology of reproductive organs. In the present case, however, for both identified adverse effects, results from repeated dose studies would be of limited relevance, due to the importance of sensitive exposure windows and such studies have therefore not been included in the present evaluation. Indications of endocrine disruption in repeated dose studies and targeted endocrine studies are supportive evidence for the mode of action behind the observed effects, and are presented in more detail in annex II.

An overview of observed effects on fertility and sexual function in rats, rabbits and mice is presented in tables 46, W and Z, and is summarised below. The potential for tebuconazole to cause adverse effects on fertility and reproductive performance was investigated in a two generation reproductive toxicity rat study (OECD TG 416, version 1983) (B.6.6.1.1/01), as well as in several other regulatory studies. In addition a range of toxicity studies from the open literature were included in the weight of evidence. While most of the published studies were not compliant with OECD guidelines or GLP, many of them included relevant toxicity targets, some of which were not included in the submitted OECD and GLP compliant studies.

Dystocia and prolonged gestation:
Statistically significant increases in gestational length have been seen in two developmental studies with tebconazol. A US-EPA guideline developmental neurotoxicity study in SD rats (B.6.6.2.1.2/01), showed a mean increase in gestation length of 0.5 days (2%) at the highest tested dietary dose of 65.9 mg/kg bw/day. A 0.94-day (4%) increase in gestation length was also seen in a published reproductive toxicity study at an oral gavage doses of 100 mg/kg bw/day in Wistar rats (B.6.6.3/07). Gestation length was unaffected at the lower doses in these two studies (22 mg/kg bw/day & 50 mg/kg bw/day, respectively), and likewise, no effect on gestation length was seen in another published developmental study in Wistar rats testing doses up to 50 mg/kg bw/day (B.6.6.3/04). Surprisingly, the highest dietary dose of 1000 ppm in the two-generation study (corresponding to 95 and 111 mg/kg bw/ day in F0 and F1 females respectively) showed no effect on gestation length (B.6.6.1.1/01).

In the two studies affecting gestation length, reductions in maternal body weight were seen during gestation, but changes in maternal body weight are generally not considered to influence gestation length, as determined from studies on feed restriction (Carney et al. 2004). Interestingly, increased gestation length has also been seen in reproductive toxicity studies with other azole fungicides, providing supporting evidence of adverse substance-related effects on sexual function and fertility caused by tebuconazole exposure during gestation.

Clear signs of dystocia was seen in the same two studies (B.6.6.2.1.2/01 and B.6.6.3/07), and possibly also in the two-generation study (B.6.6.1.1/01). In B.6.6.2.1.2/01 two maternal deaths/moribund sacrifices (GD 22 or 23) at 1000 ppm corresponding to 66-125 mg/kg bw/day were related to dystocia. This was also observed in published study (B.6.6.3/07) (Wistar rats), two dams in the 100 mg/kg bw/d group were unable to give birth due to dystocia. These effects are consistent with adverse effects on pregnancy due to alterations of steroid hormones, which was also shown in the latter study. In particular, the seven-fold increase in late-gestation progesterone levels seen in the latter study is likely causative of dystocia. The reductions in maternal body weight gain during pregnancy is not considered to influence the ability to give birth, and effects are thus not secondary to systemic toxicity. In the B.6.6.1.1/01 study, a death of one dam (F0) in the 1000 ppm group was possibly related to dystocia. This dam was found moribund before birth, and when opened there were foetuses in both uterine horns, and the placentas in one horn were found to be very thick, beige coloured and hard. Dystocia was not seen in the F1 generation.

This information on dystocia and prolonged gestation is considered relevant, and sufficient for

classification for fertility. Even though the two-generation study showed no changes in gestation length and dystocia in only one dam, the observations in the US EPA DNT study and in the published studies serve as substantial evidence for these effects. In particular, the DNT study (B.6.6.2.1.2/0) used 25 mated females per dose group and the same dietary dose of tebuconazole as the 2-generation study (B.6.6.1.1/01). This may indicate strain differences. The findings from B.6.6.3/07 may indicate that effects of gavage exposure is more marked in these Wistar rats than seen with dietary exposure in the two-generation study also in Wistar rats. The relevance of these findings is strengthened by the occurrence of similar effects with other azole fungicides.

Effects on the reproductive system of pubertally exposed male rats

Changes in weights and histology of male reproductive organs (epididymis, LABC, prostate, seminal vesicle, testis) were seen following pubertal exposure (B.6.8.3.1.2/01). These effects were mainly seen at dose levels where offspring body weights were decreased but also occurred at doses where only minimal signs of systemic toxicity were seen.

8.10.3 Comparison with the CLP criteria

For the purpose of classification for reproductive toxicity, substances are allocated to one of two categories. Within each category, effects on sexual function and fertility, and on development, are considered separately. In addition, effects on lactation are allocated to a separate hazard category.

Category	Criteria
Category 1	*Known or presumed human reproductive toxicant* *Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).*
Category 1A	*Known human reproductive toxicant* *The classification of a substance in this Category 1A is largely based on evidence from humans.*
Category 1B	*Presumed human reproductive toxicant* *The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development 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. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.*
Category 2	*Suspected human reproductive toxicant* *Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.* *Such effects shall have been observed 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 the other toxic effects.*

As presented below, the DK RMS concludes that:

Classification in Repr. Category 1A is not supported as it should be based on human data and there is no information about the potential toxicity of tebuconazole for humans.

Classification in Repr. Category 1B for effects on fertility is considered appropriate for classification of tebuconazole, as the CLP criteria are fulfilled by the available information on toxicity of tebuconazole providing clear evidence of adverse effects that are not considered to be secondary non-specific effects of other toxic effects (see description below). There is no mechanistic information that raises doubt about the relevance of the effects for humans.

Classification in Repr. Category 2 is not appropriate as there is clear evidence from animal studies of sufficient quality and effects are not considered to be secondary non-specific effects of other toxic effects.

Rationale for classification of tebuconazole in Repr. 1B for fertility

The main adverse effects of tebuconazole considered potential relevant for classification for fertility are 1) dystocia and prolonged gestation, and 2) effects on the reproductive system of perinatally exposed males.

According to ECHA 2017 guidance on CLP criteria (Annex I : 3.7.1.3), fertility effects includes but Is not limited to «*alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems* ».

A thorough evaluation of the evidence for adverse effects and possible influences of other toxic effects as well as human relevance needs to be considered.

To aid the evaluation, the following issues are addressed :

a) Data overview for fertility effects and other possibly related effects
b) Mode of action analysis considering the plausibility that observed effects are specific and human relevant
c) Considerations on presence or absence of concomitant/associated marked systemic effects
d) Severity and irreversibility of effects
e) Conclusion in relation to CLP criteria

Data overview – fertility effects of tebuconazole

1) Dystocia and prolonged gestation.

Prolonged gestation	Increased gestational length was seen in high dose groups of two oral gavage studies (B.6.6.2.1.2/01 and B.6.6.3/07), but not at the lower doses in these two studies, not at lower doses in a published study B.6.6.3/04, and not at a similar dietary dose in a two-generation study (B.6.6.1.1/01).
Dystocia	Dystocia was seen in three studies in one or two animals per dose group (B.6.6.1.1/01, B.6.6.2.1.2/01, B.6.6.3/07). These effects are consistent with adverse effects on pregnancy due to observed marked alterations of steroid hormones (B.6.6.3/07).

2) Effects on the reproductive system of perinatally exposed males. See detailed descriptions in section for adverse effects on sexual function and fertility and annex II.

Altered reproductive function in males	Pubertal exposure caused delay in PPS, decreased testosterone levels, decreased weight of seminal vesicle, dorsolateral prostate, epididymis, LABC and pituitary, macroscopic effects in prostate, seminal vesicle, LABC and testis (B.6.8.3.1.2/01). In contrast several studies investigating effects on reproductive organs after adult exposure did not see effect in rats, mice, dogs, and rabbits.
Altered testicular and epididymal function in adult male offspring	Pubertal exposure resulted in decreased weight of epididymis, dorsolateral prostate and LABC and macroscopic effects in testis, prostate, seminal vesicles and LABC (B.6.8.3.1.2/01). In addition testosterone and estradiol levels were altered in adult rats exposed during late puberty (B.6.8.3.1.2/02), corroborating findings from developmental studies showing functional impairment of fetal Leydig cells (B.6.6.3/07). Sperm motility was not affected in two studies from the open literature investigating sperm in adult offspring after developmental exposure to tebuconazole in doses up to 50 mg/kg bw/d (B.6.6.3/07 and B.6.6.3/05). Sperm count was not affected in two studies form the open literature investigating sperm counts in adult offspring after developmental (B.6.6.3/07) and developmental + pubertal exposure up to 60 mg/kg bw/d (B.6.6.2.1.2/01)

Mode of action analysis

Increased gestation length and dystocia**:**

The observed effects on these endpoints may be related to the observed endocrine disrupting activity of tebuconazole described in annex II. There is clear evidence that tebuconazole is an aromatase inhibitor inducing marked reductions in reproductive hormone levels (see also annex II).

A mode of action can be postulated showing a relationship between disruptions in steroidogenesis, reduced serum estradiol levels and adverse outcomes. This mode of action is further substantiated in annex II.

MIE	KE1	AO
Disrupted steroidogenesis including inhibition of aromatase	Altered blood hormone profile including levels of estradiol and progesterone	Increased gestational length and dystocia

Dose- and temporal concordance between key events of the postulated MoA are included in Section annex II.

Effects on the reproductive system of perinatally exposed males:

MIE	KE1	KE2	KE3	AO
Disrupted steroidogenesis	↓ testosterone levels	Reduced AR signalling in target tissues	Altered reproductive development	Altered male reproduction

Considerations on presence or absence of marked systemic effects
For fertility effects it is noted that (ECHA 2017 CLP guidance setion 3.7.2.2.1): " *Adverse effects on fertility and reproductive performance seen only at dose levels causing marked systemic toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma) are not relevant for classification purposes. There is no established relationship between fertility effects and less marked systemic toxicity. Therefore it should be assumed that effects on fertility seen at dose levels causing less marked systemic toxicity are not a secondary consequence of this toxicity*."

For tebuconazole, the observed maternal effects are not related to marked systemic toxicity. Maternal effects on body weight gain in early pregnancy and reduced food consumption is considered as less marked systemic toxicity and is seen at the doses affecting the endpoints 1) dystocia and prolonged gestation, 2) effects on the reproductive system of pubertally exposed males.

DK RMS concludes that effects of tebuconazole on these endpoints within the category fertility are not secondary consequences of systemic toxicity.

Severity and irreversibility of effects
Effects on the endpoints 1) dystocia and prolonged gestation are considered irreversible and severe. Effects on 2) the reproductive system of perinatally exposed males are seen with exposure to high doses in the pubertal period (B.6.8.3.1.2/01). They include delay in PPS and VO, decreased weight of seminal vesicle, dorsolateral prostate, epididymis, LABC and pituitary, macroscopic effects in prostate, seminal vesicle, LABC and testis. In the two-generation study using lower doses (B.6.6.1.1/01), no adverse effects on male reproductive function were noted. However, the following deviations from the current study design for a TG 416 (2001) were identified: lack of measurement of oestrus cycle and sperm parameters, vaginal opening, preputial separation, anogenital distance, weight of uterus, epididymides, prostate, seminal vesicles with coagulating glands. Hence severity

and irreversibility of reproductive development was not investigted sufficiently in this study.

The reversibility of male reproductive effects of pubertal exposure have not been investigated, and data on sperm count and motility following pubertal exposure to high doses have not been investigated. There is thus a data gap in this regard, and the available data are not considered sufficient to conclude on severity and irreversibility for this effect.

Conclusion in relation to CLP criteria – fertility
The main adverse fertility effects 1) dystocia and prolonged gestation and 2) effects on the reproductive system of pubertally exposed males have been assessed and compared with the CLP criteria by the DK RMS and the conclusions are as follows:

- The observed dystocia and prolonged gestation seen in rats in the absence of marked maternal systemic toxicity, supports a classification as Repr. 1B (CLP) for this effect.

- The observed effects on the reproductive system of pubertally exposed males are not considered sufficient for classification for fertility on its own, however the observed effects indicates a potential and in the absence of more elaborated data investigating these end points in up to date OECD compliant studies, these have been included in a WoE approach.

On this basis, classification of tebuconazole for toxicity to fertility in category 1B (Repr 1B; H360F) is proposed.

8.10.4 Adverse effects on development

Table 50: Summary table of animal studies on adverse effects on development

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL mg/kg bw/day (for parent, offspring and for developmental effects) - target tissue/organ - critical effects at the LOAEL	Reference
Rat			
Two-generation, dietary GLP OECD test guideline no. 416 (1983) (older guideline) Rat Bor: WISW (SPF Cpb) 25 males and 25 females/dose.	Tebuconazole, batch FL 132 (mixed batches), 95.2 %. ppm: 0, 100, 300 and 1000 Equivalent to (mg/kg bw/d): 0, 9.1 – 11.1, 27.8 – 33.9 and 94.8 – 111.4 in F and 0, 7.1 – 9.2, 21.6 – 27.1 and 72.3 – 97.2 in M. Exposure from 17 weeks premating, during mating, gestation, lactation of F1 and continued until PND21 of the F2 generation.	**NOAEL:** Parental and offspring: 300 ppm. Equivalent to: 21.6 – 27.1 mg/kg bw/d (males) and 27.8 – 33.9 mg/kg bw/d (females). **LOAEL:** Parental and offspring: 1000 ppm. Equivalent to: 72.3- 97.2 mg/kg bw/d (males) and 94.8-111.4 mg/kg bw/d (females). **Effects at the LOAEL** Maternal: Decreased food consumption was not significant (8-11% of control, no change in first generation females), slightly retarded weight gains for parents and decrease in body weight (less than 10% and not considered toxicologically relevant in parents). Developmental: adverse effects on pre- and postnatal offspring in the first generation (lower litter size, indicating possible postimplantation loss; increased postnatal mortality). Reduced birth weight (less than 10%, F1 and F2 generations) & bw for pups during development (15-25% at some ages). Organ weight decrease (absolute liver (12%) & kidney 7-10%) weight, not relative) secondary to decreased body weights (5-9%) in F1B parentals. For more details **see table W and Z**.(annex III)	Vol 3 B.6.6.1.1/01

Developmental toxicity, oral gavage GLP Comparable to OECD test guideline no. 414 (1981) Rat, female mated WISW, 25/dose	Tebuconazole, batch 16007/83, 93.4 % 0, 10, 30, 100 mg/kg bw/d Exposure from GD 6-15	**NOAEL** Maternal:10 Developmental: 30 mg/kg bw/d **LOAEL** Maternal:30 Developmental:100 mg/kg bw/d **Effects at the LOAEL** Maternal: Reduced body weight gains during treatment (marginal at this dose) no change in maternal body weight. Developmental: Increased number of external malformations, higher incidence of post-implantation losses and decreased foetal body weight. For more details **see table W and Z**.(annex III)	Vol 3 B.6.6.2.1.1/01
Developmental toxicity, oral gavage GLP OECD test guideline no. 414 (1981) Rat Wistar/HAN, mated female, 25/dose	Tebuconazole, batch no. 20, 98.3 % 0, 30, 60, 120 mg/kg bw/d Exposure from GD6-15	**NOAEL** Maternal: 30, Developmental: 60 mg/kg/day **LOAEL** Maternal: 60, Developmental: 120 mg/kg/day **Effects at the LOAEL** Maternal: Reduced body weight gain and feed intake and increased liver weights (relative 8%, absolute 9% at 60 mg/kg). It is noted that the values for body weight gain corrected for uterus weight were not significantly lower at the high dose during pregnancy. Developmental: Higher incidence of resorptions, reduced ossification, decreased foetal weight and an increased incidence of skeletal variations and anomalies. For more details **see table W and Z**.(annex III)	Vol 3 B.6.6.2.1.1/02
Maternal toxicity in pregnant rats, oral gavage GLP OECD test guideline not specified – investigative study Rat Wistar Hsd Cpb:WU, mated female, 20/dose	Tebuconazole, batch 278679012 98.5 / 98.6 % 0, 120 mg/kg bw/d Exposure from GD6-15	**NOAEL** Not applicable – investigative study **LOAEL** Not applicable – investigative study **Effects at the LOAEL** Effects on body weight (gain), food and water consumption (decreased) and on clinical signs of toxicity (piloerection, increased urination), specific toxic effects were seen in the liver (slight increase (13%) in weight, accompanied by histopathology) and adrenal gland (vacuolation of zona fasciculate and zona glomerulosa cells, 8/10 females).	Vol 3 B.6.6.2.1.1/03
Developmental neurotoxicity (DNT), oral dietary GLP Not in accordance with OECD test guideline (US-EPA, OPPTS 870.6300)	Tebuconazole, batch 603-00l 3, 96.0 – 96.9 % ppm: 0, 100, 300, 1000. Equivalent to (mg/kg	**NOAEL** Parental and developmental: 300 ppm Corresponds to 22 and 41.3 mg/kg/d during gestation and lactation, respectively. **LOAEL** Parental and developmental: 1000 ppm Corresponds to 65 and 125.4 mg/kg/d during gestation and lactation, respectively.	Vol 3 B.6.6.2.1.2/01

Rat Sprague-Dawley rats (Crl:CD®BR VAF/Plus®), 25 presumed pregnant rats per dosage group	bw/d) Gestation days 6-21: 0, 8.8, 22.0 and 65.0; Lactation days 1-12: 0, 16.3, 41.3 and 125.4. Exposure from GD6 to lactation day 11.	**Effects at the LOAEL** Maternal: Reduced body weight (16% during gestation and 6-12% days 1-13 of lactation) and feed consumption. Developmental: Increased mortality, decreased number of live born (6% compared to control), decreased viability index (6%), reduced pup weight and body weight gain, reduced brain weight (7-10%), delay in vaginal patency, and decrease in cerebellar thickness. For more details **see table W and Z**.(annex III)	
Developmental neurotoxicity (DNT), perinatal dosing, gavage, dams and pups Not to GLP Not in accordance with OECD test guideline Rat, Sprague-Dawley (strain: Tac: N(SD)fBR), ≥15/dose	Tebuconazole, no information about batch number, 97.4 % 0, 6, 20, 60 mg/kg bw/d Exposure from GD 14 to PND 7	**NOAEL** Maternal: 20, Developmental: 20 mg/kg/d **LOAEL** Maternal: 60, Developmental: 60 mg/kg/d **Effects at the LOAEL :** Maternal: Maternal weight gain during pregnancy was reduced (16%, 60 mg/kg bw/d group). The reduced litter weight partly explains the reduced maternal weight gain. Developmental: Decreased pup viability and pup body weights, altered learning in the spatial cognitive task and a number of organ weight changes at the highest dose tested (increased liver (10-12%), decreased kidney (9%, middle dose only in females, 8% in males), increased male spleen (26%) weights). Tendency towards decreased number of live pups in 60 mg/kg bw/d group on PND 0 (p=0.07). The number of dead pups per litter was significantly increased in the 60 mg/kg bw/d. At birth, the the pup weight was reduced in the high dose group. For more details **see table W and Z**.(annex III)	Vol 3 B.6.6.2.1.2/01
Developmental toxicity, dermal GLP US-EPA 83-3 (1984) complies with OECD TG 414. Rat, WISW (SPF Cph), 25/dose	Tebuconazole, batch 16012/86, 97.4 % 0, 100, 300, 1000 mg/kg bw/d Exposure from GD6-15	**NOAEL** Maternal: 1000, Developmental: 1000 mg/kg/d **LOAEL** NA **Effects at the LOAEL** No systemic effects recorded.	Vol 3 B.6.6.2.1.3/01
Developmental toxicity, dermal (limit test) GLP OECD test guideline no. 414 (1981) Rat, WIST HanIbm (SPF), 25/dose	Tebuconazole, batch 816196048, 96.2 or 95.8 % 0, 1000 mg/kg bw/d Exposure form GD6-15	**NOAEL** Maternal: 1000, Developmental: 1000 **LOAEL** NA **Effects at the LOAEL** No systemic effects recorded.	Vol 3 B.6.6.2.1.3/02
Developmental toxicity, oral gavage	Tebuconazole 98% Control; 24 dams of which 19 were	**NOAEL** Maternal: 50 mg/kg/d, Developmental: ND **LOAEL** Maternal: 100, Developmental: 50 mg/kg/d	Vol 3 B.6.6.3/07

