Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

4-methylimidazole

EC Number: 212-497-3
CAS Number: 822-36-6

CLH-O-0000007050-88-01/F

Adopted

26 November 2021

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during 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: 4-methylimidazole
EC number: 212-497-3
CAS number: 822-36-6
Dossier submitter: Norway

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
09.04.2021	France		MemberState	1
Comment received				
We agree with the conclusion that 4-methyl-1H-imidazole is presumed to have carcinogenic potential for humans, justifying a classification Cat 1B (H350). There are two studies available performed on two different species (rats and mice) with strong reliabilities; both are similar to OECD TG 451 guidelines. The first one is a NTP, 2-year cancer bioassay, on rats. The incidence of mononuclear cell leukemia in high dose females was significantly greater than that in the controls, and slightly exceeded the historical control range. There was also a slight, non-significant, increase in incidence of mononuclear cell leukemia in males. The second study is a NTP, 2-year cancer bioassay, on mice. The incidences of alveolar/bronchiolar adenoma in all exposed groups of females, alveolar/bronchiolar carcinoma in the high dose males group, and alveolar/bronchiolar adenoma or carcinoma (combined) in the high dose males group and in the medium and high dose females groups were significantly greater than in the control group. They clearly exceeded the historical control range. Furthermore, the incidence of alveolar epithelium hyperplasia in the medium dose females group was significantly greater than that in the controls. Histologically, this lesion was considered a morphologic continuum to adenoma. They are sufficient evidence of carcinogenic activity of the 4-methyl-1H-imidazole with the induction of alveolar/bronchiolar tumours in mice (increased incidence of benign and malignant neoplasms (even combined) in both sexes of a single species in a well-conducted study). In addition, the slight induction of mononuclear cell leukemia observed			

in rats in single sex (females) at the highest dose provides supportive evidence. Finally, the modes of action leading to increased tumour incidences in rats and mice are not identified and their relevance for humans is assumed.
Dossier Submitter's Response
Thank you for your support.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
29.03.2021	Germany		MemberState	2
Comment received				
The DE CA generally supports the conclusions to classify 4-methylimidazole for carcinogenicity. In the absence of mechanistic data convincingly demonstrating a speciesspecific mode of action, human relevance has to be assumed. Mononuclear cell leukemia (MNCL) in the female rat and lung tumours in mice in both sexes justify classification as Carc. 1B, H350. • In mice, the number of benign and malignant lung tumours in both sexes increases with higher doses of 4-methylimidazole. The incidences are well above the range of the historical control data in females and only slightly above the historical control data in males in the highest dose group. • A dose-dependent increase in mononuclear cell leukemia was observed in rats. In the highest dose group, mononuclear cell leukemia was observed in 40% of the female rats. This incidence is just above the range of the historical control data of 38%. With the lung tumours in the mouse (both sexes) and the mononuclear cell leukemia in the female rat the criteria for classification as Carc. 1B, H350, are formally fulfilled. While classification into Category 1B has been proposed by the DS, the data may also justify a Category 2 classification, e.g. for the following reasons: • Tumour type and background incidence: Both tumour types, mononuclear cell leukemia in the ageing rat and lung tumours in B6C3F1 mice, commonly occur spontaneously and not treatment-related. The incidence of mononuclear cell leukemia in the female rat only very slightly exceeds the range of historical controls. In addition, clinical findings such as neuronal effects were observed in the maximum dose group of female rats, indicating that the animals' maximum tolerable dose was exceeded. Then, the increased number of animals with mononuclear cell leukemia could be a secondary effect. The authors should further discuss this possibility. Furthermore, the relevance of mononuclear cell leukemia in rats for human carcinogenicity has been discussed with the overall conclusion that the tumour type is not relevant (Anonymous et al., 2005). According to ECHA´s guidance on the application of the CLP criteria, the "appearance of only spontaneous tumours, especially if they appear only at high dose levels, may be sufficient to downgrade a classification from Category 1B to Category 2, or even no classification". The tumour type may, hence, be negligible for the purpose of classification. • Progression of lesions to malignancy: Statistical significant increases of tumour incidences in mice were mostly noted for benign neoplastic lesions (adenomas) with the exception of the high dose male group. • Mode of action and its relevance for humans: As stated by the DS, the human relevance of alveolar/bronchiolar adenoma and carcinoma in mouse models has been questioned. The MoA leading to lung tumour formation following 4-methylimidazole exposure, however, is unclear. 4-methylimidazole is considered non-mutagenic by the DS. The				

existence of a threshold for carcinogenic effects is likely.
Considering the abovementioned factors, classification in the suspected Category Carc. 2, H 351, may also be a possibility. Therefore, it would be helpful to have a slightly broader discussion as to why the DS prefers Category 1B over Category 2. A comparative analysis of factors justifying either of the two categories may be supportive.

