Committee for Risk Assessment

RAC

Annex 1

Background document

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

1,2,4-triazole

EC Number: 206-022-9
CAS Number: 288-88-0

CLH-O-0000001412-86-270/F

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

Adopted

15 March 2019

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

1,2,4-triazole

EC Number: **206-022-9**

CAS Number: **288-88-0**

Index Number: **613-111-00-X**

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DG 5/ **Department of Product Policy and chemical Substances / Management of Chemical Substances**
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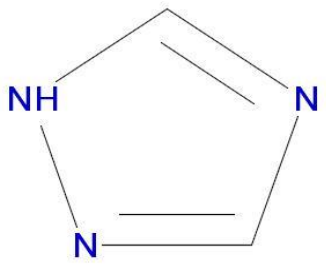
Version number: 2 **Date: February 2018**

CONTENTS

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)	1H-1,2,4-triazole
Other names (usual name, trade name, abbreviation)	1,2,4-triazole
ISO common name (if available and appropriate)	/
EC number (if available and appropriate)	206-022-9
EC name (if available and appropriate)	1,2,4-triazole
CAS number (if available)	288-88-0
Other identity code (if available)	/
Molecular formula	C2H3N3
Structural formula	
SMILES notation (if available)	N1C=NC=N1
Molecular weight or molecular weight range	69.0653
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	NA
Description of the manufacturing process and identity of the source (for UVCB substances only)	NA
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 99.5%(W/W)

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)**

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)
1,2,4-triazole (EC n° 206-022-9)	≥ 99.5%(W/W)	Acute Tox. 4*, H302 Eye Irrit.2, H319 Repr.2, H316d***	Acute Tox. 4, H302 Eye Irrit.2, H319 Repr.2, H316d***

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 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
No impurities present at ≥0.3% W/W which contribute to the classification of the substance Confidential				

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 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
No additives					

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
1,2,4-triazole (EC n° 206-022-9)	94.0% (W/W)	Not relevant for classification		Embryotic study performed in rats (Renhof M., 1988a)
1,2,4-triazole (EC n° 206-022-9)	95.3%(W/W)	Not relevant for classification		Embryotic study performed in rats (Renhof M., 1988b)
1,2,4-triazole (EC n° 206-022-9)	No information available	Not relevant for classification		-Deveopmental toxicity study in rats (Wickramaratne, 1987) -Subacute toxicity (30d) in rats (Anonymous, cited in US EPA memorandum, 2006)
1,2,4-triazole (EC n° 206-	98.5%(W/W)	Not relevant for classification		Chronic toxicity study (12 months)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
022-9)				**in rats (Wahle B.S., 2010)**
1,2,4-triazole (EC n° 206-022-9)	**≥ 99.5%(W/W)**	**Not relevant for classification**		**All other studies in section 10.10**

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	613-111-00-X	1,2,4-triazole	206-022-9	288-88-0	Acute Tox. 4* Eye Irrit.2 Repr.2	H302 H319 H361d***	GHS08 GHS07 Wng				
Dossier submitters proposal	613-111-00-X	1,2,4-triazole	206-022-9	288-88-0	**modify to** Acute Tox. 4 **Repr. 1B**	 H302 H360FD	GHS08 GHS07 Dgr				
Resulting Annex VI entry if agreed by RAC and COM	613-111-00-X	1,2,4-triazole	206-022-9	288-88-0	Acute Tox. 4 Eye Irrit.2 Repr.1B	H302 H319 H360FD	GHS08 GHS07 Dgr				

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

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Data conclusive but not sufficient for classification	No
Flammable gases (including chemically unstable gases)	Data conclusive but not sufficient for classification	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Data conclusive but not sufficient for classification	No
Flammable liquids	Hazard class not applicable	No
Flammable solids	Data conclusive but not sufficient for classification	No
Self-reactive substances	Data conclusive but not sufficient for classification	No
Pyrophoric liquids	Hazard class not applicable	No
Pyrophoric solids	Data conclusive but not sufficient for classification	No
Self-heating substances	Data conclusive but not sufficient for classification	No
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	No
Oxidising liquids	Hazard class not applicable	No
Oxidising solids	Data conclusive but not sufficient for classification	No
Organic peroxides	Data conclusive but not sufficient for classification	No
Corrosive to metals	Data lacking	No
Acute toxicity via oral route	Acute tox. 4, H302	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification	No
Acute toxicity via inhalation route	Data lacking	No
Skin corrosion/irritation	Data conclusive but not sufficient for classification	No
Serious eye damage/eye irritation	Eye Irrit. 2, H319	No
Respiratory sensitisation	Data Lacking	No
Skin sensitisation	Data conclusive but not sufficient for classification	No
Germ cell mutagenicity	Data conclusive but not sufficient for classification	No
Carcinogenicity	Data lacking	No
Reproductive toxicity	Repr.1B, H360FD	Yes
Specific target organ toxicity-single exposure	Data conclusive but not sufficient for classification	No
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification	No
Aspiration hazard	Data lacking	No
Hazardous to the aquatic environment	Data conclusive but not sufficient for classification	No

Hazard class	Reason for no classification	Within the scope of public consultation
Hazardous to the ozone layer	Data lacking	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The DSD classification was agreed by the TC C&L in 1996 (Dir. 67/548/EEC) :

Xn; R22
Xi; R36
Repr. Cat. 3; R63

The current CLP classification is a translation thereof and is included in annex VI (ATP00) :

Acute tox. 4*, H302
Eye Irrit. 2, H319
Repr. 2, H361d

In the meanwhile new data were generated by means of a 2-genaration study in rats following OECD 416 guideline (Young A.D. and Sheets L.P., 2005). Wistar rats were exposed via their diet to either 0, 250, 500 or 3000 ppm of 1,2,4-triazole from 10 weeks before mating to day 21 of lactation. Body weights in both sexes reduced significantly during different exposure periods. In conjunction with body weights disturbances, absolute and relative ovarian weights were significantly increased at 3000 ppm in P0 females. Furthermore, changes in the number of corpora lutea were noted. Also modifications of sperm parameters, significantly modified at 3000 ppm, were reported. Moreover for some endpoints (% of normal spz, % of detached spz) changes were also noted at a lower dose levels. In females, reproductive data changes were seen. The most important was the fertility index which was severely decreased (7.1 % at 3000 ppm vs 76.7% in control).

NOAEL (parental toxicity) : 500 ppm
NOAEL (fertility) : < 250 ppm based on the sperm parameters
NOAEL (developmental toxicity) : 500 ppm

The 2-generation study provides also clear evidence of adverse effects on fertility. Together with the histopathological findings in the testis in the subacute (Wahle B.S., 2004a) and chronic toxicity study (Wahle B.S., 2004b) a classification as Repr. 1B F is warranted.

Severe developmental disturbances (increased cleft palate, incidence of cryptorchism, increase of pre- and post-implimantation loss, increased incidence of runts) were observed in 2 developmental toxicity studies in rats (Renhof M., 1988a &1988b). Furthermore a dose-related increase in incidence of dead or resorbed conceptuses per liter was observed in rabbits (Hoberman, 2004).
Such severe effects warrant a classification as Repr. 1B D.

BE CA performed a substance evaluation on 1,2,4-triazole and the draft decision was discussed during MSC-54 (June 2016). Initially, two request for information were proposed :- the H295R Steroidogenesis Assay in vitro (OECD TG 456), and 2) an Extended One-Generation Reproductive Toxicity Study (EOGRTS; OECD TG 443) in rats, oral route with extension of cohort 2A and 2B (DNT cohorts), without extension of cohort 1B to mate the F1 animals to produce the F2 generation and without cohort 3 (DIT).
Based on the discussions during the MSC-54 it was concluded that the best way forward was the submission of a CLH dossier for reproductive toxicity, more particular Repro. 1B, H360FD. The request for steroidogenesis assay was maintained.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level.

Justification is not required (art. 36 of CLP regulation 1272/2008/EC) because the substance is toxic to reproduction and new data require a change in the existing entry.

5 IDENTIFIED USES

use as interemediate

use as fertilizer

6 DATA SOURCES

REACH registration dossier

JMPR, 2008, Triazole fungicide metabolites (1,2,4-triazole; triazole alanine; triazole acetic acid), 437-490

US EPA memorandum, 2006, 1,2,4-Triazole, Triazole Alanine, Triazole Acetic Acid: Human Health Aggregate Risk Assessment in Support of Reregistration and Registration Actions for Triazole-derivative Fungicide Compounds, 1-94.

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid at 20°C and 1013 hPa	IUCLID Dataset Substance, 2000	experimental study
Melting/freezing point	120-121°C	O'Neil MJ, 2006	experimental study
Boiling point	decomposed before boiling at 260°C (at 1013 hPa)	O'Neil MJ, 2006	
Relative density	1.13g/cm^3 (1130g/L) at 153°C and 1.39 g/cm^3 at 20°C	P. Jimenez, 1989	Literature data
Vapour pressure	0.22 Pa at 20°C 80.4 Pa at 25°C	Unnamed, 2001 Unnamed, 2009	OECD TG 104 (effusion method vapour pressure balance) EPI Suite estimation
Surface tension	na		
Water solubility	730 g/L at 25°C 4244 g/L at 25°C	Vlasov O. N., Sukhovs S.I., 1988	OECD TG 105 (water solubility: flask method) EPI Suite estimation
Partition coefficient n-octanol/water	25° C, pH 5: log Pow = -0.62 25°C, pH 7: log Pow = -0.71 25° C, pH 9: log Pow = -	Unnamed, 2005	OECD TG 107 (flask shaking method)

Property	Value	Reference	Comment (e.g. measured or estimated)
	0.68 -0.58.	Unnamed, 2010	EPI Suite stimation
Flash point	139.1 °C	Unnamed, 2009	QSAR estimation
Flammability	The test substance melted when approached by the ignition flame. The substance did not burn down or burn up.	Unnamed 2010	EU Method A.10 (Flammability - Solids)
Explosive properties	Non explosive	Unnamed 2010	EU Method A.14 (Explosive properties)
Self-ignition temperature	Doesn't need to be conducted for solids with malting point <160°C		
Oxidising properties	Non oxidising : substance does not contain oxygen, fluorine or chlorine		
Granulometry	no particles smaller than 78µm	Unnamed, 2011	OECD Guideline 110 -ISO 13317-2 (Fixed Pipette Method)
Stability in organic solvents and identity of relevant degradation products	Data waiving justified based on experience in handling and use of 1,2,4-triazole		
Dissociation constant	pKA =10.00 at 22°C	Unnamed, 2011	OECD Guideline 112 (Dissociation Constants in Water)
Viscosity	study technically not feasible		

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not evaluated in this dossier.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

10.1.1 Non-human information

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

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral toxicity Following OECD TG 401	in rats (Wistar) 5/sex/dose	1,2,4-triazole (purity >98%) Vehicle : bi-distilled water	Oral (gavage) Doses : 1000, 1500 and 2000 mg/kg bw	LD50 : 1320.39 mg/kg	Registration dossier (study report, 1989)
Acute oral toxicity Following OECD TG 423	in rats (Wistar) 15/sex/dose	1,2,4-triazole Technically pure Vehicle : distilled water and Cremophor EL	Oral (gavage) Doses : 100 (only ♀), 250, 500, 1000 (30♂ and 15♀), 1250, 1500, 1750, 1850 (only ♂), 2000 (15♂ and 30♀) and 2500 (14♂ and 15♀) mg/kg bw	LD50 (females) : 1648 mg/kg LD50 (males) : 1650 mg/kg	Thyssen and Kimmerle, 1976. Cited in JMPR, 2008
Acute oral toxicity Following OECD TG 423	in rats (Crl:CD BR) 3 males/dose	1,2,4-triazole Purity : not specified Vehicle : methylcellulose	Oral (gavage) Doses : 500 and 5000 mg/kg bw	LD50 : >500 and <5000 mg/kg	Procopio and Hamilton, 1992. Cited in JMPR, 2008

10.1.2. Human information

No information available

10.1.3. other relevant information

No information available

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

In an acute oral toxicity study performed following the OECD guideline 401 (Registration dossier (study report, 1989)), groups of 5 male and 5 female rats were exposed by gavage to 1,2,4-triazole at a concentration of 1000, 1500 and 2000 mg/kg bw.

At the mid and high dose group, mortality was observed : in males : at 1500 mg/kg bw, 2 rats died after 24h and 2 after 48h and at 2000 mg/kg bw, 4 died after 24h and 1 after 48h and in females : at 1500 mg/kg bw, 3 rats died after 24h and 2 after 72h and at 2000 mg/kg bw, 4 died after 24h and 1 after 48h.

According to the results, the LD50 is of 1320 mg/kg bw.

In an acute oral toxicity study performed following the OECD guideline 423 (Thyssen and Kimmerle, 1976. Cited in JMPR, 2008), groups of 15 male and 15 female rats were exposed by gavage to 1,2,4-triazole at a concentration of 100 (only ♀), 250, 500, 1000 (30♂ and 15♀), 1250, 1500, 1750, 1850 (only ♂), 2000 (15♂ and 30♀) and 2500 (14♂ and 15♀) mg/kg bw.

According to the registration dossier, the LD50 for females is 1648 mg/kg bw and for males is 1650 mg/kg bw.

In an acute oral toxicity study performed following the OECD guideline 423 (Procopio and Hamilton, 1992. Cited in JMPR, 2008), groups of 3 male rats were exposed by gavage to 1,2,4-triazole at a concentration of 500 and 5000 mg/kg bw.

Mortality was observed at the high dose. At this level, all rats died within 10 minutes.

According to the results, the LD50 is of > 500 and < 5000 mg/kg bw.

10.1.2 Comparison with the CLP criteria

According to the CLP Regulation (EC 1272/2008) the classification of a substance in category 4 for oral acute toxicity is based on a LD50 between 300 and 2000 mg/kg bw.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the results of the studies (Registration dossier (study report, 1989) and Thyssen and Kimmerle, 1976. Cited in JMPR, 2008), the LD50 is between 1320 and 1650. Another supporting study revealed a LD50 of > 500 and < 5000 mg/kg bw. All these obtained LD50 values fulfil the criteria for the acute toxicity in category 4.

According to all of these results, a classification as Acute Tox. 4, H302 is warranted. Based on table 3.1.1 of the CLP regulation, an ATE of 1320 mg/kg bw is warranted.

10.2 Acute toxicity - dermal route

Not evaluated in this dossier.

10.3 Acute toxicity - inhalation route

Not evaluated in this dossier.

RAC evaluation of acute toxicity
Summary of the Dossier Submitter's proposal The Dossier Submitter (DS) assessed 3 acute oral toxicity studies in rats. In the first study rats were exposed by gavage to 1,2,4-triazole at doses of 1000, 1500 and 2000 mg/kg bw. Mortality was observed at the mid and high dose group. The DS concluded that the LD_{50} was 1320 mg/kg bw. In the second study rats were exposed by gavage to 1,2,4-triazole at doses of 100 (only

females), 250, 500, 1000 (30 males, 15 females), 1250, 1500, 1750, 1850 (only males), 2000 (15 males and 30 females) and 2500 (14 males and 15 females) mg/kg bw. The DS concluded that the LD_{50} was 1648 mg/kg bw for females and 1650 mg/kg bw for males.

In the third study rats were exposed by gavage to 1,2,4-triazole at doses of 500 and 5000 mg/kg bw. All animals of the high-dose group died within 10 minutes. Based on the results, the DS concluded that the LD_{50} was in the range of >500 and < 5000 mg/kg bw.

According to the first two studies the LD_{50} values were between 1320 and 1650 mg/kg bw. The supporting study revealed an LD_{50} of >500 and <5000 mg/kg bw. The DS concluded that the LD_{50} values fulfilled the criteria for acute oral toxicity category 4 and therefore proposed a classification as Acute Tox. 4, H302 with an ATE value of 1320 mg/kg bw.

Comments received during public consultation

Comments on acute oral toxicity were received from three Member State Competent Authorities (MSCAs). Two MSCAs supported the proposed classification as Acute Tox. 4; H302. One MSCA recommended a discussion on the ATE value. The DS responded that the lowest LD_{50} was chosen as the ATE value.

Assessment and comparison with the classification criteria

Table: Summary of acute oral toxicity studies (modified from Table 9 of the CLH report)

Method, guideline	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**LD50 value**	**Reference**
Acute oral toxicity Following OECD TG 401	Rats (Wistar) 5/sex/dose	1,2,4-triazole Purity > 98% Vehicle: bidistilled water	Oral (gavage) Doses: 1000, 1500 and 2000 mg/kg bw	LD_{50}: 1320 mg/kg bw	Registration dossier (study report, 1989)
Acute oral toxicity Following OECD TG 423	Rats (Wistar) 14, 15 or 30 rats/sex/dose	1,2,4-triazole Technically pure Vehicle: distilled water and Cremophor EL	Oral (gavage) Doses: 100 to 2500 mg/kg bw	LD_{50} (female): 1648 mg/kg LD50 (male): 1650 mg/kg	Thyssen and Kimmerle, 1976. Cited in JMPR, 2008
Acute oral toxicity Following OECD TG 423	Rats (Crl:CD BR) 3 males/dose	1,2,4-triazole Purity not specified Vehicle: methylcellulose	Oral (gavage) Doses: 500 and 5000 mg/kg bw	500 < LD_{50} <5000 mg/kg	Procopio and Hamilton, 1992. Cited in JMPR, 2008

The first acute oral toxicity study (REACH registration dossier, Study report, 1989) was performed according to OECD TG 401. Five rats/sex/dose were exposed by gavage to 1,2,4-triazole at doses of 1000, 1500 and 2000 mg/kg bw. Mortality was observed as

follows:

- 1500 mg/kg bw males: 2 rats died after 24h, 2 rats died after 48h
- 1500 mg/kg bw females: 3 rats died after 24h, 2 rats died after 72h
- 2000 mg/kg bw males: 4 rats died after 24h, 1 rat died after 48h
- 2000 mg/kg bw females: 4 rats died after 24h, 1 rat died after 48h

An LD_{50} value of 1320 mg/kg bw was calculated.

The second acute oral toxicity study (Thyssen and Kimmerle, 1976) was performed according to OECD TG 423. In groups of 15 males and 15 females, rats were exposed by gavage to 1,2,4-triazole at doses of 100 (only females), 250, 500, 1000 (30 males, 15 females), 1250, 1500, 1750, 1850 (only males), 2000 (15 males and 30 females) and 2500 (14 males and 15 females) mg/kg bw. After treatment the animals were observed for 14 days. Mortality was seen at 1250 mg/kg bw and higher doses (1h to 12 days after dosing). The oral LD_{50} value is 1648 mg/kg bw for females and 1650 mg/kg bw for males.

The third acute oral toxicity study was also performed according to OECD TG 423. In groups of 3 males, rats were given 1,2,4-triazole at a single dose of either 500 or 5000 mg/kg bw. All animals of the high-dose group died within 10 minutes after dosing; there was no mortality at 500 mg/kg bw. The LD_{50} value is within the range of > 500 mg/kg bw and < 5000 mg/kg bw. As only two doses were tested and the LD_{50} value cannot be calculated, this study is regarded as a supporting study.

Conclusion

The results of two acute oral toxicity studies show that the LD_{50} value for 1,2,4-triazole is between 1320 and 1650 mg/kg bw. The supporting study revealed an LD_{50} value within the range of $500 < LD_{50} < 5000$ mg/kg bw. The CLP criteria for acute oral toxicity category 4 is given as $300 < LD_{50} < 2000$ mg/kg bw. **RAC agrees with the DS that classification as Acute Tox. 4; H302 is warranted with an ATE value of 1320 mg/kg bw**, since this is the lowest calculated LD_{50} value.

10.4 Skin corrosion/irritation

Not evaluated in this dossier.

10.5 Serious eye damage/eye irritation

Not evaluated in this dossier.

10.6 Respiratory sensitisation

Not evaluated in this dossier.

10.7 Skin sensitisation

Not evaluated in this dossier.

10.8 Germ cell mutagenicity

Not evaluated in this dossier.

10.9 Carcinogenicity

Not evaluated in this dossier.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

10.10.1.1. Non-human information

Table 12: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
2-generation reproductive toxicity study in rats (Wistar) (30/sex/dose) Oral (diet) Following OECD TG 416 GLP	1,2,4-triazole (purity ≥99,9 %) Doses : 0, 250, 500 and 3000 ppm Exposure : through 10 weeks premating period to lactation D21 Vehicle : ethanol	No treatment-related effects were observed concerning deaths or clinical signs at any dietary dose level. Bw of males and females decreased significantly during different exposure periods (see table 13). <u>P0 :</u> Brain weight was significantly reduced at 3000 ppm in both sexes in P0. In conjunction with brain weight, degeneration/necrosis was observed in the cerebellum. Absolute and relative ovarian weights were statistically significantly increased at 3000 ppm in P0 females. Furthermore, changes in the number of corpora lutea was noted. Modification of sperm parameters observed and which were significantly modified at 3000 ppm. Moreover for some endpoints (% of normal spz, % of detached spz) changes were also modified at lower dose level. In females, reproductive data also modified. The most important was the fertility index with a severe decrease (7.1 at 3000 ppm vs 76.7% in control). NOAEL (parental toxicity) : 500 ppm NOAEL (fertility) : < 250 ppm based on the sperm parameters NOAEL (developmental toxicity) : 500 ppm	Young A.D. and Sheets L.P., 2005 (cited in JMPR, 2008)
Subacute toxicity study in mice (CD1[ICR]/BR) (15/sex/dose) Oral (feed) No OECD guideline	1,2,4-triazole (purity 99.9 %) Doses : 0, 50, 250, 500 and 2000 ppm Exposure :	No treatment-related modification of the mortality, clinical signs, bw, clinical chemistry and organ weight Histopathological evaluation : slight testicular degeneration in 5 out of 15 males at the highest dose level, minimal to slight spermatid degeneration/depletion/asynchrony, focal tubular atrophy NOAEL (males) : 500 ppm NOAEL (females) : 2000 ppm	Wahle B.S., 2004a (cited in JMPR, 2008)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
GLP	4 weeks Vehicle : ethanol		
Subacute toxicity study in rats (strain unknown) Oral Non-guideline	1,2,4-triazole (purity unknown) Doses : 0, 8, 57 and 400 mg/kg bw/d Exposure : 30-days Vehicle : unknown	At the highest dose level, a lower bw was noted and a few clinical signs were observed such as staggering, tremors and hunched posture At 57 mg/kg bw/d : slight hematological changes observed At 8 mg/kg bw/d : lower adrenal weight NOAEL : < 8 mg/kg bw/d No more information available	Anonymous (cited in US EPA memorandum, 2006)
Subchronic toxicity study in rats (Wistar) (15/sex/dose) Oral (feed) Similar to OECD TG 408 No GLP	1,2,4-triazole (purity 99.6 %) Doses : 0, 100, 500 and 2500 ppm Exposure : 3 months Vehicle : 90 % pre-mix with ultrasil VN 3	2 males and 2 females exhibited slight convulsion at 2500 ppm Terminal bw was significantly lowered at 2500 ppm. Decreased absolute testis weight at the highest dose level however no histopathological modification observed NOAEL : 500 ppm	Bomhard E. et al., 1979 (cited in JMPR, 2008)
Subchronic toxicity study in mice (CD-1[ICR]/BR) (20/sex/dose) Oral (feed) Following US EPA OPPTS 870.3100 GLP	1,2,4-triazole (purity 99.9%) Doses : 0, 500, 1000, 3000 and 6000 ppm Exposure : 90 days Vehicle : ethanol	Higher incidence of tremors at 6000 ppm A significant decrease in bw was observed in males at 3000 and 6000 ppm and at 6000 ppm in females. Brain weight was statistically modified at 6000 ppm in both sexes and at 3000 ppm in males. In conjunction with this modification, an increased incidence of Purkinje cell loss was observed at the highest dose level. Testis weight was significantly decreased at the highest dose and in conjunction, some histopathological changes were observed such as increased incidence of apoptotic like bodies, of spermatid degeneration and of tubular atrophy. Higher incidence of exfoliated germ cells and debris in the lumen of the epididymal duct at 6000 ppm. NOAEL (males) : 1000 ppm	Wahle B.S., 2004b (cited in JMPR, 2008)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		NOAEL (females) : 3000 ppm	
Combined subchronic toxicity / neurotoxicity screening study in rats (Wistar) (20/sex/dose) Oral (feed) Following OECD TG 408 and 424 GLP	1,2,4-triazole (purity 99.9 %) Doses : 0, 250, 500, 3000 and 1000/4000 ppm Exposure 90 days Vehicle : ethanol	A lower bw was seen at the 2 highest dose levels. Organ weight examination showed a lower brain weight at 3000 ppm in both sexes and also in males at 1000/4000 ppm. In conjunction, degeneration/necrosis on the cerebellum and degeneration of some nerve fibers were observed. At the 2 highest dose levels, a slight increased number of corpora lutea was noted. The FOB revealed some effects such as tremors, gait incoordination, … at the 2 highest dose levels. NOAEL : 500 ppm	Wahle B.S. and Sheets L.P., 2004 (cited in JMPR, 2008)
Chronic toxicity study in rats (Crl:Wi(han)) (20/sex/dose) Oral (feed) Following OECD TG 452 GLP	1,2,4-triazole (purity ≥98.5 %) Doses : 0, 125, 375, 1000 and 2000 ppm Exposure : 12 months Vehicle : ethanol	A slight decrease of bw and bwg was seen at the 2 highest doses. The histopathological examination revealed a significant higher incidence of Purkinje cells loss at 2000 ppm. No effects were observed during oestrous cycle and sperm analysis. NOAEL : 375 ppm	Wahle B.S., 2010

10.10.1.2. Human information.

No information available

10.10.1.3. Other relevant information

No information available

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

In a two-generation reproductive toxicity study performed following the OECD guideline 416 (Young A.D. and Sheets L.P., 2005), groups of 30 male and 30 female rats were given diets containing 1,2,4-triazole at a concentration of 0, 250, 500 or 3000 ppm (See table 15 for the dosing in mg/kg bw/d during the different exposure periods).