Not GLP Pregnant female Wistar rats, 20-24/dose group	pregnant and 6 were sacrificed at GD 21 50 mg⁄kg bw/d; 19 pregnant dams of which 7 for GD 21 section 100 mg/kg bw/d: 18 pregnant of which 8 for GD 21. Exposure from GD 7 to GD 21 and from PND1-16. .	**Effects at the LOAEL** Maternal : reduced maternal weight gain (29-35% at high dose). Reduced dam weight (8% reduction at high dose GD 21), no change in dam weight adjusted for litter and uterine weight. Developmental: increased post-implantation loss and postnatal death, alterations in testicular testosterone (50 and 100 mg/kg) and progesterone (100 mg/kg). Altered endocrine tissue development in the offspring with virilised females with longer AGD and feminized males with increased nipple retention on PD 13 at both 50 and 100 mg/kg. A LOAEL of 50 mg/kg bw/d can be identified from this study based on effects on sex steroid hormone and increased nipple retention in the male offspring. Increased absolute fetal liver weight (8%) at 100 mg/kg bw/d. For more details and percent changes **see table W and Z**.(annex III)	
Developmental toxicity, oral gavage Not GLP Pregnant female Wistar rats	Tebuconazole 98%, control (10 dams of which 6 were pregnant) and one dose of 50 mg⁄kg bw/d (9 dams). Exposure from GD 7 to GD 21. Caesarean sections were performed on dams at GD 21.	**NOAEL** Maternal: ND, Developmental: ND **LOAEL** Maternal: 50, Developmental: 50 mg/kg/d **Effects at the LOAEL** Maternal: decrease in estradiol in dams. Developmental: increased frequency of post-implantation loss, increase in testicular progesterone levels in male foetuses (possible indicator of demasculinization of male foetuses). No effects on AGD. For more details **see table W and Z**.	Vol 3 B.6.6.3/08
Developmental toxicity, oral gavage Not GLP Pregnant female Wistar rats	Tebuconazole 98.5%, Oral, rat 0, 12.5, 50 mg/kg bw/day Exposure from GD7 to PND16. 15, 8 and 6 dams with viable litters in each exposure group.	**NOAEL** Maternal: 50 mg/kg/d, Developmental: ND **LOAEL** Maternal: ND, Developmental: 12.5 mg/kg/d **Effects at the LOAEL** Maternal: None Developmental: at 12.5 and 50 mg/kg bw/d: females: ↑ AGD (PND1, 7-8%), at 50 mg/kg bw/d: males: ↑ nipple retention (0 in ctrls vs 1.6 in exposed). No effects on male reproductive organ weights at PND16. For more details **see table W and Z**.(annex III)	Vol 3 B.6.6.3/04 Vol 3 B.6.6.3/05 Vol 3 B.6.6.3/06
Rabbit			

Developmental toxicity, oral gavage GLP Comparable to OECD test guideline no. 414 (1981) Rabbit, Himalayan CHBB:HM, 15/dose	Tebuconazole, batch 16007/83, 93.4 % 0, 3, 10, 30 mg/kg bw/d Exposure from GD6-18	**NOAEL** Maternal: 30, Developmental: 10 mg/kg/d **LOAEL** Maternal: >30, Developmental: 30 mg/kg/d **Effects at the LOAEL** Maternal: - Developmental: Increased resorptions. For more details **see table W and Z**.(annex III)	Vol 3 B.6.6.2.2.1/01
Developmental toxicity, oral gavage GLP OECD test guideline no. 414 (1981) Rabbit Chinchilla rabbits, CHIN, hybrids, SPF quality, 16/dose	Tebuconazole, batch 16002/85, 98.2 % 0, 10, 30, 100 mg/kg bw/d Exposure from GD6-18	**NOAEL** Maternal: 30, Developmental: 30 mg/kg/d **LOAEL** Maternal: 100, Developmental: 100 mg/kg/d **Effects at the LOAEL** Maternal: Decreased food consumption and reduced body weight gain. Developmental: Increased post-implantation losses and an increase in malformations and anomalies. For more details **see table W and Z**.(annex III).	Vol 3 B.6.6.2.2.1/02
Developmental toxicity, oral gavage GLP OECD test guideline no. 414 (1981) Chinchilla rabbits (CHbb: CH, Hybrids, SPF quality), 16/dose in the main study and 5/dose in the supplementary study	Tebuconazole, batch 816196048, 96.3 - 96.8 % 0, 10, 30, 100 mg/kg bw/d Exposure from GD 6-18	**NOAEL** Maternal: 30, Developmental: 10 mg/kg/d **LOAEL** Maternal: 100, Developmental: 30 mg/kg/d **Effects at the LOAEL** Maternal: Decreased food consumption and body weight gain. Developmental: Increased post-implantation loss, reduced foetal weight and increased incidence of malformations at the two highest doses. For more details **see table W and Z**.(annex III) NOAEL of 10 as agreed in the previous RAR.	Vol 3 B.6.6.2.2.1/03
Developmental toxicity, oral gavage GLP Comparable to OECD test guideline no. 414 (1981) Rabbit CHB-W (chinchilla) rabbits, 14 controls – 15 dosed animals	Tebuconazole, batch 278679012, 98.5 % 0, 100 mg/kg bw/d Exposure from GD6-19	**NOAEL** NA **LOAEL** NA - only one dose tested. **Effects at the tested dose:** Maternal: Reduced food consumption, weight loss (day 6-10 p.c. only) and decrease in overall corrected bw gain in dams however not statistically significant. Developmental: Decreased foetal weight	Vol 3 B.6.6.2.2.1/04

		For more details **see table W and Z**.	
Mouse			
Developmental toxicity, oral gavage GLP OECD test guideline no. 414 (1981) Mouse NMRI/ORIG Kisslegg, 25/dose	Tebuconazole batch 1616002/84, 93.6% (+ 5.5% symmetric isomer) 0, 10, 30, 100 mg/kg bw/d Exposure from GD6-15	**NOAEL** Maternal: 100, Developmental: 10 **LOAEL** Maternal: N/A – no maternal toxicity recorded at the top dose, Developmental: 30 **Effects at the LOAEL** Maternal: N/A – no maternal toxicity recorded at the top dose. Developmental: Increased number of runts. For more details **see table W and Z**.(annex III)	Vol 3 B.6.6.2.3.1/01
Developmental toxicity, oral gavage GLP OECD test guideline no. 414 (1981) Used as supplementary information on maternal toxicity Mouse NMRI/ORIG Kisslegg, 10/dose	Tebuconazole, batch 16012/86, 97.4 % 0, 10, 20, 30, 100 mg/kg bw/d Exposure from GD6-15	**NOAEL** Not reliable for setting NOAEL (small group size) **LOAEL** Not reliable for setting NOAEL (small group size) **Effects at the LOAEL** Maternal toxicity: Decreased body weight gain, increased liver weights (though not statistically significant) and associated histopathological changes. For more details **see table W and Z**.(annex III)	Vol 3 B.6.6.2.3.1/02
Developmental toxicity, oral gavage GLP OECD test guideline no. 414 (1981) Mouse NMRI KFM-HAN (outbred, SPF quality), main study 35/dose (subgroup of 10/dose), supplementary study 30/dose (subgroup of 7/dose)	Tebuconazole, batch 816196048, 95.8 - 96.8 % Main study: 0, 10, 30, 100 mg/kg bw/d Supplementary study: 0, 1, 3 mg/kg bw/d Exposure from GD6-15	**NOAEL** Maternal: 100, Developmental: - **LOAEL** Maternal: -, Developmental: 10 **Effects at the LOAEL** Maternal: Liver effects seen at 30 and 100 mg/kg bw/d were considered adaptive. No adverse effects at any dose. Developmental: Total incidence of malformations (open eye, runts, cleft palate) was increased at the low dose of 10 mg/kg bw/d. For more details **see table W and Z**.(annex III) Both agreed at PRAPeR Expert Meeting 49 (2-6 June 2008)	Vol 3 B.6.6.2.3.1/03
Developmental toxicity, dermal GLP	Tebuconazole, batches 16002/85, 98.1 % and 816896061, 96.1 %	**NOAEL** Maternal: 100, Developmental: 300 **LOAEL** Maternal: 300, Developmental: 1000	Vol 3 B.6.6.2.3.2/01

Test guideline: Pesticide Assessment Guidelines Subdivision F. Hazard Evaluation: Human and Domestic Animals, EPA, § 83-3, Teratogenicity Study, revised edition (1984). Mouse NMRI KFM- HAN mice (Outbred SPF Quality), 30-34/dose	0, 100, 300, 1000 mg/kg bw/d Exposure from GD6-15	**Effects at the LOAEL** Maternal: Liver effects (fatty changes and induction of mixed-function oxidase activities), but neither body- nor liver weights were significantly different from controls. Developmental: Increased incidence of cleft palate and supernumerary ribs. For more details **see table W and Z**.(annex III)	

Table 51: Summary table of human data on adverse effects on development

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 52: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Please refer to section for adverse effects on sexual function and fertility and annex II for effects in pubertal assays				

8.10.5 Short summary and overall relevance of the provided information on adverse effects on development

Developmental toxicity

The potential for tebuconazole to adversely affect development was investigated in several regulatory developmental toxicity studies (the majority in compliance or comparable to relevant OECD test guidelines), in the rat, rabbit and mouse. There are also eight publications (three *in vitro* investigations and five *in vivo* targeted developmental toxicity studies in rats) of potential relevance to developmental toxicity.

Three main adverse developmental effects of tebuconazole are considered as critical for the classification on developmental toxicity : 1) postimplantation loss and perinatal death, 2) fetal/pup growth impairment, and 3) external malformations including cleft palates.

An overview of observed effects is presented here, followed by more detailed information on studies in rats, rabbits and mice.

Developmental effect overview

1) Developmental exposure to tebuconazole, typically at doses above 50 mg/kg bw/day, clearly and consistently affects fetal and postnatal survival.

In some but not all studies (studies referred to with author and year, reference to study summary in volume 3 and the study ID matrix number used for that study in Appendix E), reduced maternal weight gain is seen at the doses causing fetal death. Reduced maternal weight gain may result from systemic toxicity or from endocrine disruption of pregnancy, including reduced growth of offspring and fetal death. In addition, effects on liver and adrenals have been observed in other studies, and these effects are considered mild compared to the severity of development effects. For every study, these issues need to be addressed, and a case-by-case evaluation is necessary to evaluate whether the observed maternal weight changes may be a cause of the

adverse effects including fetal death or not. If the maternal effects can be considered mild compared to the developmental effects, these cannot be explained as secondary to maternal toxicity. This is also supported by two feed restriction studies on the rat and rabbit (Fleeman, 2005 and Cappon, 2005), which clearly showed that severe weight loss or decrease in body weight gain induced minor changes in skeleton development but had no effects on viability or malformations in the rat. Ten regulatory studies using oral exposure route, report significant increase in embryonic/fetal death or post-implantation loss, or reduced litter size (B.6.6.1.1/01, B.6.6.2.1.2/01, B.6.6.2.1.1/01, B.6.6.2.1.1/02, B.6.6.2.1.1/03, B.6.6.2.2.1/01, B.6.6.2.2.1/02; B.6.6.2.1.3/02, B.6.6.2.2.1/03 and B.6.6.2.3.1/03). Importantly, several studies included doses where fetal death was observed with no or only minor reduction of maternal body weight gain. Specifically, B.6.6.1.1/01 identified reduced litter size in a rat two-generation study (statistically significant at first mating), at a dose not affecting maternal body weight.

A mouse developmental toxicity study showed significant post-implantation loss and reduced litter size without effect on maternal body weight gain (B.6.6.2.3.1/03). In a rat developmental toxicity study, increased postimplantation loss/increased number of resorptions was seen at a dose of 120 mg/kg bw/d which also reduced maternal body weight gain, but this reduced weight gain could be fully explained by smaller total litter weight (B.6.6.2.1.1/02). In addition, B.6.6.2.1.2/01 showed slightly reduced number of live born pups and reduced viability index, at a dietary dose (up to 65 mg/kg bw/d during gestation) causing slightly reduced maternal body weight gain and food consumption both not considered to be treatment related due to no dose-dependency. B.6.6.2.3.1/01 reported no significant effect on fetal death at doses up to 100 mg/kg bw/d, but data indicates increased numbers of resorptions as well as post-implantation loss in the high dose group without any changes in maternal body weight gain. In a few of the rat studies the doses causing fetal death (120 and 100 mg/kg bw/d) also caused reduced maternal weight gain which could not fully be explained by a lower litter weight (B.6.6.2.1.1/03, B.6.6.2.1.1/01). In rabbits, the fetal death was often seen at doses also reducing maternal body weight gain (B.6.6.2.2.1/01, B.6.6.2.2.1/02, B.6.6.2.2.1/03).

In the open literature additional studies showed increased post implantation loss at 50 mg/kg bw/d in a developmental study (B.6.6.3/08) not showing any change in maternal body weight gain, and at 100 mg/kg bw/d in a perinatal study (B.6.6.3/07) showing reduced maternal body weight gain during pregnancy, but no significant change in adjusted body weight change (bw minus uterine weight at caesarean section). No effect was observed in one open literature study at doses up to 50 mg/kg bw/d (B.6.6.3/04, ID 52) and in one rabbit study at 100 mg/kg bw/d (B.6.6.2.2.1/04, ID 26). Additionally B.6.6.2.1.2/01 reports tendencies or nominal effects on litter size at the highest doses tested (60 mg/kg bw/d), though not statistically significant. Studies using dermal exposure route (B.6.6.2.1.3/02, B.6.6.2.1.1/01, B.6.6.2.3.2/01) report no effects on fetal death/reduced litter size.

In studies with continued exposure during the lactation period, postnatal offspring survival is also adversely affected by tebuconazole, as two studies show a clearly decreased litter viability (B.6.6.2.1.2/01, B.6.6.2.1.2/01).

2) There is clear and consistent evidence that oral exposure to tebuconazole causes growth impairment in both fetuses and pups. Nine out of 14 developmental toxicity studies show reduced offspring weights (B.6.6.1.1/01, B.6.6.2.1.1/01, B.6.6.2.1.1/02, B.6.6.2.1.1/03, B.6.6.2.2.1/02, B.6.6.2.2.1/03, B.6.6.2.2.1/04, B.6.6.2.3.1/01, B.6.6.2.3.1/03), while the studies which do not show this effect had either used dermal exposure route (B.6.6.2.1.3/02, B.6.6.2.1.1/01, B.6.6.2.3.2/01) or an exposure of 50 mg/kg bw/d or below (B.6.6.2.2.1/01, B.6.6.3/08), which seems to be an approximate threshold for effect on this endpoint. In addition, effects were seen across species (rat, rabbit and mice) confirming the growth retardation effect of tebuconazole exposure. In rabbits, in B.6.6.2.2.1/02 was observed a marginal decreased fetal body weight (6 % change compared to control), which correlated with slightly retarded ossification. As described for fetal death/postimplantation loss, not all studies showed changes in maternal weight gain at effective doses, and in some studies the reduced maternal weight gain could be explained by smaller litter weight.

3) There is also evidence that oral exposure to tebuconazole causes external malformations including cleft palates in several studies.

In rats developmental effects such as skeletal anomalies and increased incidence of total external malformations and microphthalmia was seen in two developmental toxicity studies using higher doses of around 100 - 120 mg/kg bw/d (B.6.6.2.1.1/01, B.6.6.2.1.1/02). Presence of cleft palate was rare in these

studies and not related to exposure. In one study on dermal toxicity, one case was seen in a control group (B.6.6.2.1.1/01).

In rabbits, in study B.6.6.2.2.1/02 was observed a slightly increased incidence of external malformations, including cleft palate, malrotation of hind limb, enlarged fontanelle, hemimelia and agenesis of claws at 100 mg/kg bw/d (total incidence of 33.3 compared to 0 in control). Cleft palate was seen in 1.1% of the fetuses in 6.7% of the litters at the high dose.

In oral studies in mice, developmental toxicity (delayed ossification) started to occur from 30 mg/kg bw/d, becoming more severe (reduced foetal weight and slightly increased incidence of external malformations such as cleft palate, exencephaly and tail abnormalities) at 100 mg/kg bw/d. In B.6.6.2.3.1/01 the number of foetuses with cleft palate was increased (> 10 % change compared to control). Cleft palate is a common malformation in this strain of mice. However, incidence at 100 mg/kg bw/day were outside of the range of the HCD provided (table 53). Other malformations and anomalies (face malformations, kinked and shortened tail, dilation of brain ventricles, vertebral asymmetry, spinal dysplasia, rib fusion, partial aplasia of parietal bone) were also increased above controls and historical control data (> 10 % change compared to control at 100 mg/kg bw/day). In B.6.6.2.3.1/03 the total incidence of malformations (open eye, runts, cleft palate) was increased already at the low dose of 10 mg/kg bw/d. B.6.6.2.3.2/01 showed in a dermal study in mice an increased incidence of cleft palate and supernumerary ribs at 1000 mg/kg bw/day. Malformations were seen across species (rat, rabbit and mice), cleft palates only in mice and rabbits confirming effect of tebuconazole exposure.

Study overview by species

Rat

The potential developmental toxicity of tebuconazole was investigated in two standard guideline oral developmental toxicity studies, one new investigative (not to OECD test guideline) maternal toxicity study, two oral developmental neurotoxicity studies (not to OECD test guideline) and two studies via the dermal route (conducted to OECD test guidelines), and three published studies. In addition, the two-generation study in rats described above is relevant for evaluation of developmental toxicity.