Dossier Submitter's Response

We agree that based on the available data, classification of 4-methylamidazole may be considered a borderline case between Cat.1B and Cat.2.

However, the DS proposal is primarily based on the significant increases of benign and/or malignant lung tumours observed in both males and females in the mouse study. According to the CLP Annex I, 3.6.2.2.3 an increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can provide sufficient evidence of carcinogenicity in experimental animals. The DS considers the findings of statistically significantly increased incidences of alveolar/bronchiolar adenomas in female mice, alveolar/bronchiolar carcinomas in male mice and benign and malignant neoplasms combined in both sexes to constitute sufficient strength of evidence for classification in Carc. Cat. 1B. The carcinogenic activity clearly exceeded the historical control data from NTP, progression to malignancy was observed, and both sexes were affected. Although, statistical significance was shown only for high dose males, a dose-related increase in carcinomas was suggested for both sexes (4%, 8%, 8%, 16% in males and 6%, 0%, 4% and 14% in females in the ctrl, low, mid and high doses, respectively). According to the CLP Annex I, 3.6.2.2.3, if the substance has been shown to cause malignant tumours this will usually constitute sufficient evidence of carcinogenicity supporting Cat.1B. Furthermore, DS considered available data related to the hypothesis that 4-methylimidazole induces lung tumours by mouse specific CYP2F2 activation, but found this hypothesis not to be supported. Thus, for 4-methylimidazole the MoA for lung tumourigenesis was considered unknown and human relevance of the tumour increase was assumed.

The rat data was regarded as weakly supporting evidence in the overall assessment of the strength and weight of evidence due to the high spontaneous incidence of mononuclear cell leukemia in F344 rats. An increased incidence above historical control levels was only observed in female rats and at a dose in which body weigh gain was also markedly reduced compared to controls. The high spontaneous incidence of the tumour type and the toxicity observed in the form of reduced body weight gain and neurotoxicity makes the data challenging to evaluate. Still, a non-significant, increase in tumour incidence compared to study controls (32% vs 18%) was also seen in the mid dose females, an earlier onset of mononuclear cell leukemia was observed in the high dose group and mortality was not significantly decreased in exposed females all in all creating some concern for carcinogenicity of 4-methylimidazole also in rats.

It is the opinion of the DS that the findings in the mouse NTP study warrants a classification in Cat.1B although only weak support for carcinogenicity was observed in the rat NTP study.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
17.03.2021	Netherlands		MemberState	3

Comment received

NL-CA agrees classification for Carc. 1B is warranted. 1B. There is sufficient evidence from the well-conducted NTP study with mice to warrant classification.

We would like point out that in female rats a statistically significantly increased incidence of mononuclear cell leukemia was observed only at the highest dose (5000 ppm). At this dose level, a lower body weight of -35% was also observed. This could be a result of the carcinogenicity or of general toxicity (MTD reached). Other effects were noted as well but the severity is unclear from the report and therefore it is difficult to assess if the MTD has been reached. The DS may want to reflect on this. Considering this type of carcinogenicity often occurs in this strain, the single sex/species aspect and unclear general toxicity at the highest dose level, there are quite some uncertainties surrounding this effect and therefore it should be considered as barely supportive/limited evidence.

This leaves the alveolar/bronchiolar adenoma/carcinoma in mice. The effect is much clearer and observed in both sexes. Incidence of adenomas are statistically significantly increased in females in all dose groups, carcinomas in males (highest dose group) and the combined incidence is increased in both sexes in a dose dependent manner. It is noted that the carcinomas are also increased in the high-dose females although not to a statistically significant level.