For females, the exposure period began 10 weeks before mating, and continued through mating, gestation, and lactation. During lactation, dietary levels were reduced to maintain a more constant dosage (mg/kg/day) throughout the study. Following the weaning of their litters on lactation D21, each dam was sacrificed. Due to the low fertility at the highest dose (3000 ppm), this dose level was stopped after gestation in P-gen females an therefore no animal was exposed to this dose level in the F1-generation. Males were only exposed during a premating period of 10 weeks.

Table 15 : dose level by group in mg/kg bw/d (Young A.D. and Sheets L.P., 2005)

	Phase of study	250 ppm in mg/kg bw/d	500 ppm in mg/kg bw/d	3000 ppm in mg/kg bw/d
♂	Premating (P-gen)	15.4	30.9	188.6
	Premating (F1-gen)	16	32	NA
♀	Premating (P-gen)	17.5	36.2	217.9
	Gestation (P-gen)	18.6	38.6	231.7[a]
	Lactation (P-gen)	19.3	38.7	NA
	Premating (F1-gen)	18.9	37.5	NA
	Gestation (F1-gen)	17.4	34.4	NA
	Lactation (F1-gen)	20.3	35.8	NA

[a] *: based on only 2 pregnant females*

No treatment-related effects were observed concerning deaths or clinical signs at any dietary dose level. Bw of males and females was significantly modified during different exposure periods (see table 16).

Table 16 : body weight data in grams for P and F1 animals (Young A.D. and Sheets L.P., 2005)

	Phase of study	0 ppm	250 ppm	500 ppm	3000 ppm
	P D0	294.0	291.6	298.4	299.7
♂	P terminal bw	473.1	460.7	456.1	419.4*
	P BWG	179.1	169.1	157.7	119.7
	F1 D0	266.2	254.3	250.6*	/
	F1 terminal bw	464.5	440.8*	426.6*	/
	F1 BWG	198.3	186.5	176.0	/
	P D0	206.1	206.8	209.2	209.5
♀	P premating-mating (D70)	244.1	244.9	239.5	233.4*
	P gestation (D20)	345.3	340.9	340.0	284.7**[a]
	P lactation (D21)	284.2	287.4	287.4	/
	P terminal bw	277.2	283.1	280.9	245.1*[a]
	P BWG	71.1	76.3	71.7	35.6[a]
	F1 D0	172.3	166.7	169.1	/
	F1 premating-mating (D70)	236.2	227.5	230.8	/
	F1 gestation (D20)	323.8	313.3	311.8	/
	F1 lactation (D21)	281.4	267.8*	271.2	/
	F1 terminal bw	277.2	262.9*	265.7	/
	F1 BWG	104.9	96.2	96.6	/

** : p≤0.05* *** : p≤0.0* *1*[a] : based only on 2 dams

Males and females of the F0-generation at the highest dose had a significantly lower terminal bw (473.1/277.2, 460.7/283.1, 456.1/280.9 and 419.4*/245.1* g respectively at 0, 250, 500 and 3000 ppm in males/females) and lower absolute brain weight (2.092/1.955, 2.075/1.941, 2.044/1.951 and 2.006*/1.853* g at 0, 250, 500 and 3000 ppm in males/females, respectively) compared with the control group. Several other organs also showed modifications at this highest dose such as ovaries (0.058/0.058, 0.059/0.057, 0.055/0.054 and 0.067*/0.071* g (left/right ovaries) respectively at 0, 250, 500 and 3000 ppm), thyroid, and liver. In addition to the brain weight changes, minimal to marked degeneration/necrosis was observed in 30 out of 30 males and in 28 out of 30 females. Moreover, in the ovaries, changes in number of total corpora lutea was observed (24.9, 23.0, 15.6 and 41.3 at 0, 250, 500 and 3000 ppm, respectively). Finally, the histopathological examination of the uterus revealed a higher incidence of dilatation (14* females at 3000 ppm vs 4 females in control group).

During this study, male and female fertility parameters were analyzed and revealed some modifications in P-generation (see table 17 and 18). Only two litters containing one female pup each were produced by the F0-generation at 3000 ppm.

Table 17 : Sperm parameters in the P-generation (Young A.D. and Sheets L.P., 2005)

	Sperm motility		Total sperm count		Sperm morphology		
	% motile	% progressive	Epididymis	Testis	% normal	% abnormal	% detached
0 ppm	76.2	55.9	58.2	72	98.7	0.8	0.5
250 ppm	78.9	56.5	57	63.1*	98.1	1	0.8
500 ppm	78.9	56.4	65.7	64.4	97.0*	1.4*	1.6*
3000 ppm	78.9	57.3	43.2*	61.2*	95.7*	1.5*	2.8*

* : $p \leq 0.05$

Table 18 : Reproductive data from the P-generation (Young A.D. and Sheets L.P., 2005)

	Nb of estrous cycle	Estrous cycle length (d)	Mating index (%)	Fertility index (%)	Nb of implantations	Duration of gestation	Mean nb of live pups	Sex ratio (% males)	Viability index
0 ppm	3.6	4.2	100.0	76.7	265	22.3	233	54.1	96.2
250 ppm	3.8	4.2	100.0	83.3	310	22.0	279	55.4	97.1
500 ppm	3.4	4.4	96.7	86.2	279	22.2	260	50.7	99.6
3000 ppm	3.6	4.2	93.3	7.1**	3	23.5	2	/	100.0

* : $p \leq 0.05$, ** : $p \leq 0.01$

At the highest dose level, the number of live pups was severely decreased. At this dose level, mean F1-pups bw was significantly decreased at D7 but this mean value was calculated using the data of the only 2 live pups (16.5, 15.8, 15.7 and 9.1** g respectively at 0, 250, 500 and 3000 ppm).

Due to low fertility at 3000 ppm, further testing with this dose in the next generation was not performed.

The trend to lower terminal bw observed at the P-generation was also observed during the F1-generation (464.5/277.2, 440.8*/262.9* and 426.6*/265.7 respectively at 0, 250 and 500 ppm in males/females). No significant changes were observed during organ weight examination.

During this F1-generation, no significant changes were shown in male and female reproductive parameters (see table 19 and 20). However, a slight decreasing trend in fertility index and number of implantations was observed.

Table 19 : Sperm parameters in the F1-generation (Young A.D. and Sheets L.P., 2005)

	Sperm motility	Total sperm count	Sperm morphology

	% motile	% progressive	Epididymis	Testis	% normal	% abnormal	% detached
0 ppm	87.1	63.9	49.2	69.2	98.1	1.1	0.8
250 ppm	87.8	65.7	NE	NE	NE	NE	NE
500 ppm	89.5	67.6	48.6	68.3	97.9	1.4	0.7

* : $p \leq 0.05$

Table 20 : Reproductive data in the F1-generation (Young A.D. and Sheets L.P., 2005)

	Nb of estrous cycle	Estrous cycle length (d)	Mating index (%)	Fertility index (%)	Nb of implantations	Duration of gestation	Mean nb of live pups	Sex ratio (% males)	Viability index
0 ppm	3.7	4.1	100.0	93.3	304	22.1	280	48.7	99.7
250 ppm	3.7	4.1	100.0	86.7	300	21.9	287	47.3	98.8
500 ppm	3.8	4.1	96.7	86.2	273	21.8	260	40.6	95.6

According to the results, the NOAEL for the parental toxicity was set at 500 ppm based on lower bw and degenerative findings observed in the cerebellum at the highest dose level. The NOAEL for fertility could not be determined based on the reduction in testicular sperm counts noted at 250 ppm. Moreover, the NOAEL for developmental toxicity is 500 ppm which was the highest dose allowing assessment of the developmental effects as the 3000 ppm dose level was not further tested due to the low number of pups obtained.

In a 28-day repeated dose toxicity study (Wahle B.S., 2004a), groups of 15 male and 15 female mice received diets containing 1,2,4-triazole at a concentration of 0, 50, 250, 500 or 2000 ppm (corresponding to 0, 9, 47, 90 and 356 mg/kg bw/d in males and 0, 12, 60, 120 and 479 mg/kg bw/d in females).

No treatment-related effects were observed on survival, clinical signs, bw, food consumption, clinical chemistry parameters or on organ weights.

Nevertheless, the histopathological evaluation revealed some modifications in testes and in epididymis as reported in the table 21.

Table 21 : Incidence of testicular and epididymal lesions in mice (Wahle B.S., 2004a)

Observed effects		Dietary concentration in ppm				
		0	50	250	500	2000
Epididymis	Incidence of aspermia	0/15	0/15	0/15	1/15	0/15
	Incidence of exfoliated germ cells/debris	0/15	1/15 (1)	1/15 (3)	0/15	3/15 (2)
Testis	Testicular degeneration	3/15	ND	ND	ND	5/15
	Incidence of apoptotic-like bodies	2/15 (1)	4/15 (1)	1/15 (1)	3/15 (1)	5/15 (1)
	Incidence of spermatid degeneration/depletion/asynchrony	1/15 (1)	1/15 (1)	1/15 (1)	0/15	5/15 (1.4)
	Incidence of focal tubular atrophy	1/15 (1)	2/15 (1)	1/15 (2)	2/15 (2)	4/15 (1.8)

() : average severity score (1 minimal to 5 severe)

Based on the results, the NOAEL was 500 ppm in males (equivalent to 90 mg/kg bw/d) and 2000 ppm in females (equivalent to 479 mg/kg bw/d).

In a 30-day repeated dose toxicity study (anonymous cited in US EPA memorandum, 2006), rats were orally exposed to 1,2,4-triazole at a concentration of 0, 8, 57 or 400 mg/kg bw/d.

A few effects were revealed during this study such as a lower bw and some clinical signs at the highest dose level. Slight hematological changes were noted at 57 mg/kg bw/d and a lower adrenal weight was seen at 8 mg/kg bw/d. No further data were reported.

Based on the poorly available data, the NOAEL was < 8 mg/kg bw/d.

In a 90-day repeated dose toxicity study (Bomhard E. et al., 1979), groups of 15 male and 15 female rats were exposed to 1,2,4-triazole at a concentration of 0, 100, 500 or 2500 ppm (equivalent to 0, 7.79, 37.85 and 212.30 mg/kg bw/d in males and to 0, 10.23, 54.20 and 266.69 mg/kg bw/d in females).

The mortality and the food consumption were regarded but no modifications were observed. In the highest dose group, 2 males and 2 females exhibited slight temporary convulsions. Bw and bwg parameters in the low and mid dose level were in the same range than the control group whereas, in the highest dose group, there was a significantly lower bw in males for the entire study period and in females at the majority of the observation dates. The mean initial bw was of 82, 82, 82 and 82 g in males and of 78, 78, 78, and 78 g in females, both at 0, 100, 500 and 2500 ppm, respectively. The terminal bw was 335, 342, 344 and 306** g in males and 195, 195, 187 and 184* g in females respectively at 0, 100, 500 and 2500 ppm.

The absolute testis weight was decreased at the highest dose (3418, 3308, 3247 and 3215* mg respectively at 0, 100, 500 and 2500 ppm). However, no histopathological lesions were observed in this organ.

According to the results, the NOAEL was 500 ppm (corresponding to 37.85 mg/kg bw/d in males and to 54.20 mg/kg bw/d in females).

In a 90-day repeated dose toxicity study (Wahle B.S., 2004b), groups of 20 male and 20 female mice were exposed orally (via diet) to 1,2,4-triazole at a concentration of 0, 500, 1000, 3000 or 6000 ppm (corresponding to 0, 80, 161, 487 and 988 mg/kg bw/d in males and to 0, 105, 215, 663 and 1346 mg/kg bw/d in females). Moreover, additional groups of 15 males and 15 females were exposed for 28 days and then killed for hepatic enzymes analysis.

No treatment-related effects were observed on mortality. However, an increased incidence of tremors was observed in both sexes at the highest dose level (0/0, 0/0, 0/0, 1/2 and 11/2 respectively at 0, 500, 1000, 3000 and 6000 ppm in males/females). And during this clinical observation at this dose, a higher incidence of yellow staining and rough coat were also noted in males. Furthermore, the analysis of hepatic enzymes profiles showed an increased activity of ECOD, EROD, ALD and GLU-T in both sexes at 6000 ppm.

Bw were decreased through the study in both sexes as reported in table 22.

Table 22 : Bw at D84 (Wahle B.S., 2004b)

		0 ppm	500 ppm	1000 ppm	3000 ppm	6000 ppm
Bw at D84 (in g)	♂	37.3	37.0	36.4	34.9*	31.3*
	♀	29.1	28.4	28.4	28.7	26.6*
Total bwg (in g)	♂	3.1	3.6	1.7	1.1*	-3.1*
	♀	3.5	3.1	3.0	2.7	0.9*

* $p \leq 0,05$

Concerning the organ weight and the histopathological examination, brain and testes exhibited modifications. The absolute brain weight was significantly decreased in both sexes at 6000 ppm and also in males at 3000 ppm. In addition, an increased incidence of Purkinje cell loss was observed in both sexes at the highest dose. Furthermore, absolute testis weight was significantly decreased at 6000 ppm. In conjunction with this change, histopathological modifications were observed including increased incidence of apoptotic-like bodies, of spermatid degeneration/depletion/asynchrony and of tubular atrophy. These changes were generally dose-dependent in incidence and severity. Moreover, the epididymal histopathological examination revealed also a higher incidence of exfoliated germ cells and debris in the lumen of the duct at 6000 ppm.

Table 23 : Organ weight and histopathological findings (Wahle B.S., 2004b)

			0 ppm	500 ppm	1000 ppm	3000 ppm	6000 ppm
Term. Bw (in g)		♂	36.9	35.8	34.9*	33.9*	30.5*
		♀	28.1	27.9	28.0	27.9	26.0*
Brain	Abs. weight (in g)	♂	0.488	0.491	0.476	0.465*	0.445*
		♀	0.485	0.489	0.483	0.475	0.451*
	Rel. weight (in %)	♂	1.328	1.378	1.365	1.376	1.462*
		♀	1.737	1.756	1.731	1.717	1.734
	Incidence of Purkinje cell loss	♂	0/20	0/20	0/20	0/20	15*/20 (1.7)
		♀	0/20	0/20	0/20	0/20	10*/20 (1.3)
Testis	Abs. weight (in g)		0.253	0.247	0.233	0.233	0.219*
	Rel. weight (in %)		0.688	0.692	0.669	0.687	0.719
	Incidence of apoptotic-like bodies		4/20 (1.0)	4/20 (1.3)	7/20 (1.1)	11*/20 (1.3)	12*/20 (1.2)
	Incidence of spermatid degeneration/depletion/asynchrony		1/20 (1.0)	0/20	0/20	5/20 (1.4)	15*/20 (2.0)
	Incidence of tubular atrophy		0/20	0/20	2/20 (1.5)	3/20 (1.0)	10*/20 (1.8)
Epididymis	Incidence of exfoliated germ cells/debris		0/20	0/20	0/20	0/20	10*/20 (2.5)

() : average severity score (1 minimal to 5 severe); * $p \leq 0.05$

Based on the results of the study, the NOAEL was 1000 ppm in males (corresponding to 161 mg/kg bw/d) and 3000 ppm in females (corresponding to 663 mg/kg bw/d).

A combined 90-day repeated dose toxicity study and neurotoxicity study (Wahle B.S. and Sheets L.P., 2004) was performed following the OECD guidelines 408 and 424. Groups of 20 male and 20 female rats were exposed to 1,2,4-triazole at a concentration of 0, 250, 500, 3000 or 1000/4000 ppm (corresponding to an average daily intake over about 14 weeks at the precited nominal dietary concentrations of 0, 16, 33, 183 and 210 mg/kg bw/d in males and 0, 19, 41, 234 and 275 mg/kg bw/d in females). The dose of 1000 ppm has been modified after 4 weeks to 4000 ppm. Concerning the highest dose level, the daily intake value shows

the average of approximately 4 weeks of exposure at 1000 ppm and approximately 10 weeks of exposure at 4000 ppm. The mean daily intake for 1000/4000 ppm animals through week 4 was 85 + 3 and 95 + 3, for males and females respectively while the mean daily intake values until the end of the study was 248 + 16 and 329 + 21, for males and females respectively.

No treatment-related effects were observed on mortality, food consumption, hematology and urine analysis parameters.

A functional observational battery (FOB) examination revealed changes at the two highest dose levels such as ungroomed appearance, red nasal stain, urine stain muscle fasciculations, gait incoordination, decreased activity in open field in males and tremor and decreased rearing in both sexes at 3000 ppm. Red nasal stain, decreased activity in the open field and increased splayfoot were seen in males, urine stain in females and ungroomed appearance, muscle fasciculations, tremor, gait incoordination, decreased rearing and uncoordinated righting in both sexes at 1000/4000 ppm dose level.

Bw was unaffected up to 500 ppm dose level however at the two highest doses a decrease was observed in both sexes (table 24).

Evaluation of clinical chemistry parameters revealed a slight decrease of serum triglyceride concentration in 3000 and 1000/4000 ppm dose levels and a slight increased activity of the hepatic enzymes such as N-demythylase, O-demythylase, ECOD, EROD, ALD, EH, GS-T and GLU-T. Moreover, a significant decrease in TSH concentration was seen in males at 500, 3000 and 1000/4000 ppm (4.68*, 4.58* and 4.14* ng/ml vs 6.35 ng/ml in control group). No organ weight change or histopathological modification confirmed these clinical chemistry changes. The only organ weight which was significantly disturbed was the absolute brain weight (in males : 1.94* g and 1.92* g respectively at 3000 and 1000/4000 ppm vs 2.05 g in control group, in females : 1.78* g and 1.81 g respectively at 3000 and 1000/4000 ppm vs 1.91 g in control group). In conjunction of these brain weight changes, the necropsy revealed some histopathological changes such as an increased incidence of degeneration/necrosis of the brain level 7 (9 out of 10 males at 1000/4000 ppm and 10 out of 10 at 3000 ppm vs 0 out of 10 in control group and 10 out of 10 females at the 2 highest dose level vs 0 out of 10 in control group), some increased incidence of degeneration of nerves (sciatic nerve left and right, tibial nerve left and right, sural nerve left and right).

A lower uterus weight was also observed but it was not statistically significant (0.611, 0.568, 0.602, 0.521 and 0.491 respectively at 0, 250, 500, 1000/4000 and 3000 ppm).

A slight increase in number of corpora lutea was observed in females at 3000 pmm and 1000/4000 ppm (see table 24).

Table 24 : Body weight data and corpora lutea information (Wahle B.S. and Sheets L.P., 2004)

		0 ppm	250 ppm	500 ppm	3000 ppm	1000/4000 ppm
Bw (D0) (in g)	♂	265.6	267.4	267.0	267.1	266.1
	♀	181.2	181.4	180.7	179.9	182.7
Bw (D91) (in g)	♂	437.9	439.7	443.0	407.9*	401.9*
	♀	245.1	246.9	244.4	231.7*	233.0
Bwg (D0 - D91) (in g)	♂	172.3	172.2	176.0	140.8*	135.9*
	♀	63.9	65.5	63.7	51.8*	50.3*
Total corpora lutea		33	NE	33	41	40
Recent cycle corpora lutea		16	NE	17	21	19

NE : not evaluated; * $p \leq 0.05$

According to the results, the NOAEL for this combined study was set at 500 ppm.

In a chronic repeated dose toxicity study performed following the OECD guideline 452 (Wahle B.S., 2010), groups of 20 male and 20 female rats were exposed to 1,2,4-triazole during 12 months at a concentration of 0, 125, 375, 1000 and 2000 ppm (corresponding to 0, 6.9, 21, 58 and 113 mg/kg bw/d in males and 0, 8.3, 26, 71, 136 mg/kg bw/d in females). Furthermore, additional groups of 10 animals/sex/group were exposed to analyze neurotoxicity parameters.

No treatment-related effects were observed in the mortality, clinical signs, food consumption, hematology, clinical chemistry, organ weight, gross pathology, estrous cycle staging and sperm analysis examinations.

A lower bw and bwg were observed in both sexes at the 2 highest dose groups (BWG (D0 - D343) : 293/116 g at 1000 ppm, 294/115 g at 2000 ppm vs 318/144 g in control group in males/females, respectively).

Neurological assessment, comprising a FOB and a motor activity examination, was performed and revealed no treatment-related effect at any dietary dose level in both sexes.

The histopathology examination showed changes in the brain at the highest dose level. The lesion was characterized as an increased incidence of Purkinje cells loss within the vermis.

Table 25 : Incidence of Purkinje cell loss (Wahle B.S., 2010)

	0 ppm		2000 ppm	
	Toxicology group	Neurotoxicology group	Toxicology group	Neurotoxicology group
Males	0/20	0/10	10/10	6/10
Females	0/20	0/10	14/20	7/10

According to the results, the NOAEL was established to be 375 ppm for both sexes based on the lower observed bw and bwg.

10.10.3 Comparison with the CLP criteria

As there are no epidemiological studies available, Cat. 1A is not warranted.

According to the CLP Regulation (EC No 1272/2008) the classification of a substance in category 1B for reproductive toxicants *"is largely based on data from animals 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"*.

In the 2-generation reproductive toxicity study performed in rats (Young A.D. and Sheets L.P., 2005), almost complete infertility was observed at the top dose (fertility index: 76.7, 83.3, 86.2 and 7.1 % at 0, 250, 500 and 3000 ppm, respectively) in the P generation only, considering the highest dose was not tested in F1. Therefore, clear evidence of an adverse effect on fertility at the top dose was shown. The adverse effect occurred together with other toxic effects. At the highest dose, mild to moderate brain cerebellar degeneration/necrosis was observed in both sexes in the parental generation. Nevertheless, the cerebellum is not involved in the reproductive axis (hypothalamic-pituitary-gonadal axis). Furthermore, maternal body weight during gestation period was statistically significantly reduced at the highest dose however, this modification could be explained by the low number of pregnant females (since 28 out of 30 dams were not pregnant). The adverse effects on fertility are not considered to be secondary non-specific consequences of systemic toxicity since systemic toxicity appeared to be minimal. Further more, adverse effects on fertility are supported by other effects observed in this study : increased incidence of uterus dilatation, reduction in epididymal sperm counts and reduction of normal sperm morphology percentage can also explain the fertility adverse effects.

Furthermore, a few studies revealed histopathological modifications in testis. In a subacute toxicity study (Wahle B.S., 2004a), an increased incidence of spermatid degeneration/depletion/asynchrony was observed without any other signs of toxicity (no effects on survival, clinical signs, bw or organ weigth). This effect was confirmed by a subchronic toxicity study (Wahle B.S., 2004b) in which a statistically significant higher incidence of spermatid degeneration/depletion/asynchrony was also noted. In this last study, an increased incidence of tremors, yellow staining and rough coat were observed in males at the highest dose, the bw was also modified however these effects are not considered severe enough to explain the important modifications observed in testis and epididymis. In a combined 90-day repeated dose toxicity study and neurotoxicity study (Wahle B.S. and Sheets L.P., 2004), a lower uterus weight and a slight increased number of corpora lutea were observed at the 2 highest dose levels (3000 ppm and 1000/4000 ppm). Simultaneously, a bw change was noted which was significant only at 3000 ppm).

