Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

imidazole

EC number: 206-019-2
CAS number: 288-32-4

CLH-O-0000002699-59-03/A2

Adopted

10 September 2013

ANNEX 1 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON IMIDAZOLE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

ECHA has compiled the comments received via the internet that refer to several hazard classes and entered them under each of the relevant categories/headings as comprehensively as possible. Please note that some of the comments might occur under several headings, when splitting the information provided is not reasonable.

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Last data extracted on 04.02.2013

Substance name: Imidazole
EC number: 206-019-2
CAS number: 288-32-4

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
31.01.2013	Germany		MemberState	1
Comment received				
The German-CA supports the following classification of imidazole according to CLP Regulation as Acute Tox., 4, H302, Skin Corr. 1C, H314, Eye damage Cat. 1, H318. The proposal to also harmonize for the hazard class Acute toxicity, Skin corrosion/irritation and Serious eye damage/eye irritation beyond the harmonisation of the classification for CMR properties of imidazole is supported.				
Dossier Submitter's Response				
We agree with the German-CA (remark: Repr. Cat 1B, H360D is missing in the first sentence of the comment).				
RAC's response				
Thank you for your comment. Based on the German CA's specific comment on reproductive toxicity, we assume that they also agree with the Dossier Submitters proposal to classify imidazole as Repr. 1B; H360D.				

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	France		MemberState	2
Comment received				
We do not agree with the classification proposal on skin corrosion.				
Dossier Submitter's Response				
Based on the results on of a skin corrosion test with 1 and 4 hrs exposure under occlusive exposure conditions and signs of necrosis after 4 hrs only, we propose classification as skin corrosive Cat 1C according to CLP Annex I, Table 3.2.1.				
RAC's response				
RAC agrees with the Dossier Submitter that the available data best supports classification in sub-category 1C (see response to specific comments below).				

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	Belgium		MemberState	3
Comment received				
Firstly we would like to thank the Dossier Submitter for the clear analysis of the hazards				

related with the use of imidazole. At the same time we would like to stress that some parts of the dossier were not so clearly reported (more specifically, the copy-pasting of the same information in various chapters of the proposal). Toxicokinetics Remark regarding data from study no. 1 (human information): Table 13 presents data obtained during both single and multiple oral administration of imidazole-2-hydroxybenzoate. According to the information provided under the table, A stands for the results obtained after the 1st dose, whereas B should represent the values collected after the 10th dose. However, as it appears from the experimental procedure description, there was no administration of the 10th dose. In this aspect presented text is inconsistent with experimental description in the IUCLID file. Remark regarding the proposed conclusions: Rapid and quantitative absorption of imidazole was concluded after the oral administration. Imidazole was found to metabolize in the liver to the main metabolites hydantonin and hydantonic acid, which was proven after single intravenous dose. In the study presented for oral administration, hydantonin and hydantonic acid were found to be under the detection limit. Taking into account the significance of diversity of the administration routes investigated, it seems not to be appropriate to directly link results obtained in the studies of concern.
Dossier Submitter's Response
Toxicokinetics, Study no. 1: We apologize if the description in the CLH report is not sufficiently clear. For clarification, the exact dosage schedule is cited from the publication: "For the multiple dosing study in crossover they received day 1 (either tablets or drops in crossover) 48 h after the single dosing 3 x 1 tablets or 3 x 40 drops daily. Days 2 and 3 dosing continued. Day 4 last day of dosing only the first dose of the day was administered. " This explains that altogether (3 x 3) + 1 = 10 doses were administered. Proposed conclusions: We agree with the comment. We suggest adding that the two metabolites hydantoin and hydantoic acid were observed in rats after i.v. injection.
RAC's response
Thank you for the above clarifications and proposed amendments.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	Belgium		MemberState	4
Comment received				
Data presented by the Dossier Submitter, in both in vitro and in vivo studies, clearly indicate that imidazole has no mutagenic properties. Consequently, no classification for germ cell mutagenicity is required.				
Dossier Submitter's Response				
We agree with the comment				
RAC's response				
RAC agrees.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	Sweden		MemberState	5
Comment received				
The Swedish CA supports the proposed classification of Imidazole (CAS number 288-32-4)				

regarding developmental toxicity as a reproductive toxicant category 1B (hazard statement H360D).
In an OECD TG 414 prenatal study, Imidazole caused developmental toxicity in the presence of minimal maternal toxicity at the high dose level. The developmental toxicity was revealed as an increased incidence of external and skeletal malformations, a decreased fetal viability and a slight decreased fetal weight. No one-or two-generation studies have been performed. The available data from a repeated dose 90 days (gavage) toxicity study in rat revealed no effects on reproductive organs, sperm parameters or estrous cycling. However, the available data set is not sufficient for a conclusion regarding classification for fertility.

