Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the 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

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

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Substance name: 2-methylimidazole
EC number: 211-765-7
CAS number: 693-98-1
Dossier submitter: Sweden

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
06.04.2017	Belgium		MemberState	1
Comment received				
- In physicochemical properties, water solubility is not given even though a reference to the registration dossier is made. A mentioned WS would be welcome. - TK studies: an explanation concerning the guideline used should be added (OECD testing guidelines? Similar to OECD guidelines? EPA guidelines?) - Same for some studies in table 10b: is the "oral repeated dose toxicity study" related to a specific OECD guideline? - Reliabilities are mentioned for all studies except in table 11. - Table 11: please specify the dose (500 mg/kg bw/d) at which maternal toxicity was observed as a reduction of body weight gain (GD0-20). - Table 11: first study mentioned (OECD 421): "slightly, but not statistically significant, decreased pup mean body weight and body weight changes were recorded during lactation". It is not clear at which dose these modifications were observed, please specify. Plus, concerning the aneurysms, as it is not mentioned how many pups per litter were examined, the numbers (0-2-14-42) should be given as percentages to facilitate the interpretation.				
Dossier Submitter's Response				
Thank you for your comments. Specific responses are given below. Water solubility: According to the Registrant(s) the water solubility of the test item is 267 g/L at 20°C and pH 11.5. In IARC monongraphs 101 the estimated water solubility is 8.09 x 10^4 mg/L at 25°C Test guidelines used in toxicokinetic studies: No test guidelines for the toxicokinetics studies have been indicated in the registration. Evaluation of tissue distribution and metabolism of the test substance appears not to				

have been performed in studies 1, 2 and 4 as indicated below, i.e. not in line with OECD TG 417.

1. Johnson, J.D. et al 2002. National Toxicology Program (2004b). No test guideline. Male and female rats (number of animals unknown) received a single gavage dose of 25, 50 or 100 mg/kg bw 2-methylimidazole. Postdose plasma samples were analyzed for 2-methylimidazole, and the results were used to calculate toxicokinetic parameters.

2. National Toxicology Program (2004b). No test guideline. Male and female mice (number of animals unknown) received a single gavage dose of 25, 50 or 100 mg/kg bw 2-methylimidazole. Postdose plasma samples were analyzed for 2-methylimidazole, and the results were used to calculate toxicokinetic parameters.

3. Sanders, J.M. et al., 1998. No test guideline. The disposition of radiolabelled 2-methylimidazole was investigated following peroral administration of 5, 50 and 150 mg/kg bw to male rats (number of animals unknown), or intravenous administration of 5 mg/kg. Urine, faeces, expired air, tissues, bile. 4, 8, 12, 24 and 48 hours for urine and feces collection, 4, 8, 12 and 24 hours for air sampling. For tissue examinations, animals were sacrificed at 48 hours postdosing. Tissues were also collected from rats sacrificed 2 hours following oral administration of 50 mg/kg bw and from rats sacrificed at 0.25, 0.5, 1, 2, 4, 6, 8 and 12 hours following intraperitoneal administration of 5 mg/kg. Biliary excretion of 2-methylimidazole radioactivity was investigated following tail-vein injections of 5 mg/kg bw to 3 rats. Metabolites in urine by HPLC.

4. Johnson, J. D. 2002. National Toxicology Program (2004b). No test guideline. The toxicokinetics of 2-methylimidazole were studied in male and female rats (15/sex/dose) after a single intravenous dosage of 10 mg/kg bw. Blood samples were analyzed for 2-methylimidazole and the results were used to calculate toxicokinetic parameters.

Table 10b: Test guidelines used in oral repeated dose toxicity studies:

- Study report (Report date 1975-12-29), Oral repeated dose toxicity study in Rat (Sprague-Dawley). Not in compliance with GLP. Equivalent or similar to OECD TG 407 (Repeated dose 28-day Oral Toxicity in Rodents) according to Registrant.

- NTP (2004a), Chan et al., 2006, Oral repeated dose toxicity study in Fischer 344 rat (5/sex/dose). GLP-compliant. Only 15 days of administration. Not equivalent or similar to any OECD TG.

- NTP (2004a), Chan et al., 2006, Oral repeated dose toxicity study in B6C3F1 mouse (5/sex/dose). GLP compliant. Only 15 days of administration. Not equivalent or similar to any OECD TG.

Table 11, Reliabilities of studies according to Registrant:

- BASF SE (2013a) GLP compliant OECD Guideline 421(Reproduction/Developmental Toxicity Screening Test), Reliability 1.
- BASF SE (2013b) GLP compliant modified reproduction/developmental screening study, Reliability 1.

