Assessment of developmental toxicity effects in studies with 3-Methylpyrazole (CAS# 1453-58-3; EC# 215-925-7) for C&L purpose

Reference: CLH report, version number, dated 12/02/2019

Proposal for Harmonised Classification and Labelling Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

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Scope of assessment:

The substance 3-Methylpyrazole has been self-classified by the registrant (see for comparison table 2 of CLH report) into:

Acute Tox. 4, H302
Skin Corr. 1B, H314
Eye Dam. 1, H318
Repr. 2, H361

The Belgian Competent Authority (BCA) made a proposal for classification of 3-Methylpyrazole (see for comparison table 5 of its CLH report) into:

Acute Tox. 4, H302
Skin Corr. 1B, H314
Eye Dam. 1, H318
STOT RE 1, H372 (lung)
Repr.1B, H360D

Comparison of the proposed versus the self-classification reveals first, that 3-Methylpyrazole is classified additionally into STOT RE 1, H372 (lung) and divergent from self-classification into Repr.1B, H360D instead of Repr. 2, H361.

Whereas classification into STOT RE 1, H372 (lung) is of little practical relevance for the registrant and will therefore not further been discussed and commented, classification into Repr.1B, H360D is of utmost significance for the registrant, i.e. this document will therefore cover the assessment of developmental toxicity effects in studies with 3-Methylpyrazole.

Actually has BCA based its proposal for classification into Repr.1B, H360D on the results of 4 developmental studies (see for comparison table 13 of its CLH report), even though 5 developmental toxicity studies and one three-generation toxicity study are available with 3-Methylpyrazole (see for comparison below Nos (1) through (6) of chapter "*Description of developmental toxicity studies*" overview table).

It is noted, that a study named "*Anonymous, 1984*" and discussed in the CLH report is not available to the registrant (see below No (3)); further it is noted, that an additional drinking water teratology study is available to the registrant (see below No (5)), but it is not mentioned in the CLH report.
It needs also to be emphasized, that an additional three-generation study is available which has also not been mentioned in the CLH report but will be described for completeness of documentation.

This assessment aims for a thorough evaluation of all observed adverse developmental effects, whether or not classification into category 1B (instead of self-classification into category 2) for developmental toxicity could be justified, considering the legal requirements of the CLP Regulation (EC) No 1272/2008; available at:
https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008R1272&from=en and taking into account ECHA`s "*Guidance on the Application of the CLP Criteria*", Version 5.0, July 2017; available at: https://echa.europa.eu/documents/10162/23036412/clp_en.pdf.

Therefore this document describes first ALL available developmental toxicity studies and provides for each a short conclusion on basic items like study design, purity of test substance, conformity to respective guidelines and GLP prerequisites.
Thereafter a summarizing discussion of these observed results from all available studies is provided.

Summarized conclusions on the study results are then aligned with the legal provisions for classification criteria according to the CLP regulation for appropriateness of either classification into proposed category 1B or self-classification into category 2.

For the latter process also information on structurally related substances will shortly be described, upon which final conclusions will be drawn.

Description of developmental toxicity studies:

In the following, a short description of all developmental toxicity studies is presented.

Parameters not affected according to the study results of below described studies, like for reproductive data (such as conception rate, mean number of corpora lutea, implantation sites, pre- and post-implantation loss, number of resorption and viable foetuses) are not explicitly mentioned to keep this document at a reasonable size.

It is emphasized that below table provides for overview purposes a compilation with the basic findings of all studies described and discussed below.

(1) In a developmental toxicity study in rats (BASF, 1992; Study report: 30R0464/91071 (01/10/1992); "*Anonymous 1992*" as cited in CLH report) female rats were treated by gavage at dose levels of 0, 15, 45 and 90 mg/kg body weight (bw) per day [in the following mg/kg bw/d] through gestational days 6 to 15.

In the highest dose group, a distinctly reduced food consumption and statistically significantly lower body weights, impaired body weight gains, and statistically significant reduced uterus weights were observed in dams.
At this dose level, fetal malformations in soft tissues (in the urogenital tract and/or in the cardiovascular system) and skeletal malformations were observed at embryotoxic effects with regard to reduced body weights.

In the mid dose group, markedly reduced food consumption (days 6-13) and statistically significantly lower body weights and impaired body weight gains (days 6-8) occurred.
At this dose level, no fetal malformations were observed, but only significantly lower fetal body weights.

No effects were observed at the lowest dose level of 15 mg/kg bw/d neither in dams nor in pups, i.e. this dose is defined the NOAEL in this study.

