Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

2-methylimidazole

EC Number: 211-765-7
CAS Number: 693-98-1

CLH-O-0000001412-86-178/F

Adopted
5 December 2017

5 December 2017

CLH-O-0000001412-86-178/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: **2-methylimidazole**

EC Number: **211-765-7**

CAS Number: **693-98-1**

The proposal was submitted by **Sweden** and received by RAC on **27 October 2016.**

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

PROCESS FOR ADOPTION OF THE OPINION

Sweden 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 **21 February 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **7 April 2017**.

ADOPTION OF THE OPINION OF RAC

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

Co-Rapporteur, appointed by RAC: **Ralph Stahlmann**

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 **5 December 2017** 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			Specific Conc. Limits, M-factors and ATE	Notes
					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	No current Annex VI entry										
Dossier submitter's proposal	613-RST-vW-Y	2-methylimidazole	211-765-7	693-98-1	Repr. 1B	H360Df	GHS08 Dgr	H360Df			
RAC opinion	613-RST-vW-Y	2-methylimidazole	211-765-7	693-98-1	Repr. 1B	H360Df	GHS08 Dgr	H360Df			
Resulting Annex VI entry if agreed by COM	613-RST-vW-Y	2-methylimidazole	211-765-7	693-98-1	Repr. 1B	H360Df	GHS08 Dgr	H360Df			

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

Sexual function and fertility

The Dossier Submitter (DS) noted that no effects on male or female fertility or mating indices were recorded in the available Reproduction/Developmental toxicity screening test (OECD TG 421, GLP; BASF 2013a) at dose levels up to and including 500 mg/kg bw/d by oral gavage. No adverse histopathological findings were recorded on examination of the testes, ovaries and epididymides or on the weight of the testicles (no other reproductive organs were analysed in the study). However, at the highest dose level, an increase in mean duration of pregnancy (to 22.5 days, statistically significant) was observed. The recorded duration was however comparable between the test substance-treated groups and the control group (i.e. between 21.9 and 22.5 days). Two top dose dams died during or shortly after parturition (postnatal days (PND) 2 and 3), both showing signs of complicated parturition, preceding death. As a consequence, their pups either died or had to be killed for humane reasons. There were no pathological findings that could explain these deaths.

The DS considered that the available data from repeated dose toxicity studies do not raise concerns for effects on the integrity of the male and female reproductive organs.

The available data indicated to the DS that there is some evidence for an adverse effect on the process of parturition. In the OECD TG 421 study, two dams in the top dose group died during or shortly after parturition. Although no clear effect was seen on the group mean duration of pregnancy, the severity of the finding as such is considered to be high, especially since the recorded level of general toxicity in the top dose group females was considered to be mild. No abnormal clinical findings were recorded for the other top dose dams during the study, or for the period up until start of parturition for the two dams that were found dead. The observed mortalities were not considered to be secondary to non-specific toxicity but were instead considered to be specifically related to the process of parturition. On this basis, the DS proposed classification as Repr. 2; H361f.

Development

For examination of developmental effects two studies were available, a Reproduction/ Developmental toxicity screening test in Wistar rats (OECD TG 421, GLP; BASF, 2013a) and a follow-up study (GLP; BASF 2013b) according to a modified developmental toxicity protocol, with dosing of only females from gestation day (GD) 6 until PND 3. The follow-up study was conducted because developmental toxicity (dissecting aneurysm of the great vessels of the heart) was observed at all dose levels in the original study. In addition, a decreased pup viability index at PND 4 and a decreased live birth index were recorded in the original study. No maternal toxicity was seen at the low and intermediate dose levels whereas a reduced gestational body weight gain (-18% as compared to controls) and a statistically significant decrease in mean body weight (7% less than the controls at PND 0) were recorded at the top dose level.

