Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

3-methylpyrazole

EC Number: 215-925-7
CAS Number: 1453-58-3

CLH-O-0000006718-63-01/F

Adopted

5 December 2019

5 December 2019

CLH-O-0000006718-63-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **3-methylpyrazole**

EC Number: **215-925-7**

CAS Number: **1453-58-3**

The proposal was submitted by **Belgium** and received by RAC on **14 February 2019.**

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

PROCESS FOR ADOPTION OF THE OPINION

Belgium has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **4 March 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **3 May 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Ralf Stahlmann**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **5 December 2019** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	3-methylpyrazole	215-925-7	1453-58-3	Repr. 1B Acute Tox. 4 STOT RE 1 Skin Corr. 1 Eye Dam. 1	H360D H302 H372 (lung) H314 H318	GHS08 GHS07 GHS05 Dgr	H360D H302 H373 (lung) H314		oral: ATE = 500 mg/kg bw	
RAC opinion	TBD	3-methylpyrazole	215-925-7	1453-58-3	Repr. 1B Acute Tox. 4 STOT RE 2 Skin Corr. 1 Eye Dam. 1	H360D H302 H373 (lung) H314 H318	GHS08 GHS07 GHS05 Dgr	H360D H302 H373 (lung) H314		oral: ATE = 500 mg/kg bw	
Resulting Annex VI entry if agreed by COM	TBD	3-methylpyrazole	215-925-7	1453-58-3	Repr. 1B Acute Tox. 4 STOT RE 2 Skin Corr. 1 Eye Dam. 1	H360D H302 H373 (lung) H314 H318	GHS08 GHS07 GHS05 Dgr	H360D H302 H373 (lung) H314		oral: ATE = 500 mg/kg bw	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

3-Methylpyrazole has no existing entry in Annex VI of the CLP Regulation but is registered under REACH and currently self-classified as Acute Tox. 4; H320, Skin Corr. 1B; H314, Eye Dam. 1; H318 and Repr. 2; H361. In addition to the above hazard classes, STOT RE was considered in the CLH dossier.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Oral Route

The Dossier Submitter (DS) proposed to classify 3-methylpyrazole as Acute Tox. 4; H302, based on a GLP compliant OECD TG 423 acute oral toxicity study in rats, with an LD_{50} between 300 and 2 000 mg/kg bw (Anonymous 12, 2012). The DS also proposed to set a converted Acute Toxicity Estimates (ATE) of 500 mg/kg bw based on the acute oral toxicity range.

Dermal Route

There are no acute dermal toxicity studies available for 3-methylpyrazole. Therefore, this endpoint was not assessed in the CLH report.

Inhalation Route

Based on the negative results of one non-GLP, non-guideline, acute inhalation toxicity study, which was performed similarly to OECD TG 403, but with limitations in reporting (Anonymous 13, 1988), the DS proposed 'no classification' of 3-methylpyrazole for acute toxicity via inhalation.

Comments received during public consultation

One MSCA supported the DS proposal for 'no classification' for acute toxicity classification.

Assessment and comparison with the classification criteria

Oral Route

In a GLP compliant OECD TG 423 acute oral toxicity study, three female rats per group were exposed to 300 or 2000 mg/kg bw of 3-methylpyrazole via gavage (Anonymous 12, 2012). At the lower dose, no deaths and no other effects were observed. At 2000 mg/kg bw, all animals died within 7 days after exposure. Clinical signs consisted of reduced motility and muscle tone, ataxia, dyspnoea, and dorsal position.

Inhalation Route

One non-GLP acute inhalation toxicity study in Wistar rats (5/sex/concentration) is available, which was performed similarly to OECD TG 403, but with limitations in reporting (Anonymous 13, 1988). There were no details on the size of the test chamber, the concentration of the test

substance in the chamber was not verified, and the purity of the test substance was not given. 3-methylpyrazole was applied as a gas for 4 hours, at concentration levels up to 28110 mg/m^3 with no further information provided. However, RAC notes that the substance is a liquid and has a low vapour pressure (182 Pa at 20 °C). It is therefore unlikely that it was applied as gas. As the CLH report states the used vehicle as "air", RAC assumes that it was tested as an aerosol. The DS considered the study valid with a reliability score of 2. There were no mortalities, clinical signs or findings at necropsy at any of the concentration levels.

