Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Penconazole

EC Number: 266-275-6

CAS Number: 66246-88-6

ECHA/RAC/CLH-O-0000002679-61-01/F

Adopted

11 July 2012

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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 the Regulation (EC) No 1272/2008 (CLP Regulation), the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling of

Substance Name: **Penconazole** (1-[2-(2,4-dichloro-phenyl)pentyl]-1H-1,2,4-triazole)

EC Number: **266-275-6**

CAS Number: **66246-88-6**

The proposal was submitted by **Germany**
and received by RAC on **09 December 2010**

The proposed harmonised classification

	CLP Regulation (EC) No 1272/2008	**Directive 67/548/EEC**
Current entry in Annex VI CLP Regulation	-	-
Current proposal for consideration by RAC	Acute Tox. 4 H302 Aquatic acute 1 H400 Aquatic chronic 1 H410 M=1	Xn; R22 N; R50/53
Resulting harmonised classification (future entry in Annex VI of CLP Regulation)	Acute Tox. 4 H302 Aquatic acute 1 H400 Aquatic chronic 1 H410 M=1	Xn; R22 N; R50/53 N; R50/53: C ≥ 25% N; R51/53: 2,5% ≤ C < 25% R52/53: 0,25% ≤ C < 2,5%

PROCESS FOR ADOPTION OF THE OPINION

Germany 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/consultations/harmonised_cl/harmon_cl_prev_cons_en.asp*** on **17 January 2011**. Parties concerned and MSCAs were invited to submit comments and contributions by **3 March 2011**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Olivier Le Curieux-Belfond** (until end of February 2012)

Co-rapporteur, appointed by RAC: **Zhivka Halkova**

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

The RAC opinion on the proposed harmonised classification and labelling has been reached on **11 July 2012,** in accordance with Article 37(4) of the CLP Regulation, giving parties concerned the opportunity to comment. Comments received are compiled in Annex 2.

The RAC Opinion was adopted by **consensus**.

OPINION OF RAC

The RAC adopted the opinion that **Penconazole** should be classified and labelled as follows:

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

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)		
613-317-00-X	Penconazole (1-[2-(2,4-dichloro-phenyl)pentyl]-1H-1,2,4-triazole)	266-275-6	66246-88-6	Acute Tox. 4 Repr. 2 Aquatic Acute 1 Aquatic Chronic 1	H302 H361d H400 H410	GHS07 GHS08 GHS09 Wng	H302 H361d H410		Acute M= 1 Chronic M=1	

Classification and labelling in accordance with the criteria of Directive 67/548/EEC

Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits	Notes
613-317-00-X	Penconazole (1-[2-(2,4-dichloro-phenyl)pentyl]-1H-1,2,4-triazole)	266-275-6	66246-88-6	Xn; R22 Repr. Cat. 3; R63 N; R50/53	Xn; N R: 22-50/53-63 S: (2)-36/37-46-60-61	N; R50/53: C ≥ 25% N; R51/53: 2.5% ≤ C < 25% R52/53: 0.25% ≤ C < 2.5%	

SCIENTIFIC GROUNDS FOR THE OPINION

Penconazole is an active substance in the meaning of Directive 91/414/EEC (DSD) and therefore subject to harmonised classification and labelling (Article 36(2) of the Regulation (EC) 1272/2008 (CLP Regulation)).

The initial proposal of the dossier submitter included classification for acute toxicity category 4 and environmental hazard classification for aquatic acute category 1 and aquatic chronic category 1. In addition, RAC assessed other endpoints, notably the classifications for toxicity for reproduction that were previously recommended by EFSA experts (EFSA 2008) and which were also raised during the public consultation.

Acute toxicity

Summary of Dossier submitter's proposal

The dossier submitter proposed to classify Penconazole as Acute Tox. 4 (H302) according to the Regulation (EC) 1272/2008 (CLP Regulation) and Xn; R22 (Harmful if swallowed) according to Directive 67/548/EEC (DSD). The classification and labelling proposal for acute toxicity was based on four oral studies, one inhalation and one dermal study. Two out of four acute oral studies on Penconazole were below the 2000 mg/kg bw threshold for classification. A study performed according to a protocol similar to OECD Guideline No. 401 (Bathe, 1980) on male and female rats resulted in the oral LD_{50}s of 1486 and 3831 mg/kg bw /day, respectively. The LD_{50} for male oral exposure is thus below the threshold for classification. Female mortality data from the study were inconclusive because there was no clear dose response relationship. Given the large difference in male and female LD50 values, the meaningfulness of a combined (male, female) oral LD_{50} estimate can be questioned.

In another study, performed according to a protocol similar to OECD Guideline No. 401 (Kobel, 1981), the LD_{50} for male and female rabbits were 645 - 1321 mg/kg resulting in combined acute oral LD_{50} of 971 mg/kg.

The dossier submitter's proposal not to classify and label Penconazole for dermal or inhalation toxicity was based on low toxicity in both the acute dermal toxicity study (rat L LD_{50} > 3000 mg/kg bw) and the inhalation toxicity study (rat LD_{50}> 4.05 mg/l).

Comments received during public consultation

Several comments supported the dossier submitter's classification and labelling proposal for acute toxicity.

RAC assessment and comparison with criteria

No classification or labelling is required for acute dermal toxicity (rat LD_{50} > 3000 mg/kg bw) or inhalation toxicity (rat LD_{50} > 4.05 mg/l).

Based on the results of the acute oral LD_{50} in rabbits and rats, Penconazole is considered 'harmful if swallowed' and should be classified as Acute tox. 4 – H302 according to the Regulation (EC) 1272/2008 (CLP Regulation) and Xn; R22 according to the Directive 91/414/EEC (DSD). Classification and labelling is not required for acute dermal or inhalation toxicity.