Conclusions:
BCA concluded (*citation*) *"a significant decrease of the fetal body weight…observed at the mid dose level (45 mg/kg bw/d)…cannot be explained by any parental toxicity"*, while (contrary to BCA`s evaluation!) "*marked*" maternal toxicity, with reduction of food consumption and

statistically significant reduction of body weights, were present in dams according to the original study report.

This study can be defined the key study (also GLP prerequisites were followed) and a score of 1 according to Klimisch *et al.* can be assigned according to its guideline design and documentation.

It is noted, that very high purity 3-Methylpyrazole was used for the test substance (i.e. 99.9 %).

It is further noted, that by gavage application, indirect effects (i.e. reduced uptake of drinking water when respectively applied *ad libitum*) due to the very unpleasant palatability of 3-Methylpyrazole were avoided.

(2) In a study of Bleyl D.W.R. (1990) (named "*Bleyl D.W.R (1990)*" as cited in CLH report), when administering 3-Methylpyrazole at dose levels of 0, 20, 40, 80 and 160 mg/kg bw/d to female rats on (only) the 10th and 11th day post coitum, no maternal toxic effects were found.

Likewise, no embryotoxicity was detectable; the urogenital syndrome (uni- or bilateral renal agenesis, in part hydronephrosis, incomplete differentiation of the uterus) were observed at 80 mg/kg bw/d in the living fetuses.

Postnatally, the live birth and viability index were significantly reduced at 160 mg/kg bw/d.

According to the publication, the substance was administered in aqueous solution as 2.5 mL/250 g body weight, i.e. administration by gavage is the most explainable way of substance administration, even though it is not specified.

Conclusions:
The substance was administered only on two days, such, that any reduction in food consumption or body weights of dams could not be adequately determined.
No embryotoxicity was observed as well.

Teratogenic effects with regard to urogenital malformations were however clearly identified at both the highest dose levels (i.e. 80 and 160 mg/kg bw/d), together with (partly) absence of kidneys, the latter adverse effect of which has not been reported in any of the other developmental toxicity studies.

The quality of this study may be assigned score 2 to 3 "reliable with restrictions" to "not reliable" respectively, because study design is quite "unusual" and no GLP prerequisites were obviously followed; further it is clearly emphasized, that the purity of 3-Methylpyrazole has not been reported, i.e. it is unknown, the fact of which is also noted by BCA, even though not commented.

(3) A study named as "*Anonymous 17 (1984)*" in CLH report is not available to the registrant and therefore the study results and any interpretation as summarised in the CLH report (see following *citation*) could not fully be evaluated: *"In another developmental toxicity study (anonymous 17 (1984)), maternal toxicity was observed at the highest dose level (400 mg/kg bw/d). However, a significant increase incidence of fetal malformation (urogenital syndrome) was already observed at 200 mg/kg bw/d. Moreover, an increase incidence of cleft palate and a significant increase incidence of post-implantation loss were observed at 400 mg/kg bw/d."*

From the data compilation as of table 13: "*Summary table of animal studies on adverse effects on development*" of the CLH report, maternal toxicity (significant body weight change) was observed at the 200 mg/kg bw/d dose level; at 400 mg/kg bw/d four rats died already prematurely.

Conclusions:

Clear maternal toxicity was observed at both dose levels of 200 and 400 mg/kg bw/d (for the upper level of 400 mg/kg bw/d premature deaths were observed!) which "paralleled" adverse effects in the offspring already observed in the (BASF, 1992) study according to No (1) (i.e. significantly lower fetal body weights and severe alteration of the urogenital tract), but doses more than twice the height of those in the BASF (1992) developmental toxicity study in rats were used.

The quality of this study is difficult to assess because of unavailability of the original study report and may be assigned as "not reliable", a score 3 according to Klimisch *et al*; also because of the facts, that obviously no GLP prerequisites were followed, dosing regime was not continuous (only for days 4, 10, 13 and 18) and further, the purity of 3-Methylpyrazole is not known, the fact of which is also noted by BCA, even though not commented.

(4) In a developmental toxicity study (NTIS (National Technical Information Service): OTS05191233, 06/04/1990; cited as "*Anonymous 18, 1989*" in CLH report), female rats were given 3-Methylpyrazole (in corn oil) by gavage at doses of 0, 25, 100, 175 and 225 mg/kg bw/d during pregnancy from day 6 to day 18.

100 % mortality (including moribund state) was observed in the highest dose group. 6 out of 8 animals died in the 175 mg/kg bw/d dose group; in the remaining 2 rats complete resorptions were observed.

