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Dortmund, 21 October 2022

Substance name: penconazole (ISO); 1-[2-(2,4-dichlorophenyl)pentyl]-1H-1,2,4-triazole
CAS Number: 66246-88-6
EC Number: 266-275-6
Submitted by Norway

Current entry in Annex VI of CLP Regulation
Acute Tox. 4, H302,
Repr. 2, H361d,
Aquatic Acute 1, H400,
Aquatic Acute 1, M-factor=1,
Aquatic Chronic 1, H410,
Aquatic Chronic 1, M-factor=1

Proposed future entry in Annex VI of CLP Regulation
Acute Tox. 4, H302,
Repr. 2, H361d,
STOT RE 2, H373,
Aquatic Acute 1, H400,
Aquatic Acute 1, M-factor=1,
Aquatic Chronic 1, H410,
Aquatic Chronic 1, M-factor=1
Oral ATE: 971 mg/kg bw

Carcinogenicity:

We support the proposal to re-discuss to what extent these three available long-term studies are sufficient to exclude a carcinogenic potential of penconazole. From our point of view, taking into account all available information, studies can be considered sufficient to conclude on classification. However, it is noted that further information is still requested for the purpose of the PPP assessment.

Mutagenicity:

Findings observed in the available *in-vitro*- and *in-vivo*-studies on genotoxicity of penconazole do not indicate a relevant genotoxic potential. Classification for mutagenicity is not warranted.

However, we noted that some of the available data are not fully reliable (supplementary only) to conclude on clastogenicity and aneugenicity: The available *in vitro* assay on chromosome aberration is of limited reliability (number of scored cells too low) and because of the statistically significant increase over the concurrent negative control in one test concentration in one experiment, the result is not clearly negative when evaluated strictly according to OECD TG 473 (2016). The available *in-vivo*-micronucleus test in mice is of limited reliability as well because of insufficient numbers of scored cells. Nevertheless, a negative *in vitro* micronucleus test is available to support the *in vivo* result on clasto- and aneugenicity. Further confidential data generated using technical penconazole spiked with relevant impurities is considered reliable and supports the conclusion.

Reproductive toxicity:

The DS' proposal for Repr. 2, H361d is supported.

Justification: In a weight-of-evidence approach, the observed developmental effects should be taken into consideration to classify penconazole (Repr. 2, H361d). This is also in line with the current harmonised classification. Furthermore, other triazoles are classified for developmental effects; possible similarities could support the weight-of-evidence approach.

Acute toxicity:

We agree with the proposal that classification for acute oral toxicity (oral, dermal and inhalation) is required for penconazole and the existing entry in Annex VI of the CLP Regulation should be retained. The oral ATE of 971 mg/kg bw is agreed with but rounding to 1000 mg/kg bw may be appropriate. For acute dermal and inhalation toxicity, no classification is required.

Skin corrosion/irritation:

We agree with the proposal that classification for skin corrosion/irritation is not required for penconazole.

Serious eye damage/eye irritation:

Effects observed in the available eye irritation study were below the trigger for classification as an eye irritant. Thus, we agree with the proposal that classification for serious eye damage/eye irritation is not required for penconazole.

Skin sensitization:

We agree with the proposal that based on effects observed in the available GPMT (sensitisation rate of 15%), classification for skin sensitisation is not required for penconazole.

Specific target organ toxicity – single exposure:

We agree with the proposal that data are conclusive but not sufficient for classification for STOT SE.

Specific target organ toxicity – repeated exposure:

We notice that the dossier submitter (DS) did not supply new information on this human health endpoint and that RAC has already evaluated this data in the year 2012. At that time, RAC concluded that classification for STOT RE was not required even though hepatotoxicity had been observed.

The DS, however, now requests a re-discussion on RAC's conclusion. Concretely, in the DS's view „it should be rediscussed whether the observed cases with fibrosis in dogs should be considered as isolated cases."

The hepatic fibrosis occurred in a one-year study in dogs on which RAC commented as follows[1]:

„[…] *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*.[…]"

Overall, the DE CA agrees with the DS that the liver is a target organ in rats, mice and dogs, and that the dog appears to be the most sensitive species.

