Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

1-vinylimidazole

EC Number: 214-012-0
CAS Number: 1072-63-5

CLH-O-0000001412-86-130/F

Adopted

9 December 2016

9 December 2016

CLH-O-0000001412-86-130/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-vinylimidazole**

EC Number: **214-012-0**

CAS Number: **1072-63-5**

The proposal was submitted by **BASF SE** and received by RAC on **8 January 2016.**

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

PROCESS FOR ADOPTION OF THE OPINION

BASF SE 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 **17 February 2016**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **4 April 2016**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Anne-Lee Gustafson**

Co-Rapporteur, appointed by RAC: **Betty Hakkert**

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 **9 December 2016** by **consensus**.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling				
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)	Specific Conc. Limits, M-factors	Notes
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	1-vinylimidazole	214-012-0	1072-63-5	Repr. 1B	H360D	GHS08 Dgr	Repr. 1B	H360D		
RAC opinion	TBD	1-vinylimidazole	214-012-0	1072-63-5	Repr. 1B	H360D	GHS08 Dgr	Repr. 1B	H360D	Repr. 1B; H360D: C ≥ 0,03%	
Resulting Annex VI entry if agreed by COM	TBD	1-vinylimidazole	214-012-0	1072-63-5	Repr. 1B	H360D	GHS08 Dgr	Repr. 1B	H360D	Repr. 1B; H360D: C ≥ 0,03%	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

1-Vinylimidazole is used only in industrial settings as a monomer for further polymerization. The polymerized products are used in several applications including: lubricants, coating additives, emulsifiers, polymers for metal ion filtration and in both home and personal care applications.

1-Vinylimidazole is not currently classified according to CLP Regulation (EC) No. 1272/2008.

The present opinion only addresses reproductive toxicity since this was the sole endpoint that was evaluated by the dossier submitter (DS) in their proposal.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The assessment of reproductive toxicity is based on the results from a GLP compliant combined repeated dose toxicity study with a reproduction/developmental toxicity screening test performed according to OECD test guidelines (TG) 422 (BASF SE, 2013). In this study 10 rats/sex/group were given 1-vinylimidazole by gavage at dose levels of 0 (vehicle, drinking water only) 5, 15 and 35 mg/kg bw/d. The treatment covered a two-week pre-mating period and a mating period in both sexes. In females, the treatment was continued during the entire gestation period as well as approximately 2 weeks after parturition. In total, males were dosed for 30 days and females for 50 days. The offsprings were sacrificed on post-natal day (PND) 4. All pups, including the pups that were stillborn or died during the first 4 days, were when possible examined externally and their organs were assessed macroscopically.

Sexual function and fertility

There was no effect on male or female mating index (100% in all groups). One male in each group did not generate F1 pups, thus the male and female fertility index was reduced to a similar extent in all groups. The non statistically significant effects that were recorded for high dose females for the endpoints number of mating days (3 days as compared to 1.9 in controls), post-implantation loss (11.6% as compared to 3.5% in controls) and duration of gestation (22.9 days as compared to 22.2 days in controls) were all considered as being of no biological relevance by the DS. There was no effect on the mean number of implantations per dam or on the gestation index (see Table 13 in the background document for further details).

The mean relative weight of the testis in the high dose males was slightly increased (+13% as compared to the controls) whereas there was no effect on the weight of the epididymides (the testis and the epididymides are the only reproductive organs that should be weighed in an OECD TG 422 study). Histopathological examination of the sex organs (testes, epididymides, seminal vesicles, ovaries, uterus and vagina) at the termination of the study revealed no treatment-related changes in the parental animals.

The mean number of F1 pups (dead + live) delivered per dam was not affected (11.1, 10.1, 11,8 and 10.4 pups/dam in the control, low, intermediate and high dose group, respectively). The number of live born pups was however considerably reduced in the high-dose group (70 as compared to 88 in the control group) also resulting in a reduced live birth index (98.9, 100, 94.3

and 74.5% in the control, low, intermediate and high dose group, respectively). Moreover, the number of stillborn pups was significantly increased in the high-dose group (1, 0, 6, 24 pups/dam for 0, 5, 15, 35 mg/kg bw/d, respectively). According to the DS, the increased number of stillborn pups can be explained by the teratogenic effects at the high dose (which is described below in the developmental toxicity section) and according to the DS the NOAEL for fertility should be set at the highest dose tested, 35 mg/kg bw/d. Signs of systemic toxicity revealed as piloerection and semi closed eyes on the first days of dosing (both sexes), reduced food consumption (both sexes), and reduced body weight gain (only in males) was noted at the intermediate and high dose levels during the pre-mating period in the parental generation. According to the DS, 1-vinylimidazole does not affect fertility and the available data does not meet the criteria for classification in Category 1A, 1B or 2 for adverse effects on sexual function and fertility.