RAC notes that some Material Safety Data Sheets list an LC_{50} of 719 mg/m^3 in rats. However, since no study information is available to RAC, an evaluation of this value is not possible.

Conclusion on classification

The oral LD_{50} was between 300 and 2000 mg/kg bw. These are the boundaries for Acute Tox. 4 classification. RAC concurs with the DS to set a converted ATE of 500 mg/kg bw based on the acute oral toxicity range, and to classify 3-methylpyrazole as **Acute Tox. 4; H302**.

The highest concentration level (28.11 mg/L) used in the one available negative acute inhalation toxicity study was above the upper boundary for Acute Tox. 4 classification (5.0 mg/L for dusts and mists); therefore, **no classification of 3-methylpyrazole for acute toxicity via inhalation is warranted**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

There are no animal or human data available on the skin corrosive/irritative properties of 3-methylpyrazole.

One *in vitro* skin irritation study (EpiDerm™) is available (Anonymous 14, 2011), which was performed with 98.1 % pure 3-methylpyrazole and with no deviations from OECD TG 431. Based on the results of this study, the DS concluded that 3-methylpyrazole should be classified as Skin Corr. 1; H314 without sub-categorisation. RAC notes that in Table 5 of the CLH report the proposed classification is indicated as Skin Corr. 1B, which is the current self-classification, therefore it is likely a mistake.

Comments received during public consultation

One MSCA considered classification of 3-methylpyrazole as Skin Corr. 1 without sub-categorisation appropriate.

Assessment and comparison with the classification criteria

As no human data or data from animal testing are available for the skin corrosion/irritation endpoint, classification is based on the results of a guideline compliant (OECD TG 431) *in vitro* assay (EpiDerm™) (Anonymous 14, 2011). In this test, 50 µL of pure (98.1 %) 3-methylpyrazole was applied to reconstructed human epidermis (Rhe) tissues for three minutes or one hour. A negative and a positive control were also conducted, but not further specified. Relative absorbance values (*i.e.* cell viability) were 73.8 % after three minutes exposure (absolute values: 1.482, 2.009, and 0.586 for test substance, negative control, and positive control, respectively), and 14.9 % after one hour exposure (absolute values: 0.281, 1.883, and 0.456 for test substance, negative control, and positive control, respectively).

A substance is identified as corrosive in the EpiDerm™ test when cell viability is reduced to 50 % or more after three minutes exposure, and to under 15 % after one hour exposure. If cell viability is less than 25 % after three minutes exposure, sub-categorisation to category 1A is possible. With a cell viability of 25 % or more after three minutes exposure, a substance falls within subcategories 1B or 1C, but a discrimination between these two is not possible. As there are no other data available for skin corrosive properties to evaluate if category 1B or 1C is appropriate, RAC concurs with the DS to classify 3-methylpyrazole as **Skin Corr. 1; H314** without sub-categorisation.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

There are no animal or human data available for this endpoint. Based on the positive results of an OECD TG 437 bovine corneal opacity and permeability test (BCOP) (Anonymous 15, 2011), the DS concluded that 3-methylpyrazole should be classified as Eye Dam. 1; H318.

Comments received during public consultation

One MSCA agreed that classification as Eye Dam. 1; H318 is justified.

Assessment and comparison with the classification criteria

In an *in vitro* BCOP test following OECD TG 437, 750 µL of pure (98.1 %) 3-methylpyrazole were applied to three excised bovine corneas for 10 minutes. Controls included not specified positive and negative controls. The *in vitro* irritancy score (IVIS) was 85.73 for the test substance, 215.79, and -0.216 for positive, and negative controls, respectively. According to the test guideline, substances with an IVIS above 55 should be classified as Eye Damage 1.

