Committee for Risk Assessment

RAC

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/F

Adopted

10 September 2013

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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 (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:

Chemicals name: imidazole

EC number: 206-019-2

CAS number: 288-32-4

The proposal was submitted by **industry** and received by the RAC on **18 December 2012.**

In this opinion, all classifications are given firstly in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS) and secondly, according to the notation of 67/548/EEC, the Dangerous Substances Directive (DSD).

PROCESS FOR ADOPTION OF THE OPINION

Industry 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 **18 December 2012**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **1 February 2013**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by RAC: **Andrew Smith**

Co-rapporteur, appointed by RAC: **Lina Dunauskiene**

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

The RAC opinion on the proposed harmonised classification and labelling was reached on **10 September 2013** and the comments received are compiled in Annex 2.

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

OPINION OF THE RAC

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

Classification and labelling in accordance with the CLP Regulation

	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 state-ment Code(s)	Suppl. Hazard statement Code(s)
Current Annex VI entry	613-319-00-0	-	-	-	-	-	-	-	-
Dossier submitters proposal	613-319-00-0	Imidazole	206-019-2	288-32-4	Repr. 1B Acute Tox. 4 Skin Corr. 1C Eye Dam. 1	H360D H302 H314 H318	GHS05 GHS07 GHS08	H360D H302 H314 Dgr	
RAC opinion	613-319-00-0	Imidazole	206-019-2	288-32-4	Repr. 1B Acute Tox. 4 Skin Corr. 1C	H360D H302 H314	GHS05 GHS07 GHS08	H360D H302 H314 Dgr	
Resulting Annex VI entry if agreed by COM	613-319-00-0	Imidazole	206-019-2	288-32-4	Repr. 1B Acute Tox. 4 Skin Corr. 1C	H360D H302 H314	GHS05 GHS07 GHS08	H360D H302 H314 Dgr	

Classification and labelling in accordance with the DSD

	Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits
Current Annex VI entry	613-31 9-00-0	-	-	-	-	-	-
Dossier submitters proposal	613-31 9-00-0	Imidazole	206-019-2	288-32-4	Repr. Cat 2; R61 Xn; R22 C; R34	T; C R: 61-22-34 S: 26-36/37/39-45-53	
RAC opinion	613-31 9-00-0	Imidazole	206-019-2	288-32-4	Repr. Cat 2; R61 Xn; R22 C; R34	T; C R: 22-34-61 S: 45-53	
Resulting Annex VI entry if agreed by COM	613-31 9-00-0	Imidazole	206-019-2	288-32-4	Repr. Cat 2; R61 Xn; R22 C; R34	T; C R: 22-34-61 S: 45-53	

SCIENTIFIC GROUNDS FOR THE OPINION

RAC general comment

The hazard classes assessed by the RAC are those for which the Dossier Submitter (industry) provided a justification in the CLH dossier for action needed at community level (as required in Article 36(3) of the CLP Regulation):

- Reproductive toxicity
- Acute toxicity
- Skin corrosion/irritation
- Eye corrosion/irritation

HUMAN HEALTH HAZARD ASSESSMENT

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The Dossier Submitter proposed to classify imidazole as Acute Tox. 4; H302 (CLP) and Xn; R22 (DSD). This conclusion was based on two acute toxicity studies conducted in the rat by the oral route (BASF SE, 1956a and BASF SE, 1956b). No data was available by the inhalation or dermal routes.

In the key study (BASF SE, 1956a), rats (≤5/sex/dose) were administered 500, 700, 1000, 1260, 2000, 4000 or 5000 mg/kg bw imidazole (100% purity) via gavage. The LD_{50} was reported as 970 mg/kg bw. Clinical signs included convulsions, disequilibria with lateral posture, apathy and accelerated respiration.

A similar LD_{50} value (960 mg/kg bw) was also reported in a supporting study (BASF SE, 1956b), conducted under similar test conditions, but with a lower purity substance (95% imidazole). No details on clinical effects were provided for this study.

Comments received during public consultation

Two MSCAs supported the proposed classification.

Assessment and comparison with the classification criteria

In two non-guideline acute oral toxicity studies, the LD_{50} values were estimated at 970 mg/kg bw and 960 mg/kg bw in rats. These values fall within the criteria for classification as Acute Tox. 4; H302 (CLP; 300< LD_{50} ≤ 2000 mg/kg bw) and Xn; R22 (DSD; 200< LD_{50} ≤ 2000 mg/kg bw).