Table 11, clarification of doses and incidences:

- Maternal toxicity was observed at 500 mg/kg bw/d as a reduction of body weight gain (GD 0-20).

- Slightly, but not statistically significant, decreased pup mean body weight and body weight changes were recorded during lactation at 500 mg/kg bw/day.

- The number of affected pups with aneurysms (gross pathology) were 0, 2, 14 and 42 in the control, low, intermediate and high dose group, respectively in the OECD TG 421 study in rat. The corresponding number of pups with aneurysms confirmed at histopathological examination were 0, 2, 14 and 37. The number of pups examined for each dose group is not available to the dossier submitter, however, it is stated in the registration that all pups (pups scheduled for sacrifice on PND 4; stillborn pups and pups that died before PND 4; and moribund pups that were sacrificed before PND 4) were examined externally and eviscerated, with special attention to heart and aortic vessels. Based on the given mean number of delivered pups and the assumed number of litters (8, 10, 10, 10; female fertility index and the gestation index were 100% in treated groups and 80% in control) the total number of pups (live + dead) in the dose groups were 94, 119, 115, 111. Thus, the fetal incidences are 0, 1.7, 3.5, 33.3% at 0, 50, 150 and 500 mg/kg bw/day.

RAC's response

The clarification relating to the effects described in Table 11 is helpful and the additional information is included in the opinion.

Date	Country	Organisation	Type of Organisation	Comment number
22.03.2017	Germany		MemberState	2

Comment received

In section 6, table 8 of the CLH report the value for the water solubility is missing. The corresponding value shoud be amended.

Dossier Submitter's Response

Please see reponse to comment number 1.

RAC's response

Noted, but does not impact on the classification assessment.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
31.03.2017	France		MemberState	3

Comment received

Fertility :
Given the data presented, this is considered a borderline case between the category 2 and no classification for fertility endpoint since it is only based on 2 deaths of dams. The proposal must be better argued based on the following elements:
- The decrease of spermatids head could be considered relevant for classification despite the fact that the number of spermatozoa is not affected.
- From the table 9.10.1, it is not clear why stillborn pups are mentioned under title "fertility, parturition and sexual function"
- Could you please further explain why the mortalities could not rather be due to a general maternal toxicity (statistically significant decrease of body weight gain found)?
- Could you please try to explain the discrepancies between the adverse effects (on sperm and testis) found in the 90 day- study on rat (NTP 2004) and the absence of adverse effects on reproductive organs in others studies?
- It seems that there is a reporting problem with the table p16 on sperm analysis. The

highest dose is not reported whereas it has been analysed.

Development :
FR agrees with the classification proposal for developmental toxicity
The LOAEL claimed for developmental toxicity (< 2 mg/kg) in the section 9.10.5 seems however questionable regarding the data presented.

Dossier Submitter's Response

Fertility

Thank you for your comments. Specific responses are given below.

- It seems that there is a reporting problem with the table p16 on sperm analysis.The highest dose is not reported whereas it has been analysed.
- The decrease of spermatids head could be considered relevant for classification despite the fact that the number of spermatozoa is not affected.
- Could you please try to explain the discrepancies between the adverse effects (on sperm and testis) found in the 90 day- study on rat (NTP 2004) and the absence of adverse effects on reproductive organs in others studies?

See below for an updated version of of the sperm analysis table that was presented in table 10b of the CLH report (changes in bold, information has been taken from table E1 in the NTP 2004a reference). Unfortunately, the epididymal data that was presented in the NTP study was limited i.e. there was no information on the total number of sperm in the cauda epididymis and there was no information on the actual measured data (number of spermatozoa/ml of the cauda epididymis suspension). Since the number of sperms contributes to the weight, reporting of the relative number of sperm (i.e. number of sperms/g of tissue) should be avoided since it may mask a true reduction in sperm count. In the present study the weight of the epididymis was reduced in the high dose males [-30% as compared to controls, $p<0.05$]. Therefore, we calculated the data for the total number of spermatozoa/epididymis. In contrast, both the measured as well as derived data was available for spermatid head counts.

Dose (mg/kg bw/day)	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 susoension)	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/ **g** 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%]**
* Inserted by the DS, no statistical analysis was performed.				