Sedation effects observed in several acute toxicity studies would possibly justify an additional classification for narcotic effects with STOT SE 3 – H336. However, sufficient

details, e.g. on severity and duration of effects were not available to assess the need for classification.

Irritation

Summary of Dossier submitter's proposal

The dossier submitter did not propose classification and labelling for irritation. The justification not to classify was based on one skin irritation (OECD 404) and one eye irritation study (OECD 405) in rabbits.

Comments received during public consultation

One comment supported the dossier submitter's proposal not to classify Penconazole for irritation.

RAC assessment and comparison with criteria

Penconazole is not irritating to the skin but produced slight eye irritation in rabbits. However, the low severity of the response (e.g. Redness Conjunctiva average score after 24, 48 and 72 hours was respectively 1, 1 and 1) does not meet the criteria for classification laid down in DSD or the CLH Regulation.

Corrosivity

Summary of Dossier submitter's proposal

Classification for corrosivity was not proposed based on lack of evidence.

Comments received during public consultation

No comments were received on this endpoint.

RAC assessment and comparison with criteria

No data were provided to RAC on this endpoint and no conclusion is made on the classification and labelling.

Sensitisation

Summary of Dossier submitter's proposal

Classification for sensitisation was not proposed by the dossier submitter.

Comments received during public consultation

One Member State supported not to classify and proposed to clarify the comparison with the criteria.

RAC assessment and comparison with criteria

According to the Guinea pig maximisation test (OECD Guideline No. 410), Penconazole induced skin sensitisation in 3/20 animals (control = 0/10), which is less than the 30% positive responses required for classification under Regulation (EC) 1272/2008 (CLP Regulation) and R48/22 under the Directive 91/414/EEC (DSD). . RAC agrees the data do not warrant classification for sensitization.

Repeated dose toxicity

Summary of Dossier submitter's proposal

The dossier submitter did not propose to classify Penconazole for repeated dose toxicity. Among the reported repeated dose toxicity studies on rats (three studies), mice (two studies) and dogs (two studies), the dog appeared to be the most sensitive species. In a study conducted according to a protocol similar to OECD Guideline No. 409 (Gfeller, 1984), the derived 90-day NOAEL for males and females were 3.1 and 3.3 mg/kg bw/d (100 ppm), respectively. The associated LOAELs of 16.9 and 16.7 mg/kg/d were based on hepatotoxicity effects: Inflammatory cell infiltration, necrosis, clear dose-dependent increase in liver weight. Also, an increase in the activities of alkaline phosphatases γ-GT, AST, and ALT was observed. However, most of these signs were not severe**.** Furthermore, when incidence is estimated by pooling males and females, the single incidence of necrosis appears to be an isolated case: 1/8 after 90 days, 0/8 after 1 year.

In this study it was also observed in high dose males' group a moderate to marked reduction in spermatogenic activity, characterised by atrophy of the seminiferous epithelium associated with formation of giant cells, and absence of spermatozoa in the epididymis (which contained cellular debris). However, the 5000-ppm dose is largely above the MTD that was estimated around 2500 ppm. In the lower/intermediate/high dose groups, some decreases in relative gonad weights were also observed, but the observations were inconsistent compared to control: +23%, -4% and -27% for males and -35%, -8%, -16% in females at the 90-day time point. On the other hand, the liver weight increase was clearly dose-dependent: +1, +15, +75% for males and +8, +24, +88% for females.

In a 90-day oral rat study, conducted according to OECD Guideline No. 408 and with Penconazole with a purity of 98.7% (Hiles, 1987a), evidence of hepatotoxicity was also found. Observations include dose-related centrilobular hypertrophy of hepatocytes (in males 0/15, 3/15, 12/15 and 15/15 for 300, 500, 1000 and 2400 ppm, weaker in females), hepatocellular degeneration around the central vein, and an increase in the incidence of hepatocytic vacuolisation (in males 0/15, 1/15, 5/15 for 500, 1000 and 2400 ppm, weaker in females). The derived NOAELs for male and female rats were 23.2 and 28.3mg/kg bw/day (300 ppm), respectively. The LOAELs were 37.5 and 45.2 mg/kg bw/day (500 ppm). A very similar picture was also observed in mouse liver: dose-dependent increase in absolute and relative liver weight (statistically significant from 500 pmm in males and 2400 in females), centrilobular hypertrophy of hepatocytes (in males 0/15, 3/15, 6/15 and 14/15 for 300, 500, 1000 and 2400 ppm), and hypertrophic hepatocytes around the central vein with some vacuolar (2400 ppm, males only) (Hiles, 1987b, according to guideline similar to OECD guideline No. 408).

Comments received during public consultation

France commented that some severe liver changes are noted at 500 ppm in dog studies (necrosis in 1 male out of 4 in the 90-day study and fibrosis in the 1-year study) and hepatic degeneration is also observed in one rat 90-day study at 1000 ppm (72 mg/kg bw/d) and the effective dose level of 500 ppm (16.9-18 mg/kg bw/d) is below the guidance value. Based on this France proposed to add classification for repeated dose toxicity, i.e. STOT RE. 2 H373 under the Regulation (EC) 1272/2008 (CLP Regulation) and R48/22 under the Directive 91/414/EEC (DSD).