In the follow-up study, no apparent maternal toxicity was recorded and there were no treatment related effects on litter size, number of stillborn pups, on postnatal survival or on pup weight. Gross pathology examination of the pups revealed macroscopic dilations of the great vessels at the base of the heart (aorta, ductus arteriosus, pulmonary trunk and carotid artery). Mostly these effects were correlated microscopically with dissecting aneurysms. Predominant locations were the ductus arteriosus and aorta. No aneurysms were observed in male and female control pups. The incidence in the low dose group was slightly higher as compared to the background incidence reported from the same lab (0.2%; Treumann *et al*., 2011).

Histopathology also revealed the presence of intramural haemorrhages that were not detected macroscopically. There was no dose–response relationship in the distribution of pups with haemorrhages and the incidence in 2-methylimidazole treated groups were similar to the incidence recorded in the control group. Individual pups had either aneurysms or haemorrhages, but never both lesions together.

Imidazole, a substance that has a harmonised classification as Repr 1B; 360D (7th ATP), is a known impurity in 2-methylimidazole. However, considering that the stated purity of 2-methylimidazole used in the studies reported is 99.8%, the contribution from imidazole in the test substance used in the studies was at maximum 0.2%. This is below the concentration limit for classification of imidazole as a developmental toxicant. Furthermore, based on the available data, 2-methylimidazole is clearly more potent ($LOAEL_{developmental\ toxicity}$ = ≤2 mg/kg bw/d) than imidazole ($NOAEL_{developmental\ toxicity}$ = 60 mg/kg bw/d and $LOAEL_{developmental\ toxicity}$ = 160 mg/kg bw/d; see ECHA, 2013). Therefore the DS concluded that it is highly unlikely that a possible impurity of imidazole (up to a maximum concentration of 0.2%) had any impact on the developmental toxicity that was recorded in the two studies with 2-methylimidazole.

The DS concluded that classification as Repr. 1B; H360D is warranted since the evidence for developmental toxicity is considered to be clear. Dissecting aneurysm of the vessel at the base of the heart was detected in rat pups in two separate studies. The adverse effect was seen at dose levels down to 10 mg/kg bw/d, and possibly at 2 mg/kg bw/d. In addition pup viability was decreased during the first days of lactation (viability index at PND 4 was 59% as compared to 100% in controls) at 500 mg/kg bw/d. The recorded effects are relevant for humans, and are not considered to be non-specific effects secondary to maternal toxicity.

Adverse effects on or via lactation

Pups were only followed until PND 4 and this limits the assessment of possible effects on or via lactation. There was no information on whether the compound is transferred to the milk. The available database did not support classification for effects on or via lactation.

DS Conclusion on classification

Based on available data the DS concluded that classification in Repr. 1B; H360Df is warranted.

Comments received during public consultation

Comments were submitted by three Member States Competent Authorities (MSCA) and one Company/Manufacturer.

Sexual function and fertility

One MSCA agreed with the DS's proposal to classify 2-methylimidazole in Category 2 for effects on sexual function and fertility.

One MSCA considered this to be a borderline case between Category 2 and no classification because the evidence supporting classification is limited to the deaths of two dams. This MSCA asked the DS to explain why the mortalities could not be due to a general maternal toxicity (statistically significant decrease in bodyweight gain).

The DS responded that: "The mortalities occurred at PND 2 and PND 3, where the mean body weight gain was reduced by 18% in the top dose group compared to control. During GD 0-20 the mean body weight gain was -18.1 % as compared to control. At lactation day 0 the mean body weight was statistically significantly reduced by 7.4 % as compared to control. The effects (mean values) on body weight and body weight changes are not considered severe. We do not have the full study report and can therefore not look into individual data of the two dams dying on PND 2 and 3. Nevertheless, in a study by Carney et al (2004) determining the effects of feed restriction in rat during in utero and postnatal life on standard reproductive toxicity and developmental immunotoxicity end points, reductions in maternal body weights down to 32% in feed restricted rats (as compared to control) during gestation and the lactation period did not cause any mortality or treatment-related clinical effects in the dams."