Dossier Submitter's Response

We agree with the comment, although the available information from the 90-day study does not indicate a fertility impairing effect of Imidazole.

RAC's response

Thank you for the support for the proposal to classify imidazole as Repr. 1B; H360D.
As no changes in reproductive parameters were observed in the 90-day repeated dose toxicity study, RAC considers that the available data do not support classification for fertility. However, RAC notes that the available data do not allow for an assessment of whether pup development, mating behaviour or sexual maturation would have an adverse effect on fertility.

Date	Country	Organisation	Type of Organisation	Comment number
31.01.2013	Germany		MemberState	6

Comment received

Overall, the German-CA supports the proposal to classify imidazole as Repr. Cat. 1B, H360D.
However, some clarification, revision and amendment (e.g. comparison with criteria) of chapter 4.11 is recommended:

Table 20/page 47:
The data on "total resorptions" should be reported separately for (i) early and for (ii) late resorption, as it appears that fetal death rather than significant embryolethality was induced by the test substance.

Top of page 49:
Given that the effects on the endpoint maternal body weight gain (gestational days 17-20) are not considered an indication of maternal toxicity, the statement that "the administration of 180 mg imidazole /kg bw/d to pregnant rats induced adverse effects on dams" needs clarification. Similarly, the statement of "clear signs of developmental toxicity" should be amended for substance-induced fetal death and fetal growth retardation in addition to teratogenic effects.

4.11.4 Summary and discussion of reproductive toxicity:
This chapter is not a summary but just a copy of chapter 4.11.1.1 and of parts of chapter 4.11.2.1. and should be compressed for the essentials.

Bottom of page 50:
Given that the effects on the endpoint maternal body weight gain (gestational days 17-20) are not considered an indication of maternal toxicity, the statement that "the administration of 180 mg imidazole /kg bw/d to pregnant rats induced adverse effects on dams" needs clarification. Similarly, the statement of "clear signs of developmental toxicity" should

include be amended for substance-induced fetal death and fetal growth retardation in addition to teratogenic effects.

Page 51, 4.11.5 Comparison with criteria:
Comparison with the criteria in particular of the CLP regulation is missing. In addition, it is recommended to provide a comparison between the severity of the maternal toxicity and the severity of the findings in the offspring for the submission to RAC, as is advised in the Guidance on the application of the CLP criteria.
(http://echa.europa.eu/documents/10162/13562/clp_en.pdf)

Dossier Submitter's Response

Please find below our comments to the remarks and proposals from the German CA:

Table 20/page 47:
The data on "total resorptions" should be reported separately for (i) early and for (ii) late resorption, as it appears that fetal death rather than significant embryolethality was induced by the test substance.

Comment: We agree with the comment. The value for late resorptions was significantly increased at the high dose (3.1 ± 2.76 versus 0.1 ± 0.29 in controls). There was no statistically significant change for the early resorptions in any of the dosed groups compared to the controls.

Top of page 49:
Given that the effects on the endpoint maternal body weight gain (gestational days 17-20) are not considered an indication of maternal toxicity, the statement that "the administration of 180 mg imidazole /kg bw/d to pregnant rats induced adverse effects on dams" needs clarification. Similarly, the statement of "clear signs of developmental toxicity" should be amended for substance-induced fetal death and fetal growth retardation in addition to teratogenic effects.

Comment: We agree with the comment that the distinct reductions of the weight gain of the high dose animals towards the end of the study are a direct consequence of the high rate of resorptions and the distinctly lower mean fetal body weight at this dose level (as mentioned on page 45 of the CLH report). Further substance-related signs of maternal toxicity occurred (e.g. vaginal haemorrhage on one animal) and, specifically at initiation of dosing, the dams showed significant impairments in food consumption (-13% compared to control on days 6-8 p.c.) and impaired body weight gains (45% below the control on days 6-8 p.c.). These findings are described in the same chapter if the CLH report on page 46. The diminished body weight gain of the high dose rats at initiation of treatment with corroborative reductions in food consumption reflects a direct adverse effect on the dams. The weight of the "embryo-/fetal component", i.e. the implants, does not affect the weight of the mothers considerably at this stage of the gestation period.