As compared to the controls, the number of spermatids/ml of the suspension [-14%, $p<0.01$] as well as the number of spermatids/testis [-14.5%, $p<0.01$] was statistically significantly decreased in the high dose group. The lack of an effect on the number of

spermatids/g testis is most likely related to the recorded lower weight of the testis in the high dose males [absolut:-14% as compared to controls, p<0.01, see table 10b]. When sperm are released from the testis, they are neither motile nor capable of fertilizing an oocyte. By the time they reach the cauda epididymis (where sperms are stored), the sperm have acquired progressive forward motility and fertilizing ability. A reduction in homogenization-resistant spermatids should be reflected by a decrease in epididymal sperm count if sufficient time has elapsed between release of the reduced numbers of sperm from the testis and the time point for analysis of sperm number in the cauda epididymis. The duration of the 14-week study is sufficiently long to be able to detect also post-testicular effects on sperm and the entire spermatogenesis. Analysis of the cauda epididymis showed that the total number of spermatozoa/cauda epididymis was reduced [-20%, statistical analysis not performed] i.e. effects on sperm count, of a similar degree, was observed both at the testicular and the epididymal level in the 14-week rat repeated dose toxicity study. However, there was no effect on sperm motility and no adverse findings were recorded at the histopathological examination of the epididymis. An increase in the incidence (9/10 as compared to 2/10 in the controls) of testis degeneration was seen in the high dose group. The mean severity score of the lesion was lower (1.2) as compared to control (2.5), indicating that the severity of lesions in 8 out of the 9 high dose animals was minimal and consequently not justifying classification.
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-week and 2 years) or in the rat 2-year combined chronic and carcinogenicity study (see table 10b in the CLH report for details). No effect on male functional fertility was recorded in the reproductive/developmental toxicity screening test were only effects on the post-testicular part of the spermatogenesis is assessed in the mating trial.
In conclusion, the relevance of the recorded reduction in sperm count that was recorded in the 14-week repeated dose toxicity study in rat (but not in the equivalent mouse study) is considered to be of low toxicological significance and classification for adverse effect on male reproduction is not warranted.

- From the table 9.10.1, it is not clear why stillborn pups are mentioned under title "fertility, parturition and sexual function"

The data from the OECD TG 421 Reproduction/Developmental toxicity screening test is reported twice, both in table 10a (Adverse effects on sexual function and fertility) and in table 11 (Adverse effects on development). The purpose for placing the data on number of stillborn pups in table 10a together with the data on post-implantation losses, was to facilitate the interpretation of Gestation index for the reader and to show that although gestation index was 100%, live birth index was reduced at high dose.

- Could you please further explain why the mortalities could not rather be due to a general maternal toxicity (statistically significant decrease of body weight gain found)?

The mortalities occurred at PND 2 and PND 3, where the mean body weight gain was reduced by 18% in the high 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. Moreover, gestation length was statistically significantly increased in the feed restricted rats, but was within the laboratory historical control range and there were no reported effects on parturition. Thus, the decrease (-18% compared to control) in maternal body weight during gestation and lactation in the high dose group in the screening study is not considered to have influenced the observed complications in relation to parturition or the mortalitites.

Development
Thank you for your support.

Regarding the LOAEL (< 2 mg/kg) in the follow up study:
Setting of LOAEL for classification purpose is not required. Nevertheless, the reasoning of LOAEL ≤ 2 mg/kg is the following (and in line with the Registrant): Aneurysm was clearly an adverse effect in the modified reproduction/developmental screening study (BASF SE, 2013b) and is considered to be a rare and severe malformation. Macroscopically, the incidence of aneurysm in control group in the current study was zero. In treated groups there was a dose-dependent increase in the incidence of aneurysms: 1, 4, and 5 pups affected at 2, 10 and 50 mg/kg bw/day, respectively. These findings were confirmed by microscopic evaluation: 1 (0.5%), 3 (1.2%), 3 (1.3%) at 2, 10 and 50 mg/kg bw/day, respectively. The incidence at the lowest dose tested (2 mg/kg bw/day) was slightly above the background incidence reported from the same lab (from 16 different reproductive toxicity studies at the BASF laboratory in 2008) at 0.2% (2 animals out of 1016 animals in total; no range given; Treumann et al., 2011). No NOAEL could be set. Therefore, a LOAEL ≤2 mg/kg bw/day is considered appropriate.

RAC's response

Thank you for the clarification.

Date	Country	Organisation	Type of Organisation	Comment number
06.04.2017	Belgium		MemberState	4

Comment received

BECA agrees with the DS proposal to classify 2-Methylimidazole as Repr. 1B H360Df.

2-methylimidazole caused severe parturition effects (undelivered pups, uncut umbilical cords, lack of nursing of newborns) in an OECD TG 421 study, leading to two maternal deaths in the group exposed to 500 mg/kg bw/d. Considering the complicated parturition was not secondary maternal toxicity, it has to be taken into account as a treatment-related effect on female reproduction. Unfortunately, no other study confirmed the results as no other study were related to reproduction/development or gave multigenerational data. Then BECA considers that it cannot be excluded that these effects on reproduction are relevant and they should be taken into account to classify the substance.