According to the CLP Guidance a classification as Repro 1B for adverse effects on sexual function and fertility is warranted based on the above mentioned severe effects observed in the available studies, which cannot be related to a general toxicity.

The CLP regulation (EC No 1272/2008) also states that "*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*". Cat.2 is not recommended considering the clear impact of 1,2,4-triazole on the fertility, as showed above. It is considered that the effects observed are sufficiently convincing to propose a cat. 1B.

10.10.4 Adverse effects on development

10.10.4.1 Non-human information

Table 26 : Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Embryotoxicity study in rats (Bor : Wisw (SPF Cpb)) (25 females/group) Oral EPA OPPTS 83-3 guidance GLP	1,2,4-triazole (purity 94,0 %) Doses : 0, 100 and 200 mg/kg bw/d Exposure : GD 6-15 Vehicle : cremophor-EL emulsion 0.5 %	<u>Dams :</u> No mortality and no clinical signs observed. At the highest dose, bw was decreased at GD 20 and the bwg was significantly modified. No significant changes were seen in the number of dams fertilised or in the number of implantation sites per dam, but a significant increase in post-implantation loss (0.5, 0.3 and 6.3** at 0, 100 and 200 mg/kg bw/d, respectively) was observed only in the highest dose level leading to a high rate of resorptions of 53 %. The mean number of corpora lutea per dams was significantly increased (14.2* at 200 mg/kg bw/d vs 13.6 in control group). <u>Foetus :</u> The mean number of foetus per dams was significantly decreased at the highest dose	Renhof M., 1988a

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		(5.5** at 200 mg/kg bw/d vs 12.0 in control group). The mean foetus weight was significantly reduced at the 2 tested doses (3.55, 3.06** and 2.35** g at 0, 100 and 200 mg/kg bw/d, respectively). The mean placental weight was significantly decreased (0.59, 0.52** and 0.49** g at 0, 100 and 200 mg/kg bw/d, respectively). The number of foetus per litter with malformations was increased at the highest dose (0.80* vs 0.29 in control group) NOAEL (maternal toxicity) : 100 mg/kg bw/d NOAEL (developmental toxicity) : < 100 mg/kg bw/d	
Embryotoxicity study in rats (Bor : Wisw (SPF Cpb)) (25 females/group) Oral EPA OPPTS 83-3 guidance GLP	1,2,4-triazole (purity 95.3 %) Doses : 0, 10, 30 and 100 mg/kg bw/d Exposure : GD 6-15 Vehicle : cremophor-EL emulsion 0.5 %	<u>Dams :</u> No mortality observed Bwg during exposure period was significantly reduced at the highest dose (28.2, 25.4, 26.8 and 21.8*g at 0, 10, 30 and 100 mg/kg bw/d, respectively). No modification in the number of implantations per dams. <u>Foetus :</u> A significant increased incidence of runts was observed at the highest dose (0.33, 0.23, 0.53 and 2.21** at 0, 10, 30 and 100 mg/kg bw/d, respectively) A significant lower foetal weight was observed at the highest dose (3.25 vs 3.58 g in control group). No dose-related increased incidence of malformation. NOAEL (maternal toxicity) : 30 mg/kg bw/d NOAEL (developmental toxicity) : 30 mg/kg bw/d	Renhof M., 1988b
Developmental study in rats (Wistar) (10 females/dose) Oral Non-guideline, non-GLP	1,2,4-triazole (purity : no information available) Doses : 0, 25 and 100 mg/kg bw/d Exposure : GD 7 through 17 Vehicle : no information available	<u>Maternal observation :</u> Bw not affected <u>Offspring observation :</u> No effects were observed on litter weight (PND 1 and 5) and on number of live and dead pups (PND 1 and 5) NOAEL(maternal toxicity) : 100 mg/kg bw/d NOAEL (developmental toxicity) : 100 mg/kg	Wickramaratne, 1987 (cited in JMPR, 2008)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		bw/d	
Prenatal developmental toxicity study in rabbits (NZW) (25females/dose) Oral (gavage) Following OECD TG 414 GLP	1,2,4-triazole (purity 99.9 %) Doses : 0, 5, 15, 30 and 45 mg/kg bw/d Exposure : GD 6-28 Vehicle : aqueous 0.5 % carboxymethylcellulose	<u>Dams :</u> Mortality : at the highest dose, 5 females were sacrified due to their moribund condition. The bwg over the entire gestation was significantly reduced at the highest dose (0.37** g vs 0.65 g in control group). However, the maternal bw at GD 29 was not modified significantly. A significant lower gravid uterine weight was observed at the highest dose (0.46** kg vs 0.56 kg in control group). No modification in the number of coropora lutea, the number of implantations, the litter size, the incidence of early and late resorptions. <u>Foetus :</u> The live foetal bw was significantly reduced at the highest dose (39.46** g vs 44.35 g in control group). Moreover, a higher incidence of alterations of the urogenital system was observed at the highest dose. NOAEL (maternal toxicity) : 30 mg/kg bw/d NOAEL (developmental toxicity) : 30 mg/kg bw/d	Hoberman, 2004 (cited in JMPR, 2008)
2-generation reproductive toxicity study in rats (Wistar Hannover) (30/sex/dose) Oral (diet) Following OECD TG 416 GLP	1,2,4-triazole (purity 99,9 %) Doses : 0, 250, 500 and 3000 ppm Exposure : through 10 weeks premating period to lactation D21 Vehicle : ethanol	No treatment-related effects were observed concerning deaths or clinical signs at any dietary dose level. Bw of males and females was statistically modified during different exposure periods (see table 37). <u>P0 :</u> Brain weight was significantly reduced at 3000 ppm in both sexes in P0. In conjunction with brain weight, degeneration/necrosis was observed in the cerebellum. Absolute and relative ovarian weights was significantly increased at 3000 ppm in P0 females. Furthermore, changes in the number of corpora lutea was noted. Modification of sperm parameters were observed. In females, reproductive data were also modified. No treatment-related effects were observed concerning the sex ratio, the viability index, the mean litter or pup weights and the	Young A.D. and Sheets L.P., 2005

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		micropathology evaluation of the pups. NOAEL (parental toxicity) : 500 ppm NOAEL (fertility) : < 250 ppm based on the sperm parameters NOAEL (developmental toxicity) : 500 ppm	

10.4.2. Human information

No information available

10.4.2.3 Other relevant information

No information available

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

An embryotoxicity study has been performed in rats (Renhof, 1988a, cited in JMPR, 2008) with 1,2,4-triazole of 94% purity. Impurities were not specified but the study was performed with the same batch as the Renhof, 1988b –study for which 2 impurities were identified. Those impurities have a harmonized classification and labeling : Repr.1B, H360D *** and Repr. 2, H361d***resp. and can contribute to the classification if their concentration is ≥ 0.3% (Repr. 1B) or ≥3% (Repr.2). Analytical analysis of the batch used in the Renhof, 1988b , confirmed that >0.3% of the impurity with HCL Repr.1B was available, but <3% of the impurity with HCL Repr.2.

25 females per group were orally exposed to 1,2,4-triazole at doses of 0, 100 or 200 mg/kg bw/d from gestational day 6 to 15.

All animals survived during the study and no maternal treatment-related clinical signs or food consumption changes were observed. The maternal bw evaluation revealed a slight tendency to decrease which was confirmed by the bwg data (see table 29).

Table 29 : Bw and bwg data (in g) (Renhof, 1988a, cited in JMPR, 2008)

	Bw at GD 0	bw at GD 6	bw at GD 15	bw at GD 20	bwg during exposure period	bwg during entire pregnancy	Mean gravid uterus weight	Adjusted maternal body weight gain
0 mg/kg bw/d	204.6	221.5	250.9	301.4	29.3	96.8	66	30.8
100 mg/kg bw/d	203.8	222.5	249.8	295.7	27.4	91.9	57.74	34.16
200 mg/kg bw/d	203.2	220.3	241.8	263.6	21.5*	60.4**	27.16	33.24

* p < 0.05 **p<0.01

Concerning the pregnancy parameters, there were no treatment-related effects.

The fetal evaluation exhibited a lower bw and a lower placental weight at 100 and 200 mg/kg bw/d. Furthermore, the incidence of runts was significantly higher at 100 and 200 mg/kg bw/d. In addition of these modifications, the number of surviving fetuses per dam was reduced and the incidence of fetuses with malformations (undescended testicle, cleft palate and hydronephrosis) was increased at the highest dose level. Moreover, the number of resorptions per litter was increased at the highest dose level (53.2 % vs 3.9 % in controls). (See table 30 and table 31)

Table 30 : Intrauterine development parameters (Renhof, 1988a, cited in JMPR, 2008)

	0 mg/kg bw/d	100 mg/kg bw/d	200 mg/kg bw/d
Nb of corpora lutea per dam	13.6	13.9	14.2*
Nb of implantation per dam	12.5	12.2	11.8
Nb of runts per litter	0.24	2.84*	4.96**
Number of fetuses per dam	12.0	11.9	5.5**
Number of male/female fetuses per dam	5.9/6.1	6.0/5.9	3.1**/2.4**
Nb of post-implantation loss per dam	0.5	0.3	6.3**
Mean fetuses weight (in g)	3.55	3.06**	2.35**
Mean placental weight (in g)	0.59	0.52*	0.49**
Fetuses per litter with minor skeletal deviations	2.67	4.32*	2.24
Fetuses per litter with malformation	0.29	0.63	0.80*

* p < 0.05 **p<0.01

Table 31 : Observed malformations (Renhof, 1988a, cited in JMPR, 2008)

	0 mg/kg bw/d	100 mg/kg bw/d	200 mg/kg bw/d
Total incidence of undescended testicle	2/253 (0.8 %)	11/226 (4.9 %)	6/138 (4.3 %)
Incidence per litter of undescended testicle	2/21 (9.5 %)	7/19 (36.8 %)	5/25 (20 %)
Total incidence of hydronephrosis	1/253 (0.4 %)	1/226 (0.4 %)	7/138 (5.1 %)
Incidence per litter of hydronephrosis	1/21 (4.8 %)	1/19 (5.3 %)	6/25 (24 %)
Total incidence of cleft palate	0/253	0/226	4/138 (2.9 %)
Incidence per litter of cleft palate	0/21	0/19	3/25 (12 %)

Based on the results, the NOAEL for maternal toxicity was 100 mg/kg bw/d however the NOAEL for developmental toxicity was inferior to the low dose group level which was 100 mg/kg bw/d.

Contribution of the impurity (HCL : Repr.1B and conc. >0.3%) to the classification :

In the key developmental toxicity study (reliability 1) no statistical significant differences were observed in the incidences of fetal morphological anomalies, the overall incidences were approximately 1.3 (control), 2.2, 0.6, and 1.3% for the respective groups.

A NOAEL of 50 mg/kg bw/d was established, based on decreases in fetal body weight.

The average male and female fetal weight was statistically significantly reduced at 100 and 200 mg/kg bw/day. The number of fetuses per litter and fetal viability were not affected. The sex distribution was not changed. The incidence of external, skeletal or visceral malformations and variations was not increased at any dose. In this study, the rat conceptus was more sensitive than the adult to the adverse effects of formamide administered orally throughout the embryo/fetal period of gestation.

The developmental NOAEL was 50 mg/kg bw/d was established, based on decreases in fetal body weight. Teratogenicity was not seen, the NOAEL was therefore 200 mg/kg bw/day (NTP, 1998).

No cleft palate was observed in the study performed with the Repr. 1B impurity. This demonstrates that this impurity exerts another mode of action than in the Renhof study(1988a). It can be concluded that the severe malformation (cleft palate) was due to 1,2,4-triazole and not to the impurity.

Furthermore the increased incidence of cleft palates in rat has also been observed in response to exposure to other triazoles like propiconazole, cyproconazole and epoxiconazole.

Therefore we can conclude that the Renhof (1988a) study is adequate and reliable for the classification of 1,2,4-triazole for adverse effects on development.

A second embryotoxicity study has been performed in rats (Renhof, 1988b, cited in JMPR, 2008) with 1,2,4 triazole (purity 95,3%). Impurities were not specified.

25 females by group were given 1,2,4-triazole at doses of 0, 10, 30 or 100 mg/kg bw/d from gestational day 6 to 15.

Concerning maternal evaluation, all animals survived during the study and no treatment-related clinical signs or food consumption changes were observed. However, the bwg was significantly lower at 100 mg/kg bw/d during the exposure period than in the control group (21.8* g vs 28.2 g).

Considering the pregnancy parameters, there were no treatment-related effects.

Concerning intrauterine development, up to 30 mg/kg bw/d dose level the examined parameters were unaffected. Nevertheless, at the highest dose level, a few parameters were disturbed such as fetal weight which was significantly decreased and simultaneous greater incidence of runts. Moreover, at this dose level, a slight increase in the number of malformations was observed. However, it affected only one fetus for each type of malformation and therefore was considered not treatment-related. (See table 32 and table 33)

Table 32 : Effects on intrauterine development (Renhof, 1988b, cited in JMPR, 2008)

	0 mg/kg bw/d	10 mg/kg bw/d	30 mg/kg bw/d	100 mg/kg bw/d
Nb of implantation per dam	11.6	10.5	11.4	10.6
Nb of runts per litter	0.33	0.23	0.53	2.21*
Number of fetuses per dam	11.0	10.1	10.6	9.5
Number of male/female fetuses per dam	6.5/4.5	5.1*/5.0	6.0/4.6	5.0*/4.5
Mean fetuses weight (in g)	3.58	3.59	3.53	3.25**
Mean placental weight (in g)	0.56	0.56	0.57	0.56
Fetuses per litter with minor skeletal deviations	2.00	2.41	2.84	2.42
Fetuses per litter with malformation	0.05	0.05	0.05	0.17

* $p < 0.05$ **$p<0.01$

Table 33 : Incidence of malformation (Renhof, 1988b, cited in JMPR, 2008)

	0 mg/kg bw/d	10 mg/kg bw/d	30 mg/kg bw/d	100 mg/kg bw/d
Total no. examined foetuses	231	222	202	228
Microphtalmia, bilateral	1	0	0	0
Microphtalmia, right side	0	1	0	1

Microphtalmia, left side	0	0	0	1
False posture of right hind leg	0	0	1	0
Anophtalmia	0	0	0	1
Dysplasia and asymmetry of body of vertebrae	0	0	0	1

According to the results, the NOAEL for maternal and developmental toxicity was set at 30 mg/kg bw/d.

In a non-guideline developmental toxicity study (Wickramaratne, 1987, cited in JMPR, 2008), non-GLP, 10 females rats were exposed to 1,2,4-triazole (purity and vehicle not reported) at doses of 0, 25 or 100 mg/kg bw/d from day 7 to 17 of gestation.

Examined maternal parameters were restricted to bw and no change was observed.

Offspring observation was also restricted to only few parameters such as litter weight of live pups on PND 1 and 5 and the number of live and dead pups on these days. During these examinations, no effects were noted.

Based on the available results, the NOAEL for maternal and developmental toxicity was established to be 100 mg/kg bw/d.

In a prenatal developmental toxicity study (Hoberman, 2004, cited in JMPR, 2008), following OECD guideline 414, 25 pregnant female rabbits were given 1,2,4-triazole by gavage at a dose of 0, 5, 15, 30 or 45 mg/kg bw/d from gestational days 6 to 28.

At the highest dose, 5 out of 25 females were sacrificed between gestational day 16 and 24 due to their moribund condition which consisted of severely decreased food consumption and bw already observed at gestational day 7, decreased motor activity, soft and/or liquid faeces. Among surviving rabbits, there were no significant changes on bw, food consumption and gross pathology. At the end of the exposure period (GD 29), the bw was of 4.04, 3.95, 3.93, 4.00 and 3.76 kg at 0, 5, 15, 30 and 45 mg/kg bw/d, respectively. During the organ weight examination, a significant decrease of gravid uterine weight was observed at the highest dose (0.56, 0.54, 0.51, 0.53 and 0.46** kg respectively at 0, 5, 15, 30 and 45 mg/kg bw/d).

Table 34 : bw data (Hoberman, 2004, cited in JMPR, 2008)

	0 mg/kg bw/d	5 mg/kg bw/d	15 mg/kg bw/d	30 mg/kg bw/d	45 mg/kg bw/d
Bw at GD29 (kg)	4.04	3.95	3.93	4.00	3.76
Gravid uterine weight (kg)	0.56	0.54	0.51	0.53	0.46**
Corrected maternal bw	3.48	3.40	3.42	3.46	3.31[a]

[a] : excludes values for rabbits that were moribund sacrificed or prematurely delivered

The litter averages for corpora lutea, implantations, litter size, live fetuses, dead fetuses, early and late resorptions, percent of dead or resorbed conceptuses, and percent live male fetuses were comparable among all groups (See table 35).

Table 35 : Litter observations (Hoberman, 2004, cited in JMPR, 2008)

	0 mg/kg bw/d	5 mg/kg bw/d	15 mg/kg bw/d	30 mg/kg bw/d	45 mg/kg bw/d
No. dams examined	25	24	24	25	19

Corpora lutea	9.8	9.8	9.9	10.2	9.8
Implantations	9.0	9.0	8.8	9.3	9.0
Early resorption (n)	1	2	4	10	6
Late resorption (n)	7	8	7	3	8
Litter size (n)	8.7	8.6	8.3	8.8	8.3
Live fetuses (n)	217	207	199	218	157
Dead fetuses (n)	0	0	0	1	0
Percent of dead or resorbed conceptuses	3.1	4.7	4.8	6.4	7.0
Percent live male fetuses	59.0	56.9	53.2	56.6	60.6

Concerning the fetal observations, bw were significantly lower at the highest dose than the control group (See table 36). Up to 30 mg/kg bw/d, no gross external, soft tissue or skeletal fetal alterations were observed. However, at 45 mg/kg bw/d, a few alterations of the urogenital system were detected. At this dose, 3 fetuses from 1 litter had one or two low set and small kidneys, 2 fetuses from 2 litters had an absent kidney.

Table 36 : Fetal observation (Hoberman, 2004, cited in JMPR, 2008)

	0 mg/kg bw/d	5 mg/kg bw/d	15 mg/kg bw/d	30 mg/kg bw/d	45 mg/kg bw/d
Live fetal bw (in g)	44.35	43.42	43.82	42.48	39.46**
Male bw (in g)	44.92	43.91	44.25	42.39	39.65**
Female bw (in g)	42.92	42.79	43.64	42.20	38.70*

* $p < 0.05$ **$p<0.01$

Based on the results of the study, the NOAEL for maternal and developmental toxicity was 30 mg/kg bw/d.

In a two-generation reproductive toxicity study following OECD guideline 416 (Young A.D. and Sheets L.P., 2005), groups of 30 male and 30 female rats were given diets containing 1,2,4-triazole at a concentration of 0, 250, 500 or 3000 ppm (See table 37 for the dosing in mg/kg bw/d during the different exposure period). The exposure period began 10 weeks before mating, and continued through mating, gestation, and lactation.

Table 37 : Dose level by group in mg/kg bw/d (Young A.D. and Sheets L.P., 2005)

	Phase of study	250 ppm in mg/kg bw/d	500 ppm in mg/kg bw/d	3000 ppm in mg/kg bw/d
♂	Premating (P-gen)	15.4	30.9	188.6
	Premating (F1-gen)	16	32	NA
♀	Premating (P-gen)	17.5	36.2	217.9
	Gestation (P-gen)	18.6	38.6	231.7[a]
	Lactation (P-gen)	19.3	38.7	NA
	Premating (F1-gen)	18.9	37.5	NA
	Gestation (F1-gen)	17.4	34.4	NA
	Lactation (F1-gen)	20.3	35.8	NA

[a] *: based on only 2 pregnant females*

No treatment-related effects were observed concerning deaths or clinical signs at any dietary dose level. Bw of males and females was statistically modified during different exposure periods (see table 38).

Table 38 : Body weight data in grams for P and F1 animals (Young A.D. and Sheets L.P., 2005)

	Phase of study	0 ppm	250 ppm	500 ppm	3000 ppm
♂	P adults (D119)	477.5	465.1	460.6	426.6**
	F1 adults (D98)	461.9	435.2*	428.4**	/
♀	P premating-mating (D70)	244.1	244.9	239.5	233.4*
	P gestation (D20)	345.3	340.9	340.0	284.7**[a]
	P lactation (D21)	284.2	287.4	287.4	/
	F1 premating-mating (D70)	236.2	227.5	230.8	/
	F1 gestation (D20)	323.8	313.3	311.8	/
	F1 lactation (D21)	281.4	267.8*	271.2	/

[a] : based only on 2 dams; * $p < 0.05$ **$p<0.01$

Males and females of the F0-generation at the highest dose had a significantly lower terminal bw (473.1/277.2, 460.7/283.1, 456.1/280.9 and 419.4*/245.1* g at 0, 250, 500 and 3000 ppm in males/females, respectively) and lower absolute brain weight (2.092/1.955, 2.075/1.941, 2.044/19.51 and 2.006*/1.853* g respectively at 0, 250, 500 and 3000 ppm in males/females) compared with the control group. Several other organs also showed modifications at this highest dose such as ovaries (0.058/0.058, 0.059/0.057, 0.055/0.054 and 0.067*/0.071* g (left/right ovaries) at 0, 250, 500 and 3000 ppm, respectively), thyroid, liver. In addition to the brain weight change, minimal to marked degeneration/necrosis was observed in 30 out of 30 males and in 28 out of 30 females. Moreover, in the ovaries, changes in number of total corpora lutea were observed (24.9, 23.0, 15.6 and 41.3 respectively at 0, 250, 500 and 3000 ppm). Furthermore, the histopathological examination of uterus revealed a higher incidence of dilatation (14* females at 3000 ppm vs 4 females in control).

For litters of 250 and 500 ppm dose level, live birth, viability, mean litter sizes, sex ratios and clinical signs were not modified in the treated compared to the control groups. Furthermore, the gross necropsy was similar between all dose levels.

Bw and bwg changes were not observed in pups at the F1-generation however the bw at the F2-generation examined at PND 0 and 21 were reduced compared with control. (See table 39)

Table 39 : Body weight of pups in the F1 and F2-generation (number of litters) (Young A.D. and Sheets L.P., 2005)

		F1-generation				F2-generation		
		0 ppm	250 ppm	500 ppm	3000 ppm	0 ppm	250 ppm	500 ppm
D0	♂	6.3	6.0	6.2	/	6.3	6.0*	5.8**
	♀	6.0	5.6	5.9	5.4	6.0	5.6**	5.5*
	♂ + ♀	6.2 (22)	5.9 (25)	6.1 (25)	5.4 (2)	6.2 (27)	5.8** (26)	5.7** (25)
D7	♂	17.0	16.1	16.2	/	16.9	16.1	16.1
	♀	16.1	15.5	15.4	9.1**	16.3	15.6	15.8
	♂ + ♀	16.5 (22)	15.8 (25)	15.7 (25)	9.1** (2)	16.6 (27)	15.9 (26)	16.0 (24)
D21	♂	52.0	50.2	50.5	/	51.2	47.5**	48.4*

	♀	49.4	47.9	47.6	/	49.4	45.9**	46.7*
	♂ + ♀	50.7 (22)	49.1 (25)	48.4 (25)	/	50.2 (27)	46.8** (26)	47.6* (24)

According to the results, the NOAEL for the parental toxicity was 500 ppm based on lower bw and degenerative findings observed in the cerebellum at the highest dose level. The NOAEL for fertility could not be determined based on the reduction in testicular sperm counts noted at 250 ppm. Moreover, the NOAEL for developmental toxicity was set at 500 ppm which was the highest dose allowing assessment of the developmental effects as the 3000 ppm dose level was not further examined due to the low number of pups.

10.10.6 Comparison with the CLP criteria

As there are no epidemiological studies available, Cat. 1A is not warranted.

Category 2 is not supported as we consider the evidence strong enough to warrant a classification in cat. 1B as it is shown below.