Therefore, based on the available data, RAC agrees with the Dossier Submitter's proposal to classify imidazole as Acute Tox. 4; H302 (CLP) and Xn; R22 (DSD). No data were available on acute toxicity via the inhalation and dermal routes, therefore, no classification was proposed

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The Dossier Submitter proposed to classify imidazole as Skin Corr. 1C; H314 (CLP) in and C; R34 (DSD). This proposal was based on an occluded patch test in rabbits exposed to imidazole (0.5 ml aqueous paste) for either 1-hour (4 animals or 4-hours (2 animals) (BASF SE, 1979a). The skin reactions observed after 1-hour and 4-hours exposure are detailed in Tables 2 and 3 below.

Table 1 Skin reactions after 1-hr exposure

Animal Number	Score (average of day 1, 2 and 8)/ Observations		
	Erythema	**Oedema**	**Observations**
1	2.67*	<1	Necrotic spots on skin surface at the end of the observation period (day 8)
2	0	0	Desquamation at the end of the observation period (day 8)
3	1.33	1.33	Necrotic spots at the end of the observation period (day 8)
4	1.67*	1.33	Necrotic spots on skin surface and desquamation at the end of the observation period (day 8)

*not fully reversible within the 8-day observation period.

Table 2: Skin reactions after 4-hr exposure

Animal Number	Score (average of day 1, 2 and 8)/ Observations		
	Erythema	**Oedema**	**Observations**
1	4*	2*	Comprehensive parchment-like skin necrosis at the end of the observation period (day 8)
2	4*	2.33*	Comprehensive leather-like skin necrosis at the end of the observation period (day 8)

*not fully reversible within the 8-day observation period.

Comments received during public consultation

Two Member State Competent Authorities (MSCAs) expressed their support for the Dossier Submitter's proposal. However, one MSCA suggested that classification in sub-category 1B would be more appropriate because the skin lesions observed after 1-hour exposure (necrotic spots and desquamation) had not fully reversed by the end of the 8-day observation period.

In response, the Dosser Submitter stated that residual signs observed after 1-hour exposure were identified (by macroscopic pathological investigation) as superficial lesions that do not constitute full thickness destruction, unlike the clear effects observed after 4-hours exposure.

Assessment and comparison with the classification criteria

In a skin irritation/corrosion study (BASF SE, 1979a), corrosive lesions (described as comprehensive leathery or parchment-like necrosis) were observed after 4-hours exposure(2 animals) to 80% aqueous paste imidazole (0.5 ml). One hour exposure (4 animals), resulted in mild erythema (average scores 0-2.67) and oedema (0-1.33), but the lesions (superficial necrotic spots and/or desquamation) observed at the end of the observation period (day 8) were confirmed not to constitute full thickness destruction of the skin tissue. Therefore, RAC confirms that the available data meet the criteria for classification as Skin Corr. 1C; H314 (corrosion occurs after > 1-hour - ≤ 4-hours exposure) and C; R34 (corrosion occurs after > 3-mins - ≤ 4-hours exposure) in accordance with CLP and DSD, respectively.

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

The Dossier Submitter proposed to classify imidazole as Eye Dam. 1; H318 in accordance with the CLP Regulation. No classification was proposed in accordance with the DSD.

This proposal was based on an acute eye irritation/corrosion test in rabbit eyes exposed to 0.1 g unchanged imidazole (99% purity) (BASF SE, 1979b). The eye reactions observed in this study are detailed in Table 4 below:

Table 4-

Animal Number	Score (average of 24, 48 and 72 hours)/ Observations				
	Cornea	**Iris**	**Conjunctivae**	**Chemosis**	**Secretion**
1	2	1	2	2	2.33
2	2	1	2	2	1.33
3	2	1	2	2	3

All reactions had not fully reversed by the end of the study. The grade 2 reddening/swelling of the conjunctiva was accompanied by chemosis, which increased to grade 3 by the end of the 8-day observation period. Corneal opacity (grade 2) also persisted to day 8 and affected more than three-quarters of the cornea. Therefore, the Dossier Submitter concluded that the irreversible tissue damage and persistent large size cornea opacity indicate that imidazole is severely irritating to corrosive to the rabbit eye.

Comments received during public consultation

One MSCA expressed their support for the Dossier Submitter's proposal. However, another Member State noted that the ECHA guidance indicates that classification for eye irritation/corrosion is not required for substances already classified as Skin Corr. 1C.