Regarding the second comment we agree with the proposal to amend "fetal death and fetal growth retardation" in the statement on developmental toxicity.

4.11.4 Summary and discussion of reproductive toxicity:
This chapter is not a summary but just a copy of chapter 4.11.1.1 and of parts of chapter 4.11.2.1. and should be compressed for the essentials.

Comment: We agree with the comment that this chapter could be shortened and some study details could be omitted here.

Bottom of page 50:

Given that the effects on the endpoint maternal body weight gain (gestational days 17-20) are not considered *an indication of maternal toxicity, the statement that "the administration of 180 mg imidazole /kg bw/d to pregnant rats induced adverse effects on dams" needs clarification. Similarly, the statement of "clear signs of developmental toxicity" should include be amended for substance-induced fetal death and fetal growth retardation in addition to teratogenic effects.*

Comment: See our remarks above on the same topic (**Top page 49**)

Page 51, 4.11.5 Comparison with criteria:
Comparison with the criteria in particular of the CLP regulation is missing. In addition, it is recommended to provide a comparison between the severity of the maternal toxicity and the severity of the findings in the offspring for the submission to RAC, as is advised in the Guidance on the application of the CLP criteria.

Comment: We agree with the suggestion to amend a comparison with the CLP classification criteria and for the severity of effects between maternal toxicity and findings in the offspring which demonstates that the adverse effect on development was not a secondary non-specific consequence of other toxic effects.

RAC's response

Thank you to the Dossier Submitter for providing this additional information and comparison with the classification criteria. This information has been considered in the RAC opinion document.

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	France		MemberState	7

Comment received

We cannot conclude on the classification for the fertility due to the lack of appropriate studies.
We do not agree with the proposed NOAEL for prenatal development at 60 mg/kg bw/d (BASF SE, 2002b) because we observe a statistically significant increase in visceral variations at 60 mg/kg bw/d. Thus we propose a NOAEL at 20 mg/kg bw/d. However, it does not impact on the classification proposal.

Dossier Submitter's Response

We do not agree with the comment on the NOAEL for prenatal development. The oral administration of 180 mg/kg bw/d caused clear effects on fetal morphology in the presence of some maternal toxicity. However, the test substance showed no treatment-related adverse effects on fetal morphology at the low and mid dose (20 and 60 mg/kg bw/d).

In the following, the soft tissue variations are discussed in more detail. The mean percentages of affected fetuses/litter with total soft tissue variations amounted to 6.4% (control), 9.2% (20 mg/kg bw/d), 22 .7%* (60 mg/kg bw/d) and 27.1 %* (180 mg/kg bw/d; * $p \leq 0.05$). Two soft tissue variations occurred predominantly in the form of dilated renal pelvis and/or ureters and were detected in each group including the controls, but were statistically significantly increased at the mid and high dose (mean affected fetuses in %/litter: 6.4±16.25%, 9.02±17.02%, 21.2 ±28.22 and 26.0±35.6% for controls, low, mid and high dose, respectively).
These values are compared with the respective historical control data for total soft tissue variations (mean affected fetuses/litter: range 4.4 - 22.2%; mean 11.6%; 142 litters and 614 fetuses evaluated in studies conducted in a time frame of 6 months before the study). It becomes now obvious, that soft tissue variations occurred in the high dose group at

statistically significantly increased rates and at frequencies being clearly outside historical control ranges. Thus, a substance-induced origin concerning the occurrence of soft tissue variations in test group 3 (180 mg/kg bw/d) is conceded. The borderline increase of soft tissue variations at 60 mg/kw/d, however, is considered as a by chance finding due to the fact, that the respective values were within the range of the historical control data and, thus, reflect the usual biological variation inherent in the strain of rats used for this experiment.

RAC's response

Thank you to the Dossier Submitter for providing the historical control values for soft tissue variations. These have been presented and considered in the RAC opinion.
We note the French CA's comment, but emphasise that the NOAEL does not impact on the classification and labelling of imidazole.