Body weight reduction of the dams was observed in the 100 mg/kg bw/d dose group; the average weight of the fetuses was greatly reduced and one fetus showed malformations (micrognathia and cleft palate).
No effects were observed in the dams and fetuses at the lowest dose level of 25 mg/kg bw/d.

Even though this study is well documented and the level of information seems to be sufficient for evaluation, it is noted, that no GLP prerequisites were obviously followed and the purity of the test substance is not known, therefore a score of 2 to 3 by Klimisch *et al.* may be assigned.

Conclusions:
General toxicity effects seen in this study are similar to those seen in the BASF study according to No (1). The dose levels at which significant effects were observed are also similar. Except for the highest dose levels of 225 and 175 mg/kg bw/d, which were not tolerated by the treated animals in this study.

The effects observed at the dose level of 100 mg/kg bw/d from this study versus 90 mg/kg bw/d in the BASF study are comparable; body weight changes and higher resorptions in dams were "paralleled" with a severe decrease of the fetal body weight, but only one cleft palate was observed but no other malformations like urogenital.
One might speculate on any influence of the rat strain, but no information is provided for this item.

No effects were observed at dose level of 25 mg/kg bw/d in this study versus 15 mg/kg bw/d in the BASF study according to No (1), respectively, elucidating a dose range from no-effects to subtoxic effects for maternal and fetal toxicity.

(5) A drinking water teratology study in Fischer 344 Rats (Dow Chemical Company, NTIS: TS0537366, 23/10/1990) is not mentioned in the CLH report. This study is a guideline GLP study (even though from 1990), is well documented and the purity of the test substance (i.e. 99.5 %) has been reported; therefore it is sufficiently documented to be used for this assessment.
The study can be assigned score 1 according to Klimisch *et al.* and comprises therefore also a key study.

In this study, pregnant rats were administered 0, 10, 50 and 100 mg of 3-Methylpyrazole/kg bw/d in drinking water *ad libitum* from day 6 to 15 of pregnancy.

Maternal toxic effects (substantially decreased body weight or body weight gain and food consumption) were observed obviously because of unpalatability of the test substance in drinking water at doses of 50 and 100 mg/kg bw/d.
Significantly reduced fetal body weights with regard to embryotoxicity were observed.

With regard to developmental effects, delays for ossification were observed at 50 and 100 mg/kg bw/d. These effects can be considered as secondary effects because of the inedibility of drinking water for the dams (see also statement of the authors of the study) (i.e. 21 % decrease in consumption at 50 mg/kg bw/d, 33 % at 100 mg/kg bw/d; no reduction at 10 mg/kg bw/d); teratogenic effects were not observed.

Dose-dependent fetal weight reduction was observed at all dose levels (4.3 % at 10 mg/kg bw/d, 11.7 % at 100 mg/kg bw/d), but since the litter size at 10 mg/kg bw/d was increased, the fetus weight decrease was considered a secondary effect.

Conclusions:
The findings in this study with regard to "parallelism" of maternal with embryotoxicity are also similar to the findings observed in the BASF study according to No (1) and the NTIS study according to No (4): reduced fetal body weight at doses producing maternal toxicity, no teratogenic effects (except for one pup in NTIS study) were observed besides delays at ossification of the cervical and thoracic vertebral centra.

(6) There is also a three-generation study in Wistar rats available, which is also not mentioned in the CLH report. Although this study is considered as "not reliable" (score 3 according to Klimisch *et al.* because of missing information on design of the study, no GLP status, unknown purity of test substance and its poor documentation), the study can be used as a piece of evidence in the overall evaluation of the significance of the developmental effects for C&L purposes.

3-Methylpyrazole was continuously administered via feed (5, 25 and 125 mg/kg diet, corresponding approximately to 0.3, 2 and 10 mg/kg bw/d) over 3 generations to Wistar rats.
The mating of the P generation took place for the first time after 12 weeks of substance application (F1a generation) and then again after another 8 weeks (F1b generation). The mating of the F1b generation was again for the first time after 12 weeks of the substance application (F2a generation) and then again after a further 8 weeks (F2b generation). This regime was repeated using the F2b generation to generate the F3a or F3b generation.

The following findings were observed:
No effect of application of 3-Methylpyrazole on mortality, health status, and behavior was observed in parental animals (P, F1 and F2 generation). The body weight development was temporarily retarded in P and F1 generation; in the F2b generation it improved.

At 125 mg/kg diet, the fertility index (number pregnant dams with the number of mated females) at the first mating of the P and F2b generation was significantly reduced. This dose administration to lactating mothers led to impairment of postnatal viability of pups.
There were no external malformations in pups observed.