Furthermore, pronounced histopathological effects on the great vessels of the heart were observed in two studies: indeed, 2-methylimidazole induced aneurysms in 0, 2, 14 and 37 pups of dams exposed to 0, 50, 150 and 500 mg/kg bw/d, respectively, in an OECD TG 421 and in 0, 1, 3 and 3 pups of dams exposed to 0, 2, 10 and 50 mg/kg bw/d, respectively, in a GLP-compliant modified reproduction/developmental screening study. Aneurysms were observed in a dose-dependent way, with a similar incidence at the same dose (50 mg/kg bw/d) in the two studies. Moreover, significantly decreased pup viability index (99, 98, 97 and 59 %) and live birth index (100, 100, 97, 90 %) were seen in the OECD 421 study at 0, 50, 150 and 500 mg/kg bw/d, respectively.

For all these reasons, BECA agrees to classify 2-methylimidazole as Repr. 1B H360Df since reproductive effects were obvious, dose-related and it cannot be excluded that these effects are relevant to humans.
Dossier Submitter's Response
Thank you for your support.
RAC's response
RAC agrees with this interpretation of the data.

Date	Country	Organisation	Type of Organisation	Comment number
04.04.2017	Germany	BASF SE	Company-Manufacturer	5
Comment received				
We support the proposed classification for developmental toxicity, category 1B (Repr. 1B, H360D). However, we disagree with the suggested classification for fertility, category 2 (Repr. 2, H361f). The proposal for fertility is based on the death of two dams at the high dose (HD) group, which might have been caused by problems during parturition. However, it is not clear from the available data, if these signs of complicated parturition could have been, at least partly, a consequence of the known general systemic toxic potential of 2-methylimidazole at this HD level. The scope of examinations of this OECD test guideline 421 screening study is not sufficient to differentiate between these two options. Although the duration of gestation in the HD value (22.5 days) was slightly, but statistically significantly increased, the mean duration of gestation was comparable between the test substance-treated groups and the control group (i.e. between 21.9 and 22.5 days). The observed gestation time was similar, when compared to the historical control data for this rat strain from OECD screening studies (range 21.6 –22.4 days) of the same laboratory and was still within the historical control range collected from multi-generation studies (range 21.5 – 22.5 days) [BASF SE, 2013]. Females #136 and #140 of the HD group died postpartum. The first female showed insufficient maternal care, a non-consumed placenta and died at PND2. The second one had undelivered pups palpable in the dam´s abdomen, the umbilical cord was not cut and pups were not properly nursed at PND0. The dam and all pups were found dead at PND1. During clinical observation, there were no obvious severe findings in the HD group and in the two animals which 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, the HD female 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 (GD0-20) and the body weight at lactation day 0 were also statistically significantly reduced: -18.1 % or -7.4 %, respectively, compared to controls [BASF SE, 2013]. These findings in the HD animals might be hints for systemic toxicity, which was already observed in the available repeated dose toxicity studies: In 4-weeks and 14-weeks repeated dose toxicity studies in rats the NOAELs were well below 500 mg/kg bw/d. In Sprague Dawley rats reduced total protein (≥ 100 mg/kg bw/d), increased absolute and relative weights of thyroid glands (≥ 200 mg/kg bw/d, males) and relative liver weights (≥ 400 mg/kg bw/d; females) were observed after 4-weeks administration (LOAEL 100 mg/kg bw/day based on reduced total protein in males and females, no NOAEL reported) [BASF AG, 1975]. No histopathological abnormalities were reported. Fisher rats showed increased kidney and testis weights (≥ 40 mg/kg bw/d, males), diffuse thyroid follicular hyperplasia (≥ 80 mg/kg bw/d, males, ≥ 160 mg/kg bw/d in				

females), mild anemia (≥ 160 mg/kg bw/d in males and females) and decreased T3 and T4 and increased TSH (160 mg/kg bw/d in males and females) after 14-weeks administration (LOEL 40 mg/kg bw/d, NOAEL 80 mg/kg bw/d, LOAEL 160 mg/kg bw/d) [Chan et al., 2006].
Therefore, from these findings it can be assumed that there was some systemic toxicity present at the high dose level of 500 mg/kg bw/d in the dams (target organs: liver, kidney, thyroid glands), although not specifically investigated in the OECD421 study. It cannot be fully excluded, that this general toxicity might have contributed to the observed problems during and shortly after parturition in the two dams which died at PND1 and 2. Thus, the specificity of this finding with regard to a fertility impairing effect cannot finally be judged.
In conclusion, the currently available data do not support a category 2 for impaired fertility and sexual function in addition to the current self-classification as a reproductive toxin category 1B (H360D).