RAC assessment and comparison with criteria

The reported liver changes can be considered as only adaptive responses to the increased metabolic load. Although some liver changes at 16.9/16.7 (M/F) mg/kg bw/day

(500 ppm) in dog studies could be considered as severe, they appear as isolated cases: necrosis in 1 male out of 4 in the 90-day study and also fibrosis in 1 male out of 4 when the study was prolonged to 1-year. A similar interpretation can be made for the hepatic degeneration observed in one rat 90-day study at 72 mg/kg bw/d (1000 ppm). Although the effective dose levels in both dogs and rats are within the $10 < C \leq 100$ mg/kg body weight/day range, RAC's conclusion is that a classification for specific target organ toxicity is not required under the CLP Regulation or DSD.

Mutagenicity

Summary of Dossier submitter's proposal

The dossier submitter did not propose classification and labelling for mutagenicity. The proposal was based on five in vitro studies and on a micronucleus test (OECD 474) in mouse, which all were reported to give negative results.

Comments received during public consultation

The UK supported no classification for mutagenicity.

RAC assessment and comparison with criteria

Penconazole had no effects in any mutagenicity tests performed. In vitro, it induced neither gene mutations in bacterial or mammalian cells (Chinese hamster), nor chromosome aberrations in CHO cells, nor unscheduled DNA synthesis in rat hepatocytes. Furthermore, a bone marrow micronucleus test revealed no evidence for clastogenic or aneugenic activity in vivo. It is concluded that classification for genotoxicity is not required for Penconazole.

Carcinogenicity

Summary of Dossier submitter's proposal

Classification and labelling of Penconazole for carcinogenicity was not proposed by the dossier submitter. The proposal was based on one study in rats (OECD 453) and two studies in mice (OECD 451 and 453).

Comments received during public consultation

The UK noted that the top dose tested in each carcinogenicity study was low and that the maximal tolerated dose was not achieved in rats. The UK agreed that the available information does not support classification for carcinogenicity.

RAC assessment and comparison with criteria

Three carcinogenicity bioassays have been performed with Penconazole. In two of these studies (Basler 1985a and b), one in rats and one in mice, the highest dose was 300 ppm (equals 15.3 mg/kg bw/d (M) and 16.6 mg/kg bw/d (F) and 40.8 mg/kg bw/d (M) and 35.7 mg/kg bw/d (F) for rats and mice, respectively). No adverse findings, including tumours, were seen in these studies. However, as no toxicity was seen at the top dose, it was concluded that the doses were too low and the studies can only be considered supportive. In the third study in mice (Milburn 2004) a top dose of 1500 ppm, equal to 178 mg/kg bw/d (M) and 222 mg/kg bw/d (F), was used. This dose caused clear toxic effects but no tumours.

The negative result of the Milburn 2004 study together with the supportive studies Basler 1985a and b indicates no carcinogenic potential of Penconazole. Therefore, classification for carcinogenicity is not required.

Toxicity for reproduction

Summary of Dossier submitter's proposal

The dossier submitter did not propose classification and labelling for reproductive toxicity.

Effects on fertility

Two studies on the impact of Penconazole on fertility were reported. The first one, a 2-generation study in rats (Tif:RAIf(SPF) (Fritz, 1983)) was generally consistent with OECD guideline 416. The results of this study indicated slight toxicity of Penconazole at the 2000 ppm level (146 and 166 mg/kg bw/d in males F_0 and F_1, respectively and 202 and 227 mg/kg bw/d in females F_0 and F_1, respectively) for both the F_0 and F_1 generation: reduction in body weight gain and food consumption during pre-mating and pregnancy. In addition, increased duration of pregnancy or delayed parturition in F_0 and F_1 dams were associated with maternal death and/or litter loss at birth.

These effects were not seen in the second study conducted in rats (Crl:COBS CD) (Schardein, 1987) according to OECD guideline 416. No effects were noticed on pregnancy duration or pregnancy index. A statistically significant increase in relative gonad weight was considered to be related to reduce body weight.

In the 1-year study in dogs (Gfeller, 1984) (see repeated dose toxicity section) a reduction in spermatogenic activity was observed. This was accompanied by atrophy of the seminiferous epithelium associated with formation of giant cells, and absence of spermatozoa in the epididymis (which contained cellular debris). However, the signs are not considered relevant for classification, as the dose-effect relationship was not clear and was reduced during the recovery period. It was mainly present at the higher dose where systemic toxicity was recorded, based on the loss of body weight (not only a decrease in weight gain).

Developmental toxicity

Two developmental toxicity studies in rats and two in rabbits were reported, in addition to the two multi-generation studies described above.

In rats, embryotoxicity was observed as retarded skull and limb ossification and as post-implantation loss (Fritz, 1981; according to OECD guideline 414). This study was performed with the doses 0, 30, 100 and 300 mg/kg bw/d, and later a supplementary study was performed with 300 and 450 mg/kg bw/d. Maternal toxicity was seen at the highest doses but this was not sufficiently severe to explain the findings (12% decrease in corrected body weight gain, 4 % reduction in food consumption). Doses of 30 and 100 mg/kg bw/d gave neither maternal nor fetal toxicity.

In the second study in rats (Salamon, 1985; according to OECD guideline 414) the original dose selection was 5, 100 and 750 mg/kg bw/d. Due to high toxicity noted early in the study, the top dose was reduced to 500 mg/kg bw/d. At 500 mg/kg bw/d, severe maternal toxicity was also seen, including maternal death, as well as decreased body weight gain (-14%, +3% and -41% for 5, 100 and 500 mg/kg bw/d groups at study end) and food consumption (-6%, -19% and -42% for 5, 100 and 500 mg/kg bw/d on day 6). The effects seen at the top dose were similar to the effects seen in the earlier study, including retarded skull and limb ossification. Although this study may not be useful for

establishing the need to classify Penconazole, its findings were consistent with the older study. The low and mid doses caused no toxicity in neither dams nor pups and the high dose resulted in too high maternal toxicity to be conclusive. From the study protocol it seems that no dose range finding study was performed and the rationale behind the selection of doses was not clear.