Therefore, RAC concurs with the DS based on these results that 3-methylpyrazole should be **classified as Eye Dam. 1; H318**.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS summarised three short-term oral toxicity studies in mice, three sub-chronic oral toxicity studies (two in rats, one in mice) and one chronic oral toxicity study in rats (Anonymous 22, 1999). In two 28-d studies and one 90-d study in mice (Anonymous 19, 1996; Anonymous 20, 1997; Anonymous 21, 1996, respectively), histopathological alterations in the lungs of treated animals were observed. These were predominantly alterations in Clara cell (hereinafter referred to as club cells) morphology. Based on these results, the DS proposed to classify 3-methylpyrazole as STOT RE 1; H372 (lung). The DS did not specify the route of exposure.

Comments received during public consultation

Two MSCAs commented and supported classification as STOT RE, one of them in category 1 and one of them in category 2, since most of the effects were observed at doses above the guidance

value for category 1 (10 mg/kg bw/d in a 90-d study). The DS considered effects observed at 10 mg/kg bw/d in one of the mouse studies borderline and proposed to classify 3-methylpyrazole as STOT RE 1.

Assessment and comparison with the classification criteria

The studies presented by the DS are summarised in the table below and compared to guidance values for STOT RE classification.

For STOT RE 1 classification, the guidance value is C ≤ 10 mg/kg bw/d in an oral 90-d study.

For STOT RE 2 classification, the guidance values are 10 < C ≤ 100 mg/kg bw/d in an oral 90-d study.

Extrapolation of guidance values for 28-d studies was performed according to Haber's rule.

***Table**: Repeated dose toxicity studies for 3-methylpyrazole (modified from table 21 of the CLH report).*

Method, guideline, species, strain, sex, no/group, reference	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Effective doses and corresponding STOT RE category**
Short-term oral toxicity study **Mouse** (B6C3F1) 5/sex/dose **OECD TG 407** Deviation: small spacing between dose groups **GLP** Anonymous 19, 1996	Purity: 99.7 % Drinking water Conc.: 0, 900, 1 125 and 1 575 ppm (0/0, 135/173, 153/198 and 167/245 mg/kg bw/d in males/ females (m/f), respectively) Duration of exposure: **28 d**	Mortality and clinical signs: none Bwg: sign. ↓ in f of highest dose group ↑ lung weight in m and f of highest dose group Histopathology: change in lungs in all animals (karyomegaly in the epithelium of the air ducts, loss of domes in the club cells, hypotrophy of the air duct epithelia) *see Table below for number of animals affected*	LOAEL: 135/173 mg/kg bw/d (m/f) → **STOT RE 2** *(extrapolated guidance values:* *30 < C ≤ 300 mg/kg bw/d)* **No NOAEL** identified
Short-term oral toxicity study **Mouse** (B6C3F1) 5/sex/dose for main groups + 5/sex/dose for recovery groups **EU Method B.7** **GLP** Anonymous 20, 1997	Purity: 99.4 % Drinking water Conc.: 300, 900 and 1575 ppm (0/0, 70/82, 151/193 and 223/252 mg/kg bw/d in m/f, respectively) Duration of exposure: **28 d** Recovery period: 14 d	300 ppm: slight ↓ food and water consumption in f ↑ lung weight in f Moderate club cell alteration in m/f 900 ppm: tremor and hunched posture in f ↓ bwg in f and ↓ food and water consumption in m/f ↑ lung weight in m/f Moderate club cell alteration in m/f Parenchymal lung changes in a few mice	LOAEL: 70/82 mg/kg bw/d (m/f) → **STOT RE 2** *(extrapolated guidance values:* *30 < C ≤ 300 mg/kg bw/d)* **No NOAEL** identified