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	Belgium		MemberState	8

Comment received

We support the proposal of the Dossier Submitter to classify the imidazole as the Developm. Repr. Cat. 1B with regard to the findings presented in the proposal. After a detailed investigation of the presented data certain doubts arise with regard to the H-phrase selected by the Dossier Submitter. Namely, H-phrase 360D was proposed, which is referring to the statement 'may damage the unborn child' (for substances for which effects on fertility can be excluded (current CLP)). The Dossier Submitter is supporting the proposal with results obtained on the basis of tests carried out according to the OECD TG 408 guideline, where no indication of any adverse effect on the male reproductive organs and the sperm quality was found. At the same time data presented in the other non-guideline study, showing clear negative effects of the imidazole administration at the dose of 30 mg/kg bw, were questioned due to the lack of microscopical investigation as well as due to the different route of exposure applied (subcutaneous injection). Taking into account findings of the 'non-guideline' study (which can be used as a supportive findings) and due to the fact that complete fertility studies are not available, we think that no conclusions regarding effects on fertility can be drawn, but we can accept the classification Developm. Repr. Cat 1B: H360D due to the revision of the interpretation of this classification in the 4th ATP (when the other differentiation is not mentioned, this is due to evidence proving no such effect, inconclusive data or no data).

Dossier Submitter's Response

The repeated dose study over a period of 90 day does not indicate any adverse effects on the male or female reproductive organs up to the highest tested dose of 180 mg/kg bw/day (including histopathology, sperm analysis, and investigation of the oestrus cycle).
The exploratory study with subcutaneous injection suffers from several severe methodological deficiencies, so we do not consider this experiment appropriate for regulatory classification. We propose a classification regarding developmental toxicity, only (Repr. Cat 1B: H360D).

RAC's response

Thank you for the support for the proposal to classify imidazole s Repr. 1B; H360D.
As no changes in reproductive parameters were observed in the 90-day repeated dose toxicity study, RAC considers that the available data do not support classification for fertility. However, RAC notes that the available data do not allow for an assessment of whether pup development, mating behaviour or sexual maturation would have an adverse effect on fertility

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	Belgium		MemberState	9
Comment received				
Remark: The description of the experiments and experimental data should be done with more care. The results presented in the proposal vary significantly from the data incorporated in the IUCLID file. For instance, LD50 estimated on basis of data discussed in the proposal (section 4.2.1.1.) should be higher than 1000 mg/kg bw and not 970 mg/kg bw. Conclusion: Basing on the presented data, we support following classification of imidazole: Acute Tox. 4: H302.				
Dossier Submitter's Response				
We do not agree with the comment. The presented data reflect exactly the information in the original report. Based on mortality data, an LD50 value of about 970 mg/kg bw was calculated in the report.				
RAC's response				
The number of mortalities reported at each dose level is consistent between the IUCLID technical dossier and the CLH report. However, we agree with the Belgium CA that based on the number of mortalities reported at each dose level (0/5, 1/5, 2/5, 5/5, 1/1 and 1/1 mortalities at 500, 700, 1000, 1260, 2000, 4000 and 5000 mg/kg bw) the LD_{50} should be within the range of 1260 > LD50 > 1000 mg/kg bw. This does not lead to a change in the classification proposed by the dossier submitter.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	France		MemberState	10
Comment received				
☐ Skin corrosion: The classification as skin corr. 1B H314 is proposed because of the not fully reversible effects observed at the end of the observation period and after 1h of exposure (necrotic spots and desquamation).				
Dossier Submitter's Response				
We do not agree with the comment. After 1 hour of exposure under occlusive dressing, there was only mild erythema and edema. The edema resolved completely by day 8 of the post observation period. Residual signs included patchy, superficial necrotic lesions in addition to scaling. These findings were confirmed macroscopically by a pathologist after sacrifice of the animals and are not considered to represent full tissue destruction which was clearly observed after 4 hours treatment. Therefore, subcategory 1 C is proposed.				
RAC's response				
Thank you for clarifying the effects observed in the macroscopic examination. RAC agrees with the Dossier Submitter that the available data supports classification in sub-category 1C.				

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	Belgium		MemberState	11
Comment received				
We support the classification of imidazole as Skin Corr. 1C: H314, since clear evidence of				

visible necrosis of the skin tissue after exposure up to 4 hours was observed in both tested animals.
Dossier Submitter's Response
We agree with the comment.
RAC's response
Thank you for the support.