The same MSCA also suggested that the decrease in spermatid heads could be considered relevant for classification despite the fact that the number of spermatozoa was not affected. The DS noted that no effects on sperm count, testes or epididymis of relevance for the evaluation of reproductive toxicity were detected in the available mouse repeated dose toxicity studies (15 days, 14 weeks and 2 years) or in the rat 2-year combined chronic and carcinogenicity study. No effect on male functional fertility was recorded in the Reproduction/developmental toxicity screening test. Therefore the DS considered that the reduced sperm count in the 90-d study in rats was of low toxicological significance.

A third MSCA requested a more detailed justification for the proposal to classify for effects on fertility. This MSCA invited a consideration of the possible mechanism leading to abnormal parturition in Wistar rats. Since thyroid lesions were observed in F344 rats in a 90-d study, the MSCA asked whether there could be a link between disturbance of thyroid hormone levels and complications in parturition in rats. In response to the MSCA that raised this issue, the DS noted that in the screening study, the thyroid gland was not weighed and no hormonal analysis was performed. Moreover, as stated earlier, no individual data on the dams, including duration of pregnancy, were available to the DS. In the open literature, imbalances in thyroid hormone levels in humans are considered to be associated with complications during pregnancy and sequelea after delivery with adverse maternal and fetal outcomes (e.g. Cignini *et al*., 2012). However, based on the available information in the current dossier, there were no indications of a possible mechanism of the abnormal parturitions and it would be solely speculative to discuss a potential mechanism. Therefore, it is not possible to convincingly link the observed deaths of the two dams (due to complications during parturition) to effects on the thyroid gland and hormonal imbalance.

A Company/manufacturer of 2-methylimidazole disagreed with the proposal for classification in category 2, commenting that general toxicity might have contributed to the observed problems during and shortly after parturition in the two dams which died at PND 1 and 2. Thus the specificity of this finding with regard to a fertility-impairing effect cannot be judged. The stakeholder noted that the duration of pregnancy at the top dose (22.5 days) was similar to historical control data for this rat strain from OECD screening studies from the same laboratory (21.6-22.4 days). The first female that died evidently showed insufficient maternal care, a non-consumed placenta and died at PND 2. The second female had undelivered pups palpable in the dam's abdomen, the umbilical cord was not cut and pups were not properly nursed at PND 0. The dam and all pups were found dead at PND 1. During clinical observation, there were no obvious severe findings in the top dose

group and in the two animals that died (salivation after treatment and discoloured urine in all dams). There were also no particular macroscopic findings and no microscopic findings in ovaries, but no other inner organs were examined. However, dams in the top dose group showed statistically significant reduced food consumption during the first week of premating and lactation phase (-13.5% or -20.3%, respectively) compared to the control group. The maternal body weight gain during gestation (GD 0-20) and the body weight at lactation day 0 were also statistically significantly reduced: -18.1% or -7.4%, respectively, compared to controls. These findings in the top dose animals might be hints for systemic toxicity, which was observed in the available repeated dose toxicity studies.

Development

Three MSCAs and one Company/manufacturer agreed with the proposal to classify 2-methylimidazole in Category 1B for developmental toxicity.

Two of the three MSCAs asked for clarification on the incidence at which aneurysms were observed in the screening study. The number of pups examined for each group was not available to the DS. However, based on the number of pups delivered and the assumed number of litters, the foetal incidences of this effect were calculated to be 0, 1.7, 3.5 and 33.3% at 0, 50, 150 and 500 mg/kg bw/d.

Assessment and comparison with the classification criteria

Sexual Function and Fertility

1) GLP-compliant Reproduction/developmental toxicity screening test (OECD TG 421; BASF, 2013a)

Wistar rats (10/sex/dose) were exposed to 0, 50, 150 and 500 mg/kg bw/d of 2-methylimidazole, by gavage. Males were dosed for 28 days (two weeks prior to mating, during mating (maximum two weeks), and up to the day prior to scheduled necropsy). Females were dosed from two weeks prior to mating until day four of lactation (last dose on the day prior to scheduled necropsy).