Two oral gavage studies were conducted, spanning doses of 10 -120 mg/kg bw/d up to day 15 post mating (B.6.6.2.1.1/01, B.6.6.2.1.1/02). Effects on development (increased incidence of total malformations and microphthalmia, post-implantation loss and decreased foetal weight) were seen at LOAELs of 100 and 120 mg/kg bw/d in presence of mild maternal toxicity (reduction in maternal body weight gain). Differences in NOAELs of 30 and 60 mg/kg bw/d for developmental toxicity reflected differences in study design (i.e. different doses) in the two studies. Effects on maternal body weight gain was only seen during treatment (GD 6 to 15), and varied between studies, with LOAELs of 30 and 60 mg/kg bw/d for 15-16% reduction compared to control (NOAELs 10 and 30 mg/kg bw/d). At higher doses (100 and 120 mg/kg bw/d), the reduction in maternal body weight gain could be partly or fully attributed to lower litter weights due to reduced litter size and reduced fetal weight. A third study (B.6.6.2.1.1/03) investigating maternal toxicity at high dose in more detail at GD 16 and showed that the applied dose of 120 mg/kg bw/d caused marked maternal effects, including reduced body weight gain (reduction to 62% of control value at GD 16), decreased food consumption (reduced to 82% of control at GD 16), clinical signs of toxicity (piloerection), and changes in relative but not absolute liver and adrenal weights. Fetal weight was reduced to 74% of control weights.

The reductions in maternal body weight gain may be related to reduced food intake during early days of pregnancy and/or to specific toxicity to uterine and fetal growth. Therefore, a thorough evaluation of relationships between maternal weight gain changes and fetal growth and survivial needs to be carried out. This analysis is presented for each study in Vol 3. See also section " Considerations regarding presence or absence of marked systemic effects" below, section 7.10.6. In general, reductions in maternal body weight gain in high dose groups were largest (in percent of control) in the first days of exposure. At termination of the studies, reduced fetal weights and reduced litter sizes caused reduced total litter weights, which could partly or fully explain the differences in maternal body weight gain during pregnancy indicating absence of marked systemic maternal effect according to CLH criteria Annex I: 3.7.2.4.4. In the B.6.6.2.1.1/03 study, examinations were carried out already at GD 16, and the litter weight differences were smaller than seen in

the other studies at GD 20-21 (B.6.6.2.1.1/01, B.6.6.2.1.1/02) when reduced litter weights could largely explain the observed reductions in maternal body weight gain.

In a regulatory dietary DNT study (B.6.6.2.1.2/01), developmental toxicity (pup mortality, reduced number of live born, reduced viability index, delayed vaginal patency, reduced body weight gain, reduced brain weight and decreased cerebellum thickness) was observed at the top dose of 65 - 125 mg/kg bw/d in the presence of mild maternal toxicity (reduced body weight gain (16% reduction) and food consumption (5% reduction) and prolonged gestation). Observations of two dead dams in this group are related to parturition problems (dystocia is probable cause of death according to study report). The prolonged gestation and dystocia can be considered a specific effect of the substance rather than unspecific maternal toxicity, as this is related to endocrine disruption (see Section 2.10). The number of dams with stillborn pups was slightly increased from 2 dams in control group to 5 dams in high dose group, but the number of stillborn pups was higher (2 in control and 7 in high dose group). After birth, the viability index (live pups at day 5 divided by live born pups) was significantly reduced. Based on these findings, a NOAEL of 22 - 41 mg/kg bw/d was identified for both developmental and maternal toxicity from this study.

In a two-generation study (B.6.6.1.1/01), retarded weight gains for parents and pups were seen at 1000 ppm (72 - 111 mg/kg bw/d). In mating of the F0 generation (both in cohort a and b) there were statistically significant effects showing a higher number of stillborn pups, lower viability index (i.e. pup survival from birth to PND 4), lower lactation index (i.e. pup survival from PND4-21), and a lower litter size, which could be related to postimplantation loss. In the F1 generation a dose of 1000 ppm resulted in markedly increased offspring mortality and moderately reduced offspring growth, whereas in the F2 generation there was no increase in offspring mortality but even more marked reductions in postnatal offspring growth. One dam of the 1000 ppm group died of endometritis (secondary to dystocia).

Generally, the body weight reduction seen in the offspring were more marked that the corresponding reductions on maternal weight during the lactation period (5-11% decrease), indicating that tebuconazole caused specific developmental toxicity effects in the offspring. DK-RMS considers it unlikely that all of the adverse effects observed in the offspring were unspecific consequences, secondary to maternal toxicity.

DK-RMS considers the NOAEL for reproductive toxicity in this study to be 300 ppm, and not 1000 ppm as suggested by the UK-RMS. This is consistent with a previously agreed NOAEL for reproductive toxicity and also consistent with the reproductive NOAEL provided in the study report for this 2-generation study.

A published DNT study (B.6.6.2.1.2/01) is considered unreliable by the UK RMS, but reliable with restrictions by DK RMS. Withdrawal of neuropathological findings does not question the validity of the reproductive toxicity data. At the top dose of 60 mg/kg bw/d, maternal body weight gain was significantly decreased, postimplantation loss was significantly increased, and pup birth weight was significantly decreased. In addition, this study also showed reduced pup viability (2.2 vs 0.4 dead per litter in control group). The reduced maternal body weight gain (13.8 g) can likely be explained by smaller litter weight (9.6 g) and assumed proportionally smaller uterine and amniotic fluid weights. The observed effects on postimplantation loss and pup viability are thus not attributed to maternal toxicity.

In the two dermal developmental toxicity studies, there was no maternal or developmental toxicity up to the top dose of 1000 mg/kg bw/d (B.6.6.2.1.3/01 and B.6.6.2.3.1/03). It is noted that these doses were applied to skin for 6 hours per day, and no evaluation of internal dose was performed. The lack of effects in these studies may be due to lower internal doses using this exposure route compared to oral exposure.

Three published studies using perinatal exposure of rats were published by the same group. One study showed reduced maternal body weight at GD 21, increased postimplantation loss and reduced pup birth weight at 100 mg/kg bw/d (B.6.6.3/07). This and two similar studies carried out in the same lab showed no or minor changes in maternal body weight gain or pup birth weight at 50 mg/kg bw/day (B.6.6.3/04, B.6.6.3/07, B.6.6.3/08). Postimplanation loss was seen at 50 mg/kg bw/d in two of the three studies. In the first application for approval, the UK_RMS concluded: « *An overall LOAEL of 50 mg/kg bw/d can be identified from these publications, which is consistent with the overall developmental toxicity profile from the standard regulatory studies and the NOAEL of 30 mg/kg bw/d for the rat identified from these* ».

Overall, developmental toxicity (pup mortality, reduced number of live born) was seen in rats from approximately 50-65 mg/kg bw/d (two DNT studies and two of three published perinatal exposure studies). Developmental effects increased in severity (postimplantation loss and reduced fetal weights, skeletal anomalies and increased incidence of total external malformations and microphthalmia) in two developmental toxicity studies using higher doses of around 100 - 120 mg/kg bw/d. Presence of cleft palate was rare in these studies and not related to exposure (1 case in a control group, B.6.6.2.1.1/01). The overall NOAEL for developmental toxicity in rats was 30 mg/kg bw/d.

In general the observed developmental toxicity was associated with changes in maternal body weight gain, particularly in the early part of exposure, but continuing throughout gestation. Notably, this reduced maternal body weight gain may be due to direct effects on uterine factors and fetal development. When comparing maternal weight gain during pregnancy with reductions in litter weight, it is important to note that maternal weight gain is dependent on several factors including weight of litters, uterus and amniotic fluid. The developmental effects cannot by default be consided unspecific maternal toxicity. Further discussion in relation to CLP criteria is presented below.

Rabbit

The developmental toxicity of tebuconazole was investigated in the rabbit in four studies, using two different strains (Himalayan and Chinchilla rabbits). Three of the four studies were conducted according to GLP and OECD test guidelines; the fourth study was a non-standard study investigating maternal toxicity in more detail.

Developmental toxicity (increased resorptions) started to occur from 30 mg/kg bw/d (B.6.6.2.2.1/01, B.6.6.2.2.1/02, B.6.6.2.2.1/03). At 100 mg/kg bw/d there was also decreased foetal weight and a slightly increased incidence of external malformations, including cleft palate, malrotation of hind limb, hemimelia and agenesis of claws. B.6.6.2.2.1/02 observed a marginally decreased foetal body weight (6 % change compared to control), which correlated with slightly retarded ossification. In addition, an increased incidence of external malformations occurred at 100 mg/kg bw/day, including cleft palate, malrotation of hind limb, enlarged fontanelle, hemimelia and agenesis of claws (total incidence of 33.3 at 100 mg/kg bw/day compared to 0 in control). Cleft palate was seen in 1.1% of the fetuses in 6.7% of the litters at the high dose. A dose-response pattern was seen in the incidence of skeletal findings being statistically significant at 100 mg/kg bw/day, but starting at the low dose of 10 mg/kg bw/day.

In a study B.6.6.2.2.1/03 an increased incidence of malformations is seen at 30 mg/kg bw/day, but UK-RMS argues that this is due to higher background levels in the period of performing this study. DK RMS acknowlegdes the HCD data, but have reservations for using the data to dismiss the effects based on several observations described in the following; No increased incidence of malformations were seen in concurrent controls. In Table 52 HCD ranges of 0.0-0.9 % for foetus incidence and 0.0-7.7% for litter incidence for multiple malformation are given; this seems however to be based on 1 fetus affected in one litter in 3. study from Dec 91/Jan 92 containing 13 litters and 107 fetuses. None of the other 7 studies (or 9 if two other studies are included: 2. study nov 91/jan 92 and 1.study nov 92/jan 93) from 1992 each containing from 14-16 litters reported multiple malformation.

In the concurrent control group containing 16 litters and 141 foetuses or in the low dose group (10 mg/kg bw/d; 15 litters and 142 foetuses) no external or multiple malformations were reported. The concurrent control group is of comparable size with the HCD data driving the range of 0.0-7.7%. In the concurrent study at 30 mg/kg bw/d three foetuses from three different litters were affected (one fetus with malpositioned hind legs, one fetus with arthrogryposis, and one fetus with multiple malformations) which raises concerns. At 100 mg/kg bw/day three foetuses from three different litters had multiple malformations.

Furthermore, it is also noted that it seems the HCD ranges in general have included the control group of the concurrent study which is inappropriate.

According to ECETOC (Monograph No. 31 2002) and Moore et al. 2013[1] runts are considered of high concern on their own and listed under external abnormalities and malformations. It could be discussed whether runts should be taken out of the external findings as proposed by applicant and accepted by UK-RMS in table 52.

In consideration of the large historical control database, the individual specific external malformations seem to be rare spontaneous events and typically observed in 1 foetus in one litter. It could be argued that it would seem unlikely that 3 fetuses from 3 litters with external malformations arising as spontanoues events should then be detected in the current study at 30 mg/kg bw/d and also considering that treatment related malformations are seen in the highest dose. Statistical significance does not need to be present to validate the biological significance of treatment-related effects. This is particularly true of findings with low incidence (i.e., rare malformations) or high variability, or in situations where the concurrent control data have an unusual incidence profile (OECD GD 43, 2008).

It was argued by UK-RMS that study B.6.6.2.2.1/01 gave no indications for a treatment-related effect on external malformations up to and including the highest tested dose of 30 mg/kg bw/day. However this study was of low reliability and was only accepted as supplementary information, due to the poor reporting, reduced database being available for inspection, doses tested being low (not tested up to maternal toxicity) and increased number of losses not being commented.

Table 53 Foetal findings in B.6.6.2.2.1/03

Parameter		**Control data**		**Dose (mg/kg bw/day)**		
		Historical[$]	**Study**	**10**	**30**	**100**
Number foetuses			141	142	109	119
Number litters			16	15	14	14
External findings						
Foetuses affected			0	0[1)]	3[1)]	3[1)]
Litters affected			0	0[1)]	3[1)]	3[1)]
Total incidences	F	0.0 – 1.4[1)]	0	0	**2.8** *	**2.5** *
	L	*Could not be calculated*	0	0	21.4	21.4
Hind limb malrotated Pes varus position, brachydactyly, missing phalanges	F	0.0 – 0.6	0	0	0.9	
	L	0.0 – 6.3	0	0	7.1	
Hind limb hyperflexion (Arthrogryposis without skeletal finding)	F	0.0 – 0.6	0	0	0.9	
	L	0.0 – 6.3	0	0	7.1	
Multiple malformation	F	0.0 – 0.9[c]	0	0	0.9[a]	**2.5**[b]
	L	0.0 – 7.7	0	0	7.1	**21.40**

[1] Moore et al., 2013. Guidance on classification for reproductive toxicity under the globally harmonized system of classification and labelling of chemicals (GHS). Crit Rev Toxicol, 2013; 43(10): 850–891.

Parameter		Control data		Dose (mg/kg bw/day)		
		Historical[$]	Study	10	30	100
Visceral findings						
Foetuses affected			0	0	1	1
Litters affected			0	0	1	1
Diaphragm - Hernia	F	0.0 – 0.8	0	0	0.9	0.8
	L	0.0 – 6.3	0	0	7.1	7.1
Skeletal findings						
Foetuses affected			4	4	3	5
Litters affected			2	3	3	5
Total incidences	F	*No HCD range*	2.8	2.8	2.8	**4.2**
	L	*No HCD range*	12.5	20.0	21.4	**35.71**
"Runt" (small foetus <19g without malformations)						
Number small foetuses			0	2	1	1
Small foetus	F	0.0 – 4.2	0	1.4	0.9	0.8
	L	0.0 – 25.0	0	13.3	7.1	7.1

F: % foetuses,

L: % litter,

[1)] "runt" (small foetus <19 g) without malformations listed separately since not assessed as external malformation

*/** significantly different from study controls ($p \leq 0.05$ / $p \leq 0.01$),

[$]: Historical control data range from 1989 – 1995 (18 studies, 346 litters, 3225 foetuses) (data from the same laboratory (RCC, Switzerland), on Chinchilla rabbits, SPF quality, within ± 5 years of the current study date 1992).

[a]: craniochisis, dysplastic skull, protruding tongue, kyphosis, spina bifida aperta, eventration of organs, open eye, shortened extremities, bent forepaw, brachydactyly,

[b]: meningocele or partial acephaly, omphalocele or abdominal fissure, spina bifida occipitale (2 foetuses), malposition of limbs, brachydactyly, shortened tail (1 foetus),

[c]: exencephaly, open eye, arthrogryposis, brachydactyly, omphalocele, eventration of organs, spina bifida aperta

An overall NOAEL of 10 mg/kg bw/d was identified for developmental toxicity.

Maternal toxicity (decreased body weight gain) also started to occur from 30 mg/kg bw/d, becoming more severe (body weight loss, decreased food consumption, liver and adrenal hypertrophy and increased levels of corticosteroid hormones) at 100 mg/kg bw/d. An overall NOAEL of 10 mg/kg bw/d was also identified for maternal toxicity.

The developmental effects cannot by default be consided related to unspecific maternal toxicity. Further discussion on possible relations with maternal effects is presented below.

Mouse

The developmental toxicity potential of tebuconazole was investigated in the mouse in four guideline studies; three by oral administration (one of which was limited to an investigative study of maternal toxicity) and one by dermal administration.

In the oral studies, developmental toxicity (small foetuses (runts), increased incidence of post-implantation loss and delayed ossification) started to occur from 30 mg/kg bw/d (B.6.6.2.3.1/03), becoming more severe (reduced foetal weight, reduced litter size and slightly increased incidence of external malformations such as cleft palate, exencephaly and tail abnormalities) at 100 mg/kg bw/d (B.6.6.2.3.1/03 and B.6.6.2.3.1/01).

Cleft palate was seen in two mouse studies. In one study, the number of foetuses with cleft palate was increased (> 10 % change compared to control) (B.6.6.2.3.1/01). Cleft palate is a common malformation in this strain of mice, however incidence at 100 mg/kg bw/day were outside of the range of the HCD provided (table 53). Other malformations and anomalies (face malformations, kinked and shortened tail, dilation of brain ventricles, vertebral asymmetry, spinal dysplasia, rib fusion, partial aplasia of parietal bone) were also increased above controls and historical control data (> 10 % change compared to control at 100 mg/kg bw/day).

The HCD coming only from the performing laboratory is considered more appropriate by DK-RMS. These data were obtained within a 5 year period centered around study B.6.6.2.3.1/01 study as well.

Table 54 Foetal findings in B.6.6.2.3.1/01

Parameter		Dose (mg/kg bw/day)				Historical control data range from Performing laboratory, same strain, 1983-1989 (6 studies, 133 litters, 1421 fetuses)	Historical control data range from 1983 – 1993 (11 studies, 247 litters, 2760 fetuses), data combined from 2 different laboratories (NMRI mice)
		0	10	30	100	% range	% range
Number foetuses Number litters		236 24	234 22 [b]	234 23	202 20		
External examination							
Foetuses affected		1 [c]	4	0	**13**		
Litters affected		1	2	0	**8**		
Total incidences	F	0.4 [c]	1.7	0	**6.4** *	0.0 – 1.4	0.0 – 2.5
	L	4.2	9.1	0	**40.0**	0.0 – 11.1	0.0 – 26.1
Cleft palate	F	0.4 [c]	1.7	-	**3.0**	0.0-0.97	0.0 – 2.0
	L	4.2	8.7	-	**20.0**	0.0- 5.56	0.0 – 20.0
Face malformations [d]	F	0	0	0	0.5	0.0 – 0.4	0.0 – 0.4
	L	0	0	0	5.0	0.0 – 4.2	0.0 – 4.2
Tail anomaly	F	0.4 [c]	-	-	0.5	0.0 – 0.4	0.0 – 0.4
	L	4.2	-	-	5.0	0.0 – 4.8	0.0 – 4.8
Visceral examination							
Brain ventricles dilated	F	-	-	-	0.5	0.0 – 0.4	0.0 – 0.4
	L				5.0	0.0 – 5.0	(0.0 – 5.0)

Skeletal examination							
Cerebral ventricle enlarged	F	0	0	0	0.5		
	L	0	0	0	5.0		
Vertebral asymmetry	F	0	0	0	0.5		
	L	0	0	0	5.0		
Spinal dysplasia	F	0.4 c)	-	-	1.0		
	L	4.2	-	-	5.0		
Os parietale partial aplasia	F	-	-	-	0.5		0.0 – 1.3
	L	-	-	-	5.0		(0.0 – 8.3)
Ribs fused or deformed	F	0.4 c)	0.4	-	0.5	0.0 – 0.4 c)	
	L	4.2	5.0	-	5.0	0.0 – 4.2	
"Runt" (small foetus <-2 standard deviation of the control)							
Number small foetuses		5	4	**20***	**26***		
Small foetuses	F	2.1	1.7	8.6	11.9		
	L	16.7	13.6	43.5	50.0		

F: % foetuses, L: % litter,

- no information available

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

a)Historical control data range from 1983 – 1989 (6 studies), number of females examined 133, two laboratories

b)one female had a total litter loss (7 early resorptions) and was excluded from the mean calculations (non pregnant with at least one viable foetus)

c)one foetus with multiple malformations

d)Cheilognathopalatochisis

In this study: Control group: one finding of cleft face/jaw/pale (+ other anomalies), 10 mg/kg bw group: four findings of cleft palate (2 in combination with other anomalies), and 100 mg/kg bw group: Six findings of cleft palate (one combined with micrognathia and one finding of each of the following: dilation of brain ventricle, vertebral asymmetry, kinked and shortened tail, partial aplasia of the parietal bone, rib fusion (and floating rib, spinal kink), and 2 finding of spinal dysplasia.