Method, guideline, species, strain, sex, no/group, reference	Test substance, route of exposure, dose levels, duration of exposure	Results	Effective doses and corresponding STOT RE category
		1575 ppm: tremor and hunched posture in f ↓ bw, food and water consumption in m/f ↑ lung weight in m/f Moderate to marked club cell alteration in m/f Parenchymal lung changes in a few mice Recovery: not accomplished in 14 d follow up period *see two Tables below for number of animals affected*	
Short-term oral toxicity study **Mouse** (B6C3F1) 3/sex/dose **Non-guideline** **Non-GLP** Anonymous 21, 1996	Purity: 99.77 % Drinking water Conc.: 0, 225, and 675 ppm (0/0, 47/61, and 140/173 mg/kg bw/d in m/f, respectively) Duration of exposure: **14 d**	**No treatment-related effects** No histopathology performed	**None** **NOAEL:** 675 ppm (**140/173 mg/kg bw/d**)
Subchronic oral toxicity study **Rat** (Wistar) 10/sex/dose for main groups (+ 10/sex/dose for recovery groups (28 d of recovery) **OECD TG 407 and 408** **GLP** Anonymous 22, 1999	Purity: 99.34 % Drinking water Conc.: 0 and 40 mg/kg bw/d Duration of exposure: **90 d** Recovery period: 28 d	Mortality, clinical signs and bw: **no effects** Organ weight examination: ↑ kidney and liver weights (abs. + rel.) in m but **fully reversible** at the end of the recovery period Histopathology examination (kidneys, liver and lungs): **no treatment-related effects**	**None** **NOAEL: 40 mg/kg bw/d**
Sub-chronic oral toxicity study **Rat** (Wistar) 24/sex/dose 36/sex for control group **Non-guideline** **Non-GLP** Anonymous 23, 1980	Purity: no information Gavage Conc.: 0, 0.2, 2, 20 and 200 mg/kg bw/d Duration of exposure: **90 d**	200 mg/kg bw/d: ↓ bw and food consumption in m/f Haematology and clinical biochemistry: ↑ nb. of neutrophilic lymphocytes, ASAT, ALP activity, ↓ tot. protein, albumin, glucose in m/f, and ↓ ChE activity in f Organ weight: ↓ brain, spleen, thymus and testes weight and ↑liver weight Alteration in thyroid glands	**None** **NOAEL: 20 mg/kg bw/d**

Method, guideline, species, strain, sex, no/group, reference	Test substance, route of exposure, dose levels, duration of exposure	Results	Effective doses and corresponding STOT RE category
		Liver: nucleus anisomorphism, fatty degeneration and cell death	
Sub-chronic oral toxicity study **Mouse** (B6C3F1) 10/sex/dose + 10/sex/groups for recovery groups **OECD TG 408** **GLP** Anonymous 24, 2000	Purity: 98.38 % Drinking water Conc.: 0, 5, 10, 20 and 40 mg/kg bw/d Duration of exposure: 13 w (**91 d**) Recovery period: 4 w	Mortality, clinical signs, haematology, clinical biochemistry, organ weights: **no effects** Sign. lower bw in males in all dose levels ≥ 10 mg/kg bw/d: club cell alteration Recovery group: ≥ 10 mg/kg bw/d: club cell alteration and proliferation *see Table below for number of animals affected*	LOAEL: 10 mg/kg bw/d → at upper limit for **STOT RE 1**
Chronic oral toxicity study **Rat** (Wistar) 32/sex/group **Non-guideline** **Non-GLP** Anonymous 25, 1985	Purity: no information Drinking water Conc.: 0, 10, 40 and 2000/1000 ppm (2000 ppm during w1-4 thereafter 1000 ppm w5-80) Duration of exposure: **18 months**	**High mortality rate in all groups incl. control** ≥ 10 ppm: dyspnoea, cachexia, pneumonia 2000/1000 ppm: ↓ bw (m 82.3 %, f 70.6 % of control group), food and water consumption, erythrocyte, Hb and Ht ↑ aminotransferase, leucine aminopeptidase, alkaline phosphatase, inhibition activity of cholinesterase (f), cholesterol ↑ heart, liver, kidneys, brain and thyroid weight (lung weight not recorded) Histopathology: focal alteration in liver	**Mone** (high mortality rate, pronounced systemic toxicity in all dose groups, no details reported)

Bwg: body weight gain

Table: *Club cell alterations reported in a 4-week oral toxicity study in mice (Anonymous 19, 1996), modified from Table 22 of the CLH report*