Two top dose dams died during or shortly after parturition (on PND 2 and 3). Prior to their deaths, signs of complicated parturition were observed in both dams (undelivered pups, umbilical cords not cut, newborn pups not nursed).

Effects on parental generation bodyweight were observed in top dose females only. During gestation, mean bodyweight gain was 18% lower than in controls (statistically significant). The mean maternal bodyweight was statistically significantly reduced (by 7%) on PND 0.

The only reproductive organs that were weighed were the testes and epididymis. The reproductive organs that underwent histopathological examination were the testes, epididymis and the ovaries. No adverse effects on weight or histopathology were observed in these organs.

Exposure to 2-methylimidazole had no effect on gestation index or on male and female mating and fertility indices.

The DS reported that there was a statistically significant increase in mean duration of pregnancy at the top dose (22.5 days). This was just outside the historical control data range (21.6-22.4 days) in the same laboratory for this strain and type of study. However, the DS also commented that the recorded duration was "comparable between the test substance-treated

groups and the control group (i.e. between 21.9 and 22.5 days)". Therefore the biological significance of this observation is unclear.

The number of implantation sites per dam was slightly lower at the top dose compared to the number in other dose groups (12.9, 12.8, 13.0 and 11.8 at 0, 50, 150 and 500 mg/kg bw/d, respectively). However, this effect was not statistically significant.

Under the conditions of this study, two top dose dams died during or shortly after (complicated) parturitions. In the absence of sufficient overt general toxicity in this dose group to account for these findings, RAC concurs with the DS and considers that these deaths may have been due to an adverse effect on female reproduction.

During the Public Consultation, one MSCA discussed the effects observed on sperm parameters. In addition to the screening test, information from repeated dose studies in rats and mice exposed to 2-methylimidazole is also available and is relevant for the discussion on male reproductive toxicity. Findings related to reproduction which were observed in repeated dose studies are summarised below.

1) 4-week gavage study, Sprague Dawley rats (10/sex/group; REACH registration dossier, study from 1975): doses up to 800 mg/kg bw/d. There were no effects on testicular weights. No information on the weights of other reproductive organs was provided. No adverse histopathological effects were observed on the testicles/ovaries, prostate/uterus, seminal vesicles or epididymis.

2) 15-d dietary study, Fischer 344 rats (5/sex/dose; NTP 2004a): doses up to 900 mg/kg bw/d. Testis weight was not affected by treatment. No other reproductive organs were weighed. No adverse histopathological effects on the ovary or testis were observed.

3) 90-d dietary study, Fischer 344 rats (10/sex/dose; NTP 2004a): doses of 0, 40, 80, 160, 300 or 560 mg/kg bw/d. 'Small uteri' were observed in top dose females. However, uterine weights were not recorded. In males, the changes in reproductive organ weights were as follows:

Table: Reproductive organ weight changes in male rats in a 90-d study (NTP, 2004a)

Dose (mg/kg bw/d)	0	160	300	560
Testis (g) (absolute) - Right - Left	 1.388 1.498	 1.478 1.539	 1.471 1.518	 1.247* 1.289**
Testis (g) (relative) - Right - Left	 3.78 -	 4.22** -	 4.38** -	 4.37** -
L epididymis (g)	0.4987	0.4965	0.4852	0.4341**
L cauda epididymis (g)	0.1777	0.1798	0.16512	0.1250**
Necropsy weight (g)	366 ± 7	350 ± 2	336 ± 5*	294**

* p≤0.05
** p≤0.01

As tabulated below, the incidence of testicular degeneration was significantly greater at the top dose. However, the severity of testicular degeneration was lower in top dose males than in controls and therefore this effect is not considered adverse.