In another study (TG 414), the total incidence of malformations (open eye, runts, cleft palate) was increased already at the low dose of 10 mg/kg bw/d (B.6.6.2.3.1/03).

Table 55 Foetal findings from B.6.6.2.3.1/03

Finding		**Historical control data [1]**	**Dose (mg/kg bw/day)**						
			0 [a]	**0 [b]**	**1 [b]**	**3 [b]**	**10 [a]**	**30 [a]**	**100 [a]**
External malformations									
Sum external malformations	F	0.0 – 2.5	0.3	0.5	1.7	1.6	1.3	1.5	10.4#
	L	0.0 – 26.1	3.5	4.8	20.0	16.7	10.7	4.2	46.2#
Exencephaly	F	0.0 – 0.8	0	-	-	-	0.9	0.7	5.2**
	L	0.0 – 9.5	0				10.7	4.2	19.2*
"Open eyes"	F	0.0 – 0.4	0	-	-	-	0.6	0.7	3.3**
	L	0.0 – 5.0	0				7.1	4.2	11.5*
Cleft palate (Palatoschisis)	F	0.0 – 2.00	0.0	0	1.3	0.5	0.3	0.7	3.8**
	L	0.0 – 20.0	0.0	0	15.0	5.6	3.6	4.2	26.9**
	L								

Cleft palate (Palatoschisis)[v]	F		0.7	0	2.6	0.0	0.6	1.6	6.1
	L		3.4	0	15.0	0.0	3.6	8.3	19.2
Hind limb malrotated	F	0.0 – 0.4	-	0.47	-	-	-	-	-
	L	0.0 – 4.8		4.76					
Tail small, bent, curled	F	0.0 – 0.4		0.0	0.4	1.1			0.95
	L	0.0 – 4.8		0.0	5.0	11.1			7.69
Other findings not assessed as malformations									
Number of "runts" (small foetuses <0.6g)			0	0	1[-]	0	1	2[-]	3[+]
	F		0.0	0.0	0.4	0.0	0.3	0.7	1.4
	L		0.0	0.0	5.0	0.0	3.6	4.2	7.7
Warth-like growth on forepaw									
	F		0.3	-	-	-	0.6	1.5	3.3**
	L		3.4				7.1	12.5	15.4

F: % foetuses,

L: %litter,
[a]: main study,

[b]: supplementary study,
[v]: detected at visceral examination only,

[-]: without other malformations,
[+]: with other malformations

[1]: range from 1983 – 1993 (11 studies) for same strain but HCD are combined from 2 laboratories used in the current study

[#]: no statistics performed,

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

In a dermal study, an increased incidence of cleft palate and supernumerary ribs was seen at 1000 mg/kg bw/day (top dose) associated with liver effects in the dam (B.6.6.2.3.2/01).

Table 56 Foetal findings from B.6.6.2.3.2/01

Parameter	Tebuconazole [mg/kg bw/day]				HCD#	HCD¤
	0	100	300	1000		
Number of fetuses (litters) evaluated	301 (25)	332 (25)	337 (24)	285 (25)	# #	
External examination						
Number of fetuses (litters) affected	9 (8)	10 (8)	6 (6)	15 (9)	- -	
Cleft palate (palatoschisis)	2.7 [a] (24.0)	2.4 (24.0)	1.2 (16.7)	**4.2** [b] (28.0)	0.0 – 2.0 (0.0 – 20.0)	0.0 – 2.0 (0.0 – 20.0)
Malposition of hindlimb	0.3 (4.0)	0.3 (4.0)	0.3 (4.2)	**0.7** **(8.0)**	0.0 – 0.5 (0.0 – 4.8)	0.0 – 0.5 (0.0 – 4.8)
Misshapen tail (small, bent, curled)	0.3 (4.0)		0.3 (4.2)		0.0 – 0.4 (0.0 – 4.8)	
Exencephaly	0.3 [a] (4.0)	0.3 (4.0)	- -	0.7 [b] (4.0)	0.0 – 0.8 (0.0 – 9.5)	0.0 – 0.3 (0.0 – 4.2)
Skeletal examination						
No of foetuses examined	159	171	175	**145**		
Supernumerary rib, one left	58%	62%	60%	**74%****		
Supernumerary rib, one right	48%	58%	52%	**72%****		

#Historical control data (HCD) range (Bayer and RCC) from 1983 – 1993 (11 studies, 247 litters, 2760 fetuses). The HCD are combined data from the performing laboratory in Switzerland and a laboratory in Germany. HCD is from NMRI mice.

¤ Historical control data (HCD) range from 1987 – 1993 (5 studies), same mouse strain; NMRI mice. The laboratory is the performing laboratory. No information available on breeder.

a One fetus with cleft palate and additional tail cranial bended; another fetus with cleft palate and additional exencephaly.

b One fetus with cleft palate and additional exencephaly.

c Visceral examination: no further findings were observed in any treatment group compared to findings during external examination. Findings considered related to treatment with tebuconazole are written in bold letters.

** $p \leq 0.01$

Maternal toxicity (liver toxicity) also started to occur from 30 mg/kg bw/d, becoming more severe (haematotoxicity, decreased body weight gain, reduced food consumption) at 100 mg/kg bw/d. In an earlier version of the RAR, the UK RMS based on these findings considered an overall NOAEL of 10 mg/kg bw/d for maternal toxicity in the mouse. The UK RMS noteed that in PRAPeR Expert Meeting 49 (2-6 June 2008) the following NOAELs were agreed: the maternal NOAEL was set at 100 mg/kg bw/d – since liver effects were considered by the experts as adaptive but not adverse, and the developmental LOAEL was set at 10 mg/kg bw/d – based on an increased incidence of malformations (open eye, runts, cleft palate) in the study B.6.6.2.3.1/03. The UK RMS accepted the decision made at the PRAPeR meeting and the LOAEL of 10 mg/kg bw/d was taken forward. The DK RMS agrees with this NOAEL for maternal effects and LOAEL for developmental effects (from PRAPeR Expert Meeting 49), however notes that effects on maternal body weight gain and altered liver weight and histology

during pregnancy may be a direct and specific effect related to an endocrine mode of action, rather than an unspecific secondary effect of maternal toxicity.

Thus, the observed developmental effects in mice cannot be considered related to unspecific maternal toxicity. Further discussion on possible relations with maternal effects is presented below in relation to CLP criteria.

8.10.6 Comparison with the CLP criteria

CLP criteria

For the purpose of classification for reproductive toxicity, substances are allocated to one of two categories. Within each category, effects on sexual function and fertility, and on development, are considered separately. In addition, effects on lactation are allocated to a separate hazard category.

Category	Criteria
Category 1	*Known or presumed human reproductive toxicant* *Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).*
Category 1A	*Known human reproductive toxicant* *The classification of a substance in this Category 1A is largely based on evidence from humans.*
Category 1B	*Presumed human reproductive toxicant* *The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development 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. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.*
Category 2	*Suspected human reproductive toxicant* *Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.* *Such effects shall have been observed 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 the other toxic effects.*

As presented below, the DK RMS concludes that:
Classification in Repr. Category 1A is not supported as it should be based on human data and there is no information about the potential toxicity of tebuconazole for humans.

Classification in Repr. Category 1B for effects on development is considered appropriate for classification of tebuconazole, as the CLP criteria are fulfilled by the available information on toxicity of tebuconazole providing clear evidence of adverse effects that are not considered to be secondary non-specific effects of other toxic effects (see description below). There is no mechanistic information that raises doubt about the relevance of the effects for humans.

Classification in Repr. Category 2 is not appropriate as there is clear evidence from animal studies of sufficient quality and not considered to be secondary non-specific effects of other toxic effects.

Rationale/justification classification for developmental effects of tebuconazole in Repr. 1B
The three main adverse effects of tebuconazole on developmental effects are considered as critical for the classification on developmental toxicity: 1) postimplantation loss and perinatal death, 2) fetal/pup growth impairment, and 3) external malformations including cleft palates.

Thorough evaluation of the evidence for adverse effects and possible influences of other toxic effects as well as human relevance needs to be considered.

According to Annex I: 3.7.2.4.2. of ECHA 2017 guidance on CLH criteria « *....In the interpretation of the developmental outcome to decide classification for developmental effects it is important to consider the possible influence of maternal toxicity. This is a complex issue because of uncertainties surrounding the relationship between maternal toxicity and developmental outcome. Expert judgement and a weight of evidence approach, using all available studies, shall be used to determine the degree of influence that shall be attributed to maternal toxicity when interpreting the criteria for classification for developmental effects. The adverse effects in the embryo/foetus shall be first considered, and then maternal toxicity, along with any other factors which are likely to have influenced these effects, as weight of evidence, to help reach a conclusion about classification. »*

Furthermore: « *Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies.»*

To aid the evaluation, the following issues are addressed :

a) Data overview for developmental and maternal effects
b) Mode of action analysis investigating the plausibility that observed effects are specific and human relevant
c) Considerations on presence or absence of concomitant marked systemic effects
d) Severity and irreversibility of effects
e) Conclusion in relation to CLP criteria

Data overview - development

Adverse effects on developing embryo/fetus: Overall, developmental toxicity (slightly increased incidence of external malformations (including cleft palate, open eyes, runts), post-implantation loss, reduced ossification, decreased foetal weight, skeletal anomalies, pup mortality, reduced viability index) was observed in rats, rabbits and mice. Cleft palate appeared with low incidence, but in different litters, in several independent studies with both dermal and oral exposure, and in mice and rabbits, but not rats.

Maternal effects: The observed developmental effects were in some but not all cases associated with statistically significant maternal effects on body weight gains, liver and adrenal changes.

The effects on 1) postimplantation loss and perinatal death, 2) fetal/pup growth impairment, and 3) external malformations including cleft palates are summarised in the table below.

Post-implantation loss (including fetal deaths and decreased litter size)	Several studies using rats, mice or rabbits report effects on fetal death (B.6.6.2.1.1/01, B.6.6.2.1.1/02, B.6.6.2.1.1/03, B.6.6.2.2.1/01, B.6.6.2.2.1/02, B.6.6.2.2.1/03, B.6.6.2.3.1/03) all using oral exposure route. Additionally, four studies using rats, mice or rabbits report post-implantation loss (B.6.6.2.1.1/03, B.6.6.2.2.1/01, B.6.6.2.2.1/03,.6.6.2.3.1/03). B.6.6.2.3.1/01 reports no effect, but data indicates increased numbers of resorptions as well as post-implantation loss in the high dose group. Additional open literature studies showed increased post-implantation loss in rats following gestational exposure (B.6.6.3/07, B.6.6.3/08). One study showed no significant effect (B.6.6.3/04). The studies using dermal exposure route (B.6.6.2.1.3/02, B.6.6.2.1.3/01, B.6.6.2.3.2/01) and one oral study

Decreased litter viability	(B.6.6.2.2.1/04) report no effects on fetal death. In several studies, fetal death was seen at doses not affecting maternal body weight, or reductions in maternal body weight gain during pregnancy could be attributed to lower total litter weights (see description in section for adverse effects on sexual function and fertility). In rabbit studies and a few rat studies (6.6.2.1.1/03 and B.6.6.2.1.1/01, the reduced maternal weight gain could not be fully explained by lower litter weights; nevertheless, these studies support an adverse (and severe) effect on fetal death seen in other studies without marked maternal effects. Litter size decreased in rats (B.6.6.1.1/01, B.6.6.2.1.1/02) and mice (B.6.6.2.3.1/03), and tendencies or nominal decreases were seen in two other rat studies (B.6.6.3/07, B.6.6.3/08). No effect on litter size was shown in two studies, one using dermal exposure (B.6.6.2.1.1/02) and another using a maximum dose of 50 mg/kg bw/d, which is below LOAELs in studies showing effects). Two studies show a clearly decreased litter viability (B.6.6.2.1.2/01, B.6.6.2.1.2/01), supported by two other studies showing similar effect although not statistically significant (B.6.6.2.1.1/01, B.6.6.3/07).
Litter/pup fetal weight	Nine studies show decreased litter weights (B.6.6.1.1/01, B.6.6.2.1.1/01, B.6.6.2.1.1/02, B.6.6.2.1.1/03, B.6.6.2.2.1/02, B.6.6.2.2.1/03, B.6.6.2.2.1/04, B.6.6.2.3.1/01, B.6.6.2.3.1/03). The remaining studies found no effect using dermal exposure (B.6.6.2.1.3/02, B.6.6.2.1.3/01, B.6.6.2.3.2/01) or a maximum concentration of 50 mg/kg bw/d which is below other observed effects on litter weight.
External malformations including cleft palates	Malformations were seen across species (rat, rabbit and mice), cleft palates were seen in mice and rabbits. In rats developmental effects such as skeletal anomalies and increased incidence of total external malformations and microphthalmia was seen in two developmental toxicity studies using higher doses of around 100 - 120 mg/kg bw/d B.6.6.2.1.1/01, B.6.6.2.1.1/02), whereas presence of cleft palate was rare. In mice, increased incidence of external malformations such as cleft palate, exencephaly and tail abnormalities were seen at oral doses of 100 mg/kg bw/d and dermal doses of 1000 mg/kg bw/d at ranges outside historical control ranges (B.6.6.2.3.1/01, B.6.6.2.3.1/03, (B.6.6.2.3.2/01). In one study, the total incidence of malformations (open eye, runts, cleft palate) was increased already at the low dose of 10 mg/kg bw/d.

Mode of action analysis

Postimplantation loss:
The observed developmental effects on this endpoints may be related to the observed endocrine disrupting activity of tebuconazole described in annex II. There is clear evidence that tebuconazole is an aromatase inhibitor inducing marked reductions in reproductive hormone levels (see annex II).

A mode of action can be postulated showing a relationship between disruptions in steroidogenesis, reduced estradiol levels and adverse outcomes. This mode of action is further substantiated in annex II.

MIE	KE1	AO
Disrupted steroidogenesis	↓ estradiol	post-implantation loss

Dose-concordance between key events of the postulated MoA are included in annex II.

Effects are considered to be relevant for humans. Data on post implantation losses have also been shown in a non-human primate (baboon) with another aromatase inhibitor, (letrozole). Letrozole affects maternal levels of estradiol but not progesterone in monkeys (Albrecht 2000). In rats, letrozole also induces an increase in late resorptions that was prevented by co-exposure to oestrogen (Tiboni 2009). It is recognised that rats, guinea pigs, non-human primates and humans are sensitive to the general mode of action of aromatase inhibition and that physiological species differences may

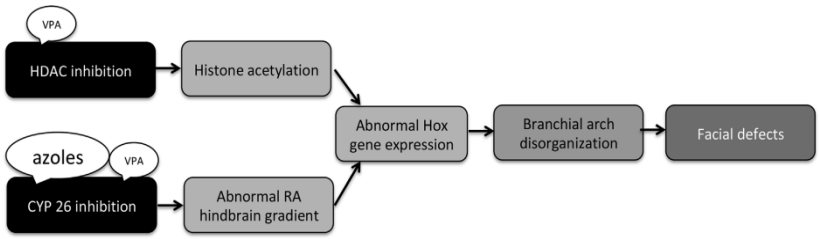
lead to various responses although through a common mechanism.

External malformations including cleft palate :
Cleft palate is a severe malformation that can be induced by chemicals if the critical dose and timing of exposure are aligned. It may be noted that some cases might be masked by the post-implantation loss and the reduced number of viable foetuses seen in several studies. An important question in the studies identifying malformations late in pregnancy or at birth is that they actually deal with the "survivors" of a cohort of malformed offspring, thus including more prevalent than incident cases (Cordier et al., 1992).

The mode of action of tebuconazole in the observed developmental alterations is not known, but for several triazoles the developmental effects are suggested to be related to altered embryonic retinoid acid catabolism, since abnormalities are confined to structures controlled by retinoid acid.

For cleft palate, an Adverse Outcome Pathway for facial defects was recently proposed by Metruccio et al., 2020 (from EuroMix EU Horizon 2020 Project). This can be considered a toxicity pathway for azoles, which includes CYP26 inhibition as MIE (Menegola et al., 2006), was the basis for developing a quantitative AOP for craniofacial malformations (Battistoni et al., 2019). An increased incidence of cleft palates in rat has been observed in response to exposure to other triazoles (e.g. propiconazole, cyproconazole and epoxiconazole).

Figure from Metruccio et al., 2020. Adverse outcome pathways (AOPs) leading to the same adverse outcome (AO, facial defects) via different key events (KEs, grey).



There is no information showing that the mechanism is not relevant for humans, and in the absence of this information, the DK RMS considers the tebuconazole induced increases in cleft palate incidences found in developmental studies of mice and rabbits as of human relevance.

Considerations regarding presence or absence of marked systemic effects
In order to decide classification for developmental effects it is important to consider whether it can be demonstrated that effects are secondary to maternal systemic effects.

In relation to effects on maternal body weight, ECHA 2017 guidance on CLH criteria specifies in Annex I: 3.7.2.4.4: « *The calculation of an adjusted (corrected) mean maternal body weight change, which is the difference between the initial and terminal body weight minus the gravid uterine weight (or alternatively, the sum of the weights of the foetuses), may indicate whether the effect is maternal or intrauterine.* »

In general, reductions in maternal body weight gain may be related to reduced food intake during early days of pregnancy and/or to specific toxicity to uterine and fetal growth. Reduced food intake in early/mid-pregnancy may be related to systemic toxicity or to direct effects on reduced uterine and fetal growth. Therefore, it is necessary on a case-by-case to clarify whether developmental effects are secondary to maternal toxicity, and a thorough evaluation of relationships between maternal weight gain changes and fetal growth and survivial needs to be carried out.