According to the CLP Regulation (EC No 1272/2008) the classification of a substance in category 1B for reproductive toxicants *"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".*

Developmental disturbances were observed in a developmental toxicity study performed in rats (Renhof M., 1988a). This study indicated an increased incidence of cleft palates (in 3/25 litters (12% litter incidence) at 200 mg/kg bw/d). In comparison with historical control data (1986-1989) only one case of cleft palate was reported in the year 1987 (litter incidence 4.17%) and only one case in 1989 (litter incidence 7.69%). Moreover, the high rate of resportions (53%) observed at this highest dose may have masked the number of malformations, which are known to be very rare in rats. Additionally, in males, the incidence of cryptorchism was above the historical values at the treated dose group (100 and 200 mg/kg) in this developmental toxicity study in rats (in 2/253, 11/226 and 6/138 pups respectively at 0, 100 and 200 mg/kg bw/d) and an increase of pre and post-implantation losses was observed (number of implantation loss per dam : 0.5, 0.3 and 6.3** respectively at 0, 100 and 200 mg/kg bw/d). In conjunction, the mean number of foetus per dams was significantly decreased. Finally, a significant dose-related decrease in mean foetus weight was seen at 100 and 200 mg/kg bw/d. These severe effects are not explained by the maternal toxicity as the bw change appear only at the highest dose level

Furthermore, in the other developmental toxicity study performed in rats (Renhof M., 1988b) a significant increased incidence of runts was noted at the highest dose level (100 mg/kg bw/d). In addition, the mean foetus weight was significantly decreased at 100 mg/kg bw/d compared to the control group. However, these effects appear at the same dose level as the bw modification.

In the prenatal developmental toxicity study in rabbits (Hoberman, 2004), an important increase in the mortality rate (20%) was observed at the highest dose (45 mg/kg bw/d). However, a dose-related increase in the incidence of dead or resorbed conceptuses per litter was already observed at 30 mg/kg bw/d (3.1, 4.7, 4.8, 6.4 and 7.0 respectively at 0, 5, 15, 30 and 45 mg/kg bw/d). This examination may have masked the number of malformations.

No conclusion about developmental toxicity can be drawn from the 2-generation toxicity study (Yound A.D. and Sheets L.P., 2005) as the severe decrease in the fertility index at 3000 ppm (corresponding to less than 235 mg/kg bw/d) lead to a premature termination of this dose level. Consequently, the highest dose for the F1-generation was 500 ppm corresponding to less than 40 mg/kg bw/d. Thus, a concern cannot be excluded.

According to all these severe effects, a classification as Repr. 1B for adverse effects on development is warranted.

10.10.7 Adverse effects on or via lactation

10.10.7.1 Non-human information

No information available

10.10.7.2 Human information

No information available

10.10.7.3 Other relevant information

No information available

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

NA

10.10.9 Comparison with the CLP criteria

NA

10.10.10 Conclusion on classification and labelling for reproductive toxicity

According to all of these available studies which revealed severe reproductive effects (in more than 1 species) as mentioned above, a classification as Repr. 1B; H360FD is warranted.

Furthermore during the MSC-54, it was concluded that the best way forward for 1,2,4-triazole was the submission of a CLH dossier for reproductive toxicity, more particular Repro. 1B, H360FD.

RAC evaluation of reproductive toxicity
EFFECTS ON SEXUAL FUNCTION AND FERTILITY **Summary of the Dossier Submitter's proposal** The current harmonised classification as Repr. 2; H361d in Annex VI of CLP is a translation of the previous classification agreed by TC C&L in 1996, that was based on the evaluation of developmental effects only. Since new studies were available, the DS decided to also evaluate the effects on sexual function and fertility. The DS considered seven studies as relevant to evaluate effects on sexual function and fertility: one two-generation reproductive toxicity study, two subacute toxicity studies,

two subchronic toxicity studies, one combined subchronic toxicity/neurotoxicity screening study and one chronic study.

In the two-generation reproductive toxicity study in rats, no treatment-related deaths or clinical signs were observed in P or F1 parental animals at any tested dose level. Males and females of the P-generation at the highest dose (3000 ppm in diet; 189 mg/kg bw/d in males and 218 mg/kg bw/d in females) had significantly lower terminal bodyweight (bw) and lower absolute brain weight compared to the control group. Also degeneration/necrosis of the cerebellum was observed at the top dose in the P-generation. In addition, several other organs such as ovaries, thyroid and liver showed weight changes at the highest dose level in the P-generation. Sperm parameters were affected in the P-generation already at the lowest dose, and the histopathological examination of the uterus revealed a higher incidence of dilatation at 3000 ppm compared to the control group. The fertility index as well as the number of implantations decreased at 3000 ppm in P generation. At the highest dose level, the number of live pups decreased. The effect on fertility was not considered to be a secondary non-specific consequence of other toxic effects because the cerebellum was not involved in the reproductive axis and because systemic toxicity was considered to be minimal. Due to low fertility at 3000 ppm, further testing with this dose in the next generation was not performed. No significant changes were shown in male and female reproductive parameters in the F1-generation. However, a slight decreasing trend in fertility index and number of implantations was observed. The NOAEL for parental toxicity was 500 ppm (31 mg/kg bw/d in males and 36 mg/kg bw/d in females). The NOAEL for fertility could not be determined based on the reduction in testicular sperm counts noted already at the lowest dose (250 ppm; 15.4 mg/kg bw/d).

In a 28-day repeated dose toxicity study in mice histopathological evaluation revealed some modifications in testis and epididymis (an increased incidence of spermatid degeneration/depletion/asynchrony) in the absence of any other signs of toxicity. The NOAEL was 500 ppm in males (90 mg/kg bw/d) and 2000 ppm in females (479 mg/kg bw/d).

In a 30-day repeated dose toxicity study in rats, lower bw and some clinical signs were noticed at the highest dose level (400 mg/kg bw/d). Based on the poorly documented data, the NOAEL was < 8 mg/kg bw/d.

In a 90-day repeated dose toxicity study in rats, two males and two females of the highest dose group exhibited slight temporary convulsions. In the highest dose group there was a significantly lower bw in males for the entire study period and in females for the majority of the study period. The absolute testis weight was decreased at the highest dose, but no histopathological lesions were observed in this organ. The NOAEL was 500 ppm (38 mg/kg bw/d.

In a 90-day repeated dose toxicity study in mice an increased incidence of tremors was observed in both sexes at the highest dose level (6000 ppm in diet; 988 mg/kg bw/d in males and 1346 mg/kg bw/d in females). The analysis of hepatic enzymes showed an increased activity of ECOD, EROD, ALD and GLU-T in both sexes at 6000 ppm. The absolute brain weight was significantly decreased in both sexes at 6000 ppm and in males also at 3000 ppm (487 mg/kg bw/d). In addition, an increased incidence of Purkinje cell loss was observed in both sexes at the highest dose. The absolute testis weight was significantly decreased at 6000 ppm. In conjunction with this change, histopathological modifications were observed including increased incidence of apoptotic-

like bodies, of spermatid degeneration/depletion/asynchrony and of tubular atrophy. The epididymal histopathological examination revealed also a higher incidence of exfoliated germ cells and debris in the lumen of the duct at 6000 ppm. The DS concluded that the effects observed in the testis and epididymis were not secondary non-specific consequences of other effects. The NOAEL was 1000 ppm in males (161 mg/kg bw/d) and 3000 ppm in females (663 mg/kg bw/d).

In a combined 90-day repeated dose toxicity study and neurotoxicity study in rats, evaluation of clinical chemistry parameters revealed a slight decrease in serum triglyceride concentrations at the 3000 and 1000/4000 ppm dose levels (183 and 210 mg/kg bw/d in males; 234 and 275 mg/kg bw/d in females) and a slightly increased activity of the hepatic enzymes. A significant decrease in TSH concentration was seen in males at 500, 3000 and 1000/4000 ppm (33, 183 and 210 mg/kg bw/d). No treatment-related effects were observed on mortality, food consumption, haematology and urine analysis parameters. At the two highest doses a decrease in body weight was observed in both sexes as compared to controls. A functional observational battery (FOB) examination revealed changes at the two highest dose levels such as ungroomed appearance, red nasal stain, urine stain, muscle fasciculations, gait incoordination, decreased activity in the open field in males and tremor and decreased rearing in both sexes at 3000 ppm. Red nasal stain, decreased activity in the open field and increased foot splay were seen in males, urine stain in females and ungroomed appearance, muscle fasciculations, tremor, gait incoordination, decreased rearing and uncoordinated righting in both sexes at the 1000/4000 ppm dose level. The absolute brain weight was statistically significantly reduced at two highest doses in males and at the top dose in females. Necropsy of the brain revealed some histopathological changes (degeneration/necrosis) in the dorsal cerebellum in both sexes at the two highest doses and an increased incidence of degeneration of the sciatic nerve, tibial nerve and sural nerve. A slightly but not statistically significantly reduced uterus weight was also observed. A slight increase in number of corpora lutea was observed in females at 3000 ppm and 1000/4000 ppm. The NOAEL for this combined study was 500 ppm (33 and 41 mg/kg bw/d in males and females, respectively).

In a chronic repeated dose toxicity study in rats, no treatment-related effects were observed in clinical signs, food consumption, haematology, clinical chemistry, organ weight, gross pathology, estrous cycle staging and sperm analysis examinations. The histopathological examination of the cerebellum showed an increased incidence of Purkinje cell loss within the vermis at the highest dose level (2000 ppm). The NOAEL was 375 ppm for both sexes 21 and 26 mg/kg bw/d in males and females, respectively) based on the observed lower bw and body weight gain (bwg).

The DS concluded that a classification as Repr. 1B; H360F for adverse effects on sexual function and fertility was warranted, because almost complete absence of fertility was observed at the top dose in the P-generation of a 2-generation study in rats (the highest dose was not tested in the F1-generation). Treatment-related increases in the incidences of uterus dilatation, reduction in epididymal sperm counts and some histological findings (reduction in the percentage of normal sperm morphology) in the P-generation of the 2-generation study and the effects observed in the 28-day and 90-day studies in mice as well as in the combined 90-day repeated dose toxicity study and neurotoxicity study in rats were considered to support the classification of 1,2,4-triazole for the adverse effects on sexual function and fertility in category 1B.

Comments received during public consultation

Three MSCAs commented on effects on fertility; all three supported the proposed classification as Repr. 1B; H360F.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility were investigated in seven animal studies (table below, modified from Table 12 of the CLH report). The two-generation reproductive toxicity study in Wistar rats (Young and Sheets, 2005) is regarded as the main study.

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

Method, guideline, deviations if any, species, strain, sex, No./group	**Test substance, dose levels, duration of exposure**	**Results**	**Reference**
Two-generation reproductive toxicity study in rats (Wistar) 30/sex/dose Following OECD TG 416 GLP	1,2,4-triazole (purity ≥ 99,9%) Doses: 0, 250, 500 and 3000 ppm (males: 0, 15, 31 and 189 mg/kg bw/d; females: 0, 18, 36 and 218 mg/kg bw/d) Exposure: Through 10 weeks premating period to lactation D21 Vehicle: ethanol	No treatment-related deaths or clinical signs were observed in P or F1 parental animals at any dose level (the top dose was not tested in the F1-generation). Terminal bw of P-males and females was significantly decreased at 3000 ppm. P-generation: Absolute brain weight was significantly reduced at 3000 ppm in both sexes in P0, degeneration/necrosis was observed in the cerebellum. Absolute and relative ovarian weights were statistically significantly increased at 3000 ppm in P females. Furthermore, changes in the number of corpora lutea were noted. Epididymal sperm count was significantly reduced at 3000 ppm; percentage of normal sperm was significantly lower with concomitant increases in the percentage of abnormal and detached sperm at 500 and 3000 ppm. Fertility index was decreased (7.1% at 3000 ppm vs. 76.7% in control). NOAEL (parental toxicity): 500 ppm NOAEL (fertility): < 250 ppm based on the sperm parameters NOAEL (developmental toxicity): 500 ppm	Young A.D. and Sheets L.P., 2005 (cited in JMPR, 2008)
Subacute toxicity study in mice (CD1[ICR]/BR) 15/sex/dose Oral (feed)	1,2,4-triazole (purity ≥ 99,9%) Doses: 0, 50, 250, 500 and 2000 ppm (males: 0, 9,	No treatment-related deaths, clinical signs, bw, clinical chemistry and organ weight changes were noticed. Histopathological evaluation: slight testicular degenerations in 5 out of 15 males at the highest dose level, minimal to slight spermatid degeneration/depletion/asynchrony, focal tubular	Wahle B.S., 2004a (cited in JMPR, 2008)

No OECD guideline GLP	47, 90 and 356 mg/kg bw/d; females: 0, 12, 60, 120 and 479 mg/kg bw/d) Exposure: 4 weeks Vehicle: ethanol	atrophy NOAEL (males): 500 ppm NOAEL (females): 2000 ppm	
Subacute toxicity study in rats (strain unknown) Oral Non-guideline	1,2,4-triazole (purity unknown) Doses: 0, 8, 57 and 400 mg/kg bw/d Exposure: 30 days Vehicle: unknown	At 400 mg/kg bw/d: Lower bw and clinical signs such as staggering, tremors and hunched posture At 57 mg/kg bw/d: Slight hematological changes At 8 mg/kg bw/d: Lower adrenal weight NOAEL: < 8 mg/kg bw/d No further information available	Anonymous (cited in US EPA memorandum, 2006)
Subchronic toxicity study in rats (Wistar) 15/sex/dose Oral (feed) Similar to OECD TG 408 No GLP	1,2,4-triazole (purity ≥ 99,6%) Doses: 0, 100, 500 and 2500 ppm (males: 0, 8, 38 and 212 mg/kg bw/d; females: 0, 10, 54 and 267 mg/kg bw/d) Exposure: 3 months Vehicle: 90% premix with ultrasil VN3	At 2500 ppm: 2 males and 2 females exhibited slight convulsions; terminal bw was significantly lowered; decreased absolute testis weight, but no histopathological changes NOAEL: 500 ppm	Bomhard E., et al., 1979 (cited in JMPR, 2008)
Subchronic toxicity study in mice (CD-1[ICR]/BR) 20/sex/dose Oral (feed) Similar to Following US EPA OPPTS 870.3100 GLP	1,2,4-triazole (purity ≥ 99,9%) Doses: 0, 500, 1000, 3000 and 6000 ppm (males: 0, 80, 161, 487 and 988 mg/kg bw/d; females: 0, 105, 215, 663 and 1346 mg/kg bw/d) Exposure: 90	At 6000 ppm: Higher incidence of tremors A significant bw decrease was observed in males (3000 and 6000 ppm) and in females (6000 ppm). Absolute brain weight was reduced at 6000 ppm in both sexes and at 3000 ppm in males (relative brain was increased in males at 6000 ppm); an increased incidence of Purkinje cell loss was observed at the highest dose level. Absolute testis weight was significantly decreased at the highest dose, histopathological changes were observed (apoptotic like bodies, spermatid degeneration, tubular atrophy). Higher incidence of exfoliated germ cells and debris	Wahle B.S., 2004b (cited in JMPR, 2008)

	days Vehicle: ethanol	in the lumen of the epididymal duct at 6000 ppm. NOAEL (males): 1000 ppm NOAEL (females): 3000 ppm	
Combined subchronic toxicity/ neurotoxicity screening study in rats (Wistar) 20/sex/dose Oral (feed) Similar to OECD TG 408 and 424 GLP	1,2,4-triazole (purity ≥ 99,9%) Doses: 0, 250, 500, 3000 and 1000/4000 ppm (males: 0, 16, 33, 183 and 210 mg/kg bw/d in males and 0, 19, 41, 234 and 275 mg/kg bw/d in females) Exposure: 90 days Vehicle: ethanol	A lower bw was seen at the two highest dose levels. Brain weight was lower at 3000 ppm in both sexes and also in males at 1000/4000 ppm; degeneration/necrosis was noted in the cerebellum. Also an increased incidence of degeneration of sciatic nerve, tibial nerve and sural nerve was reported. At the two highest dose levels, a slightly increased number of corpora lutea was noted. The FOB (functional observational battery) revealed some effects, such as tremors and gait incoordination at the two highest dose levels. NOAEL: 500 ppm	Wahle B.S. and Sheets L.P., 2004 (cited in JMPR, 2008)
Chronic toxicity study in rats (Crl:Wi(han)) 20/sex/dose Oral (feed) Following OECD TG 452 GLP	1,2,4-triazole (purity ≥ 98,5%) Doses: 0, 125, 375, 1000 and 2000 ppm (males: 0, 6.9, 21, 58 and 113 mg/kg bw/d; females: 0, 8.3, 26, 71, 136 mg/kg bw/d) Exposure: 12 months Vehicle: ethanol	A slight decrease in bw and bwg was seen at the two highest doses. The histopathological examination revealed a significantly higher incidence of Purkinje cell loss at 2000 ppm. No effects were observed on estrous cycle and sperm analysis. NOAEL: 375 ppm	Wahle B.S., 2010

In the two-generation reproductive toxicity study (Young & Sheets, 2005), performed in accordance with OECD TG 416, 30 rats/sex/dose were given diet containing 1,2,4-triazole at concentrations of 0, 250, 500 or 3000 ppm. The table below (modified from table 15 of the CLH report) shows the corresponding doses in mg/kg bw/d during the different exposure periods in P-animals. For F1-animals, similar doses were calculated (the top dose was not tested in the F1-generation).

Table: Dose levels in P-animals by group in mg/kg bw/d

	Phase of study	**250 ppm in mg/kg bw/d**	**500 ppm in mg/kg bw/d**	**3000 ppm in mg/kg bw/d**
♂	Premating (P-gen)	15.4	30.9	188.6
♀	Premating (P-gen)	17.5	36.2	217.9
	Gestation (P-gen)	18.6	38.6	231.7[a]
	Lactation (P-gen)	19.3	38.7	NA

[a]: based on 2 pregnant females only

P and F1 parental rats were exposed 10 weeks before mating, throughout mating, gestation and lactation until sacrifice. To retain a constant dosage (mg/kg bw/d) throughout the whole study, the dietary levels were reduced during lactation. Dams were sacrificed following weaning on lactation D21. No F1 offspring at 3000 ppm survived lactation. Thus, no animals were exposed to this dose level in the F1-generation. Males were exposed during a premating period of 10 weeks.

No treatment-related deaths or clinical signs of toxicity were observed in P or F1 parental animals. P males and females in the highest dose group had a significantly lower terminal bw compared to the control group (table below, from Table 16 of the CLH report).

Table: Body weight (g) for P and F1 animals

	Phase of study	**0 ppm**	**250 ppm**	**500 ppm**	**3000 ppm**
	P D0	294.0	291.6	298.4	299.7
♂	P terminal bw	473.1	460.7	456.1	419.4*
	P BWG	179.1	169.1	157.7	119.7
	F1 D0	266.2	254.3	250.6	/
	F1 terminal bw	464.5	440.8*	426.6*	/
	F1 BWG	198.3	186.5	176.0	/
	P D0	206.1	206.8	209.2	209.5
♀	P premating-mating (D70)	244.1	244.9	239.5	233.4*
	P gestation (D20)	345.3	340.9	340.0	284.7**[a]
	P lactation (D21)	284.2	287.4	287.4	/
	P terminal bw	277.2	283.1	280.9	245.1*[a]
	P BWG	71.1	76.3	71.7	35.6[a]
	F1 D0	172.3	166.7	169.1	/
	F1 premating-mating (D70)	236.2	227.5	230.8	/
	F1 gestation (D20)	323.8	313.3	311.8	/
	F1 lactation (D21)	281.4	267.8*	271.2	/
	F1 terminal bw	277.2	262.9*	265.7	/
	F1 BWG	104.9	96.2	96.6	/

*: $p \leq 0.05$ **: $p \leq 0.01$ [a]: based on 2 dams only

In addition, the study revealed a decrease in the absolute weight of the brain and other

organs of the P-generation at the highest dose. The absolute brain weight was reduced in the highest dose animals compared to controls (2.092/1.955, 2.075/1.941, 2.044/1.951 and 2.006*/1.853* g at 0, 250, 500 and 3000 ppm in males/females, respectively, *significantly different from controls; $p \leq 0.05$). Furthermore, mild to moderate degeneration/necrosis was observed in the cerebellum in the animals of the highest dose (30/30 males and 28/30 females). Ovary weights were also modified in the highest dose animals (0.058/0.058, 0.059/0.057, 0.055/0.054 and 0.067*/0.071* g (left/right ovary, *significantly different to controls; $p \leq 0.05$) at 0, 250, 500 and 3000 ppm). Moreover, changes in the total number of corpora lutea were observed (24.9, 23.0, 15.6 and 41.3 at 0, 250, 500 and 3000 ppm).

Sperm parameters were analysed during the study and revealed some modifications in the P-generation. P males at the highest dose had a significantly lower epididymal sperm count compared to controls. P males at 500 and 3000 ppm had a significantly lower percentage of normal sperm with concomitant increases in the percentage of abnormal and detached sperm (table below, modified from Table 17 of the CLH report). Sperm motility was not affected.

Table: Sperm parameters in the P-generation

	Sperm motility		**Total sperm count**		**Sperm morphology**		
	% motile	**% progressive**	**Epididymis**	**Testis**	**% normal**	**% abnormal**	**% detached**
0 ppm	76.2	55.9	58.2	72	98.7	0.8	0.5
250 ppm	78.9	56.5	57	63.1*	98.1	1	0.8
500 ppm	78.9	56.4	65.7	64.4	97.0*	1.4*	1.6*
3000 ppm	78.9	57.3	43.2*	61.2*	95.7*	1.5*	2.8*

*: $p \leq 0.05$

The P-generation in the highest dose group produced only two litters containing one female pup each. No pup of this dose group survived lactation. The fertility index was severely decreased in the highest dose group compared to the control group (table below, modified from Table 18 of the CLH report). Due to this finding, further testing with 3000 ppm in the F1-generation was not performed.

Table: Reproductive data from P-generation

ppm	**No. of estrous cycle**	**Estrous cycle length (d)**	**Mating index (%)**	**Fertility (%)**	**No. of implantations**	**Duration of gestation**	**Mean no. of live pups**	**Sex ratio (% males)**	**Viability index**
0	3.6	4.2	100.0	76.7	265	22.3	233	54.1	96.2
250	3.8	4.2	100.0	83.3	310	22.0	279	55.4	97.1
500	3.4	4.4	96.7	86.2	279	22.2	260	50.7	99.6
3000	3.6	4.2	93.3	7.1**	3	23.5	2	/	100.0

*: $p \leq 0.05$ **: $p \leq 0.01$

Lower terminal bw were observed in the F1-generation at 250 and 500 ppm (table below). No significant changes in organ weight were observed. Furthermore, no significant changes were seen in the male and female reproductive parameters (tables below, modified from Tables 19 and 20 of the CLH report). A slight decrease in the number of implantation sites and fertility index was observed.

Table: Sperm parameters in the F1-generation

	Sperm motility		**Total sperm count**		**Sperm morphology**		
	% motile	**% progressive**	**Epididymis**	**Testis**	**% normal**	**% abnormal**	**% detached**
0 ppm	87.1	63.9	49.2	69.2	98.1	1.1	0.8
250 ppm	87.8	65.7	NE	NE	NE	NE	NE
500 ppm	89.5	67.6	48.6	68.3	97.9	1.4	0.7

*: $p \leq 0.05$

Table: Reproductive data in the F1-generation

ppm	**No. of estrous cycle**	**Estrous cycle length (d)**	**Mating index (%)**	**Fertility index (%)**	**No. of implantations**	**Duration of gestation**	**Mean no. of live pups**	**Sex ratio (% males)**	**Viability index (%)**
0	3.7	4.1	100.0	93.3	304	22.1	280	48.7	99.7
250	3.7	4.1	100.0	86.7	300	21.9	287	47.3	98.8
500	3.8	4.1	96.7	86.2	273	21.8	260	40.6	95.6

In the 28-day repeated dose toxicity study (Wahle, 2004a), 15 mice/sex/dose were given a diet containing 1,2,4-triazole at concentrations of 0, 50, 250, 500 or 2000 ppm (corresponding to doses of 0, 9, 47, 90 and 356 mg/kg bw/d in males and 0, 12, 60, 120 and 479 mg/kg bw/d in females).

No treatment-related effects were observed on survival, bw, organ weights, food consumption, clinical signs or on clinical chemistry parameters. The only treatment-related findings were histopathological modifications in testis and epididymis (table below, modified from Table 21 of the CLH report).