References:
BASF SE, 2013: 2-Methylimidazol - Reproduction/Developmental Toxicity Screening Test in Wistar Rats Oral Administration (Gavage), unpublished report, cited in REACH registration for 2-methylimidazole, CAS no. 693-98-1 (https://echa.europa.eu/registration-dossier/-/registered-dossier/5866, last access 2017-03-30).
BASF AG, 1975: Bericht über die Prüfung von 2-Methylimidazol im 28-Tage Sondierungsversuch an der Ratte (Gavage), unpublished report, cited in REACH registration for 2-methylimidazole, CAS no. 693-98-1 (https://echa.europa.eu/registration-dossier/-/registered-dossier/5866, last access 2017-03-30).
Chan, P.C. et al., 2006. Induction of thyroid lesions in 14-week toxicity studies of 2 and 4-methylimidazole in Fischer 344/N rats and B6C3F1 mice. Arch. Toxicol., 80, 169-180.

Dossier Submitter's Response

Fertility
Thank your for your comments.

We did not have access to the full study report and thus did not have the possibility to assess individual data (including the data on gestational length) of the dams. We agree that there was some (but not severe) systemic toxicity (decreased mean body weight and mean body weight gain) present at the high dose level of 500 mg/kg bw/d in the dams, but we consider that there is no mechanistic evidence linking the mild systemic toxicity to the problems of parturition and the subsequent death. Furthermore, no adverse clinical observations during pre-mating or gestation were reported in the screening study indicating that the dams were in poor condition prior to parturition. It is also noted that no mortalities were reported in either of the two RDT studies in rat (28-day and 14-week). As discussed in the response to comment number 2, maternal toxicity manifested as decreased mean body weight and mean body weight gain did not cause any mortality or treatment-related clinical effects in the dams of a rat study determining the effects of feed restriction during in utero and postnatal life on standard reproductive toxicity and developmental immunotoxicity end points (Carney et al., 2014). In the study, reductions in maternal body weights down to 32% in feed restricted rats (as compared to control) during gestation and the lactation period were recorded. Moreover, there were no reported effects on parturition. Thus, the decrease (-18% compared to control) in maternal body weight gain during gestation and the lower body weight [-7.4% compared to controls) at lactation day 0 in the high dose group in the screening study is not considered to have influenced the observed complications in relation to parturition or the mortalitites.

There were no biological significant changes in mean gestation time, and there were no pathological findings to explain the deaths of the two dams in the screening study. The mortalities cannot be attributed to any other findings and they cannot be dismissed. Thus, based on the available data there is no clear evidence to conclude that the complicated parturition and the subsequent deaths are secondary to systemic toxicity of the dams. The available information is considered as some evidence for adverse effects on fertility and sexual function and therefore category 2 is justified. We also note that in their evaluation of the screening sudy, the Registrant(s) state that influence of the test substance on normal term delivery cannot be excluded considering the two high-dose dams which died having parturition difficulties (REACH registration at ECHA dissemination site, 2017).

Development
Thank you for your support.

RAC's response

RAC considered the argument put forward here for no classification, but agreed with the response provided by the Dossier Submitter.

Date	Country	Organisation	Type of Organisation	Comment number
22.03.2017	Germany		MemberState	6

Comment received

Fertility

We agree that the two deceased high-dose dams in the screening study (BASF 2013a) have to be taken into account when evaluating the toxic potential of 2-methylimidazole on female reproductive capacity. In our view there are, however, several issues which have not yet been reflected in the line of argument for the classification proposal. Twenty percent of the high dose-dams (2/10 animals) "died during or shortly after parturition" and had shown "signs of complicated parturition proceeding death". We think this is a rather high incidence for a screening study. In this context we would like to refer to the substance Thiacloprid which was evaluated for reproductive toxicity by RAC in 2015. Thiacloprid induced dystocia in rats with an overall incidence of 6.7 %. Despite this rather low incidence, RAC recommended to classify Thiacloprid in category 1B – H360F based on this adverse effect. Could you please explain in more detail why you think a 20%-mortality related to the process of parturition provides only some and not clear evidence for an adverse effect on fertility in the case of 2-methylimidazole?

In our opinion, a proposal for category 2 could be justified if, for example, there were concerns that the complicated parturitions and the subsequent deaths might be incidental findings, secondary to non-specific toxicity, or possibly induced by other factors than the substance itself. But in the CLH report such concerns are not expressed. On the contrary, it is stated that "the severity of the finding as such is considered to be high especially since the recorded level of toxicity in the high dose group females was considered to be mild and no abnormal clinical findings were recorded for the other high dose dams".