In a study in rabbits (Chinchilla, 20F) (Giese, 1982, according to OECD guideline 414), microphthalmia (3/125 foetuses from 3/16 litters, two in combination with internal hydrocephalus) were observed at a dose level of 150 mg/kg/day. The incidence of microphthalmia was above the historical control range given in the study report. However, a greater incidence of this finding was reported in historical control data submitted during the public consultation. A second study in rabbits (Nemec, 1985, OECD guideline 414) was conducted at a slightly higher dose level. In this study maternal toxicity was seen, but no embryotoxic or teratogenic effects.

Comments received during public consultation

Comments from several member states (Denmark, France, UK, Sweden, Spain and Austria) and one company (Syngenta) were received. The following provides an overview of the comments.

Denmark did not agree with the dossier submitter's arguments about the classification concerning reproductive toxicity and their view was that the observed effect was induced by the active substance and therefore Penconazole should be classified for effects on sexual function and fertility as Repr. 2 - H361 under the CLP Regulation (Repr. Cat. 3; R62 under DSD). In addition to this they point out that based on the effects seen in the developmental studies at high dose levels (cervical ribs in rat and microphtalmia in rabbits) Penconazole should be classified for developmental toxicity as Repr. 2 - H361 under the Regulation (EC) 1272/2008 (CLP Regulation) (Repr. Cat. 3; R63 under the Directive 91/414/EEC (DSD)).

Syngenta agreed with the dossier submitter's proposal for non-classification of Penconazole for fertility and developmental toxicity. Concerning fertility, Syngenta commented that the observed increase in dam mortality during the post-partum period was observed at the high dose level (2000 ppm) only and that the studies did not provide evidence that these effects are due to dystocia. Concerning developmental toxicity, Syngenta pointed out that increases in the incidence of cervical ribs were linked to marked maternal toxicity. Also, the incidence of bilateral microphthalmia observed in rabbits was higher than in the concurrent control group, but were within the historical control range for the test laboratory and were therefore considered not to be an effect of treatment. See further details in Annex II.

France supported no classification for fertility but warranted classification and labelling for developmental toxicity, i.e. Repr. 2 - H361d under the Regulation (EC) 1272/2008 (CLP Regulation) (Repr. Cat. 3; R63 under Directive 91/414/EEC (DSD)). However, concerning fertility, the absence of clear data to establish the mechanism of action (mechanistic studies and/or hormonal analysis were lacking) meant that endocrine disruptive effects could not be ruled out. Concerning the developmental toxicity, France added that hydrocephaly is known to be a class effect of triazoles in rabbit. Also, in one of the rat developmental studies, cervical ribs occurrence was increased at the high dose and increased incidences of variations in ribs are also observed with other triazole compounds. Furthermore, one of the main metabolites, 1,2,4-triazole (comprising 15% of the dose given) is currently classified in the EU as: Repr. Cat. 3; R63. Finally, France added that the argument relating to non-reproducible effects with a higher purity material is not acceptable, since the claimed purity of the technical material is 95%.

The UK wanted to have further discussion on classification for fertility and agreed with the dossier submitter that classification for developmental effects is not required. The UK noted that under the CLP Regulation, adverse effects on sexual function and fertility include effects on parturition; therefore, the statement that 'the finding of dystocia which only occurs in pregnant animals would not warrant a classification for fertility impairment' should be changed, since it is possible to classify for fertility on the basis of dystocia. The UK also suggested further discussion of the significance of the dystocia findings and their relevance to humans, and a possible classification for fertility, be included, particularly as other triazoles have been reported to induce this effect.

The UK also pointed out that the death of the corpus luteum of rodents leads to a fall in progesterone levels, whereas a 'functional progesterone withdrawal' in humans is affected by a repression of prostaglandin responsive genes. However, the mechanism of action of the dystocia induction explained in the report pertains to a down-regulation of the prostaglandin E3 receptor by Penconazole, which results in reduced uterine contractility. Since prostaglandin E3 is involved in myometrial contractions in humans, this mechanism of action would appear to be relevant to humans as well as rodents.

The UK commented on developmental toxicity by stating that in rats, the possible developmental effects observed were post-implantation loss, retarded bone ossification and an increased incidence of extra ribs. The first two of these effects were probably related to maternal toxicity, although more information in Table 5.9-2 would clarify this association. The third effect, extra ribs, has been reported in studies of other triazole substances. From the information provided on Penconazole, it is not clear if these were associated with maternal toxicity, so clarification of this point would be helpful. Uncertainty surrounds the developmental/teratogenic significance of supernumerary ribs, in particular their post-natal reversibility or otherwise. Generally, findings of this nature are not used as evidence for classification. In rabbits, an increased incidence of microphthalmia in one study was stated to be within the historical control range. An increased incidence of hydrocephalus occurred in one rabbit study but not in a second rabbit study or two rat studies that employed higher maximum doses.

Sweden proposed to consider classification of Penconazole as Repr. 2 (H361) according to the Regulation (EC) 1272/2008 (CLP Regulation) and Repr. Cat. 3; R62 according to Directive 91/414/EEC (DSD).. They also recommended considering whether the observed dystocia reported in both rats and rabbits, implantation loss in rats and aspermatogenesis in rats justified classification in Repr. 2. The results are further supported by the findings of histopathological changes in the testes and epididymidis from the 1 yr study in dogs.