A NOAEL of 25 mg/kg diet, corresponding to 1.9 mg/kg bw/d was established.

Conclusions:
The effects observed in this study, even though spacing between dose levels is thought to be too high and the dose levels were one order of magnitude lower than in the developmental toxicity studies, are roughly comparable to the BASF study according to No (1), NTIS study according to No (4) and drinking water study according to No (5), because the highest dose of 125 mg/kg diet produced reduced body weights in parental generations which were displayed by reduced fetal body weights, i.e. the same finding as in the developmental studies.

Discussion of results of developmental toxicity studies:

It is noted, that for clarity reasons, reference is made particularly to below table "*Overview of the results of developmental toxicity studies with 3-Methylpyrazole*".

The studies, which may be predominantly used for the assessment of adverse developmental effects are the BASF study according to No (1) and the drinking water study according to No (5), because of their high reliability score.
Thereafter the Bleyl D.W.R. study according to No (2) and the NTIS study according to No (4), need to be considered because of their less high and "unclear" reliability score.
The multi-generation study (predominantly no developmental toxicity study) according to No (6) may be used for supportive purposes, whereas study "*Anonymous 17 (1984)*" according to No (3) is more difficult to interpret because of its unavailability to the registrant and the extremely high dose levels used.

In the studies Nos (1), (4) and (5) reduced fetal body weight was associated with marked maternal toxic effects (like reduced body weight and body weight gain, reduced food or water intake and (for BASF (1992) study) decrease of uterus weight) at tolerated dose levels at which no mortality or bad health conditions were observed in dams, like particularly in study No (3).
This was observed also in the three generations at the highest dose level in the study No (6), therewith supporting the findings of the developmental toxicity studies.

Thus fetal toxicity (embryotoxicity) is clearly associated at the very same dose levels with maternal toxicity and is therefore a secondary consequence thereof.

In the key BASF study according to No (1) fetal malformations together with embryotoxicity by reduced body weights were observed only at the highest dose level associated with maternal toxicity (besides decrease of general toxicity parameters like body weight also reduction in uterus weight).
At the next lower dose level no malformations were observed even though embryo and "marked" maternal toxicity occurred, such, that at least to some extent malformations should have been occurred when directly caused by the test substance.

From this it may be concluded, that a high enough maternal toxicity and hence embryo-/fetotoxicity can cause specific developmental effects, in particular urogenital lesions which have actually only be observed in the "unusually" designed (see below) and less reliable Bleyel D.W.H study according to No (2) and the unreliable study according to No (3).

In the drinking water study according to No (5), no fetal malformations (like urogenital lesions) were observed, but delay in ossification which is commonly attributed to (severe) maternal toxicity resulting from unpalatability of the drinking water with the test substance, leading to similar (embryotoxic) effects in foetuses; these adverse effects seem therefore to comprise clear secondary effects thereof (as has also been concurrently interpreted by the study authors).

The study of Bleyl D.W.R. according to No (2) significantly differs from the typical study design for developmental toxicity studies, since test substance administration was only on 2 days of pregnancy, but may confirm the main finding of the adverse action of 3-Methylpyrazole and/or of any of its impurities because no specification on purity is reported.

It should be noted, that at dose levels comparable to those of the BASF (1992) study according to No (1) effects on kidneys were observed which did neither occur in the BASF (1992) study nor in any other developmental toxicity study.

Absence of observed maternal and embryotoxicity in this study may be due to the very "unusual" regime for test substance administration.

In the NTIS study according to No (4), no fetal malformations were observed (except for one fetus with micrognathia and cleft palate) despite clear embryo and maternal toxicity (the latter for dose levels with no mortality).

The study "*Anonymous 17, 1984*" according to No (3) does not seem to be reliable and its results are doubtful because of the very high doses used which were, based on the doses in the other developmental studies, probably not tolerable.

Moreover, test substance purity is not known and therefore it is not clear, why the mid dose of 200 mg/kg bw/d did not produce maternal toxicity when doses of about 100 mg/kg bw/ were already associated with marked maternal toxic effects in other studies (see for comparison study Nos (1), (4) and (5)). This obvious discrepancy may have been possibly caused by the "unusual" dose regime because test substance administration was not continuous (see above).

Therefore the results of this study cannot reliably be fully evaluated.
Nevertheless should the urogenital syndrome of foetuses be taken into account.

Uncertainties for adequate interpretation with regard to the study results may come from "unusual" test designs (see study Nos (2) and (3)), absence of guideline procedures followed (see study Nos (2), (3), (4) and (6)) and lack of GLP prerequisites (see study Nos (2), (3), (4) and (6)) and in particular the unknown purity of the test substance (see study Nos (2), (3), (4) and (6).
It may also be additionally noted that rat strains used in the studies were different or not reported.