Last but not least, we think that some considerations on the possible mechanism leading to abnormal parturitions in Wistar rats should be provided in the CLH report. In this context we would like to refer to the 90-day-study in Fischer 344-rats mentioned in the CLH report (NTP 2004a, Chan et al. 2006) in which thyroid lesions were induced by 2-methylimidazole. Do you think there could be a link between disturbance of thyroid hormone levels and complications in parturition in rats? And if yes, do you think this

mechanism is relevant for humans?

In summary we think that a more detailed justification for the proposal to classify 2-methylimidazole as reprotoxic in category 2 – H361f would be helpful for upcoming discussions. At least for us it is difficult at this moment to judge about the right classification based on the current CLH report.

Finally, a rather general remark:
On page 16 of the CLH report a table with sperm analysis results is provided in the third column. It looks as if the results for the high dose group (560 mg/kg bw/d) are missing. Could you please provide the missing data?

Developmental toxicity

The data in the CLH-dossier strongly indicate that classification for developmental toxicity in category 1B is justified for 2-methylimidazole. In particular, the dose-dependently increased incidences for dissecting aneurysms in pups from the original screening study (BASF 2013a) give strong evidence for the substance's developmental toxic potential.

Nevertheless, we would highly appreciate if the following details were – if possible – also provided in the dossier:

- Total number of pups for each dose group so that percentages can be calculated regarding the incidences for stillborn pups, runts, and pups affected by dissecting aneurysms.
(Some of the information is already provided in the dossier for the follow-up screening study (BASF SE 2013b).)

- Number or percentage of litters for each dose group with stillborn pups, runts, pups who died/were cannibalized post-natally, and pups affected by dissecting aneurysms.
(In general, information on the distribution of fetal findings over litters proves beneficial for data interpretation. Clustered occurrence of malformations, for example, may lower the evidence for developmental toxicity.)

- In the original screening study, mean body weight on PND 0 was reduced by 7 % in dams of the high dose group as compared to the control dams. Were these treated animals also affected by absolute body weight loss?

- In the original screening study two dams from the high dose group "died during or shortly after parturition (post-natal day (PND) 2 and 3)". In the same study and dose group several pups died (28 animals) or were cannibalized (3 animals) during lactation. The viability index PND 0-4 was therefore strongly reduced. Is there information available specifying how many litters were affected by pups who did not survive until study termination? And how many pups are to be attributed to the two dams which died before study end?

In light of the strongly and dose-dependently increased incidences for dissecting aneurysms in the pups we do not assume providing the missing data will lower the evidence for developmental toxicity. We think, however, that these additional pieces will add to the reprotoxic picture of 2-methylimidazole and will prove beneficial for a comprehensive assessment.

Finally, a rather general remark: On page 22 of the CLH report a viability index PND 0-4 of 59 % is stated for the high dose group as compared to the control group for the original screening study (BASF 2013 a). On pages 24 and 25, however, a percentage of 53 % is mentioned for the same index. Which rate is correct?
Dossier Submitter's Response
Fertility Thank you for comments. - Please explain in more detail why you think a 20%-mortality related to the process of parturition provides only some and not clear evidence for an adverse effect on fertility in the case of 2-methylimidazole? Due to the small group size and limited dimensions of the study, statistical analysis in the form of tests for "significance" are of limited value for many endpoints and therefore we consider the signal as less robust. However, we recognise that it is unusual that two dams (with parturition difficulties) die in a screening study. Based on the limited data without access to individual data of the dams that would clarify to what extent the gestational length for the dams that died during lactation was affected, and in absence of data exploring possible mechanism we consider the 20%-mortality related to the process of parturition provides some evidence. We would welcome further discussion in RAC if the available data can be considered as *clear* or *some evidence* on adverse effects on fertility. Regarding the case of Thiacloprid, dystocia was specifically investigated and found in several (at least 6) rat studies, thus the database was more robust and the evidence clear. - Considerations on the possible mechanism leading to abnormal parturitions in Wistar rats should be provided in the CLH report. Thanks for drawing our attention to the advere effects on the thyrod gland that was recorded in the 14-week dietary repeated dose toxicity study in the rat. As indicated in comment#5 and shown in the tables below (data copied or extracted from the NTP 2004a reference, available via https://ntp.niehs.nih.gov/results/pubs/shortterm/reports/abstracts/tox067/index.html) diffuse thyroid follicular hyperplasia (≥ 160 mg/kg bw/d in females) and effects on the serum levels of T3, T4 and TSH (from day 8 of dosing) was recorded in this study.