In rats, reductions in maternal body weight gain were seen at the same dose as severe and predominantly irreversible developmental effects (postimplantation loss and postnatal death, reduced fetal weights, malformations) or at the dose below. To aid this evaluation, tables comparing changes in maternal weight gains with changes in litter weights were presented for rats exposed by gavage. In

general, reductions in maternal body weight gain in high dose groups were largest (in percent of control) during the first days of exposure. At termination of the studies, reduced fetal weights and reduced litter sizes caused reduced total litter weights, which could partly or fully explain the differences in maternal body weight gain during pregnancy. Severe maternal effects (dystocia) were seen in a few animals in three studies at high doses, and are likely related to a specific endocrine mode of action, i.e. not general systemic toxicity.

In most rabbit studies, effects on maternal body weight gains and food consumption were seen at the same dose as severe and predominantly irreversible developmental effects (postimplantation loss, reduced fetal weights, malformations). No marked maternal systemic toxicity was observed at these LOAELs, but rather indications of specific endocrine effects (liver and adrenal hypertrophy) possibly related to a specific endocrine mode of action of the developmental effects.

In mice, some studies showed maternal effects at the same dose that caused developmental effects, whereas one study showed no maternal toxicity at the developmental LOAEL. Maternal effects were generally reduced maternal body weight gains and food consumption, and hepatic changes that may be adaptive or indications of specific endocrine effects related to the observed developmental toxicity. For the mouse study (oral gavage) (B.6.6.2.3.1/03), the lack of maternal toxicity at developmental toxic doses was noted. In PRAPeR Expert Meeting 49 (2-6 June 2008) the maternal NOAEL was set at 100 mg/kg bw/d – since liver effects were considered by the experts as adaptive but not adverse, and the developmental LOAEL was set at 10 mg/kg bw/d – based on an increased incidence of malformations (open eye, runts, cleft palate).

In addition to the hepatic alterations mentioned here, adrenal changes were seen in some of these studies As presented in the mode of action analysis above, the observed effects are likely due to disrupted steroidogenesis. Estrogen production mainly occurs in ovaries, and it is not clear whether effects on adrenal steroidogenesis are also part of adverse effect causation. Even if adrenals are part of the causation, DK-RMS considers that the adrenal effects are not indicative of "systemic maternal toxicity", but rather indicative of a specific endocrine mode of action.

Based upon all available information, DK RMS concludes that it cannot be demonstrated that the observed developmental effects in any of the tested species are secondary to marked systemic maternal toxicity. On the contrary, effects on dams are likely related to specific modes of action causing developmental toxicity. The maternal effects observed in the studies are considered mild compared to the developmental effects of which many are of high concern such as external malformations and cannot be explained as secondary to maternal toxicity. This is also supported by two feed restriction studies on the rat and rabbit (Fleeman, 2005 and Cappon, 2005), which clearly showed that even severe weight loss or decrease in body weight gain per see induced minor changes in skeleton development but had no effects on viability or malformations in the rat. In rabbits abortions occurred in the most severe restricted group but no malformations were observed. It is acknowledged that food restriction studies do not fully reflect effects of toxicant exposure, but nevertheless this data serves to support the conclusion that fetal death is not considered a cause of reduced maternal weight gain, It should be noted that the burden of proof lies in demonstrating that developmental effects are secondary to maternal toxicity.

Severity and irreversibility of effects

As noted above, *« classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies ».* The majority of the observed effects 1) postimplantation loss and perinatal death, 2) fetal/pup growth impairment, and 3) external malformations including cleft palates are considered irreversible and severe.

Conclusion in relation to CLP criteria – development

The three main adverse developmental effects 1) post implantation loss and perinatal death, 2) fetal/pup growth impairment, and 3) external malformations including cleft palates have been assessed and compared with the CLP criteria by the DK RMS and the conclusions are as follows:

- The observed post implantation loss and perinatal death seen in rats, rabbits and mice in

absence of marked maternal toxicity, support a classification as Repr. 1B (CLP) for this effect.

- The observed fetal/pup growth impairment seen in the absence of marked maternal toxicity, support a classification as Repr. 1B (CLP) for this effect.

- The presence of external malformations including cleft palates in the mouse and rabbit foetuses in the absence of marked maternal toxicity, support classification as Repr. 1B (CLP). Cleft palate was not observed in rats.

In an earliger previous version of the RAR , the UK RMS concluded that it is « *possible that some of the observed developmental effects observed in the three species investigated were the secondary unspecific consequences of maternal toxicity* ». DK RMS considers this argumentation not to be in line with the CLP criteria, as the necessary case-by-case evaluation was not performed demonstrating that the developmental effects were secondary to maternal toxicity. Secondly, if such demonstration had been available, it is not sufficient to demonstrate this for « *some of the observed developmental effects*» only.

On this basis, classification of tebuconazole for developmental toxicity in category 1B (Repr. 1B; H360D) is warranted.

The lowest LOAEL for developmental toxicity across all development toxicity studies was 10 mg/kg bw/d, identified in the mouse and agreed at PRAPeR Expert Meeting 49 (2-6 June 2008).

8.10.7 Adverse effects on or via lactation

Table 57: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Two generation study Acceptable	Please refer to table 46	Lactation is included. However, no measurement in breastmilk. The effects observed cannot be distinguished between the exposure occurring in utero or via lactation.	Vol 3 B.6.6.1.1/01
Developmental neurotoxicity US-EPA OPPTS 870.6300 (1996) Acceptable	Please refer to table 46	Lactation is included. However, no measurement in breastmilk. The effects observed cannot be distinguished between the exposure occurring in utero or via lactation.	Vol 3 B.6.6.2.1.2/01

Table 58: Summary table of human data on effects on or via lactation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 59: Summary table of other studies relevant for effects on or via lactation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

8.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

Exposure via lactation was included in the 2 generation study in rats (B.6.6.1.1/01) and in a development neurotoxicity study (B.6.6.2.1.2/01). However, there was no direct measurement in breastmilk to verify exposure. The results of the studies provide no indication of impaired nursing behaviour or effects on or via lactation. Furthermore, the effects observed cannot be distinguished between the exposure occurring in utero or via lactation.

8.10.9 Comparison with the CLP criteria

The classification is intended to indicate when a substance may cause harm due to its effects on or via lactation and is independent of consideration of the reproductive or developmental toxicity of the substance.

The scarce data available do not indicate effects via lactation. Hence, classification of tebuconazole for effects on or via lactation is not warranted.

8.10.10 Conclusion on classification and labelling for reproductive toxicity

Fertility

It is proposed to classify Tebuconazole Repr. 1B, H360F May damage fertility. The main adverse fertility effects 1) dystocia and prolonged gestation 2) effects on the reproductive system of pubertally exposed males have been assessed and compared with the CLP criteria by the Committee and the conclusions are as follows:

- The observed dystocia and prolonged gestation seen in rats support a classification as Repr. 1B (CLP) for fertililty for this effect.

-The observed effects on the reproductive system of developmentally exposed males are not considered sufficient for classification for fertility on its own, however the observed effects indicates a potential and in the absence of more elaborated data investigating these end points in up to date OECD compliant studies, these has been included in a WoE approach.

Development

It is proposed to classify Tebuconazole Repr. 1B, H360D May damage the unborn child.

The main adverse developmental effects 1) post implantation loss and perinatal death, 2) fetal/pup growth impairment, and 3) external malformations including cleft palates have been assessed and compared with the CLP criteria by the Committee and the conclusions are as follows:

- The observed post implantation loss and perinatal death seen in rats, rabbits and mice in the absence of marked maternal toxicity, support a classification as Repr. 1B (CLP) for this effect.

- The observed fetal/pup growth impairment seen in rats, rabbits and mice in the absence of marked maternal toxicity, support a classification as Repr. 1B (CLP) for this effect.

- The presence of external malformations including cleft palates in the mouse and rabbit foetuses in the absence of marked maternal toxicity, support classification as Repr. 1B (CLP).

Conclusion

On this basis, the classification of tebuconazole for fertility and developmental toxicity in category 1B is warranted:

Repr 1B; H360FD, May damage fertility. May damage the unborn child.

8.11 Specific target organ toxicity-single exposure

Table 60: Summary table of animal studies on STOT SE

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL**	**Reference**
Acute oral toxicity, OECD 401 (Rat, Wistar, M/F, 5/sex/dose) Acceptable	(HWG 1608) Tebuconazole Oral, fasted rats: 1000, 2500, 4500, 5000 mg/kg bw (male) 1000, 2500, 3150, 3550, 5000 mg/kg bw (female) oral, non-fasted rats, 500, 1000, 3550, 3750, 4000, 5000 mg/kg bw (male) 500, 1000, 2500, 3550, 4250, 4500 mg/kg bw (female)	Tretament-related clinical signs observed included: behavioural disturbances, breathing disturbances, motility disturbances, staggering, spastic gait, sternal, lateral recumbency, loss of hair, cramped posture, increased urine excretion and poor reflexes in males and females. The following effects were observed in animals which died during post-treatment observation period: lungs (spotted, distended), liver (patchy, pale, lobulation, enlarged), glandular stomach (reddened). No treatment-related findings were observed in animals sacrified at termination Fasted M: LD_{50}: > 5000 mg/kg bw Fasted F: LD_{50}: 3933 mg/kg bw Non-fasted M: LD_{50}: 4264 mg/kg bw Non-fasted F: LD_{50}: 3352 mg/kg bw No classification for STOT SE	Vol 3 B.6.2.1.1/03
Acute oral toxicity, Based on OECD 401 (Rat, Wistar, 5M) Acceptable	(HWG 1608) Tebuconazole 5000 mg/kg bw, single oral dose	Tretament-related clinical signs observed included: bristled fur, apathy, reduced motility, spastic gait, staggering, dyspnoea, salivation, diarrhoea. At termination abnormal findings in the liver (thin yellowish layer), lungs (patchy, dark spots) and scar like changes were observed. LD_{50}: > 5000 mg/kg bw No classification for STOT SE	Vol 3 B.6.2.1.1/02
Acute oral toxicity, JMAFF (59 Nohsan no. 3850); comparable to OECD 401	(HWG 1608 technical) Tebuconazole Oral: 1600, 2300, 3000, 3900 and	Tretament-related clinical signs observed included: sedation, abnormal gait, paralytic gait and emaciation which in male and females. At termination abnormal findings in the liver (yellow-white patchy areas) and the testis (atrophy)	Vol 3 B.6.2.1.1/01

(1987) (Rat, Sprague-Dawley, M/F 5/sex/group) Acceptable	5000 mg/kg bw (male). 730, 950, 1230, 1600, 2300, 3000, 3900 and 5000 mg/kg bw (female)	for males were observed. M: LD_{50}: 4000 mg/kg bw F: LD_{50}: 1700 mg/kg bw No classification for STOT SE	
Acute oral toxicity, OECD 401 (Mouse ICR, M/F, 5/sex/dose) Acceptable – supplementary	(HWG 1608 technical) Tebuconazole Oral: 100, 500, 1000, 1800, 2500, 3150 and 3550 mg/kg bw (male) 500, 1000, 1800, 2500, 3550 and 5000 mg/kg bw (female)	Tretament-related clinical signs observed included: behavioural disturbances, breathing disturbances, motility disturbances, staggering, spastic gait, sternal, lateral recumbency, poor reflexes in males and females. M: LD_{50}: 1615 mg/kg bw F: LD_{50}: 3023 mg/kg bw No classification for STOT SE	Vol 3 B.6.2.1.2/02
Acute oral toxicity, OECD 401 (Mouse ICR, M/F, 5/sex/dose) Acceptable – supplementary	(HWG 1608 technical) Tebuconazole Oral: 1600, 2300, 3000, 3900 and 5000 mg/kg bw (male), 3000, 3900 and 5000 mg/kg bw (female)	Tretament-related clinical signs observed included: sedation, abnormal gait, paralytic gait and hypnosis in male and females. At termination abnormal findings in the liver (yellow-white patchy areas) for males were observed. Changes in the digestive system (mucosal redness, dark reddish brown focus in the stomach, dilated lumen, yellowish contents and mucosal redness in the small intestine), lungs (dark reddish brown) and testis (atrophy) were observed in animals that died during the observation period M: LD_{50}: 2800 mg/kg bw F: LD_{50}: 5200 mg/kg bw No classification for STOT SE	Vol 3 B.6.2.1.2/01
Acute oral toxicity, OECD 401 (Rabbit, Albino, M/F, 3/sex/dose) Acceptable – supplementary	(HWG 1608) Tebuconazole (Single oral dose, 500 and 1000 mg/kg bw)	Tretament-related clinical signs observed included: a general loss of appetite. lung slightly distended, spotted; kidney slightly patchy were observed in animals sacrified at termination F & M: LD_{50}: >1000 mg/kg bw No classification for STOT SE	Vol 3 B.6.2.1.3/01
Acute dermal toxicity, OECD 402 (Rat, Wistar, M/F,	(HWG 1608) Tebuconazole 5000 mg/kg bw, single dose, (24h,	No clinical signs, skin irritation or pathological findings were observed.	Vol 3 B.6.2.2/02

5/sex) Acceptable	occlusive)	M & F: LD_{50}: > 5000 mg/kg bw No classification for STOT SE	
Acute dermal toxicity JMAFF (59 Nohsan no. 3850); comparable to OECD 402 (1987) (Rat, Sprague-Dawley, M/F 5/sex) Acceptable	(HWG 1608 Technical) Tebuconazole 2000 mg/kg bw, single dose, (24h, occlusive)	No clinical signs, skin irritation or pathological findings were observed. M & F : LD_{50}: > 2000 mg/kg bw No classification for STOT SE	Vol 3 B.6.2.2/01
Acute dermal toxicity, OECD 402 (Rat, Wistar, M/F, 5/sex) Acceptable	Tebuconazole TC 2600 mg/kg bw, single dose, (24h, occlusive)	No clinical signs, skin irritation or pathological findings were observed. LD_{50} > 2060 mg/kg bw No classification for STOT SE	Vol 3 B.6.2.2/03
Acute inhalation toxicity, OECD 403 (Rat, Wistar, M/F 5-10/sex/dose) Acceptable	(HWG 1608-technical grade) Tebuconazole particle size: 50% ≤ 5 µm Analytical concentration: 16, 49, 387, 818 mg/m³ 1 x 4 hours (aerosol) Analytical concentration: 0, 24, 60, 240 mg/m³ 5 x 6 hours (aerosol)	In the 1x4 hrs study reduced motility (lassitude) was observed in the 250, 2500, 5000 mg/m³ dose groups. In the 5 days x 6 hrs study non-specific disturbed behaviour (lassitude) was observed in all groups. There were no indications of concentration-related grossly apparent lung or organ damage in the 1x4 hrs or in the 5 days x 6 hrs study. 4 hours exposure: M & F : LC_{50}: > 0.818 mg/L (max. attainable concentration) 5 days * 6 hours exposure M&F: LC_{50}: > 0.24 mg/L No classification for STOT SE	Vol 3 B.6.2.3/02
Acute inhalation toxicity, OECD 403 (1981) (Rat, Wistar, M/F 5/sex) Acceptable	Tebuconazole white powder MMAD = 2.76 µm ± GSD (1.84 µm), 55% ≤ 3 µm (2118 mg/m³, target	No specific clinical of any toxicological significance were observed M & F : LC_{50}: > 2.118 mg/L (max. attainable concentration) No classification for STOT SE	Vol 3 B.6.2.3/03

	concentration 5000 mg/m³) 2118 mg/m³ 1 x 4 hours (solid aerosol)		
Acute inhalation toxicity, OECD 403 (1981) (Rat, Wistar, M/F 5/sex/dose) Acceptable	HWG 1608-technical grade) Tebuconazole MMAD = 1.4 µm ± GSD (1.4 µm), 100% ≤ 5 µm (Aerosol), MMAD = 12.8 µm ± GSD (1.9 µm), 8% ≤ 5 µm (Dust) Analytical concentration: 371 mg/m³ (aerosol), 5093 mg/m³ (dust) 1 x 4 hours (dust, aerosol)	No clinical signs or indications of grossly apparent lung of other organ damage were observed. M/F: LC_{50}: > 5.093 mg/L (dust) M/F: LC_{50}: > 0.371 mg/L (aerosol) (max. attainable concentration) No classification for STOT SE	Vol 3 B.6.2.3.1/01
Acute inhalation toxicity, OECD 403 (Rat, CD/crL: CD (SD), M/F 5/sex) Acceptable	Tebuconazole MMAD = 16.324 µm ± GSD (2.38 µm (5000 mg/m³) 5.00 mg/L air (limit test) 1 x 4 hours (aerosol)	No clinical signs of toxicity were observed throughout the duration of the study. No pathological findings were noted at necropsy M& F: LC_{50}: > 5.0 mg/L No classification for STOT SE	Vol 3 B.6.2.3/04
Acute intraperitoneal toxicity No guideline followed (Rat, Wistar, M/F 5-10/sex/group) Acceptable as supplementary	HWG 1608 Tebuconazole Single dose 50, 100, 500, 630, 710, 800, 900, 1000 mg/kg bw. (male) and 50, 100, 355, 400, 450, 560 mg/kg bw. (female)	Clinical signs of toxicity observed: Behavioural, breathing and motility disturbances, staggering, spastic gait, uncoordinated movements, poor reflexes, narcosis, convulsions, lateral or sternal recumbency LD_{50}: 751 mg/kg bw (males) LD_{50}: 395 mg/kg bw (females). No classification for STOT SE	Vol 3 B.6.8.2.2/01

Table 61: Summary table of human data on STOT SE

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 62: Summary table of other studies relevant for STOT SE

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Acute oral Neurotoxicity screening study, similar to OECD TG 424 GLP Rat, Fischer 344, M/F, 12/sex/group	Tebuconazole batch number 603-0013, Purity: 96.2-97.3 % Single Dose: 0, 100, 500 and 1000 mg/kg bw for males and 0, 100, 250 and 500 mg/kg bw for females Supplemental : 0, 20 and 50 mg/kg bw (males and females)	Evidence of neurotoxicity at the low dose (100 mg/kg bw) meant a supplementary study was conducted using doses 0, 20 and 50 mg/kg bw to establish a NOAEL. FOB was performed four hours after administration of the dose, with measurement of activity concluded at seven hours Brain weight not affected by treatment at any dose level at terminal sacrifice No microscopic lesions	Neurotoxicity: Increased activity in the FOB (F only) and in the figure-eight maze (both sexes) Generalised toxicity: Clinical signs of toxicity NOAEL for neurotoxicity and general toxicity: 50 mg/kg bw NOAEL for neuropathology: 1000 mg/kg bw No classification for STOT SE	Vol 3 B.6.7.1.1/01
Acute oral Neurotoxicity screening study, OECD TG 424 GLP Wistar Han, M/F, 10/sex/group	Tebuconazole batch number 2008040703, Purity: 99.3 % Single Dose: 0, 100, 500 and 1500 mg/kg bw		Neurotoxicity: Pilo-erection, hunched posture, ataxia and decreased respiratory rate confirmed by open-field arena behavioural observations in both sexes treated with 1500 mg/kg bw Generalised toxicity: Body weight losses observed at 1500 mg/kg bw in both sexes and for females treated with 500 mg/kg bw NOAEL Neurotoxicity: 500 mg/kg bw NOAEL general toxicity: 100 mg/kg bw LOAEL for neurotoxicity: 1500 mg/kg bw in males & females No classification for STOT SE	Vol 3 B.6.7.1.1/02

8.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

Specific target organ toxicity after single exposure to Tebuconazole was studied in rat, mouse and rabbit acute toxicity studies via oral, inhalation, dermal and intraperitoneal routes. In addition, two acute oral neurotoxicity studies in rat were conducted.