Taken together and summarizing are the BASF study according to No (1) and the drinking water study according to No (5) the foremost suitable ones for interpretation of potential developmental toxicity.

It seems to be a most consistent interpretation that at practically intolerable doses (i.e. 90 to 100 mg/kg bw/d) with marked maternal toxicity (like decrease of body weight and body weight gain, food (and water) consumption, decrease of uterus and increase of liver weight) and hence embryo-/fetotoxicity (like decrease of body weight) malformation can occur, even though not of the same types!

At a lower, more tolerable dose level (i.e. 45 and 50 mg/kg bw/d) where maternal toxicity still causes embryo-/fetotoxicity no or no severe malformations occur; actually malformations were of different types in both studies.
The derived NOAELs for maternal and developmental toxicity are identical and very close together for both study Nos (1) and (5).

Overall it is noted, that no consistent interpretation and evaluation of the observed adverse effects can be derived from above described developmental toxicity studies including the three-generation study.

Overview of the results of developmental toxicity studies with 3-Methylpyrazole

Parameter/Study	No (1) BASF, 1992; Study report: 30R0464/91071 (01/10/1992); "Anonymous 1992" in CLH report	No (2) "Bleyl D.W.R (1990)" in CLH report	No (3) "Anonymous 17 (1984)" in CLH report (conducted by Bleyl *et al.* in 1989 according to Bleyl *et al.*, 1990) (not known to registrant)	No (4) NTIS: OTS05191233, 06/04/1990; "Anonymous 18, 1989" in CLH report	No (5) Drinking water teratology study in Fischer 344 Rats (Dow Chemical Company, NTIS: TS0537366, 23/10/1990) (not mentioned in CLH report)	No (6) Three-generation study in Wistar rats (not mentioned in the CLH report)
Availability of the original study report to registrant	Yes	Yes	No	No	Yes	Yes
Reliability according to Klimisch *et al.*	1	2 or 3 (?)	3	2 or 3 (?)	1	3
Guideline study	Yes (OECD 414)	No	No	No	Yes (OECD 414)	No
GLP status	Yes	No	No	No	Yes	No
Study duration (administration of test substance)	Days 6-15 post coitum	On 10th and 11th day post coitum	On days 4, 10, 13 and 18	GD 6-18	GD 6-15	Over three generations
Doses (mg/kg bw/d)	0, 15, 45 and 90	0, 20, 40, 80 and 160	0, 50, 100, 200 and 400	0, 25, 100, 175 and 225	0, 10, 50 and 100	0.3, 2 and 10
Purity of the test substance	99.9 %	Not known	Not known	Not known	99.5 %	Not known
Species: rat/strain	Wistar	Ico Shoe: WIST	Wistar (acc. to CLH report)	Not mentioned	Fisher 344	Shoe: WIST
Maternal effects	90 mg/kg bw/d: body weight (BW)↓; food consumption↓; uterus weight↓ 45 mg/kg bw/d: BW↓ (statistically); food consumption↓ 15 mg/kg bw/d: no effects	No maternal toxicity	400 mg/kg bw/d: 4 rats died; post-implantation loss (75%); 200 and 400 mg/kg bw/d: BW↓	225 mg/kg bw/d: all animals died; 175 mg/kg bw/d: 6/8 animals died, 2 surviving dams without live foetuses 100 mg/kg bw: BW↓; resorptions↑	100 mg/kg bw/d: BW↓, food consumption↓ (17 %); water consumption↓ (33 %); liver weight↑ (6 %) 50 mg/kg bw/d: BW↓, food consumption↓ (12 %);	10 mg/kg bw/d: P and F1 generations: BW↓; fertility index↓ 0.3 and 2 mg/kg bw/d: F2 generation: BW↑