In the following, the DE CA would like to comment more particularly on:
1) the occurrence of hepatic necrosis in a 90-day study in dogs (1984);
2) the occurrence of hepatic fibrosis in a 1-year study in dogs (1984); and
3) the potential consideration of human data from other triazole fungicides.

ad 1)
The following table summarises some findings from the 90-day study in dogs:

90-day study, Beagle dog	**Males**				**Females**			
Penconazole (ppm)	0	100	500	5000	0	100	500	5000
Penconazole (mg/kg bw/d)	0	3.4	18.2	132	0	3.8	19.4	137
Body weight gain week -1 to 13° (%)	+27.5	+19.9	+19.1	-12.0	+29.0	+30.0	+21.3	-8.9%
Liver weight absolute (% change ctr)	-	+4.3	+20	+30	-	+15	+15	+22
Liver weight relative (% change ctr)	-	+1.6	+15	+75^{+}	-	+7.8	+24	+88^{+}
ALP (% change ctr)	-	+4	+7	+390*	-	-14	+1	+366*
GGT (% change ctr)	-	+14	+3	+1800	-	+0	+18	+932*
OCT (% change ctr)	-	-18	-18	+418*	-	+0	+20*	+480*
AST (% change ctr)	-	+0	+25	+154*		-7	+3	+143*
ALT (% change ctr)	-	-14	-8	+790*		-8	+5	+808*
Cytoplasmic vacuolisation	0/4	0/4	0/4	2/4	0/4	0/4	0/4	0/4
Inflammatory cell infiltration	0/4	0/4	1/4	4/4	0/4	0/4	0/4	4/4
Hepatocyte necrosis	0/4	0/4	1/4	4/4	0/4	0/4	0/4	4/4

°body weight gain in % of body weight prior to treatment (week -1)
* p 0.05, significant difference to control
$^{+}$ statistically significant positive trend from control to highest dose group

We note that 1/8 animals was affected by liver necrosis in the mid dose group (18 mg/kg bw/d) whereas this finding was observed in all animals (8/8) in the high dose group (132 mg/kg bw/d). Necrosis is a severe effect that may principally justify classification for STOT RE. Nevertheless, a closer characterisation of its intensity (e.g. focal, multi-focal, diffuse,…) may still be worthwhile for interpreting this finding. Considering the steep dose-response relationship, it could be concluded that liver necrosis would probably occur in more than one animal at doses > 18 mg/kg bw/d and ≤ 100 mg/kg bw/d, i.e. at doses relevant for STOT RE 2 classification. However, the observed body weight losses in animals from the high dose group makes it difficult to properly judge the incidence of liver necrosis in these animals. The negative body weight development may have enhanced the formation of liver necrosis caused by penconazole. However, it is also problematic to call the incidence of 1/8 animals as “isolated case”(RAC 2012) because, after all, 25% of the mid dose group males were affected.

Questions to the DS:

1a) Could you please re-inspect the individual raw data in order to check the severity grades for liver necrosis?

1b) Do you know appropriate historical control data regarding liver necrosis in beagle dogs?

Ad 2)

In a 1-year study (1984), six dogs/sex/group were exposed to penconazole for 52 weeks. Four dogs/sex/group were sacrificed after 52 weeks. The remaining two dogs/sex/group were used for a four-week recovery phase and sacrificed after 56 weeks only.

Since fibrosis is a chronic and irreversible morphological change and because of the small animal number/group, we decided to combine the incidences of liver fibrosis for all animals sacrificed after 52 and 56 weeks and for both sexes. The table below summarises the respective incidences.

The table clearly shows that inflammation with fibrosis in the liver did not occur as isolated case in the mid dose group (500 ppm/17 mg/kg bw/d): 2/6 males and 1/6 females were affected. Or, in other words: Inflammation with fibrosis was observed in the liver of 25% of all dogs exposed to 17 mg penconazole/kg bw/d for 52 weeks. Moreover, a clear dose-response relationship is present for both sexes.

1-year dog study with Penconazole (1984)
"Inflammation with fibrosis" in the liver: Overview on incidences

Test item intake (ppm)		0	100	500	2500/5000
Males (mg/kg bw/d)		0	3.0	16.8	108
Females (mg/kg bw/d)		0	3.2	16.5	110
Males					
	Week 52	0/4	0/4	2/4	4/4
	Week 56	1/2	1/2	0/2	1/2
	Week 52+56	1/6	1/6	2/6	5/6
Females					
	Week 52	0/4	0/4	1/4	4/4
	Week 56	0/2	0/2	0/2	1/2
	Week 52+56	0/6	0/6	1/6	5/6
Males+Females					
	Week 52	0/8	0/8	3/8	8/8
	Week 56	1/4	1/4	0/4	2/4
	Weeks 52+56	1/12	1/12	3/12	10/12
Males					
	Week 52	0%	0%	50%	100%
	Week 56	50%	50%	0%	50%
	Week 52+56	**17%**	**17%**	**33%**	**83%**
Females					
	Week 52	0%	0%	25%	100%
	Week 56	0%	0%	0%	50%
	Week 52+56	**0%**	**0%**	**17%**	**83%**
Males+Females					
	Week 52	0%	0%	37.5%	100%
	Week 56	25%	25%	0%	50%
	Weeks 52+56	**8%**	**8%**	**25%**	**83%**

Still, we are in doubt whether this finding alone can be considered as „significant toxic effect“ that warrants classification for STOT RE 2 according to table 3.9.1 of the CLP Regulation. According to the CLP Guidance *„‘significant‘ means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant“*. Regarding the occurrence of fibrotic changes, the CLP Regulation states in 3.9.2.7.3 that

„multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity“ shall be taken into consideration in the classification process.