TABLE 10
Incidences of Neoplasms and Selected Nonneoplastic Lesions in Rats in the 14-Week Feed Study of 2-Methylimidazole

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
Thyroid Gland[a]	10	10	10	10	10	10
Follicular Cell, Hyperplasia, Diffuse[b]	2 (1.5)[c]	0	8* (1.1)	10** (1.1)	10** (1.9)	10** (2.9)
Follicular Cell Cyst	0	0	0	0	0	1 (2.0)
Follicular Cell Adenoma	0	0	0	0	0	2
Testes	10	10	10	10	10	10
Degeneration	2 (2.5)	2 (1.0)	1 (1.0)	2 (1.0)	2 (1.0)	9** (1.2)
Female						
Thyroid Gland	10	9	10	10	10	10
Follicular Cell, Hyperplasia, Diffuse	0	0	0	10** (1.0)	10** (2.0)	10** (3.0)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion
[c] Average severity of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

	0 ppm	625 ppm (40 mg/kg bw/day)	1250 ppm (80 mg/kg bw/day)	2500 ppm (160 mg/kg bw/day)	5000 ppm (300 mg/kg bw/day)	10000 ppm (560 mg/kg bw/day)
Thyroid–stimulating hormone (ng/ml)[a]						
Day 8	1.04±0.11	0.89±0.15	1.74±0.31	4.39±0.73**	9.05±0.47**	7.83±0.36**
Day 29	0.38±0.09	1.13±0.66	0.91±0.10**	0.76±0.12**	2.32±0.42**	8.49±0.62**
Week 14	0.27±0.13	0.49±0.22	0.27±0.16	0.52±0.16	1.23±0.40*	7.90±0.87**
Triiodothyroxine (ng/dL)[a]						
Day 8	142.5±6.6	130.5±5.5	141.1±3.1	119.9±1.8**	81.6±2.0**	76.3±2.1**
Day 29	138.5±6.0	139.6±4.1	143.4±4.2	135.3±3.5	128.4±4.1	116.4±3.2**
Week 14	136.5±6.1	142.0±6.7	139.2±5.6	135.8±4.7	137.9±3.7	112.2±4.2**
Thyroxin (ug/dL)[a]						
Day 8	3.87±0.29	3.11±0.28*	3.64±0.19	3.01±0.16**	0.60±0.07**	0.74±0.11**
Day 29	3.44±0.31	2.33±0.36	2.92±0.28	2.23±0.24**	2.18±0.25**	0.80±0.08**
Week 14	2.57±0.28	2.17±0.20	1.94±0.28	2.16±0.30	2.42±0.22	0.79±0.12**

a) Female data only. Information extracted from Table 8 of the NTP 2004a reference.
*) Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.
**) Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

In addition, enlarged thyroid glands as well as an increased incidence (5/5 for both female and male) of mild to moderate diffuse hyperplasia of thyroid gland follicular cells was recorded at 297 and 900 mg/kg bw/day in the 15-day dietary rat RTD study (see table below, that was taken from the NTP 2004 reference which is available via https://ntp.niehs.nih.gov/results/pubs/shortterm/reports/abstracts/tox067/index.html). No hormonal analysis was carried out in this study.

TABLE 5
Incidences of Selected Nonneoplastic Lesions in Rats in the 15-Day Feed Study of 2-Methylimidazole

	0 ppm	1,200 ppm	3,300 ppm	10,000 ppm
Male				
Thyroid Gland[a]	5	5	5	5
Follicular Cell, Hyperplasia, Diffuse[b]	0	0	5**	5**
Pituitary Gland	5	5	5	5
Pars Distalis, Hypertrophy	0	0	5**	5**
Female				
Thyroid Gland	5	5	5	5
Follicular Cell, Hyperplasia, Diffuse	0	0	5**	5**
Pituitary Gland	5	5	5	5
Pars Distalis, Hypertrophy	0	0	0	5**

** Significantly different (P≤0.01) from the control group by the Fisher exact test
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion

In the screening study, the thyroid gland was not weighed and no hormonal analysis was performed. Moreover, as stated earlier, no individual data of the dams, including length of gestation, were available to the DS. Therefore, it is not possible to convincingly link the observed deaths of the two dams (due to complicated parturitions) to effects on the thyroid gland and hormonal imbalance. In the open literature, imbalances in thyroid hormone levels in humans are discussed as being associated with complications during pregnancy and sequelea after delivery with adverse maternal and fetal outcomes (e.g. 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). However, based on the available information in the current dossier, we do not have any indications of a possible mechanism of the abnormal parturitions and it would be solely speculative to discuss a potential mechanism. In CLP there is no requirement to determine the underlying mechanism for the observed adverse effect to justify classification, but it is recognized that such data may give support in a weight of evidence evaluation. Nevertheless, we consider that the observed effects in the absence of a described mechanism are sufficient to justify classification for adverse effects on fertility. Importantly, in their evaluation of the screening study, the Registrant(s) state that influence of the test substance on normal term delivery cannot be excluded considering the two high-dose dams which died having parturition difficulties (REACH registration at ECHA dissemination site, 2017).