The studies generally comply with older OECD test guidelines (TG 424, TG 401-403) and were conducted in accordance with GLP. The studies demonstrated a low to moderate acute toxic potential of tebuconazole with LD50 of 1700 mg/kg bw for oral route of exposure and LD50 and LC50 values above the classification criteria for dermal and inhalation route of exposure. Clinical signs shown after acute oral administration comprised effects on the peripheral and central nervous system besides other unspecific signs of toxicity.

In a first acute oral neurotoxicity study in Fisher 344 rats (B.6.7.1.1/01) increased activity during the FOB (females only) and in the figure-eight maze (males and females) was observed from 100 mg/kg bw in association with clinical signs of toxicity. A NOAEL for generalised toxicity and acute neurotoxicity was established at 50 mg/kg bw in this study. In a second study in Wistar rats (B.6.7.1.1/02), pilo-erection, hunched posture, ataxia and decreased respiratory rate were observed five hours after treatment during the clinical observations at dose level of 1500 mg/kg bw, with generalised toxicity (body weight loss) occurring from 500 mg/kg bw. These effects were confirmed by behavioural assessments undertaken on day 1. Altogether, these effects may be indicative of neurotoxicity. Complete regression of these signs however was evident on the day after treatment.

The different responses observed in the two studies could be due to the different strains of rats used in both studies. An overall acute neurotoxicity NOAEL of 50 mg/kg bw was established.

8.11.2 Comparison with the CLP criteria

According to the ECHA Guidance criteria a classification in STOT-SE Category 1 or 2 is not applicable, if non-lethal significant and/or severe toxic effects on target tissues/organs are not seen in acute toxicity studies up to the following guidance values:

	Category 2	**Category 1**
Oral rat	2000 mg/kg bw	300 mg/kg bw
Dermal rat or rabbit	2000 mg/kg bw	1000 mg/kg bw
Inhalation rat, dust / mist / fume	5 mg/L/4h	1 mg/L/4h

A double classification for the same effect causing lethality should be avoided. Furthermore, the ECHA Guidance specifies criteria that trigger a classification for STOT-SE Category 3. These criteria are generally independent from the aforementioned guidance values and include transient target organ effects, focusing on overt narcotic effects and respiratory tract irritation (respiratory tract irritation covers two different effects: ‘sensory irritation’ and ‘local cytotoxic effects’). Specifically, the following examples for findings from single and repeated inhalation toxicity studies are mentioned as possible triggers for a STOT-SE Category 3 classification: clinical signs of toxicity (dyspnoea, rhinitis etc) and histopathology (e.g. hyperemia, edema, minimal inflammation, thickened mucous layer) which are reversible.

Since no severe signs of specific symptoms or long-lasting effects on organs were seen and the dose criteria were not exceeded, a classification in STOT-SE Category 1 or 2 is not warranted.

Regarding a possible STOT-SE Category 3 classification for “overt narcotic effects”, the observed toxicological findings do not indicate such effects, the observed clinical signs were signs of general and unspecific acute toxicity which were reversible. Furthermore, signs of a specific respiratory tract irritation or non-specific sensory irritation were not seen. Therefore, also a STOT-SE Category 3 classification is not warranted.

8.11.3 Conclusion on classification and labelling for STOT SE

According to CLP criteria: "specific target organ toxicity (single exposure) is defined as specific, non lethal target organ toxicity arising from a single exposure to a substance or mixture. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed STOT-SE should be considered where there is clear evidence of toxicity to a specific organ especially when it is observed in the absence of lethality…".

Based on the overall acute toxicity and neurotoxicity no classification for STOT-SE is proposed.

8.12 Specific target organ toxicity-repeated exposure

Table 63: Summary table of animal studies on STOT RE

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure, Adjusted guidance values category 1 / 2 (mg/kg bw/day)	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Oral 28-day study in rats			
Comparable to OECD 407 (1981) Non-GLP Deviations from the current OECD guideline 407: Minor – these deviations are not considered to affect the validity of the study. Acceptable Male and female Wistar rats, Bor:WISW (SPF-bred) 20 animals/sex/group	HWG 1608, Tebuconazole (technical grade, 97.0%) Batch 16001/83 Oral 28-day study by gavage 0, 30, 100, 300 mg/kg bw/day Cat. 1 ≤ 30 Cat. 2 ≤ 300	NOAEL mg/kg bw/day: 30 LOAEL mg/kg bw/day: 100 ≥ 100 mg/kg bw/day: Decreased red blood cell parameters, increased liver weights and enzyme activity. 300 mg/kg bw/day: impairment of liver function (increase in the transaminase activities in plasma) and histopathological changes (sideropenia) in the spleen Effects at the LOAEL Effects on the blood profile, increased liver and spleen weights and sideropenia in the spleen at 100 mg/kg bw/day and above.	Vol 3 B.6.3.1.1/01
Oral 28-day study in mice			
Range-finding Not applicable, there are no OECD guidelines for range-finding studies Non-GLP Acceptable as a supporting study only.	HWG 1608, Tebuconazole (technical grade, 96.9%) Oral, 4 weeks, diet 0, 125, 500, 2000 ppm (M/F: 0/0, 12.6/14.1, 47.0/53.2,	NOAEL mg/kg bw/day (ppm): - LOAEL mg/kg bw/day (ppm): 12.6 / 14.1 (125) ≥ 125 ppm: Increased liver weights in females (in males only occurring at highest dose group), increased lipid accumulation in hepatocytes in both sexes ≥ 500 ppm: manifestation of liver effects: statistically significantly increased transaminases	Vol 3 B.6.3.1.2/01

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure, Adjusted guidance values category 1 / 2 (mg/kg bw/day)	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Mouse Bor: NMRI (SPF Han) 5/sex/dose	181.7/236.0 mg/kg bw/day) Cat. 1 ≤ 30 Cat. 2 ≤ 300	and alkaline phosphatase activities, reduced cholesterol and pathological findings (pale and partly patchy livers) Doses of 0, 20, 60 and 180 ppm were proposed for the chronic toxicity feeding study in mice.	
Range-finding Not applicable, there are no OECD guidelines for range-finding studies. Non-GLP Acceptable as a supporting study only. Mouse Bor: NMRI (SPF Han) 5/sex/dose	HWG 1608, Tebuconazole (technical grade, 96.9%) Oral, 8 weeks, diet 0, 500, 2000 ppm (M/F: approx. 0/0, 82/114, 329/454 mg/kg bw/day*) Additional test with 5-day feeding of 0, 125, 500, 2000 ppm for determination on enzyme induction ** extrapolated values; values for daily food intake (g/kg bw) taken from wk 1-8 in 21-months dietary study (M-020598-04-1)* Cat. 1 ≤ 15 Cat. 2 ≤ 150	NOAEL mg/kg bw/day (ppm): - LOAEL mg/kg bw/day (ppm): 82 / 114 (500) ≥ 500ppm: Increased liver weight (M/F); slight to moderate liver cell degeneration (M/F), slight vacuole formation and moderately increased fat content in the liver cells (M/F) Males only: increased slight to moderate lipid content in adrenal cortex cells 2000 ppm: individual hepatocyte necrosis was also noted and increased ferriferous pigment in the spleens in both sexes Additional test (5-days): ≥125 ppm: induction of microsomal enzyme systems in liver Doses of 0, 20, 60 and 180 ppm were proposed for the chronic toxicity feeding study in mice.	Vol 3 B.6.3.1.2/02
Oral 90-day study in rats			
OECD 408 (1981); US-EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals (1982 GLP	HWG 1608, Tebuconazole (technical grade, 98.2%) Batch 16007/83 Oral 90-day feeding study 0, 100, 400, 1600	NOAEL mg/kg bw/day (ppm) : 9 / 11 (100) LOAEL mg/kg bw/day (ppm) : 35 / 47 (400) Minor but statistically significant increase in the cytochrome P-450 in the 400 ppm dose group in both sexes. Decreased weight gain (M), and very slight histopathological changes (vacuoles) in the adrenal cortex, and very slightly increased siderin	Vol 3 B.6.3.2.1/01

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure, Adjusted guidance values category 1 / 2 (mg/kg bw/day)	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Deviations to the current OECD guideline 408 (1998): Minor – these deviations are not considered to affect the validity of the study. Acceptable Male and female Wistar (BOR:WISW) rats 10/sex/dose	ppm (M/F: 0/0, 8.6/10.8, 34.8/46.5, 171.7/235.2 mg/kg bw/day) Cat 1 ≤ 10 Cat 2 ≤ 100	accumulation in in the spleen in females at 400 ppm. Liver effects (increased microsomal enzymes) in males and increased liver weight in females and same effects as above in both sexes at 1600 ppm. Effects at the LOAEL Minor but statistically significant increase in the cytochrome P-450, growth retardation, and histopathological changes in the adrenal cortex at 400 ppm.	
Oral 90-day study in dog			
Not specified, but in accordance with OECD 409 (1981) Deviations to the current OECD guideline 409 (1998): Minor – the deviations are minimal, can be compensated by the results of other studies and thus they are not considered to affect the validity of the study. Acceptable Dog Male and female beagle (bor:beag) 4/sex/group	(HWG 1608) Tebuconazole (technical, 98.2%) Batch 16007/83 Oral 90-day feeding study 0, 200, 1000, 5000 ppm (0, 8.5, 41, 212 mg/kg bw/day) Cat 1 ≤ 10 Cat 2 ≤ 100	NOAEL mg/kg bw/day (ppm): 41 (1000) LOAEL mg/kg bw/day (ppm): 212 (5000) 1000 ppm. No treatment related effects[2]. Effects at the LOAEL (5000 ppm) Lens degeneration (opacity), reduced bodyweight and food consumption, increased thrombocyte counts and marked anisocytosis, hemosiderosis in the liver and spleen, increased spleen weights and vacuolation in the adrenal zona fasciculata at the next highest dose of 5000 ppm (equivalent to 212 mg/kg bw/day).	Vol 3 B.6.3.2.2/01
Oral 12-month dog study			
OECD 452 (1981) GLP Deviations to the current OECD guideline 452 (2009) : Minor – the deviations are minimal, can be	(HWG 1608) Tebuconazole (technical, 96.9%) Oral 12 month feeding study 0, 40, 200, 1000 (2000) ppm	NOAEL mg/kg bw/day (ppm) : 1.6 (40) LOAEL mg/kg bw/day (ppm) : 8 (200) Lens alterations (opacity) in the 200 and 1000/2000 ppm dose groups (females), and impairment of liver function (increased enzyme activity) were observed. There was a dose-related incidence of livers with increased lobulation noted	Vol 3 B.6.3.3.1/01

[2] Only effect obseved at this dose was increase in liver N-demethylase activity. This was not accompanied by changed in liver weight or histopathological findings and is therefore indicative of enzyme induction, an adaptive response

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure, Adjusted guidance values category 1 / 2 (mg/kg bw/day)	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
compensated by the results of other studies and thus they are not considered to affect the validity of the study. Acceptable Dog Male and female (1:1) beagle (bor:beag) 4/sex/group	(0, 1.6, 8, 47.3* mg/kg bw/day) *calculated: (substance intake / animal / day) / ((body weight week -1 + week 52) / 2) Cat 1 ≤ 2.5 Cat 2 ≤ 25	and intra-cytoplasmatic vacuoles in cells of zona fasciculata of adrenals were observed in the 200 and 1000 ppm groups (females) and slightly increased siderin content in the spleen. Effects at the LOAEL Lens alterations (opacity) (F), increased incidence of livers with lobulation, intra-cytoplasmic vacuoles in cells of zona fasciculata of adrenals (females) at 200 ppm, equivalent to 8 mg/kg bw/day for males and females.	
US-EPA FIFRA 83-1 (corresponds to OECD 452) GLP Deviations to the current OECD guideline 452 (2009): Minor – the deviations are minimal, can be compensated by the results of other studies and thus they are not considered to affect the validity of the study. Acceptable as a supplementary chronic toxicity study using the dog as the most sensitive species tested and with the purpose of refining the NOAEL value Dog Male and female (1:1) beagle (bor:beag) 4/sex/group	(HWG 1608) Tebuconazole (technical, 96%) Batch 16013/86 Oral 12 month feeding study 0, 100, 150 ppm (M/F: 0/0, 2.96/2.94, 4.39/4.45 mg/kg bw/day) Cat 1 ≤ 2.5 Cat 2 ≤ 25	NOAEL mg/kg bw/day (ppm) : 3 (100) LOAEL mg/kg bw/day (ppm) : 4.4 (150) Subtle hypertrophy of adrenal zona fasciculata cells in all animals of the 150 ppm group was observed. The slight enlargement was not accompanied by a change in adrenal weight and appeared to be due to an increase in the size and/or number of lipid vacuoles Effects at the LOAEL Hypertrophy of the adrenal zona fasciculata in all animals at 150 ppm (the top dose).	Vol 3 B.6.3.3.1/02
Dermal 3 wk study Rabbit			
Limit test OECD 410 (1981); US-EPA Para. 82-2 (1984) GLP	(HWG 1608) Tebuconazole (technical, 97.4%) Batch 16012/86	NOAEL mg/kg bw/day: 1000 LOAEL mg/kg bw/day: - There were no systemic effects at 1000 mg/kg bw/d, but local effects possibly due to mechanical	Vol 3 B.6.3.4/01

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure, Adjusted guidance values category 1 / 2 (mg/kg bw/day)	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Acceptable Rabbit Male and female New Zealand White rabbits, strain HC:NZW 5/sex/dose	Dermal 3-week toxicity (limit test) 0, 1000 mg/kg bw/day for 6 h/day – 5 days/week non-occlusive dressing Cat 1 ≤ 80 Cat 2 ≤ 800	irritation Effects at the LOAEL: not applicable	
Inhalation 3-wk study rat			
OECD 412 (1981) GLP Deviations to the current guideline OECD 412 (1981): Minor – this deviation is minimal and is not considered to affect the validity of the study. Acceptable Rat Wistar rats, strain Bor:WISW III 10/sex/dose	HWG 1608, Tebuconazole (technical grade, 96.2%) Batch 16001/83 Inhalation 3-weeks toxicity (6 hours/day, 5 days/week) 1.2, 10.6, 155.8 mg/m^3 air (analytical concentrations) Cat 1 ≤ 80 mg/m^3 Cat 2 ≤ 800 mg/m^3	NOAEL: 10.6 mg/m^3 air LOAEL: 155.8 mg/m^3 air Slight clinical symptoms (piloerection) and liver enzyme induction (increased N-demethylase activity in the liver) at 155.8 mg/m^3 air	Vol 3 B.6.3.4.2/01
Inhalation 28-day study Cat			
OECD 412 (1981); US-EPA Series 82-4 (1984) GLP Deviations to the current guideline OECD 412 (1981): Minor – these deviations are minimal and are not considered to affect the validity of the study.	(HWG 1608) Tebuconazole (95.8 %) Batch 16013/86 Inhalation 28-day toxicity (6 hours/day, 5 days/week) 0, 61, 309 mg/m^3 air (analytical	NOAEL: With respect to cataract development: 309 mg/m^3 air LOAEL: - No cataract inducing potential was identified Effects at the LOAEL : not applicable	Vol 3 B.6.3.4.3/01

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure, Adjusted guidance values category 1 / 2 (mg/kg bw/day)	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Acceptable with respect to examining the cataract inducing potential of the active ingredient Cat "Forest of Dean" breed of cats 4/sex/dose	concentrations) Cat 1 ≤ 60 mg/m^3 Cat 2 ≤ 600 mg/m^3		
Inhalation 6-wk study Dog			
OECD 412 (1981); Guideline 84/449/EWG, B.8 (1984) GLP Deviations to the current guideline OECD 412 (1981): Minor – these deviations are minimal and are not considered to affect the validity of the study. Acceptable with respect to examining the cataract inducing potential of the active ingredient Dog Female Beagle (Bor:Beag) 4/sex/dose.	(HWG 1608) Tebuconazole Inhalation 6-week toxicity (4 hours/day, 5 days/week) 163, 914 mg/m^3 air (high concentration equivalent to about 100 mg/kg bw/day) Cat 1 ≤ 60 mg/m^3 Cat 2 ≤ 600 mg/m^3	NOAEL: With respect to cataract development: 914 mg/m^3 air LOAEL: 914 mg/m^3 air NOAEL other effects: 163 mg/m^3 air Clinical signs -salivation, tussive noises; transient loss of appetite. No cataract development	Vol 3 B.6.3.4.4/01
Chronic and carcinogenicity studies in rats and mice	See section 2.9	See section 2.9	See section 2.9
Reproduction and developmental toxicity studies in rats and rabbits	See section 2.10	See section 2.10	See section 2.10

Table 64: Summary table of human data on STOT RE

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 65: Summary table of other studies relevant for STOT RE

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)		Reference
Subchronic neurotoxicity OECD TG 424 GLP	Tebuconazole batch number 603-0013, Purity: 96.7-98.2 %	Oral (diet) Rat/ Fischer 344 CDF(F-344) 12/sex/group Dose: 0, 100, 400, 1600 ppm (M/F: 0/0, 7.57/8.81, 29.2/34.0, 177/122 mg/kg bw/day 13-weeks Cat 1 ≤ 10 Cat 2 ≤ 100	Neurotoxicity: No neurotoxicity observed at the top dose (LOAEL>177/122 mg/kg bw/d for M and F, respectively) . Generalised toxicity: Significantly reduced body weight (up to -8 %/-7% in M/F), reduced overall body weight gain (-16 %/-20 % in M/F) and significantly reduced food consumption (up to -9 %/-14 % in M/F) at 177/122 mg/kg bw/d for M and F, respectively (LOAEL).	Vol 3 B.6.7.1.1/01
28-day immunotoxicity study	Tebuconazole batch number K689052, Purity: 97.5 %	Oral (diet) Rat/ Wistar 10 females/group Dose: 0, 100, 1000 ppm (F: 0, 8.1, 24.3, 78.4 mg/kg bw/day) Cat. 1 ≤ 30 Cat. 2 ≤ 300	No evidence of immunotoxic potential in female Wistar rats administered Tebuconazole continuously in the diet at levels up to 1000 ppm for at least 28 days. NOAEL immunotoxicity 1000 ppm	Vol 3 B.6.8.2.3/01