From the data for penconazole made available for public consultation it is not evident whether liver fibrosis in dogs was multi-focal or diffuse. We also could not identify significant functional disturbances related to liver toxicity which would be reflected in relevant changes in biochemistry or haematology parameters at the mid dose. Therefore, even though hepatic fibrosis occurred in a relevant number of test animals (and not as isolated case) at a dose principally relevant for STOT RE 2 classification (i.e. 17 mg/kg bw/d), we are not sure whether the intensity of the effect is sufficient for classification.

Question to the DS:

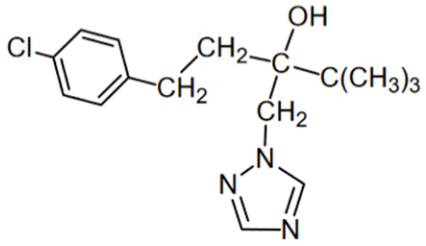
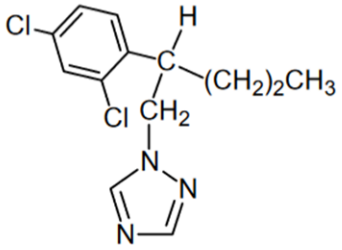
2a) Could you please re-inspect the individual raw data in order to check the severity grades for liver fibrosis?

Ad 3)

As mentioned above, the DS did not supply new information for STOT RE. However, we are wondering whether it may be worthwhile to take into consideration human data from other triazole fungicides as supportive evidence. For example, we would like to point to the publication „*Hepatotoxicity After Exposure to Tebuconazole: A Case Report and Brief Review*“ by Habibzadeh et al. (2019)[2]. Here, the authors report the case of a farmer with tebuconazole-induced hepatitis. The abstract of the publication reads as follows:

„A 48-year-old man was admitted to our hospital with complaints of productive cough, anorexia, and sweating from two weeks ago. Laboratory studies revealed an increased level of liver enzymes. The work-up of serological markers was negative for both infectious and non-infectious causes of hepatitis. He denied smoking, alcohol consumption, occupational exposures, and illicit drug use. Interestingly, he had a history of contact with an antifungal toxin called tebuconazole about 15 days before admission. In toxicological examinations using gas chromatography with a nitrogen-phosphorus detector, the cause of poisoning was revealed to be fungicide tebuconazole. To confirm the causal relationship between toxin and hepatitis, exposure avoidance and repeated testing were considered. Complete recovery was achieved in all clinical symptoms and laboratory findings. We suggest that tebuconazole-induced hepatitis should be considered in farmers exposed to this fungicide.“

Tebuconazole is structurally related to penconazole:

Tebuconazole	**Penconazole**
CAS No. 107534-96-3	CAS No. 66246-88-6
	

We acknowledge that a thorough read-across assessment would be needed in the first place in order to validate the appropriateness of human data from other triazoles. However, if such data would exist for other triazole fungicides, they might be a valuable argument to classify penconazole in STOT RE 2 according to 3.9.2.6 of the CLP Regulation:

„In exceptional cases, based on expert judgement, it is appropriate to place certain substances with human evidence of specific target organ toxicity in Category 2:
(a) when the weight of human evidence is not sufficiently convincing to warrant Category 1 classification; and/or
(b) based on the nature and severity of effects.
Dose/concentration levels in humans shall not be considered in the classification and any available evidence from animal studies shall be consistent with the Category 2 classification […]“

Question to the DS:

3a) Are you aware of appropriate human data for penconazole or other triazole fungicides which might be used to substantiate the classification proposal STOT RE 2 for penconazole?

Taken together, the DE CA is of the opinion that possibly further information will be needed in order to decide on whether classification of penconazole as STOT RE 2 hepatotoxicant is justified or not. A closer description of the liver findings observed in dogs in a 90-day study and in a 1-year study (both 1984) as well as human evidence (if available at all) might be helpful for a proper discussion.

References:

1) RAC Opinion proposing harmonised classification and labelling at EU level of Penconazole, adopted 11 July 2012, ECHA/RAC/CLH-O-0000002679-61-01/F.

2) Shahram Habibzadeh, Abbas Yazdanbod, Jafar Mohammadshahi, Nasrollah Maleki, and Sobhan Ataei (2019): Hepatotoxicity After Exposure to Tebuconazole: A Case Report and Brief Review. Hepat Mon. 2019 July; 19(7):e94548.