Spain reminded that the draft EFSA Scientific Report (2008) proposed a classification of Repr. Cat. 3; R63 and that a classification as Repr. Cat. 3; R62 should be considered. The Spanish CA considered that a classification is warranted for Penconazole as Repr. 2 (H361f) according to the Regulation (EC) 1272/2008 (CLP Regulation)and as Repr. Cat. 3 (R62) according to the Directive 91/414/EEC (DSD). This view on classification for fertility was based on prolonged gestation, dystocia and increased parturition mortality of dams and pups observed in a two generation study in rats dosed with 200 mg/kg bd/day (Fritz, 1983) and taking into account the new criteria in Regulation (EC) 1272/2008 (CLP Regulation) that considers dystocia an adverse effect on fertility. Although no similar effects were observed in a second study (Schardein, J., 1987), the rat strain used and purity of the test substance were different in that study and this could explain the different results.

Spain considered that classification for Penconazole as Repr. 2 (H361d) was warranted according to the Regulation (EC) 1272/2008 (CLP Regulation) and Xn; Repr. Cat. 3 (R63) according to the Directive 91/414/EEC (DSD). This view was based on an increased incidence of bilateral microphthalmia and internal hydrocephalus observed in a teratology

study in rabbits (Giese 1982), and an increased in the occurrence of cervical ribs at 500 mg/kg bw/d in a teratology study in rats (Salamon 1985). Besides, the formation of 1,2,4-triazole (metabolite classified as Repr. Cat. 3; R63, accounting for 15% of administered dose) also has to be taken into account.

Spain also brought up other scientific evidence supporting classification. For example, the study results on azole and triazole compounds with the same mode of action, as well as the critical role of several CYP enzymes in reproduction, support the classification of Penconazole for fertility (Repr. 2 – H361f, Regulation (EC) 1272/2008 (CLP Regulation) and Repr. Cat. 3; R62, the Directive 91/414/EEC (DSD). and for development (Repr. 2 – H361d, The CLP Regulation and Repr. Cat. 3; R63, the Directive 91/414/EEC (DSD)).
Austria stated that it seems doubtful to consider Repr. Cat 2 (H361f) under the Regulation (EC) 1272/2008 (CLP Regulation) and Repr. Cat. 3; R62 under the Directive 91/414/EEC (DSD), appropriate. However, Austria concluded that it might be appropriate to consider classification as Repr. 2, H361d.

Concerning fertility Austria commented that it is unclear whether the death of the dams observed in the 1st study (on days 0, 4 and 11 p.p. in F_0 dams and on days 2, 2 and 4 p.p. in F_1 dams) but not in the 2nd study (both studies with comparable dose ranges) is due to dystocia. According to the study author, the dams died without obvious cause. There might be a suggestion that the different findings of the 1st and the 2nd study could be attributed to differences in purity of the batches of test material used. Since the current specification for Penconazole (> 95%) is intermediate between the two test batches, no statement can be made about the possible influence of impurities. Indeed, according to the CLP Regulation, effects on parturition belong to "adverse effects on sexual function and fertility". However, it is unclear whether the deaths of the dams after parturition were due to dystocia.

It should be kept in mind that the observed toxicity in dams given 2000 ppm in the 1st study was limited to reduced body weight gain of -8% and -16% (F_0 and F_1 dams, respectively) and lower food consumption (-5% and -9% in F_0 and F_1 dams, respectively), accompanied by increased relative liver weight and hepatocellular hypertrophy. It may be that the reduction in food consumption observed was (as suggested by Austria) not sufficient to fully explain the observed reduction in BW gain. Therefore, there might be some effects which were not observed, but which caused the death of the dams following parturition.

Developmental toxicity was commented by Austria stating that all malformation types (i.e. in rats: umbilical hernia; in rabbits: bilateral microphthalmia, internal hydocephalus and cleft palate) were either seen at incidences greater than in historical control data (HCD) or no comparison to HCD is reported and all these malformations are considered to be rare. Additionally, malformations per se do not depend on maternal toxicity regarding classification and labeling.

RAC assessment and comparison with criteria

Sexual function and fertility

Penconazole administration did cause some effects on parturition and pregnancy outcome that appeared to be associated with the substance. Such findings are of relevance for this endpoint (CLP Regulation, Annex I, Section 3.3.1.3).

In a rat two-generation study (Fritz, 1983), the duration of pregnancy was prolonged and deaths of dams were seen at the time of parturition. In the F_0 generation, the number of dams with pregnancy duration of greater than 21 days was 2/20, 4/20, 6/20 and 10/19 at 0, 80, 400 and 2000 ppm; the mean duration of pregnancy was 21.1 days at 0 ppm and 21.6 days (statistically significant) at 2000 ppm. Additionally, one dam of the mid-

dose group died during delivery, with further maternal deaths occurring post parturition in the high- (3 dams) dose group. In the F1 generation, the number of dams with pregnancy duration of greater than 21 days was 4/19, 6/18, 2/17 and 14/19 at 0, 80, 400 and 2000 ppm; the mean duration of pregnancy was 21.3 days at 0 ppm and 21.8 days at 2000 ppm. The number of dams that died was in F_0 0, 0, 1 (day 0 p.p.), 3 (days 0, 4, 11 p.p.) at 0, 80, 400 and 2000 ppm; additional maternal deaths occurred in F_1 in 1 (day 19 p.p.), 0, 1 (day 0 p.p.), and 3 (days 2, 2, 4 p.p.) dams at 0, 80, 400 and 2000 ppm, respectively.