Table: Incidence of testicular and epididymal lesions in mice

Observed effects		**Dietary concentration in ppm**				
		0	50	250	500	2000
Epididymis	Incidence of exfoliated germ cells/ debris	0/15	1/15 (1)	1/15 (3)	0/15	3/15 (2)
Testis	Testicular degeneration	3/15	ND	ND	ND	5/15
	Incidence of apoptotic-like bodies	2/15 (1)	4/15 (1)	1/15 (1)	3/15 (3)	5/15 (1)
	Incidence of spermatid degeneration/depletion/asynchrony	1/15 (1)	1/15 (1)	1/15 (1)	0/15	5/15 (1.4)
	Incidence of focal tubular atrophy	1/15 (1)	2/15 (1)	1/15 (2)	2/15 (2)	4/15 (1.8)

(): average severity score of lesion (1 minimal to 5 severe)

ND, not determined

In a 30-day repeated dose toxicity study (anonymous, cited in US EPA memorandum, 2006), rats were exposed to 1,2,4-triazole at doses of 0, 8, 57 or 400 mg/kg bw/d. At the highest dose level lower bw and clinical signs were reported. A lower adrenal weight was seen at 8 mg/kg bw/d but no further data were reported.

In a 90-day repeated dose toxicity study (Bomhard et al., 1979) 15 rats/sex/dose were exposed to 1,2,4-triazole at a dietary concentration of 0, 100, 500 or 2500 ppm (corresponding to doses of approx. 0, 7.8, 37.8 and 212 mg/kg bw/d in males and 0, 10, 54 and 267 mg/kg bw/d in females). No effects on food consumption were observed at any dose level. Terminal bodyweights were reduced at the highest dose group in males and females compared to controls (table below). The absolute testis weight was decreased at the highest dose but no histological lesions were observed. Two males and two females at 2500 ppm exhibited temporary slight convulsions.

Table: Bodyweight and testis weight data

		0 ppm	**100 ppm**	**500 ppm**	**2500 ppm**
Males	Mean initial bw [g]	82	82	82	82
	Terminal bw [g]	335	342	344	306**
	Testis weight [mg]	3418	3308	3247	3215*
Females	Mean initial bw [g]	78	78	78	78
	Terminal bw [g]	195	195	187	184*

*: $p \leq 0.05$ **: $p \leq 0.01$

In a 90-day repeated dose toxicity study (Wahle *et al*., 2004b) 20 mice/sex/dose were exposed orally via diet to 1,2,4-triazole at concentrations of 0, 500, 1000, 3000 or 6000 ppm (corresponding to doses of 0, 80, 161, 487 and 988 mg/kg bw/d in males and 0, 105, 215, 663 and 1346 mg/kg bw/d in females). No treatment-related deaths were observed. An increased incidence of tremors was observed in both sexes at the highest dose level. Bw was decreased in the two highest dose groups in both sexes (table below, modified from Table 22 of the CLH report)

Table: Bw at D84 and total bwg in males and females

		0 ppm	**500 ppm**	**1000 ppm**	**3000 ppm**	**6000 ppm**
Bw at D84 (in g)	♂	37.3	37.0	36.4	34.9*	31.3*
	♀	29.1	28.4	28.4	28.7	26.6*
Total bwg (in g)	♂	3.1	3.6	1.7	1.1*	-3.1*
	♀	3.5	3.1	3.0	2.7	0.9*

*: $p \leq 0.05$

Absolute brain weights were significantly reduced at 6000 ppm in males and females and in males also at 3000 ppm. At the highest dose level an increased incidence of Purkinje cell loss was observed. Absolute testis weights were significantly decreased at 6000 ppm. Dose-dependent histopathological modifications were observed such as an increased incidence of apoptotic-like bodies, of spermatid degeneration/depletion/asynchrony and of tubular atrophy. These findings are summarised in the table below (modified from Table 23 of the CLH report).

Table: Organ weight and histopathological findings

			0 ppm	**500 ppm**	**1000 ppm**	**3000 ppm**	**6000 ppm**
Terminal bw (in g)		♂	36.9	35.8	34.9*	33.9*	30.5*
		♀	28.1	27.9	28.0	27.9	26.0*
Brain	Abs. weight (g)	♂	0.488	0.491	0.476	0.465*	0.445*
		♀	0.485	0.489	0.483	0.475	0.451*
	Rel. weight (%)	♂	1.328	1.378	1.365	1.376	1.462*
		♀	1.737	1.756	1.731	1.717	1.734
	Incidence of Purkinje cell loss	♂	0/20	0/20	0/20	0/20	15*/20 (1.7)
		♀	0/20	0/20	0/20	0/20	10*/20 (1.3)
Testis	Abs. weight (in g)		0.253	0.247	0.233	0.233	0.219*
	Rel. weight (in %)		0.688	0.692	0.669	0.687	0.719
	Incidence of apoptotic-like bodies		4/20 (1.0)	4/20 (1.3)	7/20 (1.1)	11*/20 (1.3)	12*/20 (1.2)
	Incidence of spermatid degeneration/depletion/asynchrony		1/20 (1.0)	0/20	0/20	5/20 (1.4)	15*/20 (2.0)
	Incidence of tubular atrophy		0/20	0/20	2/20 (1.5)	3/20 (1.0)	10*/20 (1.8)
Epididymis	Incidence of exfoliated germ cell/debris		0/20	0/20	0/20	0/20	10*/20 (2.5)

(): average severity score (1 minimal to 5 severe) *: $p \leq 0.05$

An additional group of 15 mice/sex was exposed for 28 days and then killed for hepatic enzyme analysis. An increased activity of EROD, ECOD, ALD and GLU-T were seen in both sexes at 3000 or 6000 ppm. These changes in enzyme activity did not correlate with changes in liver weights or histopathology and were therefore considered to be adaptive changes. A higher incidence in yellow staining and rough coat in males at 6000 ppm was noted during clinical observation.

In a combined 90-day repeated dose toxicity and neurotoxicity study (Wahle and Sheets, 2004) 20 rats/sex/dose were exposed to 1,2,4-triazole at dietary concentrations of 0, 250, 500, 3000 or 1000/4000 ppm (1000 ppm for the first 4 weeks and 4000 ppm thereafter; corresponding to doses of approximately 0, 16, 33, 183 and 210 mg/kg bw/d, respectively, in males and 0, 19, 41, 234 and 275 mg/kg bw/d in females). No treatment-related effects on food consumption, haematology and urine analysis were observed. A decreased bw was observed at the two highest dose levels in both sexes (table below, modified from Table 24 of the CLH report). A FOB examination revealed changes at the two highest dose levels such as ungroomed appearance, red nasal stain, urine stain, muscle fasciculations, gait incoordination, decreased activity in open field in males and tremor and decreased rearing in both sexes at 3000 ppm. Red nasal stain, decreased activity in the open field and increased splay foot were seen in males, urine stain in females and ungroomed appearance, muscle fasciculations, tremor, gait incoordination, decreased rearing and uncoordinated righting in both sexes at 1000/4000

ppm dose level. A slight but not significant decrease in uterus weight and slight increase in the number of corpora lutea were observed in females at 3000 and 1000/4000 ppm.

Table: Body weight data and corpora lutea information

		0 ppm	**250 ppm**	**500 ppm**	**3000 ppm**	**1000/4000 ppm**
Bw (D0) (g)	♂	265.6	267.4	267.0	267.1	266.1
	♀	181.2	181.4	180.7	179.9	182.7
Bw (D91) (g)	♂	437.9	439.7	443.0	407.9*	401.9*
	♀	245.1	246.9	244.4	231.7*	233.0
Bwg (D0-D91) (g)	♂	172.3	172.2	176.0	140.8*	135.9*
	♀	63.9	65.5	63.7	51.8*	50.3*
Total corpora lutea		33	NE	33	41	40
Recent cycle corpora lutea		16	NE	17	21	19

NE: not evaluated; *: $p \leq 0.05$

A dose-dependent decrease in thyroid stimulating hormone (TSH) was seen in males at all doses, statistically significant at 500 ppm and above. In the absence of any thyroid histopathology and changes in T3 and T4 concentrations, these decreases in TSH were considered not to be toxicologically relevant. The absolute brain weight was significantly decreased in males and females at 3000 ppm and in males at 1000/4000 ppm. Brain lesions were found in the more anterior dorsal cerebellum in males and females at 1000/4000 ppm and 3000 ppm. Also an increased incidence of degeneration of sciatic nerve, tibial nerve and sural nerve was reported.

In a chronic repeated dose toxicity study (Wahle, 2010) 20 rats/sex/dose were exposed to 1,2,4-triazole during 12 months via diet at a concentration of 0, 125, 375, 1000 and 2000 ppm (corresponding to doses of 0, 7, 21, 58 and 113 mg/kg bw/d in males, respectively, and 0, 8, 26, 71, 136 mg/kg bw/d in females). No treatment-related deaths or effects on food consumption, clinical signs, haematology, clinical chemistry, organ weight, gross pathology, estrous cycle staging and sperm analysis were observed. A lower bw and bwg were observed in both sexes at 1000 and 2000 ppm (bwg (D0-D343): 293/116 g at 1000 ppm, 294/115 g at 2000 ppm vs. 318/144 g in control group in males/females). Histological changes in the cerebellum were seen at the highest dose level. It was characterised by an increased incidence of Purkinje cell loss within the vermis.

No human data are available for evaluation.

Conclusion

Seven studies were evaluated for effects of 1,2,4-triazole on fertility and sexual function.

The two-generation reproductive toxicity study in rats (Young and Sheets, 2005) is regarded as the main study. Almost total infertility was observed at the highest dose level of 3000 ppm in the P-generation. Due to the low number of offspring, the highest dose was not tested in the F1-generation. A significantly higher number of corpora lutea was noted at the top dose in the P-generation (24.9, 23.0, 15.6 and 41.3 at 0, 250, 500 and 3000 ppm). Other adverse effects that may have contributed to infertility observed

in this study are the increased incidence of uterus dilatation at the highest dose, as well as reductions in sperm count and the number of sperm with normal morphology. These findings explain the adverse effects of 1,2,4-triazole on fertility and sexual function. Other effects at the highest dose level were cerebellar degeneration/necrosis, which were observed in both sexes of the P-generation. Since effects of systemic toxicity were minimal (the P-generation had a viability index of 100% after exposure to 3000 ppm 1,2,4-triazole) the adverse effects on fertility and sexual function are not considered to be non-specific and secondary to systemic toxicity.

Additional supporting studies showed histopathological modifications in the testes. Two studies in rats (Wahle, 2004a and 2004b) revealed an increased incidence in spermatid degeneration/depletion/asynchrony. Lower uterus weight and a slight increase in the number of corpora lutea was observed at the highest doses of a combined 90-day repeated dose toxicity and neurotoxicity study in rats (Wahle and Sheets, 2004). These effects on reproductive organs are not considered to be secondary to other types of toxicity.

Classification in category 1A is not appropriate, as no epidemiological studies are available.

Due to the pronounced impact of 1,2,4-triazole on fertility in rats, Category 2 is not considered to be appropriate.

Classification in category 1B shall be based on data from animal studies which "provide clear evidence of an adverse effect on sexual function and fertility […] in absence of other toxic effects, or if occurring together with other effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects". Based on the data on 1,2,4-triazole, the criteria for Category 1B are fulfilled and therefore **RAC agrees with the DS that 1,2,4-triazole warrants classification as Repr. 1B; H360F** for adverse effects on sexual function and fertility.

EFFECTS ON DEVELOPMENT

Summary of the Dossier Submitter's proposal

The DS assessed five studies on 1,2,4-triazole to evaluate effects on development: two embryotoxicity studies, one developmental toxicity study, one prenatal developmental toxicity study and one two-generation reproductive toxicity study.

In an embryotoxicity study (the purity of the test substance: 94.4%) in rats the foetuses exhibited a lower bw and the incidence of runts was significantly higher at 100 and 200 mg/kg bw/d than in controls. At the top dose of 200 mg/kg bw/d, the number of foetuses per dam was reduced due to an increased incidence in post-implantation losses and the incidence of foetuses with malformations (undescended testicle, cleft palate and hydronephrosis) was increased. In dams no mortalities or clinical signs were observed. At the highest dose, maternal bw was decreased at GD20 and the bwg was significantly reduced. The severe developmental effects were considered not to be secondary to maternal toxicity.

A second embryotoxicity study (the purity of the test substance: 95.3%) was conducted in rats. At the highest dose (100 mg/kg bw/d) foetal weight was significantly decreased and the incidence of runts was increased. A slight increase in the number of

malformations was observed at the highest dose (2 microphthalmia, 1 anophthalmia) compared to controls (1 foetus with bilateral microphthalmia). In dams no mortalities were observed, but the bwg during the exposure period was significantly reduced at the top dose.

Both embryotoxicity studies were performed with the same batch of the test substance. Two impurities, which have a harmonised classification and labelling as Repr.1B, H360D*** (2%) and Repr. 2, H361d*** (0.8%), respectively, were identified. The DS assessed the potential role of the impurities to the observed adverse effects on development and concluded that these effects were due to 1,2,4-triazole and not to the impurities in the tested batch and that the developmental toxicity study (Renhof, 1988a) was adequate and reliable for the classification of 1,2,4-triazole.

In a non-guideline developmental toxicity study on 1,2,4-triazole (purity and vehicle not reported) in rats, offspring observations were restricted to only few parameters such as the litter weight of live pups on PND 1 and 5 and the number of live and dead pups on these days. During these examinations, no effects were noted.

In a prenatal developmental toxicity study in rabbits on 1,2,4-triazole (purity 99.9%), conducted in accordance with the OECD TG 414, the litter averages for corpora lutea, implantations, litter size, live foetuses, dead foetuses, early and late resorptions, percent of dead or resorbed conceptuses and percent live male foetuses were comparable among all groups. Gravid uterine weight and the foetal bw were statistically significantly lower at the highest dose of 45 mg/kg bw/d compared to the controls. At this dose, a few alterations in the urogenital system were detected. Three foetuses from one litter had one or two low set and small kidneys, in two foetuses from two litters the kidney was absent. The maternal toxicity consisted of deaths (5/25 dams were sacrificed due to moribund condition), but among the surviving rabbits there were no significant changes on bw, food consumption and gross pathology in the high dose group.

In the two-generation reproductive toxicity study on 1,2,4-triazole (purity 99.9%) in rats live birth and viability indices, mean litter size, sex ratios and clinical signs were not altered in the triazole-treated groups compared to the control groups. Gross necropsy findings were similar between all dose levels. Bw and bwg changes were not observed in pups of the P-generation. The bw of the pups in the F2-generation examined at PND 0 and 21 were reduced compared to controls, and there was no maternal toxicity at this dose.

The DS concluded that the evidence was strong enough to warrant classification in category 1B (Repr. 1B; H360D).

Comments received during public consultation

Three MSCAs commented on developmental toxicity and all three supported the proposed classification as Repr. 1B; H360D. One MSCA pointed out that the decreased maternal bwg may have been related to the decreased number of foetuses and/or decreased foetal weight in the embryotoxicity studies (Renhof, 1988a and b). The DS responded that the uterus weight and thus the adjusted maternal bw was only available in Renhof (1988a).

Assessment and comparison with the classification criteria

In five animal studies, adverse effects on development were investigated (table below,

modified from Table 26 of the CLH report). The embryotoxicity study in rats (Renhof, 1988a) is regarded by RAC as the main study.

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

Method, guideline, deviations if any, species, strain, sex, No/group	**Test substance, dose levels, duration of exposure**	**Results**	**Reference**
Embryo-toxicity study in rats (Bor: Wisw(SPF Cpb)) 25 females/group Oral EPA OPPTS 83-3 guidance GLP	1,2,4-triazole (purity 94,0%) Doses: 0, 100 and 200 mg/kg bw/d Exposure: GD 6-15 Vehicle: cremophor-EL emulsion 0.5%	Dams: No mortality and no clinical signs observed. At the highest dose, bw was decreased at GD 20; bwg was significantly decreased The mean number of corpora lutea per dam was significantly increased at the highest dose group (13.6 ± 1.2, 13.9 ± 1.6 and 14.2 ± 2.2 at 0, 100 and 200 mg/kg bw/d, respectively) Developmental toxicity: No significant changes in the number of dams fertilised or in the number of implantation sites per dam, but a significant increase in post-implantation loss (0.5, 0.3 and 6.3 at 0, 100 and 200 mg/kg bw/d) was observed in the highest dose group due to a high rate of resorptions The mean number of foetuses per dam was significantly decreased at the highest dose (5.5 at 200 mg/kg bw/d) vs. 12.0 in control group) The mean foetal weight was significantly reduced at both doses (3.55, 3.06 and 2.35 g at 0, 100 and 200 mg/kg bw/d). The mean placental weight was significantly decreased (0.59, 0.52 and 0.49 g at 0, 100 and 200 mg/kg bw/d). The number of foetuses per litter with malformations was increased at the highest dose (0.80 vs. 0.29 in the control group). NOAEL (maternal toxicity): 100 mg/kg bw/d NOAEL (developmental toxicity): < 100 mg/kg bw/d	Renhof M., 1988a
Embryotoxi-city study in rats (Bor: Wisw(SPF Cpb)) 25 females/group Oral EPA OPPTS 83-3 guidance	1,2,4-triazole (purity 95,3%) Doses: 0, 10, 30 and 100 mg/kg bw/d Exposure: GD 6-15 Vehicle: cremophor-EL emulsion 0.5%	Dams: Bwg during exposure was significantly reduced at the highest dose (28.2, 25.4, 26.8 and 21.8 g at 0, 10, 30 and 100 mg/kg bw/d). No change in the number of implantations per dam. Developmental toxicity: A significantly increased incidence of runts at the highest dose (0.33, 0.23, 0.53 and 2.21 at 0,	Renhof M., 1988b

GLP		10, 30 and 100 mg/kg bw/d). A significantly lower foetal weight at the highest dose (3.25 vs 3.58 g in control group). No treatment-related increase in incidence of malformations NOAEL (maternal toxicity): 30 mg/kg bw/d NOAEL (developmental toxicity): 30 mg/kg bw/d	
Developmental study in rats (Wistar) 10 females/dose Oral Non-guideline, non-GLP	1,2,4-triazole (purity not reported) Doses: 0, 25 and 100 mg/kg bw/d Exposure: GD 7 through 17 Vehicle: not reported	Maternal observations: Bw not affected Developmental toxicity: No effects on litter weight (PND 1 and 5) or on number of live or dead pups (PND 1 and 5) NOAEL (maternal toxicity): 100 mg/kg bw/d NOAEL (developmental toxicity): 100 mg/kg bw/d	Wickramaratne, 1987 (cited in JMPR, 2008)
Prenatal developmental toxicity study in rabbits (NZW) 25 females/dose Oral (gavage) Following OECD TG 414 GLP	1,2,4-triazole (purity 99,9%) Doses: 0, 5, 15, 30 and 45 mg/kg bw/d Exposure: GD 6-28 Vehicle: aqueous 0.5% carboxy-methyl-cellulose	Dams: Mortality: at the highest dose, 5 females were sacrificed due to their moribund condition; treatment-related clinical signs were observed in four additional rabbits. The bwg over the entire gestation period was significantly reduced at the highest dose (0.37 g vs. 0.65 g in controls). However, the maternal bw at GD 29 was not modified significantly. No modification in the number of corpora lutea, the number of implantations, the litter size, the incidence of early and late resorptions. Developmental toxicity: A significant lower gravid uterine weight was observed at the highest dose (0.46 kg vs. 0.56 kg in controls). Foetal bw was significantly reduced at the highest dose (39.46 g vs. 44.35 g in controls), a higher incidence of alterations of the urogenital system was observed at the highest dose (two fetuses had small kidneys, in two fetuses kidneys were absent). NOAEL (maternal toxicity): 30 mg/kg bw/d NOAEL (developmental toxicity): 30 mg/kg bw/d	Hobermann, 2004 (cited in JMPR, 2008)
2-generation reproductive toxicity study in rats (Wistar Hannover)	1,2,4-triazole (purity ≥ 99,9%) Doses: 0, 250, 500 and 3000 ppm (males: 0, 15, 31 and 189 mg/kg bw/d; females: 0, 18, 36	For more details, see the fertility section. Dams: As the severe decrease in the fertility index at 3000 ppm (corresponding to less than 235 mg/kg bw/d) led to a premature termination of this dose level, the highest dose for the F1	Young and Sheets, 2005

	and 218 mg/kg bw/d) Exposure: Through 10 weeks premating period to lactation D21 Vehicle: ethanol	dams was 500 ppm corresponding to less than 40 mg/kg bw/d No treatment-related deaths or clinical signs were observed at any dietary dose level. In P dams the bw was decreased at certain time points at the tested top dose as compared to the controls. Developmental toxicity: 500 ppm was the highest dose allowing assessment of the developmental effects due to the low number of pups at 3000 ppm. At 250 and 500 ppm, bw of the F2-pups at PND 0 and PND 21 were statistically significantly reduced compared to controls. At 250 and 500 ppm, no treatment-related effects were observed in the sex ratio, the viability index, and the micropathological evaluation of the pups. NOAEL (parental toxicity): 500 ppm NOAEL (developmental toxicity): 500 ppm	

In an embryotoxicity study (Renhof, 1988a), 25 pregnant female rats per group were exposed orally to 1,2,4-triazole at 0, 100 and 200 mg/kg bw/d on gestational day (GD) 6 to 15. It is noted that the triazole used in this experiments had a purity of 94.0 %. No information on the impurities was given in the study report, but two impurities were identified in the same batch of 1,2,4-triazole tested in Renhof (1988b) that have a harmonised classification and labelling as Repr. 1B; H360D*** and Repr. 2; H361d***, respectively.

No maternal treatment-related changes in food consumption or clinical signs were observed. All animals survived until the scheduled sacrifice. The maternal bwg was slightly decreased at the highest dose level as shown by the bw data, but the adjusted maternal bwg was not affected (table below, modified from Table 29 of the CLH report). There were no treatment-related effects on pregnancy parameters.

Table: Maternal bodyweight and bodyweight gain (in g)

Dose (mg/kg bw/d)	**Bw at GD 0**	**Bw at GD 6**	**Bw at GD 15**	**Bw at GD 20**	**Bwg during exposure period**	**Bwg during entire pregnancy**	**Mean gravid uterus weight**	**Adjusted maternal bwg**
0	204.6	221.5	250.9	301.4	29.3	96.8	66	30.8
100	203.8	222.5	249.8	295.7	27.4	91.9	57.74	34.16
200	203.2	220.3	241.8	263.6	21.5*	60.4**	27.16	33.24

*: $p \leq 0.05$ **: $p \leq 0.01$

Foetal weight and placental weight were reduced at 100 and 200 mg/kg bw/d and the incidence of runts was statistically significantly higher at these dose levels (table below, modified from Table 30 of the CLH report). The number of surviving foetuses per dam was significantly reduced and the number of resorptions was increased at 200 mg/kg bw/d (53.2% vs. 3.9% in controls). Furthermore, the incidence of malformations such as

undescended testicle, hydronephrosis and cleft palate was increased at that dose level (tables below, modified from Table 31 of the CLH report).

Table: Intrauterine development parameters

	0 mg/kg bw/d	**100 mg/kg bw/d**	**200 mg/kg bw/d**
Number of corpora lutea per dam	13.6	13.9	14.2*
Number of implantation per dam	12.5	12.2	11.8
Number of runts per litter	0.24	2.84*	4.96**
Number of foetuses per dam	12.0	11.9	5.5**
Number of male/female foetuses per dam	5.9/6.1	6.0/5.9	3.1**/2.4**
Number of post-implantation loss per dam	0.5	0.3	6.3**
Mean foetuses weight (in g)	3.55	3.06**	2.35**
Mean placental weight (in g)	0.59	0.52*	0.49**
Foetuses per litter with minor skeletal variations	2.67	4.32*	2.24
Foetuses per litter with malformation	0.29	0.63	0.80*

*: $p \leq 0.05$ **: $p \leq 0.01$

Table: Observed malformations

	0 mg/kg bw/d	**100 mg/kg bw/d**	**200 mg/kg bw/d**
Total incidence of undescended testicle	2/253 (0.8 %)	11/226 (4.9 %)	6/138 (4.3 %)
Incidence per litter of undescended testicle	2/21 (9.5 %)	7/19 (36.8 %)	5/25 (20 %)
Total incidence of hydronephrosis	1/253 (0.4 %)	1/226 (0.4 %)	7/138 (5.1 %)
Incidence per litter of hydronephrosis	1/21 (4.8 %)	1/19 (5.3 %)	6/25 (24 %)
Total incidence of cleft palate	0/253	0/226	4/138 (2.9 %)
Incidence per litter of cleft palate	0/21	0/19	3/25 (12 %)

Regarding individual data, 3 dams (nr 2065, 2096 and 2110) exposed to the highest dose had a pup/pups with a cleft palate. The individual maternal body weight data did not indicate severe maternal toxicity and cannot explain this malformation (table below).