Dose (mg/kg bw/d) as mean daily intake		**0**	**154**	**176**	**206**
Club cell lesion	Incidence (out of 5 animals/sex) m/f	0/0	5/5	5/5	5/5
	Karyomegaly Grade 3 m/f	0/0	1/0	0/2	1/1
	Karyomegaly Grade 4 m/f	0/0	4/5	5/3	4/4
Focal hypothrophy incidence (out of 5 animals/sex) m/f		0/0	4/0	2/0	2/1
Diffuse hypotrophy incidence (out of 5 animals/sex) m/f		0/0	1/5	3/5	3/4

m – male, f - female

Table*: Club cell alterations reported in a 4-week oral toxicity study in mice (Anonymous 20, 1997) with 2-week recovery period, modified from Table 24 of the CLH report*

Dose (mg/kg bw/d) as mean daily intake		**0**	**76**	**172**	**238**
After end of exposure (4 w)					
Club cell alteration	Incidence (out of 5 animals/sex) m/f	0/0	5/5	5/5	5/5
	Grade 1 m/f				
	Grade 2 m/f			0/1	
	Grade 3 m/f		5/5	5/3	2/4
	Grade 4 m/f			0/1	3/1
After end of recovery (6 w)					
Club cell alteration	Incidence (out of 5 animals/sex) m/f	0/0	5/5	5/5	5/5
	Grade 1 m/f				
	Grade 2 m/f		3/0	0/1	
	Grade 3 m/f		2/5	5/4	2/4
	Grade 4 m/f				3/1
Club cell proliferation	Incidence (out of 5 animals/sex) m/f	0/0	5/5	5/5	5/5

	Grade 1 m/f				
	Grade 2 m/f		2/1	2/5	3/0
	Grade 3 m/f		3/4	3/0	2/5

m – male, f - female

Table: *Club cell alterations reported in a 13-week oral toxicity study in mice with 4-week recovery period (Anonymous 24, 2000), modified from Table 18 of Annex I to the CLH report*

Dose (mg/kg bw/d)		**0**	**5**	**10**	**20**	**40**
After end of exposure (13 w)						
Club cell alteration	Incidence (out of 10 animals/sex) m/f	0/0	0/0	7/4	10/10	10/10
	Grade 1 m/f			3/1	2/0	
	Grade 2 m/f			4/2	7/6	
	Grade 3 m/f			0/1	1/4	5/3
	Grade 4 m/f					5/7
After end of recovery (17 w)						
Club cell alteration	Incidence (out of 10 animals/sex) m/f	0/0	0/0	2/4	9/10	10/10
	Grade 1 m/f			1/1	0/1	
	Grade 2 m/f			1/3	7/9	
	Grade 3 m/f				2/0	9/10
	Grade 4 m/f					
Club cell proliferation	Incidence (out of 10 animals/sex) m/f	0/0	0/0	2/4	10/9	10/10
	Grade 1 m/f			2/3	2/2	3/1
	Grade 2 m/f			0/1	7/6	5/7
	Grade 3 m/f				1/1	2/2

m – male, f - female

Conclusion on classification

In four repeated dose toxicity studies in mice, alterations of club cells in the lungs of treated mice accompanied by higher organ weights were observed. Club cells are involved in the biotransformation of numerous xenobiotics. The pattern was consistent and the effects are considered severe enough for classification. No such effects were reported in rats at similar or higher doses and with longer exposure periods. RAC notes species differences in number of club cells found in the respiratory epithelium. These may explain the different findings in rats and mice, and lower the concern for humans to some extent. While club cells comprise up to 60 % of the whole tracheobronchial epithelium in mice (Pack *et al*., 1980), in rats the volume fraction for club cells ranges from 0 % in the alveolar duct to around 40 % in the proximal and terminal bronchioles (Plopper *et al.*, 1994). In humans, numbers range from 0 % in the trachea to 22 % in the respiratory bronchioles (Boers *et al.*, 1999).