- On page 16 of the CLH report a table with sperm analysis results is provided in the third column. It looks as if the results for the high dose group (560 mg/kg bw/d) are missing. Could you please provide the missing data?

Please see reponse to comment number 3.

Development
Thank you for your support and your comments.

We do not have access to the full study report of the screening study or the follow up screening study, and details on the data requested in this comment (*total number of pups for each dose group so that percentages can be calculated regarding the incidences for stillborn pups, runts, and pups affected by dissecting aneurysms; Number or percentage of litters for each dose group with stillborn pups, runts, pups who died/were cannibalized post-natally, and pups affected by dissecting aneurysms*) are not available on the REACH

Registration dissemination site. Based on the available information we have tried to clarify incidences to the extent possible below.

Fetal incidences in screening study:
- incidences stillborn pups: 0, 0, 3.5, 10% (0, 0, 4, 11)
- incidences runts: 0, 1.7, 1.7, 6% (0, 2, 2, 6)
- incidences pups affected by aneurysms: 0, 1.7, 3.5, 33.3%

Litter incidences in screening study:
We do not have data to calculate litter incidences. In high dose group, at least 7 stillborn pups were from the same litter (dam #140 that died PND 3).
In high dose group, 28 pups were found dead after birth and an additionally 3 pups were cannabilized. At least 5 pups were from the same litter (dam #140 that died PND 3). No further information is available to the dossier submitter.

Fetal incidences in the follow up study:
- incidences stillborn pups: the mean number of delivered pups per dam and the rates of liveborn and stillborn were evenly distributed about the groups (we do not have the data on the number of pups).
- incidences runts: do not have data to calculate % pup incidences; number of pups affected were 1, 2, 1, 6
- incidences pups affected by aneurysms: 0, 0.5, 1.2, 1.3% (0, 1, 3, 3)

Litter incidences in the follow up study:
We do not have data to calculate litter incidences. However, since the mean number of delivered pups per dam and the rates of liveborn and stillborn were evenly distributed about the dose groups it is not relevant with litter incidences of liveborn and stillborn pups. Moreover, there was no effect on pup viability post-natally: viability index PND 0-4 varied between 100% (control and test groups 2 and 3) and 99.6%.
Regarding the litter incidence of aneurysms, the registrant states that only single pups in each litter were affected, with one exception each in intermediate dose group (10 mg/kg bw/day) and high dose group (50 mg/kg bw/day). In intermediate dose group two female pups of the same litter and in high dose group two males of the same litter were affected. In each of these groups one pup had an aneurysm and the other had a hemorrage.

- In the original screening study, mean body weight on PND 0 was reduced by 7 % in dams of the high dose group as compared to the control dams. Were these treated animals also affected by absolute body weight loss?

Unfortunately, we do not have this information.

- In the original screening study two dams from the high dose group "died during or shortly after parturition (post-natal day (PND) 2 and 3)". In the same study and dose group several pups died (28 animals) or were cannibalized (3 animals) during lactation. The viability index PND 0-4 was therefore strongly reduced. Is there information available specifying how many litters were affected by pups who did not survive until study termination? And how many pups are to be attributed to the two dams which died before study end?

Since we did not have access to the full study report, no information specifying how many litters were affected by pups who did not survive until study termination was available to the DS. Female #140 of the high dose group had 7 stillborn pups (out of 12 pups). All pups were dead at PND 1. At least 12 of the 42 dead pups PND 0-4 (stillborn + dead + cannibalized) is therefore attributable to dam # 140.

The number of dead pups from female #136 of the high dose group is unknown to the DS. Assuming that the entire litter died (approx. 11 pups) an additional 19 pups from the 8 other litters in the high dose group were dead (stillborn, dead or cannibalized) PND 0-4. - On page 22 of the CLH report a viability index PND 0-4 of 59 % is stated for the high dose group as compared to the control group for the original screening study (BASF 2013 a). On pages 24 and 25, however, a percentage of 53 % is mentioned for the same index. Which rate is correct? The viability index PND 0-4 was 59 % according to the Registrant.
RAC's response
These clarifications are helpful. RAC agrees with the assessment of the Dossier Submitter.