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)		Reference
28-day liver mechanistic study GLP Acceptable as a mechanistic study	Tebuconazole Purity 97.5%	Oral (diet) Mice, NMRI 20/sex/group Dose: 0, 25, 500, 1500 ppm Equivalent to: M/F: 0/0, 4/5, 77/90, 231/276 mg/kg bw/d in the 28 days study and equivalent to: M/F: 0/0, 4/5, 72/87, 201/273 mg/kg bw/d in the 7-days sub-group	≥ 4/5 mg/kg bw/d: ↑ microsomal enzyme activities (M) ≥ 77/90 mg/kg bw/d: clinical chemistry changes, ↑ liver weight (M/F), enlarged (M/F) and pale liver (F), ↑ microsomal enzyme activities (F) 231/276 mg/kg bw/d: ↓ body weight (M/F), ↓ feed consumption (M/F), prominent liver lobulation (M) ≥ 4/5 mg/kg bw/d: ↑ microsomal enzyme activities (M) ≥ 72/87 mg/kg bw/d: enlarged (F) and pale livers (M/F) with white foci (M), ↑ microsomal enzyme activities (F) 201/273 mg/kg bw/d: ↓ body weight (M/F), ↓ feed consumption (M), prominent liver lobulation (M)	Vol 3 B.6.5.3/01
28-day liver mechanistic study GLP Acceptable as a mechanistic study	Tebuconazole Purity 97.5%	Oral (diet) Mice, NMRI 15/sex/group Dose: 0, 25, 500, 1500 ppm Equivalent to: M/F: 0/0, 4/5, 77/90, 231/276 mg/kg bw/d in the 28 days study and equivalent to: M/F: 0/0, 4/5, 72/87, 201/273 mg/kg bw/d in the 7-days sub-group	≥ 4/5 mg/kg bw/d: no adverse effects observed ≥ 77/90 mg/kg bw/d: ↑ liver weight (M/F), enlarged (M/F) and pale livers (F), hepatocellular hypertrophy, single cell necrosis and liver micro/macrovacuolation (M/F), interstitial mixed cell infiltrate in liver (M/F), increased liver cell proliferation (F) 231/276 mg/kg bw/d: ↓ body weight (M), ↓ feed consumption (M), prominent liver lobulation (M), increased number of mitoses (M/F), bile duct hyperplasia (M), pigment accumulation in Kupffer cells (F), increased liver cell proliferation (M) 4/5 mg/kg bw/d: no adverse effects observed ≥ 72/87 mg/kg bw/d: ↑ liver weight (M/F), enlarged (M/F) and pale liver (F), hepatocellular hypertrophy, single cell necrosis and liver micro/macrovacuolation (M/F), hepatocellular necrotic foci (M),	Vol 3 B.6.5.3/02

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)		Reference
			increased liver cell proliferation (F) 201/273 mg/kg bw/d: ↓ body weight (M/F), ↓ feed consumption (M), prominent liver lobulation (M/F) and white liver foci (M), increased liver cell proliferation (M), increased number of mitoses and interstitial mixed cell infiltrate in liver (M/F)	

8.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

The specific target-organ toxicity of tebuconazole upon repeated exposure was investigated in ten short-term oral studies (one gavage, nine dietary) in rats, mice and dogs ranging from 7-days in mice to one-year in dogs. Furthermore, one 3-week dermal study (in rabbits), and three 3- to 6- week inhalation studies (none in accordance with guidelines) were available in rats, cats and (female) dogs. Additional information is provided by chronic / carcinogenicity studies in rats and mice the findings in the parental animals and offspring in the 2-generation study and from a published DNT study. Findings observed in the developmental toxicity studies (rat, rabbit, mouse) after oral and dermal exposure are also addressed.

Oral administration

Rat:
The short-term toxicity of tebuconazole via the oral route was investigated in the rat in a standard 28-day oral gavage study and 90-day feeding study covering a dose range of 8.6 – 235 mg/kg bw/d. In rats treated orally the target organs in the 28-day gavage study and the 90-day feeding study were the liver (increased liver weight and enzyme induction) and the spleen (siderin accumulation in red pulp). Increased mortality was seen at the highest doses (171 and 235 mg/kg bw/d in males and females respectively) in the 90-day study. In the 28-day study decreased red blood cells were also seen. After 90 days, intra-plasmatic vacuoles were identified by histopathology in the zona fasciculata of the adrenal cortex.

Increases in absolute and relative liver weights were seen from 100 mg/kg bw/d in the 28-day study but no increase was observed in the 90-day study. Furthermore, effects on some clinical-chemistry parameters, which were mainly indicative of liver damage, as well as histopathological effects in the liver, were seen at 300 mg/kg bw/d in the 28-day study. In both studies, liver enzyme induction was observed, from 100 mg/kg bw/d in the 28-day study and from 35 mg/kg bw/d in males in the 90-day study.

In the spleen, increases in absolute and relative weights were seen from 100 mg/kg bw/d in the 28-day study. In addition, histopathological effects in the spleen were seen at 300 mg/kg bw/d and sideropenia was seen in females at 100 mg/kg bw/d in the 28-day study. In the 90-day study, hemosiderin accumulation in the spleen was seen at 235 mg/kg bw/d in females, the highest dose tested.

Effects on some haematological parameters (e.g. haematocrit values, haemoglobin content and MCV were decreased) were seen only in the 28-day study from 100 mg/kg bw/d.

In the adrenal cortex, increased vacuoles in the zona fasciculata was seen from 47 mg/kg bw/d in females and 172 mg/kg bw/d in males in the 90-day study.

It is noted that there were some inconsistencies in the observations made between these studies which may be reflective of the dosing regimen (gavage versus dietary) and duration of exposure.

Overall, taking into account the full range of observations, the lowest relevant subchronic NOAEL in the rat was 9 and 11 mg/kg bw/d in male and female, respectively. The LOAEL was 35 and 47 mg/kg bw/d in male and female, respectively, based on slight histopathological changes in the adrenal cortex in females in the 90-day dietary study.

Mouse:
In mice treated orally the main target organ in the 4- and 8-week dose range-finding studies was also the liver (increased liver weight, fat accumulation and enzyme induction; at higher dose groups additional pathological findings). Absolute and relative liver weights were increased in females at 14 mg/kg bw/d and above after 28 days and 82 mg/kg/day (the lowest dose tested) after 56 days. Liver weight increases in males were seen at 181 mg/kg/day (highest dose) but not 47 mg/kg/day after 28 days and 114 mg/kg/day (lowest dose) after 56 days, possibly indicating an increasing severity of response with duration of treatment.

In addition, histopathological findings (such as pale, patchy, lobulated livers and liver degeneration) were seen at 53 mg/kg bw/d in females and at 182 mg/kg bw/d in males in the first study; and at 82 and 114 mg/kg bw/d in males and females respectively in the second study. An increased lipid accumulation in the hepatocytes from 13 and 14 mg/kg bw/d (the lowest dose tested) in males and females respectively and above was also seen in the first study. Induction of microsomal enzyme systems was seen in the liver from 31 mg/kg bw/d in males and females in the second satellite study.

Effects on the spleen (pigment deposition) were seen at the top dose of 329 and 454 mg/kg bw/d in males and females respectively in the 8-week study.

Adrenal cortex cells showed an increased lipid content from 82 mg/kg bw/d in males following 8-weeks of dosing

The LOAELs recorded were 12.6/14.1 mg/kg bw/day (125 ppm) in the 4-week study and 82/114 mg/kg bw/day (500 ppm) in the 8-week study, in males and females, respectively. A subchronic NOAEL in the mouse could not be established based on these limited range-finding studies as effects were seen at the lowest tested doses; however, these studies are useful as supporting information on potential target tissues.

In an additional test, with administration of the test substance for five days induction of microsomal enzyme systems was seen in the liver from 125 ppm on in males and females.

Dog:
The repeated dose oral toxicity of tebuconazole was investigated in the dog in 90-day and two 1 yr feeding studies covering a dose range of 1.6 – 212 mg/kg bw/d. The main target organs were the liver, spleen, eyes and adrenals.

Liver histopathology (hemosiderosis and/or increased lobulation) was seen at 212 mg/kg bw/d in the 90-day study and in the first 1 yr study at 8 mg/kg bw/d.

Spleen weights and histopathological changes (hemosiderosis) were seen at 212 mg/kg bw/d, the highest dose tested, in the 90-day study. Increased spleen siderin content was seen at 47.3 mg/kg bw/d, the highest dose tested, in one of the 1 yr studies.

In the eye, ophthalmoscopic alterations (lens opacity) were observed at 212 mg/kg bw/d in the 90-day study, this finding was confirmed in the first 1-yr study, where eye lesions were seen at 8 mg/kg bw/d and above, where the opacities first appeared between week 26 and 32.

In the 90-day study the effect is clear in the high dosage group (according to study author a typical high dose phenomena) and in the 1-year study cataract appears as well in lower dosages but after longer exposure.

In special studies for cataract development by the inhalative route of exposure in cats (28 days) and dogs (6 weeks) the dosing was most probably not long enough to develop the effect.

In the adrenal cortex, increase in vacuole formation was seen at 212 mg/kg bw/d in the 90-day study; this was also seen in the second 1-yr study, at 4.4 mg/kg bw/d, the highest dose tested.

Changes in haematological and clinical-chemistry parameters were observed in all studies in dogs.

The lowest sub-chronic NOAEL, from the first 1-yr study in the dog, was 1.6 mg/kg bw/d; the LOAEL in this study was 8 mg/kg bw/d, based on effects on the eye (lens), liver and vacuoles in the adrenal cortex.

However, in the second 1-yr study in the dog, the highest NOAEL was 3 mg/kg bw/d, with a LOAEL of 4.4 mg/kg bw/d, based on hypertrophy of the adrenal zona fasiculata.

Dermal administration
In the 3-week dermal study in rabbits no systemic effects were recorded. Minimal local irritation was considered due to mechanical irritation. The NOAEL is 1000 mg/kg bw/day, the highest and only dose level in the study

Inhalation
In rats treated nose-only by inhalation to a respirable aerosol of tebuconazole at concentrations of 1.2 – 155.8 mg/m3 for three weeks, increased N-demethylase activity in the liver was the only effect observed. This is considered as an adaptive response of no toxicological significance. Hence, there was no systemic toxicity at the highest concentration tested of 155.8 mg/m3 air.

Cats (whole body) and dogs (head and nose only) have been treated by inhalation to respirable aerosols of tebuconazole at concentration of 61 – 914 mg/m3 for four (cats) or six (dogs) weeks to observe the cataract inducing potential of this way of exposure. This was to investigate the cataract inducing potential observed in dietary studies in dogs via the inhalation route of exposure. Cataract formation was not increased but the treatment caused body weight depression in the treated dogs at 914 mg/m3.

Administration of ≤309 mg/m3 to cats for 4 weeks did not lead to cataract formation or any other indications of toxicity. The overall no observable adverse effect concentration for the dogs was 163 mg/m3 air.

Conclusion
Thus, repeated dosing with tebuconazole induced several effects consistent between species; the main target organ of toxicity was the liver, with increases in liver weights, effects on some clinical-chemistry parameters (indicative of liver damage) and changes to liver histopathology consistently seen in all three species. Adverse effects on the spleen, including changes to its histopathology and adverse effects on the adrenals including changes to its histopathology (e.g. increase in lipid content and vacuolation) were also seen in all three species.

Adverse effects in the eye, such as lens degeneration and eye lesions were consistently seen in dog studies, but not in rats, cats and mice. There is no evidence that eye effects are of no relevance to humans (see discussion above). Adverse effects on body weight development and on some haematological parameters were observed in the rat and dog but not in mice.

The dog was the most sensitive species, with adverse effects being observed in more tissues (i.e. additionally in the eye) and at lower dose levels than in other species (i.e. observed LOAELs of 4.4 mg/kg bw/d in the dog compared to 13 mg/kg bw/d in mice and 47 mg/kg bw/d in the rat).

Table 66: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Not relevant				

8.12.2 Comparison with the CLP criteria

Adrenal

The adrenal findings were mainly described as minimal to moderate and are not considered to lead to an impaired organ function. Moreover, the adrenal findings were observed inconsistently across species and gender and did not increase in severity over time.

In rats, in the pubertal assay the absolute and relative weight of the adrenals was decreased in males only at doses ≥ 75 mg/kg bw/day (no histopathological examination was performed) (B.6.8.3.1.2/01). In the 2-year rat study the absolute and relative adrenal weight was reduced in females and accompanied by a reduced number of females with haemorrhagic degeneration of the adrenal cortex at 86.3 mg/kg bw/day (B.6.5.1/01). In the subchronic study in rats (B.6.3.2.1/01) the organ weight was not changed at concentrations up to and including 171.7/235.2 mg/kg bw/day in males and females, respectively. But an increased incidence in intra-cytoplasmatic vacuoles in zona fasciculata ≥ 35/47 mg/kg bw/day (more pronounced in females) was observed. Finally, no treatment-related effects on adrenal gland weight and no histopathological changes were found in the published rat developmental studies.6.6.3/07 and B.6.6.3/08 and B.6.6.2.1.2/01 and the two-generation study (B.6.6.1.1/01).

Conflicting results were reported for mice. In the 8-week range-finding study the absolute and relative adrenal weight was decreased in females at the highest tested dose of 454 mg/kg bw/day and increased lipid content in adrenal cortex cells was reported in males at ≥ 82 mg/kg bw/day. In the 21-month mice carcinogenicity study the absolute and relative adrenal weight was increased in females at the highest tested dose of 357 mg/kg bw/day but no histopathological changes were observed both in males and females.

In the mechanistic prenatal developmental toxicity study in rabbits (B.6.6.2.2.1/04), absolute and relative adrenal weights were marginally increased at the only tested dose of 100 mg/kg level (not statistically significant). Histopathology revealed a distinct hypertrophy of the zona fasciculate cells. This finding seems to be a species-specific consequence of stress in rabbits which are more sensitive to stress effects than other species, so that this is not regarded as relevant to humans.

In the subchronic dog study (B.6.3.2.2/01) and the first 12-month dog study (B.6.3.3.1/01) an increased incidence of intra-cytoplasmatic vacuoles in cells of zona fasciculata of the adrenals were found in females at 212 mg/kg bw/day (incidence 1-1-1-2) and ≥ 8 mg/kg bw/day (incidence 0-0-2-2), respectively.

In the second 12-month dog study study (B.6.3.3.1/02) hypertrophy of adrenal zona fasciculata cells in both sexes were found at the highest tested dose of 4.4 mg/kg bw/day. There were no other histopathological findings and no effect on adrenal weight in males and females in all three studies.

In three studies effects on the adrenals were oberserved within the guidance value range for classification in category 2. Increased incidence in intra-cytoplasmatic vacuoles was observed in zona fasciculata ≥ 35/47 mg/kg bw/day (more pronounced in females) in rats (1986, B.6.3.2.1/01) and at ≥ 8 mg/kg bw/day in females dogs (B.6.3.3.1/01). Additionally, hypertrophy of adrenal zona fasciculata cells in both sexes in a 12-month dog study were found at 4.4 mg/kg bw/day (B.6.3.3.1/02). These effects are typically signs of stress on the cells but considered adaptive responses and therefore not severe enough for classification.

Liver

Findings in rats after repeated exposure included mainly increased liver weights and microsomal enzyme induction which, depending of the degree, can be seen as adaptive responses not considered toxicologically relevant. In the 28-day gavage study additionally increased plasma transaminase activities and histopathological changes (increased lipid accumulation, enlarged hepatocytes, bile duct proliferation and in increased hepatocellular mitosis) indicating an influence on liver function were reported in a dose range relevant for STOT-RE category 2. However, since these findings were described as minimal to mild and did not show up in the studies with longer treatment durations, they are not considered triggering a STOT-RE classification.

The data of the repeated dose toxicity studies in mice demonstrated distinct effects on the liver which is the main target organ of tebuconazole in mice. Doses in the range relevant for STOT-RE category 1 revealed adaptive changes like microsomal enzyme induction and slightly increased organ weight and minimal to slight histopathological changes. Clear liver toxicity with marked increase in liver weight, gross pathological observations (e.g. pale and/or patchy liver), moderate to marked increased lipid storage and vacuole formation, as well as increases of alkaline phosphatase and transaminases activities in plasma were determined at higher doses relevant for STOT-RE category 2 after subacute to subchronic exposure, but not after 21 months. In addition, in two of the mechanistic studies minimal to moderate single cell and/or focal necrosis were observed in a classification relevant dose range. These findings occurred as consequence of the rodent-specific liver MOA of the phenobarbital-type which is not regarded as relevant to humans and thus also not for STOT-RE classification.

The dog was less sensitive regarding liver effects compared to rodents. Gross pathological changes (increased lobulation/irregular surface in 2/4 females), increased microsomal enzyme activities and a minimally increased ALP activity in plasma were the only findings at doses relevant for STOT-RE classification. Therefore, there are no indications in dogs triggering a STOT-RE classification.

Minimal to slight single cell and focal liver necrosis were observed in a supplementary maternal toxicity study in rabbits included in one of the developmental toxicity studies already at very low doses. However, a clear dose dependency was missing and the number of animals investigated in this supplementary study was too low (5/group) to permit definitive interpretation of these findings. Therefore, this study will not be taken into consideration for STOT-RE classification..

Red blood/spleen
In rats treated orally with tebuconazole moderately increased spleen weight and siderin accumulation in red pulp was observed at doses relevant for STOT-RE category 2 classification which is considered a response to an increase red blood cell elimination and iron recycling. This is supported by the fact that red blood cell parameters were not or only slightly affected at the respective dose levels.
Histopathological changes which were considered adverse like severe spleen sclerosis and yellow bone marrow were only seen in females in the 28-day gavage study at the highest dose of 300 mg/kg bw/day which is a bolus dose and thus not relevant for humans since the usual exposure is via diet.

Similar to rats, mice showed also slightly decreased red blood parameters and moderately increased spleen weight at doses relevant for STOT-RE category 2 classification. However, histopathological changes in spleen were limited to doses above the STOT-RE classification levels. As adaptive response to the increased red blood cell elimination the percentage of reticulocytes was moderately increased in one of the developmental toxicity studies.

In the dog, haematological changes, increased spleen weight and spleen and liver sideropenia were observed at high dose levels above STOT-RE classification levels only.