Assessment and comparison with the classification criteria

Although the recorded scores (average 24, 48 and 72 hours) for corneal opacity and iritis were below the cut off for classification as Eye Dam. 1; H318 and the study observation period was <21 days, the eye reactions did not reverse or reduce within the 8-day observation period. Accordingly, RAC agreed 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 agreed not to classify imidazole for Eye Dam. 1; H318 due to classification as Skin Corr. 1C, noting however, that classification for severe eye damage for substances already classified as Skin Corr. 1C is subject to an ongoing review of the guidance on the application of the CLP criteria.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

No one or two-generation studies were available for imidazole. However, reproductive parameters (including histopathology of the male and female reproductive organs and an assessment of sperm numbers/morphology) were assessed in a 90-day repeated dose toxicity study, conducted in rats (Wistar) via oral gavage (0, 20, 60 or 180 mg/kg bw/day) (BASF SE, 2002a). No changes were observed in the weight and histopathology of reproductive organs (including uterus, ovaries, oviducts, vagina, female mammary glands, left testis, left epididymis, prostate gland and seminal vesicles) and sperm/oestrus cycle parameters were unaffected. Therefore, the Dossier Submitter concluded that imidazole had no effect on these reproductive parameters up to 180 mg/kg bw/day.

In an earlier investigative study (Adams *et al*, 1998), increasing doses of imidazole (three doses between 10 and 300 mg/kg bw) were subcutaneously injected into adult rats (10/group). Samples of serum and testicular interstitial fluid were collected after 2 hours of exposure. Imidazole suppressed both testosterone function and testicular interstitial fluid at 30 mg/kg bw and above. However, the Dosser Submitter considered that this study was of limited relevance because: 1) subcutaneous injection does not represent a relevant route of exposure, 2) the injection site is not known, 3) only one time point was assessed and 4) no microscopic examination of the testes was performed.

The Dossier Submitter did not propose classification for fertility.

Development

The Dossier Submitter proposed to classify imidazole as Repr. 1B; H360D and Repr. Cat. 2; R61 in accordance with CLP and DSD, respectively. This proposal was based on a prenatal developmental toxicity study (OECD 414; BASF SE, 2002b) and an *in vitro* whole cell embryo test (Daston *et al*, 1989).

In the key study (BASF SE, 2002b), pregnant Wistar rats were administered (oral, gavage) 0, 20, 60 or 180 mg/kg bw/day imidazole during days 6-19 of gestation. At the top dose, signs of maternal toxicity (transient), fetotoxicity and teratogenicity were reported. These effects are described further below:

Maternal toxicity

Significant reductions in food intake (↓13% relative to controls) and body weight gain (↓45% relative to controls) were observed at 180 mg/kg bw/day on days 6-8. Reduced body weight gain (↓34% relative to controls) was also reported on days 17-20. However, the Dosser Submitter concluded that this was due to decreased gravid uterus weight, high resorption rates and a lower mean fetal body weight, rather than maternal toxicity. Terminal body weights (actual and corrected) were comparable to controls. Additional clinical signs included transient salivation (6 females) and vaginal haemorrhage (1 female).

Fetotoxicity

A statistically significant reduction in the number of live foetuses per dam was observed as a consequence of increased post-implantation loss (43.4% compared to 7.9% in the controls) at the top dose. 3/24 females resorbed all implants, producing no live foetuses at necropsy. Due to an increased number of runts, mean fetal weight was also reduced (↓14% relative to controls) at the top dose.

Teratogenicity

A statistically significant increase in the incidence of external malformations (anasarca and/or cleft palate) was observed, affecting 10% of foetuses in 7/21 litters at the top dose. No incidences of anasarca or cleft palate were reported in the control, low and mid dose groups.

The number of skeletal malformations (including shortened scapula, bent radius, bent ulna, malpositioned and bipartite sternebrae) was also increased at the top dose with 1.1, 2.3, 0.9 and 7.8% of foetuses affected per litter at 0, 20, 60 and 180 mg/kg bw/day, respectively. The incidence of affected litters was 4.5, 9.1, 4.3 and 24% at 0, 20, 60 and 180 mg/kg bw/day, respectively.

Soft tissue variations (dilated renal pelvis and ureter: 6.4, 9.2, 22.7 and 27.1% affected foetuses per litter at 0, 20, 60 and 180 mg/kg bw/day, respectively) and skeletal variations (primarily delays in ossification: 91.1, 87.2, 94.2 and 98.4 at 0, 20, 60 and 180 mg/kg bw/day, respectively) were also significantly increased in foetuses of the top dose dams. The incidence of skeletal variations slightly exceeded the historical control range at the highest dose (87.0 – 98.1%, lab, number of studies and date data were collected was not specified).

The Dossier Submitter concluded that the incidences of malformations (external, skeletal and total malformations) and several soft tissue and skeletal variations were statistically significantly increased and clearly above the historical control values. However, the historical control values for external (anasarca and/or cleft palate), skeletal and total malformations were not provided in the CLH report.