Note that the study report was not consistent in the pregnancy duration and in the timing and number of the deaths. There was no obvious cause of the deaths and clinical signs prior to the onset of parturition were not reported. Body weight gain of the F_0 females was reduced during pregnancy (not dose-related) and in the F1 high-dose females (-16% compared with the controls). The observed effects on reproduction and litter parameters (live litter size and total litter losses) were most likely secondary to the prolonged duration of pregnancy and difficulties with delivery. Effects on pregnancy duration and parturition did not occur in a second rat (different strain) two-generation study conducted with a slightly higher top dose (Schardein, 1987).

The developmental studies also provided some information on effects on pregnancy / parturition. In one rat study (Fritz, 1981), some maternal toxicity was seen (13% reduction in body weight gain, slightly reduced food consumption) at the high dose (300 mg/kg/d). In this main study, 2/25 dams in the high-dose group died on GD 21 without other signs of toxicity. In a supplementary study to investigate this unusual finding, 0/15, 4/15 and 2/15 dams at 0, 300 mg/kg/d (dosed GD 6-15) and 450 mg/kg/d (dosed GD 10-14) died on GD 21. However, it should be noted that one of the dams at 300 mg/kg/d that died showed no sign of being pregnant. No pathological findings were noted on necropsy. In all cases, deaths occurred up to 5 days after the end of treatment and about one day before natural parturition should have commenced. No such effects occurred in a second developmental toxicity study in rats (Salamon 1985), where the top dose tested (500 mg/kg/d) was maternally toxic.

Also, in one rabbit study (Nemec, 1985) there was evidence of premature parturition in all treated groups for which a relationship to substance administration could not be excluded. Five treated does delivered 1 day prior to or on the day of the scheduled caesarean section (0/18, 2/16, 2/14, 1/18 at 0, 10, 50, 200 mg/kg/d, respectively, without a clear dose-response relationship). All their foetuses were normal and necropsy findings did not indicate any treatment-related findings. The historical control incidence for premature delivery was reported to be about 3%, whereas the combined incidence in the Penconazole-treated groups in this study was about 10%. Some maternal toxicity was seen in the high dose group (mild clinical signs, body weight loss and reduced food consumption during the first week of treatment). Penconazole did not affect the duration of pregnancy or the onset of parturition in another rabbit developmental study when tested up to 150 mg/kg/d (Giese, 1982).

Total litter loss at birth or in the postnatal period was increased in the F_0 mating of a two-generation rat study (Fritz, 1983) only at the high dose, and appeared to be related to the problems with parturition that were experienced by the dams. In the same study, the main impact on the *live litter size* (which was reduced in the high-dose F_0 and F_1 groups) was dead pups at birth which, likewise, was probably a consequence of the prolonged pregnancy and difficulties in parturition. These effects, therefore, should be considered as ones on sexual function and fertility rather than developmental toxicity.

<u>Weight of evidence (WoE) considerations:</u>

Effects on duration of pregnancy and on death of dams were seen in some studies, but not in others. The inconsistency in results was observed between studies, between and

within species and within the effects. Death of dams was seen in a rat 2-generation (Fritz, 1983) and developmental study (Fritz, 1981) conducted by one laboratory in one strain of rats, but not in a 2-generation (Schardein, 1987) and developmental study (Salamon, 1985) with other strains of rats at slightly higher doses tested, nor in developmental studies with rabbits (Giese, 1982; Nemec, 1985). In the Fritz 1981 study deaths occurred about 1 day before natural parturition would have commenced. This is an unusual finding and the relevance is unknown. In the Fritz 1983 study a small number of dams died at or shortly after parturition (1 dam each at 400 mg/kg/d in F_0 and F_1 and at 2000 mg/kg/d in F_0), but others (with increased pregnancy duration) died 2 days or later after parturition. The relevance of these later deaths, which also occurred in one control dam, is not clear, but they are probably more related to maternal toxicity than to dystocia. It is further noted that the Fritz 1983 study report was not consistent in the timing and number of the deaths.

As to the duration of pregnancy, a prolonged duration was seen in the rat 2-generation study by Fritz 1983 (together with possible consequences for total litter loss and live litter size), but not in the rat 2-generation by Schardein 1987 that was conducted with a slightly higher top dose. In rabbits on the other hand, premature parturition was seen, but only in one study (Nemec, 1985), not in a second study with another strain (Giese, 1982). The relevance of the finding in the Nemec study is doubtful, given the absence of dose-response and all foetusus being normal.

Looking at all data available, the effects on pregnancy duration and on death of dams are difficult to interpret as to the need to classify them, given the inconsistencies observed in the findings and, for the 2-generation study, in the study reporting. The overall WoE consideration is that there is no clear link between the death of dams and dystocia, nor between Penconzole treatment and prolonged pregnancy. Therefore no classification for sexual function and fertility according to the CLP Regulation and DSD is warranted.

Developmental toxicity

Several findings that were possibly indicative of developmental toxicity were observed in the available studies. These are summarised and discussed below.

Increased *post-implantation loss* was recorded in two rat developmental studies. In the Fritz (1981) study, the incidences of early resorption were 4.8%. 5.9%, 8.1%, 9.0% at 0, 30, 100, 300 mg/kg/d. None of these increases was statistically significant. The increased post-implantation loss of the high-dose group occurred together with some maternal toxicity (-13% in the corrected weight gain) and this was only just above the historical control mean of 8.9%. At the mid- and low- doses, the incidences of early resorption were increased without evident maternal toxicity but were within the historical control mean. In the Salamon (1985) study, the incidences of resorptions expressed as a % of implantations were 2.2%, 4.4%, 3.6% and 18.9% at 0, 5, 100 and 500 mg/kg/d, respectively. Only the increase at the highest dose was statistically significant; at this dose, however, the maternal toxicity was considerable (death of 2/25 dams, severely reduced body weight gain (-41%), clinical symptoms that included emaciation, weakness and lethargy). At the other doses, a clear dose-related effect was not seen.