Parameter/Study	No (1) BASF, 1992; Study report: 30R0464/91071 (01/10/1992); "Anonymous 1992" in CLH report	No (2) "Bleyl D.W.R (1990)" in CLH report	No (3) "Anonymous 17 (1984)" in CLH report (conducted by Bleyl *et al.* in 1989 according to Bleyl *et al.*, 1990) (not known to registrant)	No (4) NTIS: OTS05191233, 06/04/1990; "Anonymous 18, 1989" in CLH report	No (5) Drinking water teratology study in Fischer 344 Rats (Dow Chemical Company, NTIS: TS0537366, 23/10/1990) (not mentioned in CLH report)	No (6) Three-generation study in Wistar rats (not mentioned in the CLH report)
					water consumption↓ (20 %); liver weight ↑ (4 %) due to unpalatability	
Embryo/fetotoxic effects	90 mg/kg bw/d: BW↓ 45 mg/kg bw/d: BW↓ 15 mg/kg bw/d: no effects	No embryotoxicity	400 mg/kg bw/d: placental weight↓; 200 and 400 mg/kg bw/d: BW↓;	100 mg/kg bw/d: BW↓	Fetal BW↓: all treatment groups, but clearly related to treatment at 50 and 100 mg/kg bw/d	F1 and F2 generations: 10 mg/kg bw/d: viability↓ BW↓
Developmental effects	90 mg/kg bw/d: malformations (urogenital, cardiovascular, skeletal); 15 and 45 mg/kg bw/d: no malformations	160 mg/kg bw/d: malformations (urogenital) 77 % of live foetuses; viability index: 26 % due to bilateral kidney agenesis (two kidneys missing) 80 mg/kg bw/d: 15.6 % of foetuses with uni- and bilateral kidney agenesis	Malformations (severe urogenital, skeletal): 100 mg/kg bw/d: 11 % 200 mg/kg bw/d: 46 % 400 mg/kg bw/d: 100 %	100 mg/kg bw/d: cleft lip and palate (1 fetus); no further malformations	100 and 50 mg/kg bw/d: delayed ossification of the cervical and thoracic vertebral centra; low incidence of anophthalmia and microphthalmia in all groups including controls; no other malformations	No malformations
NOAEL	Maternal and developmental: 15 mg/kg bw/d	Developmental: 40 mg/kg bw/d	Maternal: 100 mg/kg bw/d; Developmental: 50 mg/kg bw/d	Maternal and developmental: 25 mg/kg bw/d	Maternal and developmental: 10 mg/kg bw/d	Maternal and developmental: 2 mg/kg bw/d

Alignment with CLP criteria:

The CLP Regulation defines criteria for the following hazard categories for reproductive toxicants as of its table 3.7.1(a).

Table 3.7.1(a)

Hazard categories for reproductive toxicants

Categories	Criteria
CATEGORY 1	Known or presumed human reproductive toxicant Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).
Category 1A	Known human reproductive toxicant The classification of a substance in Category 1A is largely based on evidence from humans.
Category 1B	Presumed human reproductive toxicant The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or 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. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.
CATEGORY 2	Suspected human reproductive toxicant Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, 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 Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed 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 the other toxic effects.

Attention is drawn to the category 1B classification which demands (citation): "*clear evidence of an adverse effect on … or on development in the absence of other toxic effects*" (see also chapter 3.7.2.3.4. of the CLP Regulation) the fact of which does obviously not apply for 3-Methylpyrazole as has been outlined above, such that according to chapter 3.7.2.2.2. of the CLP Regulation, *"in the evaluation of toxic effects on the developing offspring, it is important to consider the possible influence of maternal toxicity (see section 3.7.2.3.4)".*

The CLP Regulation requires particularly, that several aspects of developmental toxicity need to be considered which are of direct relevance for 3-Methylpyrazole, i.e.

- availability of respective toxicity data for structural analogues (according to chapter 3.7.2.3.1. of the CLP Regulation (*citation*): *"Evaluation of substances chemically related to the substance under study may also be included, particularly …"*),

- appropriateness of information gained from adequate testing and considering particularly shortcomings and inconsistencies in test results (according to chapter 3.7.2.3.1. of the CLP Regulation (*citation*): "*The weight given to the available evidence will be influenced by factors such as the quality of the studies, consistency of results, nature and severity of effects,* …"), and

- significance of concurrent developmental, embryo/feto and maternal toxicity according to chapter 3.7.2.4.1. of the CLP Regulation (*citation*): "*Development of the offspring throughout gestation and during the early postnatal stages can be influenced by toxic effects in the mother either through non-specific mechanisms related to stress and the disruption of maternal homeostasis, or by specific maternally-mediated mechanisms.*", since (*citation*) "*Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, …*".

Information on developmental toxicity with structurally similar analogue substances:

This chapter makes reference to chapter 3.7.2.3.1. of the CLP Regulation, where evaluation of chemically related substances may be included for adequate assignment of classification for developmental toxicity.

Pyrazole (CAS# 288-13-1)

There is a developmental GLP non-guideline study available in the REACH registration dossier; available at: https://echa.europa.eu/registration-dossier/-/registered-dossier/23403/7/9/1.