Development toxicity

In the combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422, GLP-compliant), an increase in perinatal pup mortality was observed at the high dose level (35 mg/kg bw/d), i.e. live birth and pup viability indeces were 74.5% and 59.6%, respectively, as compared to 98.9% and 100% in the control group. These effects were considered to be test-substance dependent and adverse by the DS. At the gross pathological examination 4 pups (from 2 litters) in the intermediate dose group (15 mg/kg bw/d) and 8 pups (from 2 litters) in the high dose group (35 mg/kg bw/d) exhibited dilated pericardial vessels (i.e. aneurysms of the great vessels of the heart). When these macroscopic alterations were examined microscopically, histopathology revealed dissecting aneurysm in the dilated vessels (aorta, arteries or ductus arteriosus), a finding which correlated overall with the macroscopic findings. Pup body weights/weight gain were also reduced at the intermediate and high dose levels. The NOAEL for developmental toxicity was set to 5 mg/kg bw/d by the DS based on the decreased pup weights, on perinatal mortality and on the dissecting aneurysms in the great vessels of the heart that was recorded at 15 mg/kg bw/d and above.

According to the DS all these severe effects on embryofoetal development should be considered as representing "clear" and not "some" evidence for an adverse impact on development. The dossier submitter's view is that the observed effects are not secondary to the effects observed at 15 mg/kg bw/d and higher in the parental animals (slightly reduced body weight during gestation, minimal centrilobular hepatocellular hypertrophy and reduced body weights during lactation). Moreover, since there is no mechanistic information available that raises doubt about the relevance of the effects for humans, the DS concludes that classification in Repr. 1B is justified for adverse effects on development of the offspring, but the DS does not propose a specific concentration limit for developmental toxicity.

Effects on or via lactation

According to the DS, it is not possible to fully assess the effects of the test substance on or via lactation due to the experimental design of an OECD TG 422 study (i.e. pups are only studied until PND 4 and the study does not provide information on the presence of the test substance or metabolites in milk or on milk yield). The DS also considers that the recorded effects on pup viability and on pup body weight may be caused mainly by the teratogenic and fetotoxic potential of 1-vinylimidazole. Thus, based on the available data, classification for effects on or via lactation is not warranted.

Comments received during public consultation

The two MSCA who commented were both in support of the proposed classification in Repr. 1B; H360D. One of them asked for additional information on historical control data for the endpoints gestational length and mating days, and the other suggested minor amendments to the report

as well as some clarifications regarding the numbers of pups with effects on the great vessels of the heart. The DS agreed with the minor amendments proposed by the MS to clarify that the available data suggest that there is "no indication of impaired fertility" rather than "no indication of reproduction toxicity". The requested historical control data (HCD) was provided by the DS (see Annex 2).

Assessment and comparison with the classification criteria

Fertility and sexual function

In addition to the rat oral gavage combined repeated dose toxicity study with the reproduction/developmental screening test (OECD TG 422; BASF SE, 2013), data from a rat oral gavage 90-day repeated dose toxicity study (BASF AG, 1991; see background document for details) is also presented in the CLH report. However, no histopathological examination was performed on the reproductive organs, and therefore RAC is of the opinion that the 90-day repeated dose toxicity study is of no importance for the assessment of effects on fertility.

During public consultation one MSCA commented on the interpretation of the endpoints "mating days" and "gestational days". RAC agrees with the DS that the slight increase in the number of mating days recorded in the high dose females (3.0 as compared to 1.9, 2.4, 2.4 in the controls, low and intermediate dose groups, respectively) as well as the somewhat increased gestational length (22.9 days, as compared to 22.2, 22.3 and 22.6 that was recorded in the controls, low and intermediate dose groups, respectively) are of no biological relevance. Although the recorded value for gestational length is just outside the reported HCD (range: 21.6 – 22.4 days; median: 22 days) no adverse clinical signs related to dystocia were recorded for the high dose females. The number of mating days (3.0) recorded in the high dose group is very close to the median value (2.8) of the HCD (range: 1.6 – 6 days) for this endpoint. In addition, there was no effect on mating or fertility indices and no adverse findings were identified at the histopathological examination of the sex organs.