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	Belgium		MemberState	12
Comment received				
The Dossier Submitter suggested classification of the imidazole in the category: Eye damage Cat. 1: H318. Data were presented to confirm the hazards related with the application of imidazole in the eyes of tested animals. However the CLP ECHA guidance clearly indicates that additional classification for eye irritation/corrosion hazards is not required for substances already classified as Skin Corr. 1C.				
Dossier Submitter's Response				
We do not agree with the comment. According to our interpretation each endpoint needs a separate classification (if the hazard is present), although for corrosive substances Eye damage Cat 1 is implicit. Regarding labelling, H318 is not needed in addition to H314.				
RAC's response				
RAC agrees that imidazole meets the criteria for classification as Eye Dam. 1; H318. However, since imidazole is to be classified as Skin Corr. 1C, classification is not required for this endpoint according to the current guidance and practice. RAC therefore agrees not to classify imidazole for Eye Dam. 1; H318 due to classification as Skin Corr. 1C. RAC notes, however, that classification and labelling for severe eye damage for substances already classified as Skin Corr. 1C is a subject of an ongoing review of the guidance on the application of the CLP criteria.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	Belgium		MemberState	13
Comment received				
Taking into account that no data on the specific target organ toxicity investigation were presented, it can be concluded that the classification as STOT SE is not possible.				
Dossier Submitter's Response				
A classification regarding STOT SE is not proposed as there was no indication for such an effect in the acute tests.				
RAC's response				
RAC agrees.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2013	Belgium		MemberState	14

Comment received

No classification for repeated dose or specific target toxicity (STOT RE) was suggested by the Dossier Submitter. We agree with conclusion on basis of the 90-day study. However, on the basis of the results obtained in a 28-day study, the clear changes in the blood chemistry (i.e., decrease in hemoglobin, hematocrit and erythrocytes) were already seen after oral administration of 125 mg/kg bw/d of the imidazole. No numbers were provided allowing the quantitative judgment of the observed effects. Therefore we think that it could be perhaps advisable to revise whether imidazole should not be classified for STOT RE Cat. 2. This should be done by means of more detailed quantitative discussion of obtained data and on other parameters like the quality of the studies, the degree of purity of the substance used, etc..

Dossier Submitter's Response

As described in the CLH report, a GLP conforming 90 days gavage study according to OECD TG 408 showed treatment related findings at the highest dose level of 180 mg/kg bw/d, identifying the liver (minimal to centrilobular hypertrophy) and kidneys (alpha 2-microglobulin accumulation) as target organs. In addition, various parameters of blood chemistry were changed at the high dose (serum globulin, total protein, albumin, chloride).

In an older non-GLP study (experiment conducted in 1973), imidazole with a purity of about 99% was tested in a 4-week gavage study in rats at dosages of 62.5, 125, 250 and 500 mg/kg bw/d. Unfortunately there are no further analytical details for the test substance or on the stability during the administration period available.
The treatment resulted in a dose dependent decrease in hemoglobin, hematocrit and erythrocytes being statistically significant in females at 125 mg/kg bw/d (hemoglobin) and above. The extent of these effects reached up to about -10% at the high dose (see table below), but this is considered to be still in the physiological variation for this rat strain. There were no other findings in the study which could be indicative of an anemic effect. More importantly, the observation on red blood cells could not be reproduced in the more recent guideline study with a three times longer application period of 90 days and, therefore, no classification with STOT RE is proposed.

Table: 28-day study: Red blood cell parameters at the end of treatment

	Erythrocytes		Hemoglobin		Hematocrit	
Dose (mg/kg bw/d	Males	Females	Males	Females	Males	Females
Control	7.59 (0.38)	7.84 (0.35)	15.14 (0.46)	15.42 (0.39)	49.8(2.95)	50.8 (2.17)
62.5	7.46 (0.60)	7.73 (0.44)	15.20 (0.89)	15.48 (0.80)	49.6 (3.21)	50.2 (2.77)
125	6.96 (0.24)	7.33 (0.43)	14.38 (0.38)	14.38 (0.80)*	46.2 (0.84)	48.4 (3.36)
250	7.49 (0.25)	7.23 (0.30)*	14.58 (0.68)	14.06 (0.47)*	46.6 (1.52)	47.2 (1.79)*
500	7.16 (0.70)	7.14 (0.42)*	13.72 (1.07)*	13.60 (0.72)*	45.5 (3.08)*	45.8 (2.17)*

* statistically significant (t-test)

ECHA comment

Please note that classification for this endpoint was not proposed by the Dossier Submitter and no justification was given that action at community level would be needed for this endpoint. Accordingly, this endpoint was not opened for commenting during the public consultation and RAC does not discuss this endpoint in the Opinion.