Table: Testicular degeneration in male rats in a 90-day study (NTP, 2004a)

Dose (mg/kg bw/d)	0	40	80	160	300	560
Number of animals with testicular degeneration	2	2	1	2	2	9**
Group mean severity score[a]	2.5	1	1	1	1	1.2

[a] group mean severity score (grading 0-4, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked severity)
** p≤0.01

No adverse histopathological effects on the epididymis, seminal vesicle, prostate, ovary or uterus were reported.

A decrease in spermatid heads per testis and spermatid counts was observed in top dose males as shown below. However there were no notable changes on the motility or concentration of epididymal spermatozoa.

Table: Sperm parameters in male rats in a 90-day dietary study (NTP, 2004a)

Dose (mg/kg bw/d)	0	160	300	560
Spermatid heads (10^7/g testis) (10^7/testis)	8.63 ± 0.32 13.02 ± 0.83	8.74 ± 0.29 13.44 ± 0.52	8.70 ± 0.29 13.22 ± 0.52	8.63 ± 0.30 11.13 ± 0.42 [-14.5%]
Spermatid counts (mean/10^{-4} mL suspension)	65.09 ± 4.17	67.22 ± 2.59	66.09 ± 2.64	55.66 ± 2.09 [-14%]
Epididymal Spermatozoal measurements				
(Motility (%))	87.67 ± 0.36	86.88 ± 0.70	87.91 ± 0.51	87.46 ± 0.62
Conc (10^6/cauda epididymal tissue)	439 ± 25	378 ± 44	399 ± 38	487 ± 72 [+10%]
Total number of spermatozoa (10^6)/ cauda epididymis	78.01	67.96	65.88	60.88 [-20%]

In females, there was a dose-dependent increase in mean oestrous cycle length (4.30 ± 0.15, 4.61 ± 0.14, 4.65 ± 0.15 and 5.56 ± 0.41 (p≤0.01) days at 0, 160, 300 and 560 mg/kg bw/d, respectively).

4) 2-year combined dietary chronic toxicity/carcinogenicity study, F344 rats (60/sex/dose; NTP 2004b): doses up to 130 and 230 mg/kg bw/d in males and females, respectively. Reproductive organs were not weighed. After 2 years, there were similar incidences of germinal epithelium atrophy, sperm granuloma of the epididymis and cysts of the periovarian tissue in concurrent controls and treated groups. Hyperplasia of uterine endometrium was observed in 10/50, 14/50, 15/50 and 15/50 females at 0, 50, 120 or 230 mg/kg bw/d, respectively.

5) 15-d study (GLP), B6C3F1 mice (5/sex/dose; NTP 2004a): doses up to 1933 mg/kg bw/d. After limited investigation, no adverse histopathological or weight changes of reproductive organs were reported.

6) 90-d study (GLP), B6C3F1 mice (10/sex/dose; NTP 2004a): doses up to 1740 and 1860 mg/kg bw/d, respectively. Although investigation of the reproductive organs was limited, no adverse histopathological or weight changes of reproductive organs were reported. There were no

significant differences in sperm motility or on oestrous cycle length in treated animals in comparison to controls.

7) 2-year combined dietary chronic toxicity/carcinogenicity study (GLP), B6C3F1 mice (60/sex/dose; NTP 2004b): doses of up to 315 and 325 mg/kg bw/d in males and females, respectively. No dose-dependent adverse effects were observed in female reproductive organs. In males, the following effects were observed after 2 years, but not at the 6 months interim evaluation.

Table: Effects on the reproductive organs of male mice in a chronic toxicity/carcinogenicity study (NTP, 2004b)

Dose (mg/kg bw/d)	0	75	150	315
Incidence of sperm granuloma of the epididymis (%)	0	0	6	12
Incidence of germinal epithelium atrophy (%)	2	8	16	28

Summary and conclusion of the findings from the repeated dose studies

Overall, the small changes in male fertility parameters observed in repeated dose studies are considered to be a possible indication of an adverse effect of 2-methylimidazole on fertility. However, RAC concurs with the DS's conclusion that the reduced sperm count in the 90-d study in rats was of low toxicological significance. The limited findings in the repeated dose studies are not considered sufficient to support classification for effects on sexual function and fertility.