According to the guidance on application of the CLP criteria, sufficient evidence of carcinogenicity would have to be evidence of a causal relationship in (a) two or more species, (b) two or more studies in a single species or a single well conducted GLP study. The NTP study can be considered a well conducted GLP study and therefore we agree this is sufficient evidence for carcinogenicity in line with the CLP criteria warranting classification as Carc. 1B.

Dossier Submitter's Response

Thank you for your support.

We agree that the general toxicity in the female rats of the high dose group was marked, as revealed by the decrease in body weight gain and neurotoxicity. Neurotoxicic effects of 4-methylimidazole is a recognized high dose phenomenon that has been described in various animal species. The marked general toxicity observed in the high dose group females in combination with the high spontaneous incidence of the mononuclear cell leukemia in F344 rats makes the rat carcinogenicity data uncertain. DS has accordingly considered the rat carcinogenicity data as only weakly supporting evidence and based the classification proposal on the NTP mouse study as further commented in our response to DE above.

Table 5 from the NTP 2-years study included for transparency:

TABLE 5
Neurological Clinical Findings in Female Rats in the 2-Year Feed Study of 4-Methylimidazole[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm
Clonic Seizures	0/50	0/50	21/50	36/50
Excitability	0/50	2/50	9/50	50/50
Hyperactive	0/50	0/50	0/50	5/50
Impaired Gait	1/50	0/50	4/50	49/50

[a] Number of rats with clinical finding per number of rats in the exposure group

RAC's response

Noted.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
09.04.2021	France		MemberState	4

Comment received

We agree with the conclusion that 4-methyl-1H-imidazole does not warrant a mutagenic classification.

Two in vitro studies comparable to OECD guideline 471 concluded on negative results observed in Salmonella typhimurium strains TA98, TA1535 and TA97, TA98, TA100, or TA1535 (up to 5000 and 10000 µg/plate, respectively) both in the absence and in presence of exogenous metabolism.
For the third in vitro study, genotoxic chromosomal effects were observed in human peripheral lymphocytes at all concentrations, concomitantly with cytotoxicity, in chromosome aberration test. The reliability of this study is lower (3) due to various biases reported.

Concerning the in vivo studies, two studies are reliable (1) and show no effect on micronucleus induction in bone marrow of male rats or male mice or in peripheral blood in male and female mice. These studies are comparable to OECD guideline 475 and to FDA GLPR (21 CFR, Part 58), respectively. The third in vivo study concluded that 4-Methylimidazole increased the percentage of chromosomal aberrations at all concentrations after 12 h and at highest concentration after 24 h. Moreover, the mitotic index decreased in comparison with control at highest concentration for 12 h and at all concentrations for 24 h in the bone marrow cells of Swiss Albino mice. It was noticed that this study includes several biases (methodologic deviations vs TG 475 OECD guideline), therefore its reliability is low (3).

The conclusion on the comparison with the CLP criteria is clear, however the indications made on the less reliable in vitro and in vivo studies (reliability 3) could have been underlined although this information do not allow the classification of the substance as mutagenic.

Dossier Submitter's Response
Thank you for your support. We agree that there are some findings of genotoxicity in the academic studies which originates from the same research group. However, these academic studies have major deviations compared to OECD test guidelines (e.g. cytotoxicity at dose levels where genotoxicity were observed), and hence are of low reliability.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
29.03.2021	Germany		MemberState	5
Comment received				
The DE CA supports the conclusion for the endpoint germ cell mutagenicity. The data appear to be insufficient for classification. Yet, the inconsistent data in the NTP mouse bone marrow micronucleus test (1st trial positive, 2nd trial negative) together with the positive in vivo data reported by Norizadeh Tazehkand et al. (2016) give rise to uncertainty and precluding one from drawing a final conclusion regarding the mutagenic potential of 4-methylimidazole.				
Dossier Submitter's Response				
We agree that there are some findings of genotoxicity in Norizadeh Tazehkand et al. (2016), however, this study had major deviations (reliability 3), and the effects occurred together with cytotoxicity, so we did not put a lot of weight on them (potentially the observed increased genotoxicity could be an indirect consequence of high cytotoxicity). Since both the Ames tests and the NTP rat bone marrow micronucleus test were negative, and since NTP concluded that the mouse bone marrow MN assay was negative overall, no classification or labelling according to CLP criteria are justified.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
17.03.2021	Netherlands		MemberState	6
Comment received				
NL-CA agrees with the DS proposal not to classify for (germ cell) mutagenicity. The in vivo micronuclei studies were negative. The chromosomal aberration test was positive but of questionable reliability. However, no proper negative chromosomal aberration test is available. As no reliable negative mutagenicity are available for this endpoint, no classification should be proposed based on inconclusive data or data lacking. However, the DS states that no classification is proposed based on conclusive but insufficient for classification, as noted in Table 6, which is thus not correct.				
Dossier Submitter's Response				
We disagree. Since bone marrow micronucleus tests were conducted both in the rat and the mice, in addition to micronuclei in mouse peripheral blood erythrocytes (which all were negative), classification or labelling according to CLP criteria can be justified. The only weakness is that although 4-methylimidazole is rapidly absorbed and widely distributed in the body, no data for presence in bone marrow is available. This is often the case for many substances.				
RAC's response				
Noted. Please see the RAC Opinion for conclusion on whether the data is conclusive.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
09.04.2021	France		MemberState	7
Comment received				

