Committee for Risk Assessment

RAC

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

Adopted

15 March 2019

Annankatu 18, P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

15 March 2019

CLH-O-0000001412-86-270/F

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

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

Chemical name: **1,2,4-triazole**

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

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

The proposal was submitted by **Belgium** and received by RAC on **15 March 2018.**

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

PROCESS FOR ADOPTION OF THE OPINION

Belgium has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **4 June 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **3 August 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Ralf Stahlmann**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **15 March 2019** by **consensus**.

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

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	613-111-00-X	1,2,4-triazole	206-022-9	288-88-0	Repr. 2 Acute Tox. 4* Eye Irrit. 2	H361d*** H302 H319	GHS08 GHS07 Wng	H361d*** H302 H319			
Dossier submitters proposal	613-111-00-X	1,2,4-triazole	206-022-9	288-88-0	**Modify** Acute Tox. 4 Repr. 1B	**Retain** H302 **Modify** H360FD	**Retain** GHS08 GHS07 **Modify** Dgr	**Retain** H302 **Modify** H360FD		**Add** oral: ATE = 1320 mg/kg bw	
RAC opinion	613-111-00-X	1,2,4-triazole	206-022-9	288-88-0	**Modify** Acute Tox. 4 Repr. 1B	**Retain** H302 **Modify** H360FD	**Retain** GHS08 GHS07 **Modify** Dgr	**Retain** H302 **Modify** H360FD		**Add** oral: ATE = 1320 mg/kg bw	
Resulting Annex VI entry if agreed by COM	613-111-00-X	1,2,4-triazole	206-022-9	288-88-0	Repr. 1B Acute Tox. 4 Eye Irrit. 2	H360FD H302 H319	GHS08 GHS07 Dgr	H360FD H302 H319		oral: ATE = 1320 mg/kg bw	

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD EVALUATION

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	Rats (Wistar) 14, 15 or 30 rats/sex/dose	1,2,4-triazole Technically pure	Oral (gavage) Doses: 100 to 2500 mg/kg bw	LD_{50} (female): 1648 mg/kg	Thyssen and Kimmerle, 1976. Cited in JMPR, 2008

Following OECD TG 423		Vehicle: distilled water and Cremophor EL		LD50 (male): 1650 mg/kg	
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.

C*onclusion*

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.

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) No OECD guideline GLP	1,2,4-triazole (purity ≥ 99,9%) Doses: 0, 50, 250, 500 and 2000 ppm (males: 0, 9, 47, 90 and 356 mg/kg bw/d; females: 0, 12, 60, 120 and 479 mg/kg bw/d) Exposure: 4 weeks Vehicle: ethanol	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 atrophy NOAEL (males): 500 ppm NOAEL (females): 2000 ppm	Wahle B.S., 2004a (cited in JMPR, 2008)

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 days Vehicle: ethanol	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 in the lumen of the epididymal duct at 6000 ppm. NOAEL (males): 1000 ppm NOAEL (females): 3000 ppm	Wahle B.S., 2004b (cited in JMPR, 2008)
Combined subchronic toxicity/ neurotoxicity	1,2,4-triazole (purity ≥ 99,9%)	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	Wahle B.S. and Sheets L.P., 2004 (cited in JMPR, 2008)

screening study in rats (Wistar) 20/sex/dose Oral (feed) Similar to OECD TG 408 and 424 GLP	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	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	
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 <u>two-generation reproductive toxicity study</u> (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).	Renhof M., 1988a

		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	
Embryotoxi-city 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%	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, 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	Renhof M., 1988b
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.	Hobermann, 2004 (cited in JMPR, 2008)

		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	
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 and 218 mg/kg bw/d) Exposure: Through 10 weeks premating period to lactation D21 Vehicle: ethanol	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 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	Young and Sheets, 2005

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.**

ANNEXES:

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

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