Limited details were also provided for a supporting study (Daston *et al*, 1989), in which cultures of rat and mouse embryos were exposed to 30 or 60 µg/ml imidazole (no details on exposure time). Exposure to imidazole resulted in a reduced yolk sac diameter, crown rump length and decreased brain size in ≤100% of embryos. Mortality was reported as 83%.

A comparison with the classification criteria was initially missing from the CLH report, but was provided after the public consultation period (see below).

Comments received during public consultation

One Member State Competent Authority expressed their support for the Dossier Submitters proposal. Another supported the proposal but asked for some additional information/clarification and a comparison with the classification criteria. One Member State Competent Authority did not agree with the NOAEL set by the Dossier Submitter and another stated that a conclusion on the classification for fertility could not be made in the absence of a multi-generation study.

In response to these comments the Dossier Submitter provided additional information/data, which is outlined in the RCOM and summarised below (see additional key elements).

Assessment and comparison with the classification criteria

Fertility

No one- or two-generation studies were available. However, treatment with imidazole did not affect the weight or histopathology of reproductive organs (including uterus, ovaries, oviducts, vagina, female mammary glands, left testis, left epididymis, prostate gland and seminal vesicles) and/or sperm/oestrus cycle parameters in the rat 90-day repeated dose toxicity study, conducted up to 180 mg/kg bw/day (oral gavage).

An investigative study (Adams *et al*, 1998), involving the subcutaneous injection of imidazole in adult rats (10/group) suggested that imidazole may suppress male hormone secretion and testicular function. However, this study had significant flaws (administration by an irrelevant route of exposure, limited reporting detail and a lack of microscopic examination in the testes) and is consequently considered to be of limited relevance for classification and labelling.

The RAC noted that since no sexual function and fertility studies were submitted for imidazole, the available data do not allow for an assessment of whether e.g. mating behaviour or sexual maturation would have been affected and therefore whether imidazole might adversely affect fertility. |However, considering that no changes in reproductive parameters were observed in the 90-day repeated dose toxicity study, RAC agreed that the available data did not support classification for sexual function and fertility.

Development

RAC agreed with the Dossier Submitter that the following findings support the proposal to classify imidazole for developmental toxicity:

- Increased post implantation loss and reduced fetal body weight

At the top dose in the only available pre-natal developmental toxicity study, there was a statistically significant increase in post implantation loss (43% compared to 8% in the controls) and total resorption in 3/24 females. As a result, the number of live foetuses per litter was statistically significantly reduced, indicating that 180 mg/kg bw day imidazole caused fetal toxicity. In addition, an increase in the number of runts and a reduced mean fetal weight (↓14%) was observed at the top dose. As these findings occurred at a dose level that caused only minimal maternal toxicity, they are not considered to be secondary to non-specific maternal toxicity.

- Increased number of external and skeletal malformations

A statistically significant increase in the incidence of external malformations (anasarca and/or cleft palate: affecting 10% of foetuses in 7/21 litters) and skeletal malformations (including shortened scapula, bent radius, bent ulna, malpositioned and bipartite sternebrae: affecting 7.8% foetuses in 24% litters) were observed at the top dose. No incidences of anasarca or cleft palate were reported in the control, low and mid dose groups and the incidences of skeletal malformations in the other dose groups were low (incidence of affected foetuses per litter: 1.1, 2.3 and 0.9% of at 0, 20, and 60 mg/kg bw/day, incidence of affected litters: 4.5, 9.1 and 4.3% at 0, 20 and 60 mg/kg bw/day). These findings occurred at a dose causing only minimal maternal

toxicity and are consequently considered to be independent of secondary non-specific maternal toxicity.

The following findings are also considered to provide supportive information for classification:

- Increased incidence of soft tissue and skeletal variations

The incidence of total soft tissue (predominantly dilated renal pelvis and ureter) variations were significantly increased at the mid and top dose levels and skeletal variations (delays in ossification) were increased at the top dose level. These incidences were just outside of the historical control range and are consequently considered treatment related.

- Embryo toxicity in rat and mouse whole cell embryo cultures

Embryo toxicity was also observed in an *in vitro* whole embryo test, in which exposure of rat and mouse embryo to 30 and 60 µg/ml imidazole resulted in embryo lethality and abnormalities (decreased yolk sac diameter and brain size).

In conclusion, RAC agrees with the Dossier Submitter that these findings represent clear evidence of an adverse effect on development, which is not considered to be secondary to the minimal non-specific maternal toxicity observed in the rat prenatal developmental study. Therefore, RAC considers that the available data on imidazole support classification as Repr. 1B; H360D.

On a similar basis, a classification of Repr. Cat. 2; R61 is recommended under DSD.

ANNEXES:

Annex 1 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 rapporteurs' comments (excl. confidential information).