Table: Maternal body weight, number of living pups and number of pups with a cleft palate

Pregnant Rat Number	**Body Weight (g)**				**Number of living pups**	**Number of pups with cleft palate**
	GD0	GD6	GD14	GD20		
2065	193	213	238	261	3	1
2096	197	215	236	270	8	1
2110	193	212	234	248	4	2
Mean for the highest dose level (200 mg/kg bw/d)	203.2	220.8	238.4	263.6	5.5**	
Mean for the control group	204.6	221.5	244.5	301.4	12.0	

**: $p \leq 0.01$

In the second embryotoxicity study (Renhof, 1988b), 25 female rats/group were given 1,2,4-triazole at 0, 10, 30 or 100 mg/kg bw/d during GD 6-15. It is noted that the triazole used in this experiment had a purity of 95.3 %. It was the same batch as in Renhof (1988a). According to the study report, the test substance contained 2.0 % of an impurity with a harmonised classification as Repr. 1B; H360D*** and 0.8 % of an impurity with a harmonised classification as Repr. 2; H361d***. The nature of other impurities is unknown.

No maternal treatment-related changes in food consumption or clinical signs were observed. The bwg during the entire exposure period was significantly lower in the highest dose group (21.8 g vs. 28.2 g in controls). No treatment-related effects were observed on pregnancy parameters. The foetal weight was significantly reduced with a higher incidence of runts (table below, modified from Table 32 of the CLH report). The number of foetuses per litter with malformations was slightly increased at 100 mg/kg bw/d, but as the observed malformations affected only one foetus each, they were considered to be spontaneous in nature (tables below, modified from Table 33 of the CLH report).

Table: Effects on intrauterine development

	0 mg/kg bw/d	**10 mg/kg bw/d**	**30 mg/kg bw/d**	**100 mg/kg bw/d**
Number of implantation per dam	11.6	10.5	11.4	10.6
Number of runts per litter	0.33	0.23	0.53	2.21*
Number of foetuses per dam	11.0	10.1	10.6	9.5
Number of male/female foetuses per dam	6.5/4.5	5.1*/5.0	6.0/4.6	5.0*/4.5
Mean foetal weight (in g)	3.58	3.59	3.53	3.25**
Mean placental weight (in g)	0.56	0.56	0.57	0.56
Foetuses per litter with minor skeletal variations	2.00	2.41	2.84	2.42
Foetuses per litter with malformations	0.05	0.05	0.05	0.17

*: $p \leq 0.05$ **: $p \leq 0.01$

Table: Incidences of malformations

	0 mg/kg bw/d	**10 mg/kg bw/d**	**30 mg/kg bw/d**	**100 mg/kg bw/d**
Total no. of foetuses	231	222	202	228
Microphthalmia, bilateral	1	0	0	0
Microphthalmia, right side	0	1	0	1
Microphthalmia, left side	0	0	0	1
False posture of right hind leg	0	0	1	0
Anophthalmia	0	0	0	1
Dysplasia and asymmetry of body of vertebrae	0	0	0	1

In a developmental toxicity study (Wickramaratne, 1987) 10 female rats were exposed to 1,2,4-triazole at doses of 0, 25 or 100 mg/kg bw/d on GD 7-17. No change in bw or bwg was observed between treated and control animals. The offspring observations were limited to the litter weight of live pups on PND 1 and 5 and to the number of live and dead pups on these days. No effects on these parameters were reported. No specific examination for malformations was conducted.

In a prenatal developmental toxicity study (Hoberman, 2004), 25 pregnant female rabbits were exposed to 1,2,4-triazole by gavage at doses of 0, 5, 15, 30 and 45 mg/kg bw/d on GD 6-28. Between GD 16 and 24, 5 out of 25 females at the highest dose were sacrificed due to their moribund condition (decreased food consumption and bw, decreased motor activity, soft and/or liquid faeces). The examination of the surviving females revealed no significant changes on bw, food consumption or gross pathology. A significant decrease in gravid uterine weight was observed at 45 mg/kg bw/d (table below, modified from Table 34 of the CLH report). The mean number of corpora lutea and implantations in the treated groups were similar to controls, but dead or resorbed conceptuses (%) increased from 3.1 (controls) to 7.0 (45 mg/kg bw/d) (tables below, from Table 35 of the CLH report).

Table: Maternal bodyweight data

	0 mg/kg bw/d	**5 mg/kg bw/d**	**15 mg/kg bw/d**	**30 mg/kg bw/d**	**45 mg/kg bw/d**
Bw at GD29 (kg)	4.04	3.95	3.93	4.00	3.76
Gravid uterine weight (kg)	0.56	0.54	0.51	0.53	0.46**
Corrected maternal bw	3.48	3.40	3.42	3.46	3.31[a]

[a]: excludes values for rabbits that were moribund sacrificed or prematurely delivered

**: $p \leq 0.01$

Table: Litter observations

	0 mg/kg bw/d	**5 mg/kg bw/d**	**15 mg/kg bw/d**	**30 mg/kg bw/d**	**45 mg/kg bw/d**
No. of dams examined	25	24	24	25	19
Corpora lutea	9.8	9.8	9.9	10.2	9.8
Implantations	9.0	9.0	8.8	9.3	9.0
Early resorption (n)	1	2	4	10	6
Late resorption (n)	7	8	7	3	8
Litter size (n)	8.7	8.6	8.3	8.8	8.3
Live foetuses (n)	217	207	199	218	157
Foetal bw (g)	44.35	43.42	43.82	42.48	39.46**
Dead foetuses (n)	0	0	0	1	0
Dead or resorbed conceptuses (%)	3.1	4.7	4.8	6.4	7.0
Percent live male foetuses	59.0	56.9	53.2	56.6	60.6

**: $p \leq 0.01$

Foetal bw was significantly lower at 45 mg/kg bw/d compared to the controls. No

skeletal, soft tissue or gross external foetal alterations were observed at doses up to 30 mg/kg bw/d. Alterations of the urogenital tract were observed at the highest dose. Three foetuses from one litter had one or two low set and small kidneys, two foetuses from two litters had an absent kidney. However, the highest dose was associated with severe maternal toxicity (5/25 females were sacrificed due to their moribund condition) (Hoberman, 2004) and the data at that dose level was not considered for further evaluation by RAC.

In the two-generation reproductive toxicity study (Young and Sheets, 2005; more details are provided in the fertility section), no treatment-related deaths or clinical signs were observed. P males and females in the highest dose group had a significantly lower terminal bw compared to the control group. Absolute brain and ovary weights were also reduced in males and females at 3000 ppm.

The highest dose allowing assessment of the developmental effects was 500 ppm due to the low number of pups at 3000 ppm. At 250 and 500 ppm, live birth and viability indices, mean litter sizes, sex ratios, clinical signs and gross necropsy were comparable to controls in both generations. At these doses, bw and bwg changes were not observed in pups in the F1-generation. Bw of the pups in the F2-generation was reduced compared to the controls (the table below, from Table 39 of the CLH report).

Table: Body weight (g) of pups in the F1- and F2-generation

		F1-generation				**F2-generation**		
		0 ppm	**250 ppm**	**500 ppm**	**3000 ppm**	**0 ppm**	**250 ppm**	**500 ppm**
D0	♂	6.3	6.0	6.2	/	6.3	6.0*	5.8**
	♀	6.0	5.6	5.9	5.4	6.0	5.6**	5.5**
	♂+♀	6.2 (22)	5.9 (25)	6.1 (25)	5.4 (2)	6.2 (27)	5.8** (26)	5.7** (25)
D7	♂	17.0	16.1	16.2	/	16.9	16.1	16.1
	♀	16.1	15.5	15.4	9.1**	16.3	15.6	15.8
	♂+	16.5 (22)	15.8 (25)	15.7 (25)	9.1** (2)	16.6 (27)	15.9 (26)	16.0 (24)
D21	♂♀	52.0	50.2	50.5	/	51.2	47.5**	48.4**
	♀	49.4	47.9	47.6	/	49.4	45.9**	46.7*
	♂+♀	50.7 (22)	49.1 (25)	48.4 (25)	/	50.2 (27)	46.8** (26)	47.6* (24)

*: $p \leq 0.05$ **: $p \leq 0.01$

No human data were available for evaluation.

Conclusion

Five studies were evaluated for effects of 1,2,4-triazole on development.

In the developmental toxicity study in rats (Renhof, 1988a), an increased incidence of cleft palate (4 of 138 foetuses (2.9%); 3 of 25 litters (12%)) at 200 mg/kg bw/d was observed. This is above the historical control data (HCD, 1986-1989) given in the CLH-report (one case of cleft palate in 1987 (4.17% litter incidence) and one case of cleft palate in 1989 (7.69% litter incidence). The high rate of resorptions of 53.2% in the highest dose group may have masked some malformations. The incidence of undescended testicle was increased at 100 and 200 mg/kg bw/d. Additionally, the post-implantation losses were increased and the number of foetuses per dam was significantly decreased. The foetal weight was dose-dependently decreased at 100 and 200 mg/kg

bw/d. The adjusted maternal bwg was not affected.

One supporting developmental toxicity study in rats revealed a significantly increased incidence of runts and a decreased mean foetal weight at 100 mg/kg bw/d (Renhof M., 1988b). A developmental toxicity study in rabbits showed a dose-related increase in the incidence of dead or resorbed conceptuses per litter at 30 and 45 mg/kg bw/d (Hoberman, 2004). However, the highest dose was associated with severe maternal toxicity (5/25 females were sacrificed due to their moribund condition) and the data at that dose level were not considered for further evaluation by RAC.

In the two developmental toxicity studies in rats the test substance had a purity of 94.0% (Renhof, 1988a) and 95.3% (Renhof, 1988b). Two confidential impurities were identified: one impurity (impurity X) is currently classified as Repr. 2; H361d *** and occurred at a concentration of 0.8%. The second impurity (impurity Y) is currently classified as Repr. 1B; H360D*** and occurred at a concentration of 2.0%. To assess if the impurities at such concentrations could explain the observed effects in the developmental toxicity study and if the classification would not be justified for pure 1,2,4-triazole, RAC evaluated the developmental toxicity data on these substances. Impurity Y induced malformations and other embryo-/fetotoxic effects in mice, rats and rabbits. In rats, the lowest developmental NOAEL for the impurity Y was set at 50 mg/kg bw/d, based on decreased foetal body weight, and these effects occurred at lower doses than maternal toxicity. The highest dose of 200 mg 1,2,4-triazole/kg bw/day used in the developmental toxicity study corresponded to a dose of 4 mg/kg bw/day of the impurity Y. As the doses expressing developmental toxicity of the tested 1,2,4-triazole corresponded to much lower doses of the impurity Y than its lowest NOAELs for different species, RAC concludes that the malformations (cleft palate) and other adverse effects on development in rats which were described in the Renhof (1988a) study are caused by 1,2,4-triazole and not by the impurity Y. Regarding impurity X, RAC concludes that the developmental toxic doses of the tested 1,2,4-triazole corresponded to much lower doses of this impurity (1.6 mg/kg bw/day at 200 mg/kg bw/day of 1,2,4-triazole) than its lowest NOAELs for developmental toxicity in the tested species (rat and rabbit). The NOAEL for developmental toxicity in rat was set at 100 mg/kg bw/day for the impurity X. RAC concludes that the malformations (cleft palate) and other adverse effects on development in rats described in the Renhof (1988a) study are caused by 1,2,4-triazole and not by the impurity X. RAC further concludes, that the studies by Renhof (1998a and b) are reliable and adequate for classification.

Classification in category 1A is not appropriate, as no epidemiological studies are available.

Due to the clear impact of 1,2,4-triazole on development in rats, Category 2 is not considered to be appropriate.

Classification in category 1B should be based on data from animal studies which "provide clear evidence of an adverse effect on […] development in absence of other toxic effects, or if occurring together with other effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects". According to the data on 1,2,4-triazole the criteria for category 1B are fulfilled and therefore **RAC agrees with the DS that 1,2,4-triazole warrants classification as Repr. 1B; H360D** for adverse effects on development of the offspring.

Overall, for reproductive toxicity **RAC agrees with the DS that 1,2,4-triazole warrants classification as Repr. 1B; H360FD.**

10.11 Specific target organ toxicity-single exposure

Not evaluated in this dossier.

10.12 Specific target organ toxicity-repeated exposure

Not evaluated in this dossier.

10.13 Aspiration hazard

Not evaluated in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in this dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this dossier.

13 ADDITIONAL LABELLING

Not relevant.

14 ABBREVIATIONS

* : p<0.05, statistically significant

** : p<0.01, statistically significant

♂ : male

♀ : female

bw : body weight

bwg : body weight gain

Calc. : Calcium

Cl : chloride

D : day

FOB : functional observational battery

GD : gestational day

Hct : hematocrit

HDW : haemoglobin distribution width

Hgb : hemoglobin

K : potassium

MCH : mean cell hemoglobin

MCV : mean cell volume

NA : not applicable

ND : not determined

NE : not examined

NOAEL : no observed adverse effect level

PND : post-natal day

RDW : red cell distribution width

Trigl. : triglyceride

TSH : thyroid stimulating hormone

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16 ANNEXES

- *Annex I to CLH report*
- *Confidential annex to the CLH report*

Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

1,2,4-triazole

EC Number: 206-022-9

CAS Number: 288-88-0

Index Number: 613-111-00-X

Contact details for dossier submitter:

FPS Public Health, Food Chain Safety and Environment
DG 5/ **Department of Product Policy and chemical Substances / Management of Chemical Substances**
BELGIUM

Version number: 2 **Date: February 2018**

CONTENTS

1 PHYSICAL HAZARDS

Not evaluated in this dossier.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not evaluated in this dossier.

3 HEALTH HAZARDS

3.1 Acute toxicity – oral route

3.1.1.1 Acute oral toxicity

Study reference:

US EPA HPV Challenge Program, Test plan submission, July 2009, 1H-1,2,2-triazole CAS No 288-88-0

Detailed study summary and results:

Test type

According to OECD Guideline 401

Test substance

- *1,2,4-triazole*
- *Degree of purity : > 98%*
- *Batch number : Z81429*

Test animals

- *Species/strain/sex : Rat/Wistar/ male and female*
- *No. of animals per sex per dose : 5/sex/dose*

Administration/exposure

- *Mode of administration (gavage, in diet, other) : oral : gavage*
- *Doses/concentration levels : 1000, 1500 and 2000 mg/kg bw*
- *Post exposure observation period : 15 days*
- *Control group and treatment : no data*
- *Vehicle: bi-distilled water*

Results and reliability

- LD50 or LC50 value with confidence limits if calculated : 1320.39 mg/kg bw
- Number of deaths at each dose level : 1000mg/kg bw : no mortality

  In males : at 1500 mg/kg bw, 2 rats died after 24h and 2 after 2d and at 2000 mg/kg bw, 4 died after 24h and 1 after 2d.

  In females : at 1500 mg/kg bw, 3 rats died after 24h and 2 after 3d and at 2000 mg/kg bw, 4 died after 24h and 1 after 2d.

- Clinical signs: ≥ 1000 mg/kg : sedated, ventral recumbency, dyspnea
- Necropsy findings, including doses affected, severity and number of animals affected : No data available

3.1.2 Acute oral toxicity

Study reference:

Thyssen and Kimmerle, 1976. Cited in JMPR, 2008

Detailed study summary and results:

Test type

According to OECD Guideline 423

Test substance

- *1,2,4-triazole*

Test animals

- *Species/strain/sex : Rat/Wistar/ male and female*
- *No. of animals per sex per dose : 15rats/sex/dose : 100 (only ♀), 250, 500, 1000 (30♂ and 15♀), 1250, 1500, 1750, 1850 (only ♂), 2000 (15♂ and 30♀) and 2500 (14♂ and 15♀) mg/kg bw*

Administration/exposure

- *Mode of administration (gavage, in diet, other) : oral : gavage*
- *Doses/concentration levels : 100 (only ♀), 250, 500, 1000 (30♂ and 15♀), 1250, 1500, 1750, 1850 (only ♂), 2000 (15♂ and 30♀) and 2500 (14♂ and 15♀) mg/kg bw*
- *Post exposure observation period : 14 days*
- *Control group and treatment : no data*
- *Vehicle: distilled water and Cremophor EL*

Results and reliability

- *LD50 or LC50 value with confidence limits if calculated :*

  LD50 (females) : 1648 mg/kg

  LD50 (males) : 1650 mg/kg
- *Number of deaths at each dose level : no data*
- *Time of death (provide individual animal time if less than 24 hours after dosing) : no information available*
- *Clinical signs: reduction in general well-being, sedation, breathing disorders*
- *Necropsy findings : no major changes*

3.1.3 Acute oral toxicity

Study reference:

Procopio and Hamilton, 1992. Cited in JMPR, 2008

Detailed study summary and results:

Test type

According to OECD Guideline 423

Test substance

- *1,2,4-triazole*

Test animals

- *Species/strain/sex : Rat/Crl:CD BR/ male*
- *No. of animals per sex per dose : 3 male/dose*

Administration/exposure

- *Mode of administration (gavage, in diet, other) : oral : gavage*
- *Doses/concentration levels : 500 and 5000 mg/kg bw*
- *Control group and treatment : no*
- *Vehicle: methylcellulose*

Results and reliability

- *LD50 or LC50 value with confidence limits if calculated : > 500 - < 5000 mg/kg bw*
- *Number of deaths at each dose level : at 500 mg/kg bw no death and at 5000 mg/kg bw all rats died*
- *Time of death (provide individual animal time if less than 24 hours after dosing) : at 5000 mg/kg bw all rats died within 10 min*
- *Clinical signs: no effects*
- *Necropsy findings : 5000 mg/kg : reddened duodenum and reddened glandular portion of stomach*

3.2 Acute toxicity - dermal route

Not evaluated in this dossier.

3.3 Acute toxicity - inhalation route

Not evaluated in this dossier.

3.4 Skin corrosion/irritation

Not evaluated in this dossier.

3.5 Serious eye damage/eye irritation

Not evaluated in this dossier.

3.6 Respiratory sensitisation

Not evaluated in this dossier.

3.7 Skin sensitisation

Not evaluated in this dossier.

3.8 Germ cell mutagenicity

Not evaluated in this dossier.

3.9 Carcinogenicity

Not evaluated in this dossier.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 A two-generation reproductive toxicity study in rats

Study reference:

Young A.D. and Sheets L.P., 2005

Detailed study summary and results:

Test type

Following OECD TG 416

Following US EPA guideline : OPPTS 870.3800

Following GLP regulation

Test substance

- *1,2,4-triazole*
- *Degree of purity : ≥ 99.9 %*
- *Batch number : S13691*

Test animals

- *Species/strain/sex : Rats / Wistar Hannover / both sexes*
- *No. of animals per sex per dose : 30/sex/dose*
- *Age and weight at the study initiation : 9.5 weeks of age at the beginning of exposure*

Administration/exposure

- *Route of administration : oral (diet)*
- *duration and frequency of test/exposure period : P-gen and F1-gen : 10 weeks of premating period until lactation D21, daily*
- *doses/concentration levels : 0, 250, 500 and 3000 ppm corresponding to :*

	Phase of study	*250 ppm in mg/kg bw/d*	*500 ppm in mg/kg bw/d*	*3000 ppm in mg/kg bw/d*
♂	*Premating (P-gen)*	*15.4*	*30.9*	*188.6*
	Premating (F1-gen)	*16*	*32*	*NA*
♀	*Premating (P-gen)*	*17.5*	*36.2*	*217.9*
	Gestation (P-gen)	*18.6*	*38.6*	*231.7*[a]
	Lactation (P-gen)	*19.3*	*38.7*	*NA*
	Premating (F1-gen)	*18.9*	*37.5*	*NA*
	Gestation (F1-gen)	*17.4*	*34.4*	*NA*
	Lactation (F1-gen)	*20.3*	*35.8*	*NA*

[a] *: based on only 2 pregnant females*

- *vehicle: ethanol*

Description of test design:

- *details on mating procedure : mating was accomplished by co-housing one female with one male for up to 14 days. During this phase, vaginal smears were collected each morning to examine the presence or absence of sperm and/or the internal vaginal plug.*
- *premating exposure period for males and females (P and F1) : 10 weeks*
- *standardization of litters : yes. On lactation D4, each litter was adjusted (4 males and 4 females) by random selection*
- *parameters assessed for P and F1*
    - *oestrous cycle evaluation : during a 3-w period prior mating period*
    - *sperm examination : yes*

Results and discussion

NOAEL (parental toxicity) : 500 ppm based on lower bw and degenerative findings observed in the cerebellum at the highest dose level

NOAEL (fertility) : < 250 ppm based on the reduction in testicular sperm counts noted at 250 ppm

NOAEL (developmental toxicity) : 500 ppm which was the highest dose allowing assessment of the developmental effects as the 3000 ppm dose level had been stopped due to the low number of pups

For P and F1 adults (per dose):

- *time of death during the study and whether animals survived to termination : no mortality observed during the study*
- *body weight data (in grams) for P and F1 animals :*

	Phase of study	0 ppm	250 ppm	500 ppm	3000 ppm
♂	P D0	294.0	291.6	298.4	299.7
	P terminal bw	473.1	460.7	456.1	419.4*
	P BWG	179.1	169.1	157.7	119.7

	F1 D0	266.2	254.3	250.6*	/
	F1 terminal bw	464.5	440.8*	426.6*	/
	F1 BWG	198.3	186.5	176.0	/
♀	P D0	206.1	206.8	209.2	209.5
	P premating-mating (D70)	244.1	244.9	239.5	233.4*
	P gestation (D20)	345.3	340.9	340.0	284.7**[a]
	P lactation (D21)	284.2	287.4	287.4	/
	P terminal bw	277.2	283.1	280.9	245.1*[a]
	P BWG	71.1	76.3	71.7	35.6[a]
	F1 D0	172.3	166.7	169.1	/
	F1 premating-mating (D70)	236.2	227.5	230.8	/
	F1 gestation (D20)	323.8	313.3	311.8	/
	F1 lactation (D21)	281.4	267.8*	271.2	/
	F1 terminal bw	277.2	262.9*	265.7	/
	F1 BWG	104.9	96.2	96.6	/

** : p≤0.05* *** : p≤0.0* *1[a]* : based only on 2 dams

- *body weight at sacrifice and absolute and relative organ weight data for the parental animals :*
  - *P-gen : ♂ : absolute weight :*

Weight (in g)	*Term. Bw*	*brain*	*Liver*	*Sem.Ves.*	*TestisL*	*TestisR*	*Epid.L*	*EpidiR*
0 ppm	*473.1*	*2.092*	*16.482*	*1.605*	*1.837*	*1.983*	*0.739*	*0.772*
250 ppm	*460.7*	*2.075*	*16.225*	*1.593*	*1.759*	*1.848*	*0.733*	*0.740*
500 ppm	*456.1*	*2.044*	*15.968*	*1.673*	*1.878*	*1.893*	*0.763*	*0.781*
3000 ppm	*419.4**	*2.006**	*15.220*	*1.616*	*1.827*	*1.793*	*0.735*	*0.737*

* p < 0.05

Relative weight :

Weight	*Term. bw*	*brain*	*Liver*	*Sem.Ves.*	*TestisL*	*TestisR*	*Epid.L*	*EpidiR*
0 ppm	*473.1*	*0.445*	*3.470*	*0.341*	*0.387*	*0.422*	*0.156*	*0.163*
250 ppm	*460.7*	*0.452*	*3.522*	*0.347*	*0.381*	*0.402*	*0.159*	*0.161*
500 ppm	*456.1*	*0.451*	*3.504*	*0.368*	*0.413*	*0.417*	*0.168*	*0.172*
3000 ppm	*419.4**	*0.483**	*3.630*	*0.384**	*0.440**	*0.431*	*0.176**	*0.176**

* p < 0.05

  - *P-gen : ♀ : absolute weigh :*

Weight (in g)	*Term. bw*	*Brain*	*Liver*	*Uterus*	*OvaryL*	*OvaryR*
0 ppm	*277.2*	*1.955*	*11.296*	*0.625*	*0.058*	*0.058*
250 ppm	*283.1*	*1.941*	*11.959*	*0.594*	*0.059*	*0.057*
500 ppm	*280.9*	*1.951*	*12.211*	*0.594*	*0.055*	*0.054*

3000 ppm	*245.1**	*1.853**	*9.021**	*0.603*	*0.067**	*0.071**

* p < 0.05

Relative weight :