RAC notes that no one- or two-generation study is available for 1-vinylimidazole and that the design of the available screening study does not provide information on sexual maturation.

In conclusion, from the limited data available there is no indication for an effect on mating, fertility or gestation indices and no adverse effect was recorded at the histopathological examination of male and female reproductive organs. RAC therefore agrees with the DS that no effect on fertility or on sexual function was detected in the available OECD TG 422 screening study that justifies classification for effects on fertility and sexual function.

Developmental toxicity

RAC notes that no specific developmental toxicity study is available for 1-vinylimidazole and consequently the assessment of effects on embryonic, foetal, and pup development is based on the results from the rat oral gavage screening test (OECD TG 422; BASF SE, 2013). In this study, females in the high dose group consumed less food during the period of gestation and lactation as compared to the controls and the mean body weights on gestation day (GD) 20, PND 0 and on PND 4 were 7%, 11% and 20% lower, respectively, as compared to the controls. Also the intermediate dose females had a lower body weight on PND 0 (-6% as compared to controls). No consistent clinical signs were recorded during the gestational and lactational phase of the study and no adverse effects were recorded for haematological or clinical chemistry parameters. Histopathological examination revealed centrilobular hepatocellular hypertrophy (grade 1) in 9/10 high dose females correlating to the observed increased liver weight of the high dose females (+18% as compared to the controls) (see Table 1 and the background document for further information).

Table 1. Maternal effects

	Control	5 mg/kg bw/d	15 mg/kg bw/d	35 mg/kg bw/d
Number of pregnant animals	8	9	9	9
Clinical observation during lactation[1,2]	0/8	0/9	1/9 (piloerection)	2/9 (complete litter loss)
Food consumption [g/d] during gestation (d 0-20)[3]	22.2 ± 1.6	22.6 ± 1.3	20.7 ± 1.9	20.2 ± 1.4* (-9%)
Food consumption [g/d] during lactation[2] (d 1-4)	34.4 ± 4.3	31.9 ± 4.5	31.2 ± 5.9	22.8 ± 4.7** (-34%)
Body weight [g] GD 0[3,4]	231.7 ± 13.3	221.1 ± 8.0	225.3 ± 9.1	223.1 ± 12.4 (-4%)
Body weight [g] GD 20[3,4]	347.6 ± 24.9	331.9 ± 22.9	342.2 ± 29.5	324.3 ± 11.3 (-7%)
Body weight [g] at PND 0[3]	264.4 ± 16.5	258.2 ± 11.6	248.3 ± 13.2* (-6%)	235.1 ± 9.2** (-11%)
Body weight [g] at lactation day 4[3]	281.8 ± 17.0	270.8 ± 14.9	267.7 ± 14.3 (-5%)	224.2 ± 9.0** (-20%)
Relative liver weight[3] [%]	2.431 ± 0.126	2.597 ± 0.191	2.565 ± 0.145	2.869 ± 0.064** (+18%)
Hepatic centrilobular hypertrophy[1] (revealed at histopathological examination)	0/10	0/10	0/10	9/10 (Grade 1)

1) Number of affected animals/total number in group. 2) No adverse clinical signs were recorded during the period of gestation. 3) The number in brackets represents the decrease or increase as compared to the controls. 4) This data was requested by RAC, unclear if statistical analysis was performed by the DS. * $p \leq 0.05$, ** $p \leq 0.01$, statistically significant differences compared to control group.

<u>The developmental toxicity was manifested as follows:</u>

1. An increase in perinatal mortality was observed. Forty-three out of 94 pups in the high dose group were stillborn or died during the first four days after birth whereas in the control only one out of 89 control pups died perinatally. Consequently the total number of live born pups, the live birth index as well as the viability index on PND 4 and the mean viable litter size on PND 0 and PND 4 were all reduced as compared to the controls (see Table 2 for details). RAC notes that in studies that evaluated the effect of maternal feed restriction on reproductive parameters there was no effect on the occurrence of stillborn pups or on pup viability even when the maternal body weight was severely reduced (-30% as compared to controls) (Carney *et al.,* 2004). Thus RAC agrees with the DS that the observed pup mortality should not be considered as being secondary to the observed decrease in maternal body weight gain. Although effects were seen on liver weight and slight histopathological changes were seen in the high dose group, RAC is of the opinion that the serious effect seen in pups, i.e. an increased mortality and serious vascular effects, do not point towards a secondary effect.