We agree with the conclusion that 4-methyl-1H-imidazole is presumed human reproductive toxicant, justifying a classification Cat 1B (H360Fd).

In a NTP continuous breeding study, cross-over matings showed a reduction in mated/pair in the high dose exposed male rats mating with naïve females. All female rats that did not deliver were found to be non-pregnant indicating an effect on fertility in males.
Regarding the reproductive organ toxicity on rats, histopathological findings included a dose dependent increase in testicular germ cell degeneration in F0 males and a significant increase in spermatid retention at the high dose. Reduced weights of dorsolateral prostate (relative in F0), ventral prostate (relative) and seminal vesicles (relative in F0) were reported. Concerning the sperm parameters: significantly reduced sperm count in the high dose group (F0) and dose-dependent reductions in % motile sperm were observed in F0 and F1c males. For female rats, ovarian weights (absolute and relative) were significantly reduced in the high dose group and some changes in follicular counts, extended diestrus and oestrus cycle length were reported. 4-methyl-1H-imidazole affected puberty, i.e. adjusted age at vaginal opening (VO) and balanopreputial separation (BPS) were significantly increased in all exposure groups (medium dose, F1c). Markers of disturbance of sexual development were also significantly affected, i.e. number of male pups showing areola/nipples in the medium dose group.

From this study, they are evidence that 4-methylimidazole induces male reproductive toxicity. It is further supported by effects observed in the 14-week studies. In rats, the spermatid heads per testis and mean spermatid count at low dose were significantly higher than in the control group. The left epididymis and cauda epididymis weight were also significantly lower than the controls at the medium dose and the relative weight of the right testis was significantly lower than the controls at the higher dose. They are also increase incidences of animals with testicular degeneration and incidence of animals with prostate gland atrophy in the medium and high dose groups. Concerning the sperm parameters, the epididymal spermatozoal motility was significantly lower than the controls in the low dose group and the epididymal spermatozoal concentrations in the low and medium dose groups were significantly higher than the controls. In the 14-week study in mice, the relative right testis weights of males exposed to medium and high doses of 4-methylimidazole were significantly higher than the control group.

Reproductive toxicity in female rats was also suggested at the high dose groups in the continuous breeding study. These results are in agreement with the effects observed in studies of cancer in rats, where 4-methyl-1H-imidazole affects "hormonal-dependent" organs. Moreover, disturbed parturition and dystocia have been observed in high-dose F0 females. These effects can be a direct reproductive effect, in particular considering possible endocrine disruption and should not be associated with general toxicity. It provides further support that female reproductive function is also affected by 4-methylimidazole.