Weight	*Term. bw*	*Brain*	*Liver*	*Uterus*	*OvaryL*	*OvaryR*
0 ppm	*277.2*	*0.710*	*4.061*	*0.227*	*0.021*	*0.021*
250 ppm	*283.1*	*0.688*	*4.210*	*0.211*	*0.021*	*0.020*
500 ppm	*280.9*	*0.698*	*4.331*	*0.214*	*0.020*	*0.019*
3000 ppm	*245.1**	*0.758**	*3.679**	*0.247*	*0.028**	*0.029**

* p < 0.05

- *F1-gen : ♂ : absolute weight :*

Weight (in g)	*Term. bw*	*Brain*	*Liver*	*Sem.Ves.*	*TestisL*	*TestisR*	*Epid.L*	*EpidiR*
0 ppm	*464.5*	*2.066*	*15.897*	*1.506*	*1.899*	*1.879*	*0.739*	*0.711*
250 ppm	*440.8**	*2.032*	*15.461*	*1.424*	*1.803*	*1.856*	*0.714*	*0.699*
500 ppm	*426.6**	*2.002**	*14.905*	*1.481*	*1.830*	*1.818*	*0.719*	*0.706*

* p < 0.05

Relative weight :

Weight	*Term. bw*	*Brain*	*Liver*	*Sem.Ves.*	*TestisL*	*TestisR*	*Epidi.L*	*Epidi.R*
0 ppm	*464.5*	*0.447*	*3.420*	*0.328*	*0.411*	*0.407*	*0.160*	*0.154*
250 ppm	*440.8**	*0.463*	*3.513*	*0.320*	*0.411*	*0.423*	*0.162*	*0.159*
500 ppm	*426.6**	*0.472**	*3.489*	*0.349*	*0.432*	*0.429*	*0.169*	*0.167*

* p < 0.05

- *F1-gen : ♀ : absolute weight :*

Weight (in g)	*Term. bw*	*brain*	*Liver*	*Uterus*	*OvaryL*	*OvaryR*
0 ppm	*277.2*	*1.930*	*12.191*	*0.567*	*0.054*	*0.052*
250 ppm	*262.9**	*1.888*	*11.263*	*0.554*	*0.052*	*0.054*
500 ppm	*265.7*	*1.881*	*11.489*	*0.606*	*0.054*	*0.053*

* p < 0.05

Relative weight :

Weight (in g)	*Term. bw*	*brain*	*Liver*	*Uterus*	*OvaryL*	*OvaryR*
0 ppm	*277.2*	*0.700*	*4.369*	*0.210*	*0.019*	*0.019*
250 ppm	*262.9**	*0.720*	*4.275*	*0.212*	*0.020*	*0.021*
500 ppm	*265.7*	*0.711*	*4.308*	*0.230*	*0.020*	*0.020*

* p < 0.05

- *effects on sperm :*
  - *Sperm motility :*

	Doses (N)	*Mean % motility*	*Mean % progressive*
P-gen	*0 ppm (28)*	*76.2*	*55.9*
	250 ppm (27)	*78.9*	*56.5*
	500 ppm (30)	*78.9*	*56.4*
	3000 ppm (29)	*78.9*	*57.3*
F1-gen	*0 ppm (29)*	*87.1*	*63.9*
	250 ppm (29)	*87.8*	*65.7*
	500 ppm (29)	*89.5*	*67.6*

- *Total sperm count :*

	Doses (N)	*Epididymis (mean)*	*Testis (mean)*
P-gen	*0 ppm (28)*	*58.2*	*72.0*
	250 ppm (27)	*57.0*	*63.1**
	500 ppm (29)	*65.7*	*64.4*
	3000 ppm (28)	*43.2**	*61.2**
F1-gen	*0 ppm (29)*	*49.2*	*69.2*
	500 ppm (29)	*48.6*	*68.3*

* $p < 0.05$

- *Sperm morphology :*

	Doses (N)	*Mean % normal*	*Mean % abnormal*	*Mean % detached*
P-gen	*0 ppm (28)*	*98.7*	*0.8*	*0.5*
	250 ppm (27)	*98.1*	*1.0*	*0.8*
	500 ppm (29)	*97.0**	*1.4**	*1.6**
	3000 ppm (29)	*95.7**	*1.5**	*2.8**
F1-gen	*0 ppm (29)*	*98.1*	*1.1*	*0.8*
	500 ppm (29)	*97.9*	*1.4*	*0.7*

* $p < 0.05$

- *number of P and F1 females cycling normally and cycle length :*
  - *number of estrous cycle :*

    In P-gen : 3.6, 3.8, 3.4 and 3.6 respectively at 0, 250, 500 and 3000 ppm

    In F1-gen : 3.7, 3.7 and 3.8 respectively at 0, 250 and 500 ppm
  - *Estrous cycle length :*

    In P-gen : 4.2, 4.2, 4.4 and 4.2 respectively at 0, 250, 500 and 3000 ppm

    In F1-gen : 4.1, 4.1 and 4.1 respectively at 0, 250 and 500 ppm
- *Number of animals mated, number of animals with implants, mating index, fertility index :*
  - *In P-gen :*

	0 ppm	*250 ppm*	*500 ppm*	*3000 ppm*
Number of animals mated	*30*	*30*	*29*	*28*
Number of animals with implants	*23*	*25*	*25*	*2*
Mating index	*100.0*	*100.0*	*96.7*	*93.3*
Fertility index	*76.7*	*83.3*	*86.2*	*7.1***

** $p < 0.01$

- *In F1-gen :*

	0 ppm	*250 ppm*	*500 ppm*
Number of animals mated	*30*	*30*	*29*
Number of animals with implants	*28*	*26*	*25*
Mating index	*100.0*	*100.0*	*96.7*
Fertility index	*93.3*	*86.7*	*86.2*

- *duration of gestation (calculated from day 0 of pregnancy) :*

  In P-gen : 22.3, 22.0, 22.2 and 23.5 days respectively at 0, 250, 500 and 3000 ppm

  In F1-gen : 22.1, 21.9 and 21.8 days respectively at 0, 250, and 500 ppm

- *number of implantations, corpora lutea, litter size :*
  - *Total number of implantations : in P-gen : 265, 310, 279 and 3 respectively at 0, 250, 500 and 3000 ppm*

    in F1-gen : 304, 300, 273 respectively at 0, 250 and 500 ppm

- *histopathological findings: a few organs were affected :*
  - *cerebellum : at 3000 ppm in the P-generation, a mild to moderate degeneration/necrosis was observed. Moreover, a loss of Purkinje cells, white matter degeneration and gliosis were noted.*
  - *ovaries : at the P-generetaion, a statistically significant higher number of total corpora lutea were noted at the highest dose level.*
  - *uterus : an increased incidence of uterine horn dilatation was observed in the P-generation at 3000 ppm.*

For F1 and F2 pups/litters (per dose):

- *mean number of live pups (litter size) :*
  - *F1-pups : Total No. of pups born : 233, 279, 260, 2 respectively at 0, 250, 500 and 3000 ppm.*

    Litter size : 10.6, 11.2, 10.4, 1.0 respectively at 0, 250, 500 and 3000 ppm.
  - *F2-pups : Total No. of pups born : 280, 287, 260 respectively at 0, 250 and 500 ppm*

Litter size : 10.4, 11.0, 10.4 respectively at 0, 250 and 500 ppm

- *Pup clinical observation : no treatment-related effects*
- *sex ratio : Sex distribution at birth (% males)*
  - *F1-pups : 54.1, 55.4, 50.7, 0.0 respectively at 0, 250, 500 and 3000 ppm.*
  - *F2-pups : 48.7, 47.3, 40.6 respectively at 0, 250 and 500 ppm*
- *viability index :*
  - *F1-pups : 96.2, 97.1, 99.6, 100.0 respectively at 0, 250, 500 and 3000 ppm.*
  - *F2 pups : 99.7, 98.8, 95.6 respectively at 0, 250 and 500 ppm*
- *mean litter or pup weight by sex and with sexes combined :*
  - *F1-pups body weight in grams (number of litters) :*

		0 ppm	*250 ppm*	*500 ppm*	*3000 ppm*
D0	♂	*6.3 (22)*	*6.0 (25)*	*6.2 (24)*	*/*
	♀	*6.0 (22)*	*5.6 (25)*	*5.9 (25)*	*5.4 (2)*
	♂ + ♀	*6.2 (22)*	*5.9 (25)*	*6.1 (25)*	*5.4 (2)*
D7	♂	*17.0 (21)*	*16.1 (25)*	*16.2 (24)*	*/*
	♀	*16.1 (22)*	*15.5 (25)*	*15.4 (25)*	*9.1** (2)*
	♂ + ♀	*16.5 (22)*	*15.8 (25)*	*15.7 (25)*	*9.1** (2)*
D21	♂	*52.0 (21)*	*50.2 (25)*	*50.5 (24)*	*/*
	♀	*49.4 (22)*	*47.9 (25)*	*47.6 (25)*	*/*
	♂ + ♀	*50.7 (22)*	*49.1 (25)*	*48.4 (25)*	*/*

** p < 0.01

  - *F2-pups body weight in grams (number of litters) :*

		0 ppm	*250 ppm*	*500 ppm*
D0	♂	*6.3 (26)*	*6.0* (26)*	*5.8** (23)*
	♀	*6.0 (27)*	*5.6** (26)*	*5.5* (25)*
	♂ + ♀	*6.2 (27)*	*5.8** (26)*	*5.7** (25)*
D7	♂	*16.9 (26)*	*16.1 (26)*	*16.1 (22)*
	♀	*16.3 (27)*	*15.6 (26)*	*15.8 (24)*
	♂ + ♀	*16.6 (27)*	*15.9 (26)*	*16.0 (24)*
D21	♂	*51.2 (26)*	*47.5** (26)*	*48.4* (22)*
	♀	*49.4 (27)*	*45.9** (26)*	*46.7* (24)*
	♂ + ♀	*50.2 (27)*	*46.8** (26)*	*47.6* (24)*

* p < 0.05 **p < 0.01

- *Preputial separation :*
  - *F1-pups : 40.7, 41.2, 41.3 respectively at 0, 250 and 500 ppm*

- *F2-pups : 40.7, 41.8*, 41.5 respectively at 0, 250 and 500 ppm*

- *Vaginal opening :*
  - *F1-pups : 33.4, 35.3**, 35.0* respectively at 0, 250 and 500 ppm*
  - *F2-pups :33.6, 34.9, 34.2 respectively at 0, 250 and 500 ppm*
- *Anogenital distance : F2-pups : ♂ : 3.5, 3.5, 3.4 respectively at 0, 250 and 500 ppm*

  ♀ : 1.8, 1.6, 1.6 respectively at 0, 250 and 500 ppm
- *Micropathology evaluation : no treatment-related effects*

3.10.1.2 An embryotoxicity study performed in rats

Study reference:

Renhof M., 1988a (cited in JMPR, 2008)

Detailed study summary and results:

Test type

Following EPA OPPTS 83-3 guidance

Following GLP regulation

Test substance

- *1,2,4-Triazole*
- *Degree of purity : 94.0 %*
- *Batch number : 270184*

Test animals

- *Species/strain/sex : Rat / Bor : Wisw (SPF Cpb) / female*
- *No. of animals per sex per dose : 25 inseminated females*
- *Age and weight at the study initiation : weight : between 181 and 228 g, sexually mature, nulliparous*

Administration/exposure

- *Route of administration : oral (with stomach tube)*
- *duration and frequency of test/exposure period : GD 6 - 15, daily*
- *doses/concentration levels : 0, 100 and 200 mg/kg bw/d*
- *vehicle: cremophor-EL emulsion 0.5 %*

Results and discussion

NOAEL (maternal toxicity) : 100 mg/kg bw/d

NOAEL (developmental toxicity) : < 100 mg/kg bw/d

For dams :

- *number of animals at the start of the test : 25/dose*
- *Mortality : no mortality observed*

- *Clinical observation : no effects*
- *body weight data (in g):*

	0 mg/kg bw/d	*100 mg/kg bw/d*	*200 mg/kg bw/d*
BW at GD 0	*204.6*	*203.8*	*203.2*
BW at GD 6	*221.5*	*222.5*	*220.3*
BW at GD 15	*250.9*	*249.8*	*241.8*
BW at GD 20	*301.4*	*295.7*	*263.6*
Adjusted maternal bwg	*30.8*	*34.16*	*33.24*

- *body weight gain :*
  - *during administration period : 29.3, 27.4 and 21.5* g respectively at 0, 100 and 200 mg/kg bw/d*
  - *during entire pregnancy : 96.9, 91.9 and 60.4** g respectively at 0, 100 and 200 mg/kg bw/d*
- *body weight at sacrifice and absolute and relative organ weight data for the parental animals : no information available*
- *Insemination and fertilisation :*

	Number of inseminated ♀	*Fertilised ♀*		*Pregnant ♀*	
		number	*%*	*number*	*%*
0 mg/kg bw/d	*25*	*21*	*84.0*	*21*	*100.0*
100 mg/kg bw/d	*25*	*19*	*76.0*	*19*	*100.0*
200 mg/kg bw/d	*25*	*25*	*100.0*	*25*	*100.0*

- *haematological and clinical biochemistry findings : no information available*
- *number of implantations, corpora lutea, litter size :*

		0 mg/kg bw/d	*100 mg/kg bw/d*	*200 mg/kg bw/d*
Mean number of implantations per dams		*12.5*	*12.2*	*11.8*
Mean number of corpora lutea per dams		*13.6*	*13.9*	*14.2**
Mean number of foetus per dams	♂	*5.9*	*6.0*	*3.1***
	♀	*6.1*	*5.9*	*2.4***
	Total	*12.0*	*11.9*	*5.5***

* p < 0.05 ** p < 0.01

- *number of dams with abortions, early deliveries, stillbirths, resorptions and/or dead foetuses :*

		0 mg/kg bw/d	*100 mg/kg bw/d*	*200 mg/kg bw/d*
% resorption	*Early*	*0.381*	*0.316*	*0*
	Late	*3.381*	*2.474*	*52.36*

Mean number of foetus loss per dams	*0.5*	*0.3*	*6.3***
Mean runts	*0.24*	*2.84**	*4.96***

** p < 0.01

- *mean gravid uterine weight (in g) : 66, 57.737 and 27.16 g respectively at 0, 100 and 200 mg/kg bw/d*

For foetus (per dose):

- *Total number of foetus : 253, 226 and 138 respectively at 0, 100 and 200 mg/kg bw/d*
- *mean number pups (litter size) and sex ratio :*

		0 mg/kg bw/d	*100 mg/kg bw/d*	*200 mg/kg bw/d*
Mean number of foetus per dams	♂	*5.9*	*6.0*	*3.1***
	♀	*6.1*	*5.9*	*2.4***
	Total	*12.0*	*11.9*	*5.5***

** p < 0.01

- *mean foetus weight and placental weight (in g) :*

	0 mg/kg bw/d	*100 mg/kg bw/d*	*200 mg/kg bw/d*
Mean placental weight	*0.59*	*0.52***	*0.49***
Mean foetus weight	*3.55*	*3.06***	*2.35***

** p < 0.01

- *Malformation : incidence of malformation :*

	0 mg/kg bw/d	*100 mg/kg bw/d*	*200 mg/kg bw/d*	*HCD*
Undescended testicule	*2*	*11*	*6*	*/*
Cleft palate	*0*	*0*	*4*	*/*
hydronephrosis	*1*	*1*	*7*	*1*

HCD : incidence of spontaneous malformation in control group (year 1985)

3.10.1.3 An embryotoxicity study performed in rats

Study reference:

Renhof M., 1988b (cited in JMPR, 2008)

Detailed study summary and results:

Test type

Following EPA OPPTS 83-3

Following GLP regulation

Test substance

- *1,2,4-triazole*
- *Degree of purity : 95.3 %*

- *Batch number : 270/84*

Test animals

- *Species/strain/sex : Rats / Bor : wisw (SPF Cpb) / female*
- *No. of animals per sex per dose : 25 inseminated females/group*
- *Age and weight at the study initiation : weight : 182 – 213 g, sexually mature, nulliparous*

Administration/exposure

- *Route of administration : oral*
- *duration and frequency of test/exposure period : GD 6 - 15, daily*
- *doses/concentration levels : 0, 10, 30 and 100 mg/kg bw/d*
- *vehicle : cremophor-EL emulsion 0.5%*
- *post exposure observation period : until GD 20*

Results and discussion

NOAEL (maternal toxicity) : 30 mg/kg bw/d

NOAEL (developmental toxicity) : 30 mg/kg bw/d

For dams (per dose):

- *number of animals at the start of the test and matings :*

		0 mg/kg bw/d	*10 mg/kg bw/d*	*30 mg/kg bw/d*	*100 mg/kg bw/d*
Inseminated females		*25*	*25*	*25*	*25*
Fertilised females	*Number*	*21*	*22*	*19*	*24*
	%	*84.0*	*88.0*	*76.0*	*96.0*
Pregnant females	*Number*	*21*	*22*	*19*	*24*
	%	*100*	*100*	*100*	*100*

- *time of death during the study and whether animals survived to termination : no mortality observed during the study*
- *body weight data : BWG (in grams)*

	0 mg/kg bw/d	*10 mg/kg bw/d*	*30 mg/kg bw/d*	*100 mg/kg bw/d*
During exposure period	*28.2*	*25.4*	*26.8*	*21.8**
During entire pregnancy	*92.9*	*86.6*	*90.0*	*79.8**

* $p < 0.05$

- *body weight at sacrifice and absolute and relative organ weight data : no information available*
- *clinical observations: no effects*

- *number of implantations, corpora lutea, litter size : number of implantations per dams : 11.6, 10.5, 11.4, 10.6 respectively at 0, 10, 30 and 100 mg/kg bw/d*
- *number of dams with abortions, early deliveries, stillbirths, resorptions and/or dead fetuses : increased incidence of runts at the highest dose : mean runts : 0.33, 0.23, 0.53, 2.21* respectively at 0, 10, 30 and 100 mg/kg bw/d*

For foetus (per dose):

- *mean number of live pups (litter size) :*

  total number of foetus : 231, 222, 202, 228 respectively at 0, 10, 30 and 100 mg/kg bw/d

  Number of foetuses per dam : 11.0, 10.1, 10.6, 9.5 respectively at 0, 10, 30 and 100 mg/kg bw/d
- *sex ratio :*

  Number of male foetuses per dam : 6.5, 5.1, 6.0, 5.0* respectively at 0, 10, 30 and 100 mg/kg bw/d*

  Number of female foetuses per dam : 4.5, 5.0, 4.6, 4.5 respectively at 0, 10, 30 and 100 mg/kg bw/d
- *mean litter or pup weight by sex and with sexes combined : significant lower foetal weight at 100 mg/kg bw/d : 3.58, 3.59, 3.53 and 3.25** g respectively at 0, 10, 30 and 100 mg/kg bw/d*
- *external, soft tissue and skeletal malformations and other relevant alterations : foetuses with minor skeletal deviations : 2.00, 2.41, 2.84, 2.42 respectively at 0, 10, 30 and 100 mg/kg bw/d*

  Foetuses with malformations : 0.05, 0.05, 0.05, 0.17 respectively at 0, 10, 30 and 100 mg/kg bw/d

	0 mg/kg bw/d	*10 mg/kg bw/d*	*30 mg/kg bw/d*	*100 mg/kg bw/d*
Microphtalmia, bilateral	*1*	*0*	*0*	*0*
Microphtalmia, right side	*0*	*1*	*0*	*1*
Microphtalmia, left side	*0*	*0*	*0*	*1*
False posture of right hind leg	*0*	*0*	*1*	*0*
Anophtalmia	*0*	*0*	*0*	*1*
Dysplasia and asymmetry of body of vertebrae	*0*	*0*	*0*	*1*

3.10.1.4 A developmental toxicity study in rats

Study reference:

Wickramaratne, 1987 (cited in JMPR, 2008)

Detailed study summary and results:

Test type

Non-guideline study

Non-GLP study

Test substance

- *1,2,4-triazole*
- *Degree of purity : no information available*
- *Batch number : no information available*

Test animals

- *Species/strain/sex : rat / Wistar / female pregnant*
- *No. of animals per sex per dose : 10 pregnant females/dose*
- *Age and weight at the study initiation : no information available*

Administration/exposure

- *Route of administration : oral*
- *duration and frequency of test/exposure period : GD 7 through 17*
- *doses/concentration levels : 0, 25 and 100 mg/kg bw/d*
- *historical control data if available : no information available*
- *vehicle: no information available*

Description of test design:

- *Examined maternal parameters were restricted to bw on gestational days 1, 7-17 and 22.*
- *Offspring observations were litter weight of live pups on PND 1 and 5 and number of live and dead pups on these days.*
- *Evaluation of malformations was not performed.*

Results and discussion

NOAEL (maternal toxicity) : 100 mg/kg bw/d

NOAEL (developmental toxicity) : 100 mg/kg bw/d

For dams (per dose):

- *body weight data : no effects (no more information available)*

For offspring (per dose):

- *mean number of live pups (litter size) : no effects (no more information available)*
- *viability index : no effects (no more information available)*

3.10.1.5 A developmental toxicity study performed in rabbits

Study reference:

Hoberman, 2004 (cited in JMPR, 2008)

Detailed study summary and results:

Test type

Prenatal developmental toxicity study

Following OECD TG 414 and US EPA OPPTS 870.3700

GLP

Test substance

- *1,2,4-triazole*
- *degree of purity : 99.9 %*
- *Batch number : S13691*

Test animals

- *Species/strain/sex : Rabbit / New Zealand White / pregnant female*
- *No. of animals per sex per dose : 25 / dose*
- *Age and weight at the study initiation : weight 2.7 – 4.4 kg, age : 5.5 months*

Administration/exposure

- *Route of administration : oral (stomach tube)*
- *duration and frequency of test/exposure period : GD 6 through 28 (sacrified at GD 29), daily*
- *doses/concentration levels : 0, 5, 15, 30, 45 mg/kg bw/d*
- *historical control data if available : no information available*
- *vehicle: aqueous 0.5% (w/w) carboxymethylcellulose (CMC)*

Results and discussion

NOAEL (maternal toxicity) : 30 mg/kg bw/d

NOAEL (developmental toxicity) : 30 mg/kg bw/d

For maternal (per dose) :

- *time of death during the study and whether animals survived to termination : at the highest dose, 5 females sacrified due to their moribund condition (between GD 16 - 24). No other mortality reported.*
- *Clinical observations : at the highest dose, a significantly increased incidence of decreased motor activity, clear perinasal substance, ptosis, excess salivation and hyperpnea were observed. Most of these signs occurred in does which were killed during the exposure period for their moribund condition.*
- *maternal body weight data : BWG was significantly decreased at 45 mg/kg bw/d*

	0 mg/kg bw/d	*5 mg/kg bw/d*	*15 mg/kg bw/d*	*30 mg/kg bw/d*	*45 mg/kg bw/d*
Maternal bw at GD 29 (in kg)	*4.04*	*3.95*	*3.93*	*4.00*	*3.76*
Bwg for the entire gestational period (0 - 29) (in kg)	*0.65*	*0.54*	*0.52*	*0.55*	*0.37***

** $p < 0.01$

- *absolute and relative organ weight data for the parental animals :*
    - *Gravid uterine weight : significantly reduced at 45 mg/kg bw/d (0.56, 0.54, 0.51, 0.53, 0.46** kg respectively at 0, 5, 15, 30 and 45 mg/kg bw/d)*
- *number of female pregnant : 25, 24, 24, 25, 25 respectively at 0, 5, 15, 30 and 45 mg/kg bw/d*
- *duration of gestation : no effects*
- *number of implantations, corpora lutea, litter size, live births : no effects*

	0 mg/kg bw/d	*5 mg/kg bw/d*	*15 mg/kg bw/d*	*30 mg/kg bw/d*	*45 mg/kg bw/d*
Corpora lutea	*9.8*	*9.8*	*9.9*	*10.2*	*9.8*
Implantations	*9.0*	*9.0*	*8.8*	*9.3*	*9.0*
Litter size	*8.7*	*8.6*	*8.3*	*8.8*	*8.3*
Live foetuses	*217*	*207*	*199*	*218*	*157*
Dead foetuses	*0*	*0*	*0*	*1*	*0*

- *number of pre- and post-implantation loss : no effects*
- *number of dams with abortions, early deliveries, stillbirths, resorptions and/or dead fetuses :*
    - *Only 1 dead foetus was observed at 30 mg/kg bw/d.*
    - *Resorption : 0.3, 0.4, 0.4, 0.5 and 0.7 respectively at 0, 5, 15, 30 and 45 mg/kg bw.*