Some increases in early resorptions, expressed as a % of implantations, were reported in the rabbit studies. In the Giese (1982) study, these were 4.8%, 6.0%, 0.9% and 9.7% at 0, 20, 75 and 150 mg/kg/d, respectively. At the high dose, maternal toxicity in the form of reduced body weight development was noted at different time points, notably at GD 6-11 (50% reduction). In the Nemec (1985) rabbit study, the incidences of early resorptions were 6.6%, 12.5%, 1.4% and 16.4% at 0, 10, 50 and 200 mg/kg/d, respectively. It should be noted that, in the case of the high-dose group, one of the females was responsible for one third of the cases of resorptions. Maternal toxicity was evident in this group in the form of a 37% reduction in daily food consumption during

treatment (GD 7-20, but particularly marked during the first week), which was associated with weight loss over the same time period, such that there was almost no weight change throughout pregnancy. None of the findings in the rabbit studies was statistically significant and clear dose–response relationships were not apparent.

Statistically significant decreases in *pup weight* were recorded in the two multi-generation rat studies (Fritz 1983 and Schardein 1987) at the high dose (up to 16.5%, but mostly less than 10%) at PND 14 and 21, and in the Schardein study also at PND 4 and 7 (F_2 only). There was also a decrease in parental body weight and an increase in relative organ weight in pups and parents in these studies.

Incomplete/absent skeletal ossification was recorded in two rat and one rabbit developmental studies (Fritz, 1981; Salamon, 1985; Nemec, 1985) but only in the presence of maternal toxicity. Such findings are regarded as variations or delays in development. In association with maternal toxicity such effects may not merit classification. Supernumerary cervical ribs, which were reported in one rat study (Salamon, 1985), also in association with maternal toxicity, do not normally lead to classification on their own, since there is no consensus on their relevance to developmental toxicity.

Malformations were also seen in some instances. Microphthalmia and hydrocephalus occurred in one study (Giese 1983) with Chinchilla rabbits at 150 mg/kg/d. One foetus had bilateral microphthalmia alone, giving an incidence (foetal: 0.8%; litter: 6.3%) that was within the historical control range (foetal range 0-4.1%, mean 0.052%; litter range 0-12.5%, mean 1.6%, same strain and laboratory). Two further foetuses had bilateral microphthalmia in combination with internal hydrocephalus, giving incidences of 1.6% (foetal) and 12.5% (litter); these were just outside the historical control range for internal hydrocephalus (foetal range 0–0.9%, mean 0.09%; litter range 0–7.1%, mean 0.7%). The combined incidence of microphthalmia in the three affected foetuses (2.4%) was still within the range of the historical control data.

These kinds of rare malformations are unlikely to be related to the maternal toxicity observed (changes in body weight development). In this study, other severe malformations also occurred at the high dose but only as single cases. In another rabbit study but with a different strain (New Zealand White) and at a higher maximum dose (200 mg/kg/d, associated with maternal toxicity), these effects were not seen (Nemec 1985), nor did they occur in two rat developmental studies at doses up to 500 mg/kg/d. In the rat studies (Fritz 1981 and Salamon 1985) there was an increase in the number of foetuses with abnormalities but the effects on the different malformations were not consistent and were generally within the range normally seen for the laboratories; they were therefore regarded by the study authors as spontaneous occurrences.

<u>WoE considerations:</u>

Effects were seen on several variables. Post implementation loss in the form of early resorptions was seen in all developmental studies at the top dose. In one study (Salamon, 1985) the effect was clear and statistically significant, but associated with considerable maternal toxicity. In the other studies the effect was about two fold and neither consistently above historical controls nor statistically significant, and also here slight to more marked maternal toxicity was observed. However, as the effects are consistently seen in all the studies they can not be disregarded as chance findings. Pup weight was decreased postnatally in both rat multigeneration studies at the high dose. Incomplete/absent ossification occurred in two rat and one rabbit studies, and supernumery cervical ribs in one rat study, all in the presence of slight to considerable maternal toxicity.

These variations or delays in development may not warrant classification on their own, especially when associated with maternal toxicity, but here they are regarded to add to the WoE. Finally, and most important, severe malformations were seen in one study in rabbits (Giese, 1982): these were three cases of microphtalmia, two in combination with internal hydrocephalus. This effect can not be disregarded. Other severe malformations seen in the rat and rabbit studies were single cases, not consistent and within historical controls, and do thus not contribute to the WoE. Overall there are several effects on development seen and although these may each not all warrant classification on their own, the WoE of all the effects combined makes classification warranted.

Overall, adverse effects on development are seen in the studies. The effects are not pronounced and consistent in the different studies. However, it would be inappropriate to not classify, as there are effects seen in several studies and it has not been shown that these are irrelevant for humans. It should be noted that this is a borderline case for classification. As no evidence from humans is available, classification in Repr. 1A is not possible. The data are not sufficiently conclusive to place the substance in Repr. 1B. Classification for developmental toxicity as Repr. 2 - H361d according to the Regulation (EC) 1272/2008 (CLP Regulation) and Repr. Cat. 3; R63 according to the Directive 91/414/EEC (DSD) is therefore warranted.

Environmental hazard assessment

Summary of Dossier submitter's proposal

The dossier submitter proposed classification and labelling for environmental hazards as Aquatic Acute 1 and Aquatic Chronic 1 with an M-factor 1.