We agree that the observed effects suggest an anti-androgenic effect that is supported by study with imidazoles on testosterone secretion in male rats (Adams, 1998) ; although it

is regrettable that hormones have not been measured in the other studies. The observation of a reduction of neoplasms in hormone-dependent organs (mammary gland, uterus, pituitary gland…) in the carcinogenic rat study also support endocrine alterations. The classification in 1B for fertility is therefore justified by clear evidence of toxicity on the male reproductive function and some evidence on female reproductive function. The effect on viability after birth and developmental landmarks of sexual function also justify a category 2 for development.
Dossier Submitter's Response
Thank you for your support.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
29.03.2021	Germany		MemberState	8
Comment received				
The DE CA supports the conclusion for the endpoint reproductive toxicity. Based on the available data (NTP reproductive and developmental continuous breeding toxicity study in rats and NTP 90-day repeated dose toxicity study in rats and mice), the proposal to classify 4-methylimidazole into Category 1B appears to be appropriate. For the evaluation of the reproductive toxic potential of 4-methylimidazoles, a two-generation study in rats is available (NTP, 2019; Behl et al., 2020). In this study, male rats showed increasing impairment of fertility with increasing doses of 4 methylimidazole, as indicated by severely reduced litter size with poorly viable offspring. In all generations, sperm motility decreased in a dose-dependent manner, probably due to adverse effects in the epididymis of the animals. In females, dystocia, a disturbed and/or difficult birth process, occurred, resulting in the death of dams in the highest dose group, but was also observed in the next lower dose group. In addition, effects on the sexual development of the offspring such as extra nipples in males and delayed vaginal opening in females were observed. The adverse effects on both fertility and offspring development justify classification as Repr. 1B, H360Fd. A short discussion as to why the DS proposes Category 1B as opposed to Category 2 would strengthen the dossier.				
Dossier Submitter's Response				
Thank you for your support and you comments. The following are some reflections concerning the Repr Cat.1B vs Cat.2 criteria for 4-methylimidazole. For a classification in Cat.1B the data shall show clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, whereas for Cat.2 data must provide some evidence of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Cat.1. The NTP RACB study is an extensive, well documented study and it reveales clear evidence of adverse effects on fertility parameters including at lower doses not associated with parental toxicity; e.g. marked effects on reproductive performance at the high dose and at lower doses clear toxicity to male reproductive primary and secondary organs. A significantly delayed adjusted male and female puberty onset was observed from the lowest dose group. Absolute and relative ventral prostate weights were consistently reduced across generations also at lower doses and there are thus clear indications that 4-methylimidazole has anti-androgenic properties. The effects observed at lower were not				

considered secondary to maternal or other toxic effects as toxicity was low, especially at the low dose. In the DS opinion, these data clearly warrant a classification in Cat.1B for fertility.

There is no available pre-natal developmental study for 4-methylimidazole, but the RACB study has several relevant endpoints included. However, the case for adverse effects on development is considered by DS as a borderline case because the distinction between fertility effects and developmental effects is not clear-cut. In a wide sense, it is clear that 4-methylimidazole disturbs foetal and postnatal development. However, for the purpose of classification developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. Consequently, onset of puberty is for pragmatic reasons considered to be effects on fertility and not on development according to the CLP guidance. Additional signs of developmental toxicity of 4-methylimidazole are decreases in live litter size, reduced pup survival from PND1 to PND4 as well as increase in nipple retention and a significant trend toward delayed day of testicular descent. These signs all gives concern for developmental disruption. Of note, it is not known whether the reduction in litter size is caused by impaired parental fertility or implantation loss, as implantation loss was not investigated in the RACB study. The DS has proposed Repr. Cat.1B, H360Fd and considers the classification for development as borderline between Cat.1B and Cat.2. Some additional comments concerning possible read-across to support the classification for development is given in the responst to SE below.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
17.03.2021	Netherlands		MemberState	9

Comment received

NL-CA agrees with classification of Repr. 1B (H360Fd) based on a reduction of fertility, supporting evidence of effects in reproductive organs and mode of action.
The DS describes some effects that suggest possible developmental toxicity but it is not clear to what extend these effects warrant classification. Please reflect on the criteria and whether the findings are sufficient for classification. In addition, the delayed onset of puberty discussed with fertility, may also be regarded as a form of developmental toxicity.

Dossier Submitter's Response

Thank you for your support and your comments. Please see also the response to DE (above) and to SE (below) for further argumentation for classification for development. DS consideres the classification for development as borderline between Cat.1B and Cat.2. Because the CLP guidance (Annex I: 3.7.1.3) clearly states that some developmental effects, such as adverse effects on onset of puberty, is to be regarded as effects on future fertility for classification purposes the DS has proposed H360Fd. The same argumentation may be used for other ED related effects (increased nipple retention and delayed testic descent).