In one study in mice, effects were observed at a dose near the guidance value for STOT RE 1, but these effects occurred at lower incidences than in the higher dose groups and no club cell alterations were observed at the next lower dose level (5 mg/kg bw/d). Moreover, incidences and grades were lower in the recovery group, and proliferation of club cells was noted, indicating at least partial reversibility of the effect. In addition, no accompanying clinical signs were observed at 10 mg/kg bw/d. No details on the reported alterations were provided in the CLH report. Thus, RAC could not evaluate their severity. The observed effects are therefore considered not sufficient for classification as STOT RE 1. Effects in the two other studies were seen at dose levels clearly in the range of STOT RE 2 guidance values.

Given that effects seen at the upper limit of the guidance value for STOT RE 1 were not seen at the next lower dose level and were not supported by clinical signs, and since mice seem more prone to club cell effects compared to rats, RAC proposes in a weight of evidence approach to **classify 3-methylpyrazole as STOT RE 2; H373 (lung)**. RAC concurs with the DS not to specify a route of exposure as no other than oral studies are available, and the other routes of exposure cannot be ruled out.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

Two-generation or one-generation studies are not available for 3-methylpyrazole. In the repeated dose toxicity studies, no effects on reproductive organs were observed. Therefore, the DS did not conclude on classification for fertility due to a lack of data.

Development

The DS presented four developmental toxicity studies. Only one of these (Anonymous 16, 1992) followed OECD TG 414 and was conducted under GLP, while the other three provided no information on GLP status and did not follow a guideline.

In three of these studies (including the guideline compliant study), foetuses and pups showed malformations of the urogenital tract. Malformations included uni- and bilateral kidney agenesis, hydronephrosis, and malformations of great vessels.

Lactation

No data are available to assess toxicity effect on or via lactation. Therefore, the DS did not conclude on classification for fertility due to a lack of data.

Taking into account that no data on effects on/via lactation and fertility are available, and based on the developmental results above, the DS proposed to classify 3-methylpyrazole as Repr. 1B; H360D.

Comments received during public consultation

Two MSCAs and an industry representative commented. The MSCAs supported classification as Repr. 1B; H360D. The industry commenter pointed out that the presented studies had various shortcomings, and results were contradictory. In the attachment to their comment, they summarised two additional studies on developmental effects of 3-methylpyrazole (see Additional Key Elements) and one study each for the structurally related substances pyrazole and 3,5-dimethylpyrazole. Based on their discussion of the results they proposed that an OECD TG 414 study should be conducted to generate reliable data. In the meantime, classification in Category 2 should be considered.

The DS responded that assessment of the presented studies is not possible from a short summary, and furthermore, that negative results should not overrule positive results. They therefore maintained the proposed classification.

Assessment and comparison with the classification criteria

Fertility

There are no studies investigating the potential of 3-methylpyrazole to damage the fertility of animals and no human data on this endpoint. In one OECD TG 414 developmental toxicity study in rats with doses up to 90 mg/kg bw/d, mean number of corpora lutea, implantation sites, pre- and post-implantation loss, number of resorption and viable foetuses were unaffected.

During public consultation, Industry mentioned a 3-generation-study, without reference (non-guideline, non-GLP, Klimisch score 3, 10 mg/kg bw highest dose tested). A short summary was provided. However, due to the limitations of this summary, RAC could not assess this study.

Development

The DS presented four developmental toxicity studies in rats. During public consultation, industry provided an additional OECD TG 414 developmental toxicity study in rats (see Additional Key Elements). The study report of this study is publicly available; therefore, RAC included it in the assessment.

The industry commenter also summarised one developmental toxicity study each for pyrazole and 3,5-dimethylpyrazole. Since the comment did not provide any analysis of toxicokinetic data of these substances compared to 3-methylpyrazole, for a possible read-across, RAC does not consider these studies applicable for classification purposes.

The studies presented in the CLH report and the additional developmental toxicity study (Dow Chemical Co., 1990) are summarised in the table below.