Dose level (mg/kg bw/d)	*0*	*5*	*15*	*30*	*45*
Early resorption	*1*	*2*	*4*	*10*	*6*
Late resorption	*7*	*8*	*7*	*3*	*8*

    - *Percent of dead or resorbed conceptuses by litter : 3.1, 4.7, 4.8, 6.4 and 7.0 respectively at 0, 5, 15, 30 and 45 mg/kg bw/d*

For foetus/pups (per dose) :

- *mean number of live pups (litter size) : no effects*
- *sex ratio : no effects*
- *mean litter or pup weight by sex and with sexes combined : foetal body weight : significantly reduced at the highest dose*

	0 mg/kg bw/d	*5 mg/kg bw/d*	*15 mg/kg bw/d*	*30 mg/kg bw/d*	*45 mg/kg bw/d*
Live fetal bw (in g)	*44.35*	*43.42*	*43.82*	*42.48*	*39.46***
Male fetuse bw (in g)	*44.92*	*43.91*	*44.25*	*42.39*	*39.65***
Female fetuse bw (in g)	*42.92*	*42.79*	*43.64*	*42.20*	*38.70**

* $p < 0.05$ ** $p < 0.01$

- *external, soft tissue and skeletal malformations and other relevant alterations : at the highest dose, a few alterations of the urogenital system were noted such as low set, small or absent kydneys and/or absent ureter*

	0 mg/kg bw/d	*5 mg/kg bw/d*	*15 mg/kg bw/d*	*30 mg/kg bw/d*	*45 mg/kg bw/d*
Fetal incidence of low set of kidney	*0*	*0*	*0*	*0*	*3***
Fetal incidence of small kidneys	*0*	*0*	*0*	*0*	*3***
Fetal incidence of absent kidneys	*0*	*0*	*0*	*0*	*2***

** p < 0.01

3.10.1.6 A subacute toxicity study (28days) performed in mice

Study reference:

Wahle B.S., 2004a

Detailed study summary and results:

Test type

Following GLP regulation

Non guideline

Test substance

- *1,2,4-triazole*
- *Degree of purity : 99.9 %*
- *Batch number : S13691*

Test animals

- *Species/strain/sex : Mice / CD-1 [ICR]/BR / both sexes*
- *No. of animals per sex per dose : 15/sex/dose*
- *Age and weight at the study initiation : 8 weeks old*

Administration/exposure

- *route of administration : oral (feed)*
- *duration and frequency of exposure period : approximately 4 weeks, daily*
- *doses/concentration levels : 0, 50, 250, 500 and 2000 ppm. Corresponding to (in mg/kg bw/d) :*

	0 ppm	*50 ppm*	*250 ppm*	*500 ppm*	*2000 ppm*
♂	*0*	*9*	*47*	*90*	*356*
♀	*0*	*12*	*60*	*120*	*479*

- *post exposure observation period : no*

- *vehicle: ethanol*

Results and discussion

NOAEL (males) : 500 ppm

NOAEL (females) : 2000 ppm

- *mean body weight (in g) at day 28 of exposure : no effects observed*

	0 ppm	*50 ppm*	*250 ppm*	*500 ppm*	*2000 ppm*
♂	*35.4*	*35.9*	*35.5*	*35.6*	*33.8*
♀	*26.9*	*26.9*	*26.7*	*25.9*	*26.4*

- *food/water consumption : no effects observed*
- *description, severity, time of onset and duration of clinical signs : no effects observed*
- *haematological findings :*

  in males : significant decrease of Hgb in male at 250 and 2000 ppm (15.3 g/dl at 500 ppm and 15.3* g/dl at 2000 ppm vs 16.4 g/dl in control group) and statistically significant increase of HDW at 2000 ppm (1.99* vs 1.85 g/dl in control group)*

  In females : no effects observed
- *clinical biochemistry findings : no effects observed*
- *terminal body weight and absolute organ weight : no effects observed*
- *gross pathology findings : no effects observed*
- *histopathology findings : No statistically significant effects observed*

		0 ppm	*50 ppm*	*250 ppm*	*500 ppm*	*2000 ppm*
Epididymis	*Incidence of aspermia*	*0/15*	*0/15*	*0/15*	*1/15*	*0/15*
	Incidence of germ cells/debris	*0/15*	*1/15*	*1/15*	*0/15*	*3/15*
Testis	*Incidence of apoptotic-like bodies*	*2/15*	*4/15*	*1/15*	*3/15*	*5/15*
	Incidence of spermatid degeneration/depletion/asynchrony	*1/15*	*1/15*	*1/15*	*0/15*	*5/15*
	Incidence of tubular atrophy	*1/15*	*2/15*	*1/15*	*2/15*	*4/15*

- *mortality and time to death (if occurring) : 1 male in the lowest dose group (50 ppm) at day 23 of exposure, 1 female in control group at day 23 of exposure, 1 female in 500 ppm group at day 23 of exposure and 2 females in the highest dose group (2000 ppm) at day 24 of exposure.*

3.10.1.7 A subacute toxicity study (30 days) performed in rats

Study reference:

Anonymous (cited in US EPA memorandum, 2006)

Detailed study summary and results:

Test type

30-day oral toxicity study

Non-guideline

Test substance

- *1,2,4-triazole*
- *Degree of purity : no information available*

Test animals

- *Species/strain/sex : rats / no more information available*
- *No. of animals per sex per dose : no information available*
- *Age and weight at the study initiation : no information available*

Administration/exposure

- *route of administration : oral*
- *duration and frequency of test/exposure period : 30-day*
- *doses/concentration levels : 0, 8, 57 and 400 mg/kg bw/d*
- *vehicle: no information available*

Results and discussion

NOAEL : < 8 mg/kg bw/d

- *body weight and body weight changes : lower bw at the highest dose level (no more information available)*
- *food/water consumption : no information available*
- *description, severity, time of onset and duration of clinical signs : a few clinical signs were observed such as staggering, tremors and hunched posture however no more information available*
- *haematological findings : at 57 mg/kg bw/d, slight hematology changes were noted (no more information available)*
- *clinical biochemistry findings : no information available*
- *gross pathology findings : at 8 mg/kg bw/d, adrenal weight was reduced*
- *histopathology findings : no information available*
- *mortality and time to death : no information available*

3.10.1.8 A subchronic toxicity study (90 days) performed in rats

Study reference:

Bomhard E. et al., 1979

Detailed study summary and results:

Test type

Similar to OECD TG 408

Not following GLP regulation

Test substance

- *1,2,4-triazole*
- *Degree of purity : 99.6 %*

Test animals

- *Species/strain/sex : rat / Wistar / both sexes*
- *No. of animals per sex per dose : 15/sex/dose*
- *Age and weight at the study initiation : 5-6 weeks old and approximately 82 g for males and 78 g for females*

Administration/exposure

- *route of administration : oral (feed)*
- *duration and frequency of exposure period : 3 months, daily*
- *doses/concentration levels : 0, 100, 500 and 2500 ppm corresponding to (in mg/kg bw/d) :*

	0 ppm	*100 ppm*	*500 ppm*	*2500 ppm*
♂	*0*	*7.79*	*37.85*	*212.30*
♀	*0*	*10.23*	*54.20*	*266.69*

- *post exposure observation period : no*
- *vehicle: 90% pre-mix with ultrasil VN 3*

Results and discussion

NOAEL : 500 ppm

- *body weight and body weight changes : a statistically significant decrease was observed at the highest dose in both sexes*

  Initial and terminal bw (in grams)

Sex	*Study period*	*0 ppm*	*100 ppm*	*500 ppm*	*2500 ppm*
♂	*Initial bw*	*82*	*82*	*82*	*82*
	Terminal bw	*335*	*342*	*344*	*306***
♀	*Initial bw*	*78*	*78*	*78*	*78*
	Terminal bw	*195*	*195*	*187*	*184**

- *clinical signs : at the highest dose, 2 males and 2 females exhibited temporary slight convulsions*
- *haematological findings : a statistically significant lower haemoglobin, haematocrit, MCH and MCV were noted at the highest dose in males that pointed to slight anemia*
- *clinical biochemistry findings : no effects observed*
- *gross pathology findings : no effects observed*
- *organ weight : a statistically significant lower body weight was observed in both sexes at the highest dose (in males : 335, 342, 344 and 306* g respectively at 0, 100, 500 and 2500 ppm and in females :*

195, 195, 187 and 184 g respectively at 0, 100, 500 and 2500 ppm). At this dose a statistically significant testis weight decrease was also observed (3418, 3308, 3247 and 3215* mg respectively at 0, 100, 500 and 2500 ppm). Furthermore, in males, a few other organ weights were statistically modified such as a lower thymus weight, a lower heart weight, a lower lung weight (also observed in females) and a lower spleen weight.*

- *histopathology findings: no treatment-related findings*
- *mortality : no mortality observed*

3.10.1.9 A subchronic study (90 days) performed in mice

Study reference:

Wahle B.S., 2004b

Detailed study summary and results:

Test type

Following US EPA OPPTS 870.3100

Following GLP regulation

90 days toxicity study

Additional groups were performed and were sacrified after an exposure period of 28 days

Test substance

- *1,2,4-triazole*
- *Degree of purity : 99.9 %*
- *Batch number : S13691*

Test animals

- *Species/strain/sex : Mouse / CD-1[ICR]/BR / both sexes*
- *No. of animals per sex per dose : 20/sex/dose*

  + additional animals : 15/sex/group at 0, 3000 and 6000 ppm for an exposure period of 28 D and killed for hepatic enzyme analyses
- *Age and weight at the study initiation : 8 weeks old*

Administration/exposure

- *route of administration : oral (feed)*
- *duration and frequency of exposure period : 90 days, daily*
- *doses/concentration levels : 0, 500, 1000, 3000 and 6000 ppm, corresponding to (in mg/kg bw/d) :*

	0 ppm	*500 ppm*	*1000 ppm*	*3000 ppm*	*6000 ppm*
♂	*0*	*80*	*161*	*487*	*988*
♀	*0*	*105*	*215*	*663*	*1346*

- *post exposure observation period : no*

- *vehicle: ethanol*

Results and discussion

NOAEL (males) : 1000 ppm

NOAEL (females) : 3000 ppm

- *body weight and body weight changes : a significant body weight decrease was observed in males at 3000 and 6000 ppm (approximately -6 and -16 %) and in females at 6000 ppm (approximately -9 %).*

	BW at D84	*0 ppm*	*500 ppm*	*1000 ppm*	*3000 ppm*	*6000 ppm*
BW at D84 (in g)	♂	*37.3*	*37.0*	*36.4*	*34.9**	*31.3**
	♀	*29.1*	*28.4*	*28.4*	*28.7*	*26.6**
Total BWG (in g)	♂	*3.1*	*3.6*	*1.7*	*1.1**	*-3.1**
	♀	*3.5*	*3.1*	*3.0*	*2.7*	*0.9**

** $p \leq 0.05$*

- *clinical signs: at the highest dose, an increase incidence of tremors was observed in both sexes (more marked in males). Furthermore, in males a statistically significantly increase of yellow staining and rough coat were observed at this dose level.*

  Incidence of tremors :

  In males : 0, 0, 0, 1 and 11 respectively at 0, 500, 1000, 3000 and 6000 ppm

  In females : 0, 0, 0, 2 and 2 respectively at 0, 500, 1000, 3000 and 6000 ppm

- *ophthalmologic findings: no information available*
- *haematological findings: a statistically significant decrease of Hgb and a statistically significant increase of RDW and HDW were observed at 6000 ppm in both sexes*
- *clinical biochemistry findings: a significant increase of cholesterol was observed at 3000 and 6000 ppm in females. In liver tissue, an increased activities of 7—ethoxycoumarin deethylase (ECOD), 7-ethoxyresorufin deethylase (EROD) and Aldrin epoxide (ALD) were observed in the additional group exposed during 28 D to 3000 and 6000 ppm and at the highest dose after 13 weeks of exposure.*
- *organ weight :*
  - *Absolute organ weight : terminal body weight and brain weight were statistically significantly decreased in both sexes at the highest dose. Moreover in males, testis weight was also statistically significantly reduced at this dose.*

At day 89		*0 ppm*	*500 ppm*	*1000 ppm*	*3000 ppm*	*6000 ppm*
Term. BW (in g)	♂	*36.9*	*35.8*	*34.9**	*33.9**	*30.5**
	♀	*28.1*	*27.9*	*28.0*	*27.9*	*26.0**
Brain weight (in g)	♂	*0.488*	*0.491*	*0.476*	*0.465**	*0.445**
	♀	*0.485*	*0.489*	*0.483*	*0.475*	*0.451**

Testis weight (in g)		*0.253*	*0.247*	*0.233*	*0.233*	*0.219**

** p ≤ 0.05*

- *Relative organ weight :*

At day 89		*0 ppm*	*500 ppm*	*1000 ppm*	*3000 ppm*	*6000 ppm*
Term. bw (in g)	♂	*36.9*	*35.8*	*34.9**	*33.9**	*30.5**
	♀	*28.1*	*27.9*	*28.0*	*27.9*	*26.0**
Brain weight (in %)	♂	*1.328*	*1.378*	*1.365*	*1.376*	*1.462**
	♀	*1.737*	*1.756*	*1.731*	*1.717*	*1.734*
Testis weight (in %)		*0.688*	*0.692*	*0.669*	*0.687*	*0.719*

** p ≤ 0.05*

- *histopathology findings: effects were observed in brain in both sexes. Furthermore, testis and epididymis were also affected.*

			0 ppm	*500 ppm*	*1000 ppm*	*3000 ppm*	*6000 ppm*
Brain	*Incidence of Purkinje cell loss*	♂	*0/20*	*0/20*	*0/20*	*0/20*	*15*/20*
		♀	*0/20*	*0/20*	*0/20*	*0/20*	*10*/20*
Epididymis	*Incidence of exfoliated germ cells/debris*		*0/20*	*0/20*	*0/20*	*0/20*	*10*/20*
Testis	*Incidence of apoptotic-like bodies*		*4/20 (1.0)*	*4/20 (1.3)*	*7/20 (1.1)*	*11*/20 (1.3)*	*12*/20 (1.2)*
	Incidence of spermatid degeneration/depletion/asynchrony		*1/20 (1.0)*	*0/20*	*0/20*	*5/20 (1.4)*	*15*/20 (2.0)*
	Incidence of tubular atrophy		*0/20*	*0/20*	*2/20 (1.5)*	*3/20 (1.0)*	*10*/20 (1.8)*

*() : average severity of animals with lesions : 1 (minimal) to 5 (severe); * p ≤ 0.05*

- *mortality : no effects observed*

3.10.1.10 A combined subchronic toxicity / neurotoxicity screening study performed in rats

Study reference:

Wahle B.S. and Sheets L.P., 2004

Detailed study summary and results:

Test type

Combined subchronic toxicity/neurotoxicity screening study

90-day

Following OECD TG 408 and 424

Animals were also tested for functional observational battery (FOB) and motor activity (12 males and 12 females /groups) : before the exposure and again during weeks 2,4,8 and 13 of exposure. At the study termination, 10 males and 10 females / groups were killed and perfused in situ for neuropathological examination.

Test substance

- *1,2,4-triazole*
- *Degree of purity : 99.9 %*
- *Batch number : S13691*

Test animals

- *Species/strain/sex : Rat / Wistar hanover / both sexes*
- *No. of animals per sex per dose : 20/sex/dose*
- *Age and weight at the study initiation : 8 weeks old*

Administration/exposure

- *route of administration : oral (feed)*
- *duration and frequency of exposure period : 90 days, daily*
- *doses/concentration levels : 0, 250, 500, 3000 and 1000/4000 ppm, corresponding to (in mg/kg bw/d) :*

	0 ppm	*250 ppm*	*500 ppm*	*3000 ppm*	*1000/4000 ppm*
♂	*0*	*16*	*33*	*183*	*210*
♀	*0*	*19*	*41*	*234*	*275*

The dose of 1000 ppm has been changed after 4 weeks of exposure in 4000 ppm for the rest of the study period. For the highest dose level, the daily intake value shows the average of approximately 4 weeks of exposure at 1000 ppm and approximately 10 weeks of exposure at 4000 ppm. The mean daily intake for 1000/4000 ppm animals through Week 4 was 85 + 3 and 95 + 3, for males and females respectively while the mean daily intake values until the end of the study was 248 + 16 and 329 + 21, for males and females respectively.

- *post exposure observation period : no*
- *vehicle: ethanol*

Results and discussion

NOAEL : 500 ppm

- *body weight and body weight changes : a lower body weight was observed at 3000 and 1000/4000 ppm dose group. Body weight gain was also affected at these doses (approximately -18 % in ♂ and -19 % in ♀ at 3000 ppm and -21 % in both sexes at 1000/4000ppm).*

		0 ppm	*250 ppm*	*500 ppm*	*3000 ppm*	*1000/4000 ppm*
Bw (D0) (in	♂	265.6	267.4	267.0	267.1	266.1

g)	♀	181.2	181.4	180.7	179.9	182.7
BW (at D91) (in g)	*♂*	*437.9*	*439.7*	*443.0*	*407.9**	*401.9**
	♀	*245.1*	*246.9*	*244.4*	*231.7**	*233.0*
BWG (D0 - D91) (in g)	*♂*	*172.3*	*172.2*	*176.0*	*140.8**	*135.9**
	♀	*63.9*	*65.5*	*63.7*	*51.8**	*50.3**

* *p ≤ 0.05*

- *clinical signs : no effects observed*
- *Functional observational battery : effects were noted in both sexes at the 2 highest dose. These effects (tremors, gait incoordination, decreased rearing, ungroomed appearance, red nasal and lacrimal stain, muscle fasciculations, uncoordinated righting reflex) were more marked at 8 weeks than at 13 weeks of exposure. Increased incidence of foot splay was noted in males.*
- *motor activity assessment : no treatment-effects observed*
- *ophthalmologic findings : retinal degeneration was observed in 4 out of 20 males and in 2 out of 20 females at 3000 ppm, in 5 out of 20 males and in 5 out of 20 females at 1000/4000 ppm vs in 2 out of 20 males and in 0 out of 20 females in control group.*
- *haematological findings :*
    - *in males : at ≥ 500 ppm, astatistically significant decrease of haemoglobin and haematocrit were observed (Hgb : 16.0*, 15.7* and 15.8* g/dl respectively at 500, 1000/4000 and 3000 ppm vs 16.7 in control and Hct : 45.1*, 43.7* and 44.6* % respectively at 500, 1000/4000 and 3000 ppm).*
    - *In females : a statistically significant increase of platelets was observed at the 2 highest dose (911* and 903* 10*3/mm^3 respectively at 1000/4000 and 3000 ppm vs 785 10*3/mm^3 in control group). Moreover a statistically significant lower Hgb and MCV were noted at 3000 ppm (Hgb : 15.7* g/dl vs 16.3 and MCV 50.6* um³ vs 54.3 in control group).*
- *clinical biochemistry findings :*
    - *in males : statistically significant changes of CL, Trig, Calc, TSH were noted at the 2 highest doses*
    - *in females : statistically significant differences of K, CL and Calc at 3000 ppm and TSH at 1000/4000 ppm.*
- *Hepatic enzyme profile : at the 2 highest dose levels, a slightly increased activity for N-demethylase in males, O-demethylase in females and ECOD, EROD and ALD in both sexes.*
- *gross pathology findings : no effects observed*
- *organ weight (in g):*

		0 ppm	*250 ppm*	*500 ppm*	*1000/4000 ppm*	*3000 ppm*
♂	*Term.bw*	*432.6*	*428.1*	*427.6*	*385.8**	*402.8*

	Brain weight	*2.046*	*2.022*	*2.009*	*1.922**	*1.941**
	Testis weight	*3.732*	*3.660*	*3.651*	*3.621*	*3.619*
	Epidid. weight	*1.827*	*1.849*	*2.078*	*1.590*	*1.728*
♀	*Term.bw*	*239.1*	*242.5*	*234.3*	*230.9*	*226.6*
	Brain weight	*1.915*	*1.891*	*1.878*	*1.814*	*1.784**
	Ovary weight	*0.169*	*0.165*	*0.162*	*0.172*	*0.156*
	Uterus weight	*0.611*	*0.568*	*0.602*	*0.521*	*0.491*

* $p \leq 0.05$

No changes on relative organ weight observed on these organs.

- *histopathology findings : No effects observed on reproductive organs at the examined dose (0 and 1000/4000 ppm, the other dose levels were not tested). Only a slight increased number of corpora lutea at 3000 ppm and at 1000/4000 ppm levels (33, NE, 33, 41 and 40).*

  For the nervous system : degeneration of some nerve fibers were observed at the 2 highest doses. Moreover a degeneration/necrosis on the cerebellum (brain level 7) was noted at these 2 dose levels (in 9♂/10 tested and in 10♂/10 tested respectively at 1000/4000 and 3000 ppm and 10♀/10 tested at the 2 highest doses).
- *mortality : no effects observed*

3.10.1.11 A chronic toxicity study (12 months) performed in rats

Study reference:

Wahle B.S., 2010

Detailed study summary and results:

Test type

Following OECD TG 452

Folliwing GLP regulation

Test substance

- *1,2,4-triazole*
- *Degree of purity : ≥98.5 %*
- *Batch number : S4317788*

Test animals

- *Species/strain/sex : rat / Crl:Wi(Han) / both sexes*
- *No. of animals per sex per dose : 20 /sex/dose*

  + additional animals : 10/sex/group for neurotoxicology assessment
- *Age and weight at the study initiation : age : 8 w and weight : 133 – 255 g for males and 153 – 191 g for females*

Administration/exposure

- *route of administration : oral (feed)*
- *duration and frequency of test/exposure period : 12 months, daily*
- *doses/concentration levels : 0, 125, 375, 1000 and 2000 ppm corresponding to (in mg/kg bw/d):*

	0 ppm	*125 ppm*	*375 ppm*	*1000 ppm*	*2000 ppm*
Males	*0*	*6.9*	*21*	*58*	*113*
Females	*0*	*8.3*	*26*	*71*	*136*

- *post exposure observation period : no*
- *vehicle: ethanol*

Results and discussion

NOAEL : 375 ppm

- *body weight and body weight changes : lower bw and bwg at 1000 and 2000 ppm*

		0 ppm	*125 ppm*	*375 ppm*	*1000 ppm*	*2000 ppm*
BW at D 343	*Males*	*543*	*558*	*545*	*514*	*512*
	Females	*320*	*314*	*297*	*292*	*291*
BWG	*Males*	*318*	*332*	*321*	*293*	*294*
	Females	*144*	*139*	*123*	*116*	*115*

- *food/water consumption : no effects*
- *description, severity, time of onset and duration of clinical signs : no effects*
- *neurological assessments (functional observational battery and motor activity) : no statistically significant effects*
- *haematological findings: no effects*
- *clinical biochemistry findings: no effects*
- *gross pathology findings: no effects*
- *organ weight : no effects*
- *histopathology findings: statistically significant higher incidence of Purkinje cells loss within the vermis at the highest dose level*
- *mortality and time to death (if occurring) : no effects*
- *oestrous cycle : no effects*
    - *Number of oestrous cycle : 2.1, 1.4, 1.6, 2.3 and 1.6 respectively at 0, 125, 375, 1000 and 2000 ppm*
    - *Cycle length : 6.6, 5.1, 4.9*, 5.7 and 5.2 respectively at 0, 125, 375, 1000 and 2000 ppm*
- *sperm analyses : no effects*
    - *Mean % motility : 84.5, 83.5, 85.4, 86.1 and 82.3 respectively at 0, 125, 375, 1000 and 2000 ppm*

- *Mean % progressive : 58.5, 59.1, 62.1, 62.9 and 57.4 respectively at 0, 125, 375, 1000 and 2000 ppm*
- *Sperm count (sperm/g) : in testis : 37.2 at 2000 ppm vs 34.7 in control group*

  In epididymis : 103.7 at 2000 ppm vs 79.7 in control group
- *Sperm morphology : mean total number of normal sperm : 196.3 at 2000 ppm vs 197.9 in control group*

  mean total number of abnormal sperm : 3.0 at 2000 ppm vs 2.0 in control group

  mean total number of detached head : 0.7 at 2000 ppm vs 0.1 in control group

3.10.2 Human data

No information available

3.10.3 Other data (e.g. studies on mechanism of action)

No information available

3.11 Specific target organ toxicity – single exposure

Not evaluated in this dossier.

3.12 Specific target organ toxicity – repeated exposure

Not evaluated in this dossier.

3.13 Aspiration hazard

Not evaluated in this dossier.

4 ENVIRONMENTAL HAZARDS

Not evaluated in this dossier