RAC's response

Noted. The reference for adverse effects on onset of puberty to be regarded as effects on sexual function and fertility would ideally be the CLP criteria (Annex I: 3.7.1.3) and not the CLP guidance.

Date	Country	Organisation	Type of Organisation	Comment number
09.04.2021	Sweden		MemberState	10

Comment received

The Swedish CA supports the classification of 4-Methylimidazole as Repro. 1B, H360Fd based on the results from a 2-generation NTP reproductive and developmental continuous breeding (RACB) toxicity study on Sprague Dawley SD rats fed with 4-Methylimidazole in the diet as well as the supportive findings from a NTP 14-week repeated dose toxicity (RDT) study in Fischer 344 rats and B6C3F1 mice fed with 4-Methylimidazole. SE agrees with the rationale for classification into the proposed hazard classes and differentiations for the following reasons;

Fertility
☐ Significantly reduced mating performance at 5000 ppm in the RACB study (including the cross-over study).
☐ Significant/marked toxicity on F0 male reproductive organs was observed in the RACB study from histopathological examinations in absence of marked general toxicity.
☐ Adverse effects on male reproductive organs were also observed in the F1 generation in the RACB study.
☐ Effects on male reproductive organs were further supported by findings in another strain of rats in the NTP 14-week RDT study. The study also found effects on male in mice but at higher doses.

Developmental
☐ Significant reductions in total litter sizes and live litter size in the F1 generation in the RACB, despite no reduction in reproductive performance observed at doses below the highest dose administered (5000 ppm).
☐ Marked reduction in pup survival at PND1-4 in the RACB study, that is not considered as being secondary to the observed maternal toxicity (decrease in maternal body weight).
☐ Disturbed sexual development in F1 and F2 generations in the RACB study (including modest increase in male pups with retained nipples, dose-dependent trend in delayed testis descent in F1 and F2 generations, dose-dependent delay in onset of puberty in males (balanopreputial separation) and females (vaginal opening) in the F1 generation).

According to the dossier submitter, no pre-natal developmental toxicity study is available for 4-Methylimidazole. Hence, findings from the RACB study are used for the classification for developmental toxicity in category 2. Could read-across from structurally similar substances, including imidazole (CLH: Repr. 1B H360D based on pup mortality and external/skeletal malformations observed in an OECD TG 414 prenatal developmental toxicity study), 2-methylimidazole (CLH: Repr. 1B H360 Df based on dissecting aneurysm of the great vessels of the heart and pup mortality) and 1-vinylimidazole (CLH: Repr. 1B H360D based on pup mortality and vascular effects) be used in the weight of evidence determination to discuss classification in category 1B for developmental toxicity?

Dossier Submitter's Response

No test is available for 4-methylimidazole designed as a prenatal developmental toxicity study (OECD TG 414), with administration of the substance only during a certain period of the gestation. However, information about developmental toxicity can also be found in studies with continous exposure over one ore more generations.

As stated in the CLP Annex I: 3.7.1.4, "Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation...
...Developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects
can be manifested at any point in the life span of the organism. The major manifestations of
developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency"

For 4-methylimidazole, a US NTP Reproductive assessment by continuous breeding (RACB) study is available. The protocol was illustrated in the CLH report in figure 1, and also shown here: Schematic of the Reproductive Assessment by Continuous Breeding (RACB) Design. From Behl et al., 2020.