In this study, pregnant Sprague Dawley rats were exposed to the test substance (purity 98 %) per gavage at doses of 0, 1, 5, 10, 15 and 20 mg/kg bw/d on gestation days 6-15.
No mortality or remarkable clinical signs were observed in treated animals.
Body weight reduction was dose dependent at doses of 5 to 20 mg/kg bw/d. A non-statistically significant increase in mean thyroid weight (26 % compared to control) was observed at 20 mg/kg bw/d.
A slight, non-statistically significant, increase in post-implantation loss was observed at 20 mg/kg bw/d.
No other pregnancy parameters were affected.

The number of live offspring was similar to the control at all doses. A statistically significant decrease in foetal body weight was observed at the two highest doses 10 and 20 mg/kg bw/d. Cleft palate was observed in 6 foetus from 2 litters at 20 mg/kg bw/d.

NOAEL for maternal toxicity was 1 mg/kg bw/d; NOAEL for fetotoxicity was 5 mg/kg bw/d; NOAEL for teratogenicity was 15 mg/kg bw/d.

According to registrants, there were no indications for developmental toxicity at doses below maternal toxic doses. Therefore, the developmental effects observed are considered as a secondary non-specific consequence of maternal toxicity and the substance does not need to be classified as developmental toxicant.

3,5-Dimethylpyrazole (CAS# 67-51-6; EC# 200-657-5)

A GLP OECD Guideline 422 study (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test) is available in the REACH registration dossier; available at: https://echa.europa.eu/registration-dossier/-/registered-dossier/5791/7/9/1.

In this study, the substance was administered per gavage to animals at doses of 20, 60 and 200 mg/kg bw/d. Males were exposed during minimum 28 days: 2 weeks premating, during mating and up to the day prior to scheduled necropsy, and females: 2 weeks premating, during mating, gestation, and at least 4 days of lactation up to the day prior to scheduled necropsy.

Parental toxicity was observed at 60 and 200 mg/kg bw/d. At 200 mg/kg bw/d, treatment related effects on body weights, food and water consumption, functional observations, clinical pathology, macroscopic findings and microscopic findings in the thymus, liver spleen, testes and epididymides were observed. Females at 60 mg/kg bw/d had a trend towards increased water consumption and males at this dose level had toxicologically relevant liver findings at the microscopic examinations. There was no parental mortality in the study.

Toxicity to reproduction was observed at 200 mg/kg bw/d, where microscopic changes were recorded in the epididymides and testes, characterized by oligospermia, seminiferous cell debris and degeneration/depletion of spermatocytes. Lower fertility and conception indices were also observed in the 200 mg/kg bw/d exposure group.

Based on these observations the NOAEL for reproduction was determined to be 60 mg/kg bw/d and the parental NOAEL was determined to be 20 mg/kg bw/d.

Developmental toxicity was observed at 200 mg/kg bw/d, based on treatment related effects observed in pup mortality (postnatal loss) and lower pup body weights. No treatment-related changes were noted in any of the remaining developmental parameters investigated in this study, i.e. gestation index and duration, parturition, maternal care and clinical signs and macroscopy of pups.
The developmental NOAEL was determined to be 60 mg/kg bw/d.

Developmental toxicity was not seen in the absence of parental toxicity, and therefore the material is not classified as such under Regulation (EC) No. 1272/2008 or Directive 67/548/EEC.

In the endpoint summary, however, the following conclusion is made (*citation*): "*On the basis of the effects seen, the material should be classified as Repr. Cat. 2: H361: Suspected of damaging fertility or the unborn child, in accordance with Regulation (EC) No. 1272/2008.*"
This conclusion is in conflict to the previous conclusion.

Conclusions for classification of 3-Methylpyrazole for developmental toxicity:

The embryo/fetotoxic effects (i.e. reduced body weights) can be discounted, as is described in chapters 3.7.2.3.5. and 3.7.2.4.2. of the CLP Regulation, because they consistently occurred at doses associated with maternal toxicity (like (*citation* from chapter 3.7.2.3.5.) "*maternal stress and the disruption of homeostasis*") and are therefore obviously a secondary consequence thereof in the above studies. This can particularly clearly be seen in the NTIS study according to No (4), when tolerable doses were used which do not lead to death of dams.

Regarding teratogenicity, are the BASF (1992) study according to No (1) and the drinking water study according to No (5) the foremost suitable studies for interpretation of potential teratogenic effects (see above "*Discussion of results of developmental toxicity studies*").