Eyes
In dogs treated orally with tebuconazole, adverse effects in the eyes was observed in two studies. Ophthalmoscopic alterations (lens opacity and lens star) were observed at 212 mg/kg bw/d in a 90-day study. This dose is above the guidance value (GV) for STOT RE classification. These effects were however confirmed in a 1-yr study, where eye lesions were seen at 8 mg/kg bw/d and above with the first lesions observed between week 26 and 32, indicating that time and dose is essential for the development of the effect. This dose is within the guidance value range (GVR) for classification with STOT RE 2 when applying Haber's rule to adjust for the longer duration of the study (GVRoral 2.5 mg/kg bw/d < C < 25 mg/kg bw/day). In another 1-yr study in dogs, doses were only up to 4.4 mg/kg bw/d, and no effects on eyes were observed. The findings followed a dose-response pattern in the 90-day dog study, but not in the 1-yr dog study. In two special studies performed by the inhalative route of exposure in cats (28 days) and dogs (6 weeks) cataract development was investigated. No cataract formation was observed when dosing cats for 28 days for 6 hours/d, 5 d/week at 61 and 309 mg/m3 air (analytical concentrations). Likewise, no cataract formation was observed when dosing dogs for 6 weeks for 4 hours/d, 5 d/week at 163 and 914 mg/m3 air (analytical concentrations). The adverse effects seen in the eyes of dogs treated for 90 days and one year are different stages of cataract formation. A cataract is defined as a clouding of the lens, and the severity is based upon both visual examination of

the appearance as well as the degree of functional deficit (vision loss). These are not completely linear correlated (in humans), however when acquiring visual apparent opacities, these are considered as irreversible adverse effects and can only be surgically removed. Only visual examination is available for determining the severity of the cataracts seen in the dog studies. Here reported effects are described both as lens opacity and lens star as well as other anormalities in the eye lens, which are positively correlated with development of cataract. There is no subcategorisation of the effects (e.g. localisation within the lens) to further differentiate which type of cataract formation is observed and the most likely pathophysiology. Considering that tebuconazole is postulated to cause disruption in the steroidgenesis pathway, the effects seen in the eyes are potential sequlae to this disturbance. Cataract formation in humans is a highly prevalent sequlae to glucucorticoid treatment, and as such widely recognised as a side effect of disruption in th steroidgenesis. However, tebuconazole is hypothesized to cause a decrease in hormonal output from the steroidgenesis, and as such, this MoA may not be relevant. Cataracts can be a sequlae to decreased cortisol concentration, but it is a rare condition and almost only associated with a long-term severe decrease at which severe clinical symptoms such as marked fatigue appear before the development of this effect. Nevertheless, several MoA exist in which cataracts in the eyes is a symptom and/or sequale. All these have not been elucidated. Effects in the eyes were only seen in the oral dog studies, and not in cats, rats or mice. According to the study author of the 90-day study, this is a typical high dose phenomena, however this has not been confirmed and/or substantiated, and should therefore not discard the relevance of this effect when classifying tebuconazole. According to CLP, the species in which the most severe effect is observed is considered the most relevant for classification purpose. If development of cataract is connected to metabolisme pattern in the liver the rat is more comparable to humans than mice and dogs, but as the MoA cannot be established, the relevance for humans cannot be determined and the effect should per default be considered relevant for humans, as it is seen in one species consistently across studies, and in one of these studies in a dose-dependent pattern.

8.12.3 Conclusion on classification and labelling for STOT RE

Findings at doses relevant for STOT-RE category 1 were primarily observed in liver of mice after subacute exposure. Changes at these dose levels were described as minimal to slight and which were indicative of an adaptive rather than an adverse effect. No adverse effects below the guidance value for category 1 occurred in mice when the substance was administered for 8 weeks or 21 months. Therefore, tebuconazole does not meet the criteria for classification in category 1.

At higher dose levels in the range relevant for a STOT-RE category 2 classification changes in liver parameters were detected in all tested species. Liver findings at doses relevant for a STOT-RE category 2 classification included increased liver weight and microsomal enzyme activities, both are considered adaptive and reversible, thus not toxicologically adverse. Clear liver toxicity was only indicated in the subacute mouse studies and the 28-day rat gavage study. The latter was also the only study showing a significant effect in terms of effect on spleen weight and histopathological changes in spleen. However, this study was conducted with gavage administration and consideration of Cmax related effects should be taken into account in relation to the route of exposure.

Changes in red blood cell parameters and spleen findings in all other studies were considered adaptive and/or detected only above the STOT-RE classification cut-off levels.

The metabolism of tebuconazole in human is best comparable to that of rat. While the principal metabolic reactions (hydroxylation, oxidation, and conjugation) were similar in hepatocytes of humans, rats, mice and dogs the oxidation and conjugation was most similar in rat and human hepatocytes. Glucuronidation was the most important detoxification pathway in both humans and rats. In contrast mouse hepatocytes lead to different oxidised metabolites and showed less conjugation. All data indicate differences of the metabolic capability for rat and human hepatocytes versus those from dog and mouse. Therefore, especially for the assessment of liver effects the rat is the more suitable test animal species and the mice results are considered less relevant for the human health hazard classification.

In the 28-day rat study tebuconazole was applied once per day by gavage up to 300 mg/kg bw/day. Such a bolus administration results in a toxicokinetic behaviour (high peak plasma concentration, saturation

of metabolizing enzymes) which is of limited relevance for human exposure patterns. Comparable histopathological changes in liver and spleen were not observed in the other rat studies in which tebuconazole was administered by diet up to two years.

In conclusion, significant and severe effects on liver and spleen at doses below the STOT-RE classification cut-off levels were mainly restricted to mice which are considered a less relevant test species based on the differences in metabolism compared to humans or to the 28-day rat gavage study. The adverse histopathological changes in liver and spleen seen in the 28-day rat gavage study are attributed to the bolus administration.

Irreversible adverse effects in the eyes were observed in a dose dependent pattern in dogs dosed for 90 days at 212 mg/kg bw/d and in a non-dose dependent pattern in dogs dosed from 8 mg/kg bw/day for a year. Irreversible adverse effects such as cataracts are considered biologically significant and severe. Only the dose from the 1-yr study is within the guidance value range for classification for STOT RE 2, but the effects seen in the 90-day study is considered as supporting evidence. These effects are not observed in other species or in dogs dosed at lower dosage levels and/or for a shorter period of time. The effects are considered a result of both dose and time which is in agreement with general knowledge on pathophysiology of cataract formation.

In conclusion, significant and severe effects on liver and spleen at doses below the STOT-RE classification cut-off levels were mainly restricted to mice which are considered a less relevant test species based on the differences in metabolism compared to humans or to the 28-day rat gavage study. The adverse histopathological changes in liver and spleen seen in the 28-day rat gavage study are attributed to the bolus administration. Significant and severe effects on the eyes (cataract formation) in dogs at doses within the guidance value range (GVR) are considered relevant for classification as STOT RE 2.

Therefore, tebuconazole causes significant and severe effects in eyes that are relevant for humans at dose levels requiring classification as STOT-RE 2 (eyes).

8.13 Aspiration hazard

Table 67: Summary table of evidence for aspiration hazard

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

8.13.1 Short summary and overall relevance of the provided information on aspiration hazard

Not applicable.

8.13.2 Comparison with the CLP criteria

Not applicable.

8.13.3 Conclusion on classification and labelling for aspiration hazard

Not applicable.

9 ADDITIONAL LABELLING

Labelling:

Signal word:

GHS07 – Warning

GHS08 – Warning

GHS09 – Warning

Hazard statements:

Acute Tox. 4, H302, Harmful if swallowed

Repr. 1B, H360FD, May damage fertility. May damage the unborn child

STOT RE 2, H373: May cause damage to organs (eyes) through prolonged or repeated

Aquatic Acute 1, H400 'Very toxic to aquatic life'

Aquatic Chronic 1, H410 'Very toxic to aquatic life with long lasting effects'

Precautionary statements:

P280

P308+P313

P391 'Collect spillage'

P501 'Dispose of contents/container to a licensed hazardous-waste disposal contractor or collection site except for empty clean containers which can be disposed of as non-hazardous waste.'

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11 ANNEXES

Annex I: Draft RAR Volume 3, B6 (non-confidential)

Annex II: Assessment of endocrine disrupting properties (confidential)

Annex III: Table W and Z: Fertility and Sexual Function (non-confidential)

Annex VI: Historical control data of chinchilla rabbits (hybrids, SPF quality) from embryotoxicity studies (including teratogenicity) performed between 1989 and 1995) (confidential)

Annex V: Reference list (confidential)

12 ANNEX III

Table W. Fertility and Sexual function

Abbreviations: no effect = NE, statistically significant = ss, not statistically significant = nss, number = no., control = ctrl, preputial separation = PPS, vaginal opening = VO, body weight = bw, anogenital distance = AGD.

If there is no mention of significance in the text, the effect is statistically significant with a p-value ≤ 0.05

Reference	study matrix #	preimplantation loss	Bw gain during gestation	gestation length	dystochia	fertility index	hormone levels	Puberty onset
B.6.6.1.1/01	17		Reduced (5-10%) at 1000 ppm	NE	Seen in one dam (nss)	NE		
B.6.6.2.1.2/01	31		Reduced (16% during gestation and 6-12% days 1-13 of lactation) at 1000 ppm	Increased (2%, ss)	Two maternal deaths related to dystochia			Increased time to VO secondary to reduced weight gain for females (5%, control mean 31.6 High dose 33.2), NE on age at PPS.
B.6.6.2.1.2/01	55		Reduced (16%) in the 60 mg/kg/day group			NE		Vaginal opening was accelerated (5%) in the 20 mg/kg/day treated group. NE on PPS.

B.6.6.3/07 Taxvig et al 2007	54		Reduced (35%) in 100 mg/kg group	Increased (4%) in the group treated with 100 mg/kg and nss in the 50 mg/kg group (1%).	Two maternal deaths related to dystochia		GD21 testes: A 330% and 237% increase in 17α-hydroxyprogesterone at 50 mg/kg and 100 mg/kg, respectively. Testosterone levels decreased (50%) in the 100 mg/kg group. Progrestone levels increased (178%) in the group treated with 50 mg/kg. In dams: Increase in progesterone levels (638%) in the group treated with 100 mg/kg. Decrease in T3 levels (16%) NE: Testosterone levels in dams.	
B.6.6.3/04 Hass et al 2012	52		NE	NE	NE			
B.6.6.3/05 Jacobsen et al 2013	50		NE				NE	NE
B.6.6.3/06 Overgaard et al 2013	51							NE

B.6.6.3/08 Taxvig et al 2008	53		NE				Reduced estradiol (58%) in dams treated with 50 mg/kg. Male GD21 fetuses showed increase in testicular progrestone (238%) in the 50 mg/kg group. NE on fetal plasma hormone levels.	
B.6.6.2.1.1/01	18		Decrease (16% and 74%) in dam weight gain in the groups treated with 30 mg/kg and 100 mg/kg, respectively.	NE				
B.6.6.2.1.1/02	19	NE	Decreased (60% days 6-11, 29% days 6-16) at 120 mg/kg					
B.6.6.2.1.1/03	20	NSS: 31% decrease in preimplantation loss following treatment with 120 mg/kg/day (Control: 1.6, 120: 1.1)	Decreased (38%) in the 120 mg/kg/day group					

B.6.6.2.1.3/01	22		NE					
B.6.6.2.1.3/02	21	NE	NE					
B.6.6.2.2.1/01	23	NE	NE, slight tendency to reduced weight gains during the administration period in the highest dose group (30 mg/kg/day)					
B.6.6.2.2.1/02	24	NE	Reduced (38%) relative to start at days 7-25 following treatment with 100 mg/kg.					

B.6.6.2.2. 1/03	25		Reduced (unknown%) bw in the 100 mg/kg/day group during days 6-8 post coitum.					
B.6.6.2.2. 1/04	26	NE	Reduced (3242%, 1.4g in ctrls vs -86g in exposed) between day 6-10 post coitum when treated with 100 mg/kg/day. NE when comparing gain over days 6-19 or 0-19.	NE			Slightly increased concentration of 11-deoxycorticosterone and corticosterone in the adrenal tissue.	
B.6.6.2.3. 1/01	27		NE	NE				
B.6.6.2.3. 1/02	28		Decreased (32%, nss) in animals treated with 100 mg/kg/day.					

B.6.6.2.3.1/03	29		Reduced (12.5%, unknown statistical significance) from days 6-16 post coitum following treatment with 100 mg/kg/day.					
B.6.6.2.3.2/01	30		Increase in mean body weight (ss) was observed on day 12 post coitum in the group treated with 100 mg/kg/day. This finding was deemed incidental.					

B.6.8.3.1.2/01	32						Reduced (53%) testosterone levels in males from the 150mg/kg group (also reduced by 17% at 75 mg but nss). NE on TSH, T3 and T4.	Increased age at PPS (6%) and VO (8%) at 150 mg/kg. NE on estrous cycle. Bw at PPS or VO was not affected, but due to observed weight reductions (initial weight up to 10%, weight gain 8-71%) the effects were considered secondary to reduced weight.
B.6.8.3.1.2/02 Chen et al. 2019	56						Increased serum testosterone level (~175%) and lowered serum estradiol (~40%) at 100 mg/kg. NE on LH or FSH.	

Table Z. Development

Abbreviations: no effect = NE, statistically significant = ss, not statistically significant = nss, number = no., control = ctrl, preputial separation = PPS, vaginal opening = VO, body weight = bw, anogenital distance = AGD.

If there is no mention of significance in the text, the effect is statistically significant with a p-value ≤ 0.05

Reference	study matrix #	% post-implantation loss	litter size at birth	birth weight	pup bw (postnatally)	pup mortality (postnatally)	pup development

B.6.6.1.1/01	17	Increased (No. of still born pups. F1A: from 2.5% in ctrls to 7 and 9.6% in 300 and 1000 ppm groups, F1B: 4.3% in ctrls to 7.9 and 7.5% in 300 and 1000 ppm groups)	Reduced (at 1000 ppm, F1A NE, F1B 26%, F2A NE, F2B NE)	Reduced (at 1000 ppm, F1A 7%, F1B NE, F2A 10%, F2B 7%)	Reduced (at 1000 ppm, approx 8-10%)	Increased (reduced survival index prior to litter standardization (5 days after birth) at 1000 ppm, F1A 25% nss, F1B 30% ss)	NE on femur length and no abnormalities reported
B.6.6.2.1.1/01	18			Decreased (11%) in the 100 mg/kg group.			Number of pup malformations increased notably in the 100 mg/kg treated group.
B.6.6.2.1.1/02	19	Increased (380%) in the 120 mg/kg group. Not statistically assessed	Decreased (19%) number of fetuses per dam in the 120 mg/kg group	Reduced (11%) in the 120 mg/kg group		NE	Increased visceral anomalies (Fluid in thorax 0/1/0/4 for ctrl/low/mid/high doses). Increased incidence of skeletal anomalies, Includes supernumerary ribs non ossification of vertebrae and phalanges in 2/2/2/8 fetuses for con/low/mid/high doses

B.6.6.2.1.1/03	20	Increased (271%) in 120 mg/kg group	Reduced (14%, nss)	Decreased (13%) fetal weight (not completely clear whether this was measured at birth)			
B.6.6.2.1.2/01	31	Increased (No. of still born pups. 250% increase, 2 in control group vs 7 at 1000 ppm)	Reduced (6%) at 1000 ppm	NE	Reduced (bw 11-24% during lactation and 3-7% lower gain in adults) at 1000 ppm	Increased (6%, reduced survival index prior to litter standardization) at 1000 ppm	7-10% reduced absolute and relative brain weight and reduced cerebellar thickness (at PND 12 and at adult stage) at 1000 ppm NE (Auditory startle, motor activity, motor and sensory function)
B.6.6.2.1.2/01	55		Reduced (No. of dead pups/ litter at PND0 was 450% increased (0.4 vs 2.2 deaths) in the 60 mg/kg/day group. Downward trend (13% decrease, nss) in live pups/litter in the 60 mg/kg/day dose group.	Decreased (13%) in the 60 mg/kg/day group.	NE		Altered habituation, delayed spatial aquisition, and increased handling reactivity at 60 mg/kg/d. An increased approach response was obseved in females at 20 mg/kg. NE on passive avoidance test, probe, swim speed, or working memory. NE on anogenital distance.

B.6.6.2.1.3/01	22		NE	NE		NE	
B.6.6.2.1.3/02	21	NE		NE			NE, some pups had bone malformations when treated with 1000 mg/kg
B.6.6.3/07 Taxvig et al 2007	54	Increased (high dose: 27% vs ctrls: 7%).	NE	NE	NE	Increased (696%) in the group treated with 100 mg/kg	Increased AGD in males (11%) and females (38%) of the 100 mg/kg group. Increased nipple retention on PND13 from 50 mg/kg (50-75%).
B.6.6.3/08 Taxvig et al 2008	53	Increased (11%) post-implantation loss in the group treated with 50 mg/kg/day	NE	NE			NE on AGD.
B.6.6.3/04 Hass et al 2012	52	NE	NE	NE	NE	NE	Increased nipple retention (0 in ctrls vs 1.6 in exposed) at PD13 in the 50 mg/kg bw/d group. NE on male AGD, but ss increased female AGD was observed at 12.5 (7%) and 50 (8%) mg/kg. No ss effects on incidence of genital malformations.

B.6.6.3/05 Jacobsen et al 2013	50		NE		Adult male offspring showed reduced body weight (8%) following treatment with 12.5/mg/kg/day during gestation. NE at PND16.	NE	Increase in total latency (46%) and swim length (59%) was increased in the Morris Water Maze test in male offsspring in the 12 mg/kg/day group. An increase in activity (45%) was observed in female offspring exposed to 12.5 mg/kg/day.
B.6.6.3/06 Overgaard et al 2013	51						NE on *Kiss1* expression in periventricular nucleus and the arcuate nucleus
B.6.6.2.2.1/01	23	Increased (mean no. of resorptions / dam, 300%, ss, from 0.2 in ctrls to 0.8 in 30 mg/kg group)	NE	NE			NE
B.6.6.2.2.1/02	24	Increased (230%) in the group treated with 100 mg/kg		Reduced (6%, nss) following treatment with 100 mg/kg/day		NE	9% of the fetuses had malformations in the 100 mg/kg/day group (8/90 vs 0 in ctrls).
B.6.6.2.2.1/03	25	Increased (24%) in the 100 mg/kg/day group.					

B.6.6.2.2.1/04	26	NE	NE	Reduced (12%) in the 100 mg/kg group.			
B.6.6.2.3.1/01	27			Decreased mean fetal weight (4%, uncertain whether it is signficant or not)			Indications of increased incidence of malformations at 100 mg/kg/day. A total of 1 malformation in the controls, and a total of 13 malformations in the 100 mg/kg/day group.
B.6.6.2.3.1/02	28						
B.6.6.2.3.1/03	29	Increased (320%) at 100 mg/kg/day. A marginal increase (49%, nss) was also observed at 30 mg/kg/day.		Reduced (8%) following treatment with 100 mg/kg/day.			Increased incidence of malformations of 1.9% (10mg/kg/day), 3.7% (30 mg/kg/day) and 12.8% (100 mg/kg/day), statistically significant.
B.6.6.2.3.2/01	30	Increased (60%, ss) at 100 mg/kg bw/day. In the 300 mg/kg/day group a nss increased post-implantation loss was observed (28%).		NE			Treatment with 1000 mg/kg caused an increase (77%) in malformations.