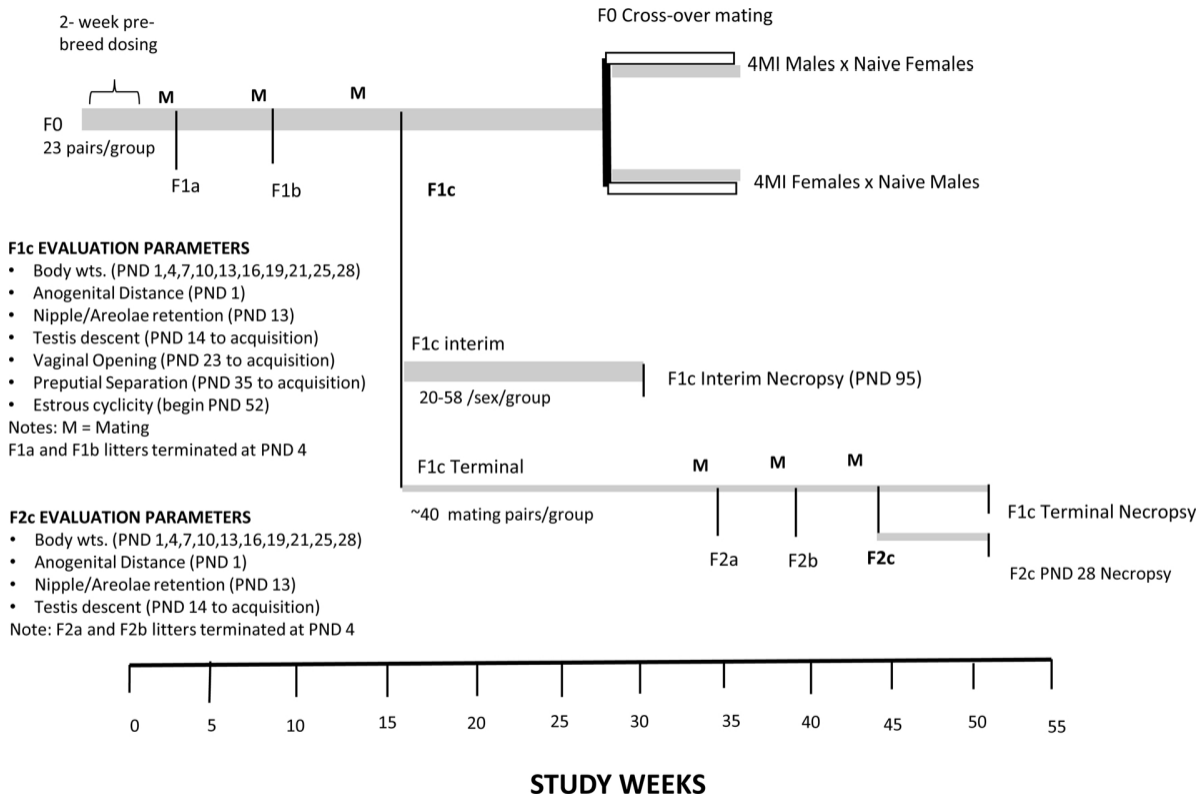

Fig. 1. Schematic of the Reproductive Assessment by Continuous Breeding (RACB) Design.

The RACB study gives information relevant to assess developmental toxicity (F2a and F2b litters were euthanized on PND 4. The F2c litter and corresponding dams were evaluated through PND 28. T). The RACB study does not include investigations of e.g. vascular effects/aneurysm in the offspring or developing fetus, but gives information about lethality and other developmental effects as shown in the figure above. Given that we have information on developmental toxicity from this animal study, read-across from other imidazoles seems unjustified. In an early draft of the CLH proposal we actually proposed Repr. 1B - H360 for 4-methylimidazole as a read-across to 2-Methylimidazole for reproductive toxicity, but when we became aware that the RACB study from NTP was to be published, we decided to delete the read-across and only use the available studies. Even if we do a read-across now, we think the information from the *in vivo* studies would

outweigh the read-across to a large extent. Still, we assume that the correct CLH for 4-methylimidazole could be Repr. 1B H360FD, but we think we do not have enough information yet to go for more than Repr. 1B H360 Fd. In practice this means that all necessary risk reduction management measures necessary for substances in cat Repr. 1B needs to be implemented, so we think there is no risk for "undermanaging" this substance even if the D is not in place.
RAC's response
The classification of 2-methylimidazole and 1-vinylimidazole as Cat. 1B for developmental toxicity was based on screening studies (OECD TG 421/422) and no prenatal developmental toxicity studies are available for these substances either. For both these substances dilated pericardial vessels were observed during gross pathological examination of pups. These gross pathological changes were then microscopically confirmed as aneurysms of the great vessels of the heart. RAC notes that in the RACB study with 4-methylimidazole the gross pathology of pups revealed no cardiovascular effects (i.e., dilated pericardial vessels were not observed). There are also differences in adverse effects on fertility between these substances. For e.g., there were no effects on the mating performance for 2-methylimidazole and 1-vinylimidazole, while that for 4-methylimidzole it was markedly reduced. Therefore, RAC considers that the read-across is not justified.