Specific fetal malformations (i.e. urogenital effects) were observed at only the highest dose level in the key BASF (1992) study according to No (1) associated with maternal toxicity, while in the reliable drinking water study according to No (5) only delays at ossifications were observed at mid and the highest doses producing also maternal toxicity. Such effects are also according to chapter 3.7.2.4.3. of the CLP Regulation (*citation*) "*minor developmental changes, …, when seen in association with maternal toxicity.*"

Nevertheless it is emphasized, that these effects are of a different kind and, therefore, based on the results of these two studies alone no clear evidence of teratogenicity of 3-Methylpyrazole can be concluded; in particular, when taking into account criteria of above-mentioned chapter 3.7.2.3.4. of the CLP Regulation, it is not clear, whether a causal relationship exists between maternal toxicity, embryo/fetotoxicity and finally teratogenicity and whether maternal toxicity and embryo/fetotoxicity (*citation* from chapter 3.7.2.3.4.) *"are likely to have influenced these effects"*.

Applying criteria of chapter 3.7.2.3.5. of the CLP Regulation, there is an indication (not to be mixed up with "*clear evidence*"), that urogenital effects might be a specific effect of 3-Methylpyrazole, even though they were associated with marked maternal toxicity (and hence embryo/fetotoxicity) in the BASF(1992) study according to No (1). On the other hand, taking the outcome of the unreliable "Anonymous 17 (1984)" study according to No (3) into account, in which an increase in urogenital malformations was observed, it is not clear whether the teratogenic effects can be a secondary consequence of maternal toxicity because of mortalities in both high dose group!

It is important to note, that this study according to the introduction presented in the BASF study was conducted by the same working group of Bleyl D.W.R. *et al.* as was the study with its unusual design with only two days of substance administration (Bleyl D.W.R. *et al.* study according to No (2)), in which severe urogenital malformations were observed. Furthermore, if urogenital effects were specific effects, it is not clear why they were not present at the mid dose level of 100 mg/kg bw/d associated also with marked maternal and embryo/fetotoxicity toxicity in the NTIS study according to No (4), while only one fetus with cleft palate was observed?

According to the criteria of chapters 3.7.2.4.1., 3.7.2.4.2. and 3.7.2.4.3.of the CLP Regulation the degree of influence of maternal toxicity on urogenital effects is doubtful based on the available pieces of evidence and therewith a clear conclusion whether teratogenic effects are specific effects of 3-Methylpyrazole cannot be made with a solid degree of certainty.
Both key studies identified (BASF (1992) study according to No (1) and drinking water study according to No (5)) and do not allow for an in-depth`s interpretation and evaluation of their divergent study results.

Supporting evidence which could be helpful for a more adequate interpretation and evaluation of these effects with regard to their causal relationship at comparable doses is not available, to the contrary it is noted, that (as far as studies are grossly comparable, which is in some cases difficult to decide because of most divergent study designs also with regard to rat strains used, absence of GLP prerequisites and unknown purity of test substance):

- reduction in uterus weight of dams was observed in the BASF (1992) study according to No (1) but in no other study,

- absence of kidneys were noted in the Bleyl D.W.R (1990) study according to No (2) and in "Anonymous 17" (1984) study according to (3) conducted also by the working group of Bleyl D.W.R *et al.* but in no other study,

- no malformations (besides one cleft palate) were observed in the NTIS study according to No (4), and

- only delayed ossification was only observed in the drinking water study according to No (5) but again in no other study.

According to chapter 3.7.2.3.1. of the CLP Regulation, information on developmental toxicity from the two structurally related Pyrazole derivatives has been taken into account to conclude in a weight-of-evidence approach on C&L of the 3-Methylpyrazole.

According to the results of these studies, reduced fetal body weight at doses associated with maternal toxicity was the main adverse effects regarding developmental toxicity.
No teratogenic effects were observed.

Taken together it becomes clear, that the available data base does not allow for unanimous classification of 3-Methylpyrazole into category Repr. 1B as has been proposed in the CLH report of BCA, actually without an extended discussion of the contradictory results and the various shortcomings of the reviewed studies.

On the other hand, are there indications for a substance specific teratogenic effect of 3-Methylpyrazole which would make it difficult to clearly substantiate a classification into category Repr. 2 only.

Considering respective study results gained from two closely related substances (i.e. Pyrazole and 3,5-Dimethylpyrazole) category Repr. 2 seems be more appropriate.

Taking into account this unsatisfactory situation for the registrant, it seems to be a valid option, to execute of a well-conducted, and possibly extended, OECD 414 study by a study design aiming for discrimination between in particular maternal and teratogenic effects.

Therefore, a developmental study in rodents by oral route of exposure is proposed by the registrant to clarify the uncertainties described above.

For the time being the existing self-classification into category Repr. 2 seems to grossly justified and adequate.