Table 2. Summary of pup data

	Control	5 mg/kg bw/d	15 mg/kg bw/d	35 mg/kg bw/d	HCD[1] (range) [median value]
Number of pregnant females	8	9	9	9	NA
Mean post implantation loss	3.53%	6.66%	3.34%	11.57%	0.7 – 14.6% [5.55%]
Mean viable litter size PND 0 (number of females)	11.0 ± 2.2 (N=8)	10.1 ± 3.1 (N=9)	11.1 ± 2.6 (N=9)	7.8 ± 3.3 (N=9)	NA
Total no. of liveborn pups (Live birth index[3])	88 (98.9%)	91 (100%)	100 (94.3%)	70** (74.5%)	93% - 100% [99%][2]
Total no. of stillborn pups (% stillborn) [no of litters]	1 (1.1%) [1]	0	6 (5,7%) [1]	24 (25.5%**) [6]	0 -7.3%

Total number of pups dying postnatally[4]	0	1	7	19	NA
Total number of pups dying perinatally[4]	1	1	13	43	NA
Mean viability index PND 4 [mean% ± SD]	100 ± 0	99.3 ± 2.2	92.3 ± 15.9	59.6 ± 43.1**	83 - 100 [99]
Mean viable litter size PND 4 (number of females)	11.0 ± 2.2 (N=8)	10.0 ± 2.9 (N=9)	10.3 ± 2.9 (N=9)	5.7 ± 4.3** (N=7)	NA
Pup weight (g) PND 1[5] (all viable pups) [mean ± SD]	7.1 ± 0.9	7.2 ± 0.8	6.1 ± 0.6* (-14%)	5.9 ± 0.7* (-17%)	NA
Pup weight gain (g) PND 1 to PND 4[5] [mean ± SD]	3.9 ± 0.8	3.8 ± 0.9	3.4 ± 0.8	2.1 ± 1.6** (-46%)	NA
Pup weight (g) PND 4[4,6] (all viable pups)	11.0	11.0	9.5	8.0	NA

1) HCD for a number of endpoints was submitted by the DS during the RAC process. 2) HCD was only provided for live birth index. 3) Not clear from the CLH report if statistical analysis was performed by the DS. 4) Parameter inserted during the preparation of the ODD – no statistical analysis. 5) The number in brackets represents the decrease as compared to the controls. 6) Calculated value based on available PND 1 data and body weight gain data. NA; no information available.
* $p \leq 0.05$, ** $p \leq 0.01$, statistically significant differences compared to control group

2. Dilation of the great vessels of the heart (i.e. dilation of one or more of the following vessels: aorta, aortic arch, subclavian artery, or the ductus arteriosus) was recorded at the gross pathological examination of 4 pups (from 2 litters) and 8 pups (from 2 litters) at the intermediate and high dose levels, respectively (see Table 3). Histopathological examination of these findings revealed "dissecting aneurysms" in the dilated vessels which correlated overall with the observed macroscopic lesion "dilation". The recorded malformations were seen in pups that were either stillborn, died during the first 4 days or were viable when all remaining pups were killed as scheduled on PND 4. It is possible that the number of affected pups are underestimated since a number of the pups that died perinatally could not be examined due to post-mortem autolysis or because they already had been cannibalized. The DS (BASF SE) provided HCD during the RAC process and in the 31 studies performed between 2007 and 2012 using the same strain of rats the only pup necropsy observation related to the great vessels of the heart was "aneurysm of the ductus arteriosus" that was observed in 2 pups, from 2 different litters, in one single study. RAC concludes that this finding is a very severe and rare malformation.

Table 3. Summary of pup pathology data

	Control	**5 mg/kg bw/d**	**15 mg/kg bw/d**	**35 mg/kg bw/d**
Number of pups evaluated[1] (numbers of litters)	89 (8)	91 (9)	106 (9)	94 (9)
Number of pups that could not be examined (due to cannibalization or post-mortem autolysis)	1	1	6	20
Gross pathological examination				
Total number of pups with dilated aorta (thereof in stillborn pups)	0	0	1 (1)	7 (4)
Total number of pups with dilated aortic arch (thereof in stillborn pups)	0	0	3 (1)	3 (2)
Total number of pups with dilated ductus arteriosus	0	0	1	0
Total number of pups with dilated subclavian artery	0	0	0	1
Total number of pups with dilated aorta/ aortic arch/subclavian artery or ductus arteriosus [number of litters]			4 [2]	8 [2]
Histopathological examination of macroscopic findings				
Number of pups with dissection aneurysm as revealed by histopathological examination)/total			2/13 & 2/12	7/12 & 1/10

number of pups in each affected litter				

1) all pups including the pups that were stillborn or died during the first 4 days were examined when possible.