Summary and Conclusion on sexual function and fertility

In the absence of evidence of overt general toxicity, RAC concurs with the conclusion of the DS, i.e. that the deaths of two dams during, or shortly after, parturition in the screening study was considered to be a specific adverse effect on female fertility that is not secondary to general toxicity. Signs of complicated parturition were observed in both dams preceding death. Therefore RAC considers that classification for effects on sexual function and fertility is warranted. It is noted that the adverse effects were seen in a single screening study with only 10 rats/sex/dose and that the evidence of an adverse effect on fertility is limited to that observed in two dams only. There is no mechanistic explanation for the findings. Overall, RAC agrees that there is some evidence for an adverse effect of 2-methylimidazole on female fertility, but that the evidence is not clear enough to support classification in Category 1B. Therefore RAC supports classification of 2-methylimidazole in **Category 2; H361f** for effects on sexual function and fertility.

Development

Two studies in rats are available.

1) <u>GLP-compliant Reproduction/developmental toxicity screening study (OECD TG 421; BASF 2013a)</u>

In the screening study, Wistar rats were exposed to 2-methylimidazole at 0, 50, 150 or 500 mg/kg bw/d, as described above in the section "Sexual Function and Fertility".

Maternal toxicity was limited to bodyweight changes at the top dose only: during gestation, mean bodyweight gain was 18% lower than in controls (statistically significant). The mean maternal bodyweight was statistically significantly reduced (by 7%) on PND 0.

As tabulated below, post-implantation losses and mean number of pups (live + dead) were unaffected by treatment. However, there was a significant increase in the number of stillborn pups, and consequently a statistically significant reduction in live birth index, at the top dose (90%

compared to 100% in controls). The live birth index was outside the historical control data range in the laboratory (93-100%). The reduced live birth index in this study was mainly attributable to a single top dose dam, who gave birth to 12 pups. Seven of these pups were stillborn and this dam died on PND 3. The litter incidence of this effect reduces concern for developmental toxicity. In addition, there was a statistically significant reduction in the viability index at the top dose (59% compared to 99% in controls). In the controls, one pup was cannibalised, whereas at the top dose 28 pups died and three were cannibalised during PND 0-4. Five of these 31 pups were born to the dam that died on PND 3.

Table: Births and pup survival in the Reproduction/developmental toxicity screening study in rats (BASF, 2013a)

	Dose (mg/kg bw/d)			
	0	50	150	500
Post-implantation losses (%)	8.2	7.5	12.0	9.2
Mean number of pups (live + dead) delivered per dam	11.8	11.9	11.5	11.1
Number of stillborn pups	0	0	4	11*
Live birth index (%)	100	100	97	90**
Viability index (PND 0-4) [a] (%)	99	98	97	59**

[a] Pup survival from postnatal day 0-4
* p≤0.05
** p≤0.01

No adverse clinical signs were observed in the pups, but 2, 2 and 6 runts were born at the low, mid and top doses, respectively. Runts were defined as pups weighing less than 75% of the mean weight of concurrent control pups. During lactation, slight non-statistically significant decreases in pup mean body weight and body weight changes were recorded at the top dose.

As shown below, a dose-related increase in the incidence of aneurysms was observed at gross pathological examination and confirmed via histopathology. The aneurysms were observed at different levels of the aorta, in the region of the ductus arteriosus and the pulmonary trunk.

Table: Incidences of aneurysms in the screening study in rats (BASF, 2013a)

	Dose (mg/kg bw/d)			
	0	50	150	500
Aneurysms (gross pathology)	0	2	14	42
Aneurysms (histopathology) (% foetal incidence)	0 0	2 1.7	14 3.5	37 33.3

In conclusion, the study shows that 2-methylimidazole is a developmental toxicant. The observation of increased incidences of aneurysms is a clear indication of a developmental effect.