Degradability

According to the OECD Guideline No. 301B, Penconazole was not found to be readily biodegradable, because no degradation occurred during 28 days whereas >70% degradation within 28 days is required to achieve this criterion.

In water/sediment systems Penconazole is dissipated primarily by partitioning to the sediment with single first order DT_{50} of 1.9-3.4 days where it subsequently degraded (whole system pseudo first order DT_{50} 505 up to >706 days) forming the major metabolite CGA 179944 that was present in the water phase (max. 17.3 % of AR after 365 days) and only accounted for a maximum of 4.8% of AR in the sediment. In aerobic laboratory soil degradation studies the overall geometric mean DT_{50} value of Penconazole was 117 days (SFO, 20 °C, pF2). In field soil dissipation studies DT_{50} values of Penconazole were in the between 67 d – 115 days (SFO). In the field, Penconazole can exhibit slow primary degradation but not ultimate mineralisation. As a result of the field and laboratory studies, Penconazole is considered as not rapidly degradable.

Bioaccumulation

Penconazole has a log Kow of 3.72. The only available experimental bioaccumulation study was performed according to EPA guideline No. 165-4 and the calculated BCFs were based on total radioactive residue. The maximum BCF of 320 for whole fish is considered more reliable estimate than the steady state BCF of 200. Both BCF values are above the Directive 91/414/EEC (DSD) limit values of 100 but lower than the Regulation (EC) 1272/2008 (CLP Regulation)limit value of 500. Penconazole is thus considered as bioaccumulative according to the Directive 91/414/EEC DSD, but not bioaccumulative according to the Regulation (EC) 1272/2008 (CLP Regulation)**.**

Ecotoxicity

In fish, LC_{50}s ranged from 1.13 to 3.8 mg/l. A chronic NOEC in fathead minnow *Pimephales promelas* was 0.32 mg/l (Surprenant, 1984; 30 days post-hatch test / internal protocol, based on measured concentrations).

In the water flea *Daphnia magna* EC_{50} was 6.75 mg/l. In this species NOEC was 0.069 mg/l (Surprenant D.C., 1984; 21-day flow through test, according to an internal method similar to US EPA (1975) Series 660/3-75-009). This NOEC was based on measured concentrations and does not need any correction for the 87.3% purity.

In the algae *Pseudokirchneriella subcapitata* ErC_{50} (72h) was 4.9 mg/l, but in the duckweed *Lemna gibba* the 14-day EC_{50} value was 0.22 mg/l (NOEC = 0.087 mg/l) (Hughes, 1985, static 14-day test according to the US EPA proposed Guidelines for Registering Pesticides). In this study, the substance purity was 87.3%, so the toxicological values were corrected to 100% active ingredient nominal concentrations.

Comments received during public consultation

Several member states (Belgium, the Netherlands, France, the UK, Sweden) commented proposed environmental hazard classification and labelling of Penconazole. All comments agreed with the proposed classification and labelling.

Most of the comments concerned editorial issues or data reporting. Some comments brought up the appropriateness of 7- and 14-days *Lemna* studies for the purpose of determining an EC_{50} and NOEC and further consideration of the results of 7-day was recommended.

Lack of analytical verification of test concentrations in some studies was commented and the low purity of the test material was recommended to be taken into account when defining threshold values (corrected values are available in the revised report in Annex 2).

Some comments concerned data that does not have relevance for classification and labelling and was recommended to be removed. Also, it was brought up that degradation and bioaccumulation have separate criteria and should be assessed independently**.**

RAC assessment and comparison with criteria

According to the Regulation (EC) 1272/2008 (CLP Regulation):

As the acute toxicity of Penconazole in *Lemna gibba* (14-day EC_{50} = 0.19 mg/l) is above 0.1 mg/l but below or equal to 1 mg/l, classification as aquatic acute category 1 – H400 and an M-factor of 1 are required.

The chronic toxicity of Penconazole in *Daphnia magna* (0.01 mg/l < water flea flow-through 21-day test NOEC = 0.069 mg/l ≤ 0.1 mg/l) is above 0.01 mg/l but below or equal to 0.1 mg/l. Since Penconazole does not meet the criteria of rapid degradation, classification as aquatic chronic category 1 – H410 and an M-factor of 1 are required.

According to the Directive 91/414/EEC (DSD):

The acute toxicity of Penconazole in *Lemna gibba* (14-day EC_{50} = 0.19 mg/l) is below or equal to 1 mg/l and Penconazole does not meet the criteria of ready biodegradability in

the OECD-301B test. Classification as N; R50/53 with the specific concentration limits as given below are required.

N; R50/53: C ≥ 25%
N; R51/53: 2.5% ≤ C < 25%
R52/53: 0.25% ≤ C < 2.5%

In addition to the data presented in the CLH report, RAC is aware that Penconazole, like other ergosterol biosynthesis inhibiting (EBI) substances, is under particular regulatory scrutiny with regard to their potential for endocrine disruption. For these substances, e.g. the (re-)approval process may generate further data from long-term fish studies like full life-cycle or sexual development tests, if requested for the underlying risk assessment.

Based on the provided data in the CLH report, RAC agrees with the dossier submitter's proposal to classify Penconazole for Aquatic acute 1 and Aquatic chronic 1 according to the CLP Regulation and N; R50/53 according to the Directive 91/414/EEC (DSD) (with the specific concentration limits as given above). However, separate M-factors, i.e. Acute M-factor 1 and Chronic M-factor 1, are warranted according to the 2nd ATP of CLP Regulation.

ANNEXES:

Annex 1	Background Document (BD)[1]
Annex 2	Comments received on the CLH report, response to comments provided by the dossier submitter and RAC (excl. confidential information)

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