In addition, a statistically significant lower pup weight on PND 1 (high dose and intermediate dose) as well as statistically significant lower body weight gain between PND 1 and PND 4 (high dose only) was recorded in the OECD TG 422 study (see Table 2 for details). RAC notes that these effects could be secondary effects because of the maternal toxicity observed in the high dose group. It is to be noted however that also in the intermediate dose group effects on body weight were observed on PND 1 pups in the absence of clear effects on the maternal animals. All in all, it is not fully clear whether the effects seen on pup weight are due to a direct or secondary effect of maternal toxicity. In view of the severe effects on blood vessels of the heart in the intermediate and high dose RAC considers it is of limited or no value to conclude whether the effects on pup body weight are primary or secondary.

Conclusion regarding classification for developmental toxicity

Since there is no evidence that 1-vinylimidazole adversely affects the development of the offspring in humans, Category 1A is not justified.

RAC agrees with the DS that classification in Category 1B is warranted based on *clear evidence* from a reliable screening study in rat of an adverse effect on the development of the offspring. The effects on development (perinatal mortality and aneurysm of the great vessels of the heart) are considered not to be a secondary non-specific consequence of the other non-specific toxic effects (effects on maternal body weight, food consumption, liver hypertrophy) that were noted in the study. RAC finds that it is notable that in view of the limited power of a screening study such serious developmental effects were observed at a rather low dose level (LOAEL 15 mg/kg bw/d).

Classification in Category 2 is not appropriate since available data is considered to be sufficiently convincing to place the substance in Category 1B, and not considered to be *some evidence* of developmental toxicity from experimental animals.

Setting of an specific concentration limit (SCL)

The DS stated in the CLH report that "A specific concentration limit for developmental toxicity is not proposed" (see section 4.11.6 of the background document). However, RAC notes that the DS did not include a justification for not proposing an SCL.

In the available OECD TG 422 developmental screening study with a limited number of animals (10 instead of 20 as is normally used for developmental studies), serious effects were seen at 15 mg/kg bw/d with a NOAEL of 5 mg/kg bw/d. Interpolation between NOAEL and LOAEL leads to ED_{10} values (30 mg/kg bw/d for the incidence (%) of pups with aneurysms; and 14 mg/kg bw/d for the incidence (%) of pup perinatal death) that RAC considers to be more close to than distant from the lower border for the medium potency group ($ED_{10} \geq 4$ mg/kg bw/d, and ≤ 400 mg/kg bw/d) for which a general concentration limit is applied (see Table 3.7.2-e in the Guidance on the application of the CLP criteria v. 4.1). RAC notes that in the present study the number of pups with aneurysm could have been underestimated since gross pathological examination only could be performed on a subset of the pups that died perinatally. This uncertainty as well as the additional uncertainties related to the inherent limited statistical and toxicological power of a screening study should be considered when assessing the need for setting an SCL. Consequently, RAC is of the opinion that the modifying factors "Type of effect/severity" and "Data availability" should, in accordance with the Guidance on the application of the CLP criteria (3.7.2.5.5), be taken into account when assigning the final potency group for 1-vinylimidazole. On the basis thereof, RAC concludes that 1-vinylimidazole should be assigned to the high potency group and that an SCL of 0.03% should be set.

Effects on or via lactation

Considering the limitations of the available screening study, where the group size is only 10 and the pups are only examined during the first four days of lactation, RAC considers that it is difficult if not impossible to properly assess the effects of 1-vinylimidazole on or via lactation. Therefore, no classification for effects on or via lactation is justified since the available information does not allow to make an assessment of potential effects on or via lactation.

Overall, RAC concludes, in agreement with the DS proposal, that based on the observed effects classification as **Repr. 1B; H360D** is justified for 1-vinylimidazole. However, contrary to the DS proposal, RAC is of the opinion that an **SCL of 0.03%** is justified because of the serious effects seen in a study with limited sensitivity close to the lower limits of no SCL.

Additional references

Additional references not included in the CLH report

Carney E.W., Zablotny C.L., Marty M.S., Crissman J.W., Anderson P., Woolhiser M., Holsapple M. (2004). The effects of feed restriction during in utero and postnatal development in rats. Toxicol. Sci. Nov; 82(1):237-49.

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).