2) <u>GLP-compliant modified Reproduction/developmental screening study (BASF, 2013b)</u>

In a second modified screening study, 25 pregnant Wistar rats per group were exposed to lower doses of 2-methylimidazole (0, 2, 10 or 50 mg/kg bw/d) by gavage from GD 6 to PND 3. No signs of maternal toxicity were observed. This follow-up study was conducted to define a NOEL for this endpoint because developmental toxicity was observed at all doses in the original study, as described above.

There were no substance-related effects on gestation index, live birth index, mean litter size at birth, number of stillborn pups, viability index on PND 4, pup bodyweight or pup bodyweight changes.

Adverse developmental effects are summarised in the table below.

Table: Developmental effects in the Reproduction/developmental screening study in rats (BASF, 2013b)

	Dose (mg/kg bw/d)			
	0	2	10	50
Number of runts	1	2	1	6
Macroscopic dilations of the great vessels at the base of the heart (aorta, ductus arteriosus, pulmonary trunk and carotid artery)	0	1	4	5
Aneurysms (number, (%)) (histopathology)	0	1 (0.5%)	3 (1.2%)	3 (1.3%)
Intramural haemorrhages (number, (%)) (histopathology)	2 (0.9%)	3 (1.4%)	1 (0.4%)	2 (0.9%)

Thus, the findings in this second study were consistent with those from the first. The macroscopic dilations of the great vessels at the base of the heart generally correlated with dissecting aneurysms identified upon histopathological investigation. There was a clear dose-dependent increase in the incidence of aneurysms in pups exposed to 2-methylimidazole during development. The incidence of aneurysms at the lowest dose (0.5%) was slightly higher than the incidence in historical controls from the same laboratory (0.2%).

Intramural haemorrhages were also observed histopathologically. However the incidences were not dose-dependent and were similar to the incidence in controls. It was reported that individual pups had either aneurysms or haemorrhages, but never both lesions together. According to the registrant, only single pups in each litter were affected, with one exception each in the mid and top dose groups. At each of these doses, there was a litter with two affected pups. Where two pups were affected in a single litter, one pup had an aneurysm and the other had a hemorrhage.

Summary and Conclusion on developmental toxicity

Since there is no evidence of 2-methylimidazole-induced reproductive toxicity in humans, classification in Category 1A is not appropriate.

Dose-related increases in the incidence of aneurysms were seen in pups in two studies, with exposure to 2-methylimidazole from doses as low as 2 mg/kg bw/d. In addition, there was a decrease in the viability index at 500 mg/kg bw/d. The observed developmental effects are not considered to be secondary to maternal toxicity because maternal toxicity was limited to bodyweight changes at 500 mg/kg bw/d. Therefore the criteria[1] for **classification in Category 1B; H360D for developmental toxicity** are met.

Effects on or via lactation

Classification for effects on or via lactation can be assigned based on:

a) human evidence indicating a hazard to babies during the lactation period; and/or
b) results of one- or two-generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or
c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

[1] Classification in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects.

There is no human evidence indicating a hazard to babies during the lactation period. During lactation in the original study, there was a statistically significant decrease in viability index (PND 0-4) at 500 mg/kg bw/d. At the top dose, 28 pups died and three were cannibalised during PND 0-4, in contrast to controls where one pup was cannibalised. Since the 28 deaths occurred during lactation, it is possible that 2-methylimidazole caused adverse effects on lactation. However, the pups may already have been compromised when they were born. Since it is not clear whether the postnatal pup deaths were due to effects on or via lactation and since there are no data available to inform on whether the substance is present in breast milk, the criteria for classification are not considered to be met.

Conclusion on classification for reproductive toxicity

RAC considers that 2-methylimidazole warrants **classification as Repr. 1B; H360Df.**

Additional references

Cignini P, Cafà EV, Giorlandino C, Capriglione S, Spata A, Dugo N. Thyroid physiology and common diseases in pregnancy: review of literature. J Prenat Med. 2012 Oct;6(4):64-71

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).