Table: *Summary of the available developmental/prenatal toxicity studies on 3-methylpyrazole (modified from Table 13 of the CLH report).*

Method, guideline, species, strain, sex, no/group **Reference**	**Test substance, dose levels duration of exposure**	**Results**	**Remarks**
Developmental toxicity study **Rat** (Wistar) 25 pregnant females/group **OECD TG 414** **GLP** Anon. 16, 1992	Purity: 99.9 % Vehicle: water Gavage Conc.: 0, 15, 45 and 90 mg/kg bw/d Duration of exposure: GD 6-15	*Dams* **90 mg/kg bw/d:** ↓food consumption significantly ↓bw significantly ↓uterus weight (69.1 g compared to 81 g in controls) corrected bwg GD6-20: -17.5 % compared controls **45 mg/kg bw/d:** ↓food consumption ↓bw corrected bwg GD6-20: -3.1% compared to controls *Foetuses* **90 mg/kg bw/d:** significantly ↓foetal bw (3.3 g compared to 3.9 g in controls) Delayed ossification, malformations of the urogenital tract, cardio-vascular system, and thoracic vertebral bodies *(see Table below)* **45 mg/kg bw/d:** significantly ↓foetal bw (3.6 g compared to 3.9 g in controls) No teratogenic effects	**Key study 1** *Changes in bwg were calculated by RAC using the reported body weights in Annex I to the CLH report, see Table below* maternal NOAEL = 15 mg/kg bw/d foetal NOAEL = 15 mg/kg bw/d
Teratogenicity study **Rat** (Fisher 344) 30 pregnant females/group **OECD TG 414** **GLP** Dow Chemical Co., 1990	Purity: 99.5 % Drinking water Targeted doses: 0, 10, 50 and 100 mg/kg bw/d Actual doses based on water consumption and body weights: 0, 10.3, 44.9, 77.3 mg/kg bw/d Duration of exposure: GD 6-15	*Dams* *Note: no information on gravid uterus weights* **100 mg/kg bw/d:** ↓water consumption (-33 % compared to controls at GD6-16) ↓food consumption (-17 % compared to controls at GD6-16) uncorrected bwg GD6-16: -31 % compared to controls **50 mg/kg bw/d:** ↓water consumption (-21 % compared to controls at GD6-16)	**Key study 2** maternal NOAEL = 10 mg/kg bw/d foetal NOAEL = 10 mg/kg bw/d

Method, guideline, species, strain, sex, no/group Reference	Test substance, dose levels duration of exposure	Results	Remarks
		↓food consumption (-12 % compared to controls at GD6-16) uncorrected bwg GD6-16: -15 % compared to controls *Foetuses* **100 mg/kg bw/d:** significantly ↓foetal bw (-11.7 % compared to controls) Delayed ossification of thoracic and cervical vertebral centra **50 mg/kg bw/d:** significantly ↓foetal bw Delayed ossification of thoracic and cervical vertebral centra **10 mg/kg bw/d:** significantly ↓foetal bw (-4.3 % compared to controls), BUT: ↑ mean litter size (9.4 foetuses/litter compared to 7.8 in controls)	
Prenatal toxicity study **Rat** (Wistar) 13, 13, 12, 14, and 6 rats at 0, 50, 10, 200 and 400 mg/kg bw, respectively **No guideline** **Non-GLP** Anon. 17, 1984	Purity unknown Vehicle: water Gavage Doses: 0, 50, 100, 200 and 400 mg/kg bw/d Exposures on: GD 4, 10, 13, and 18	*Dams* **400 mg/kg bw:** **4/6 rats died prematurely** (catarrhal enteritis and/or nephrosis) Significantly ↓bw (-22.75 % compared to controls on GD20) uncorrected bwg GD1-20: -92.4 % compared to controls Significantly ↑post implantation loss Significantly ↑resorption rate (75 % compared to 11.5 % in controls) **200 mg/kg bw:** significantly ↓bw (-8.4 % compared to controls on GD20) uncorrected bwg GD1-20: -16.7 % compared to controls ↑post implantation loss ↑resorption rate (14.9 % compared to controls 11.5 %) **100 mg/kg bw/d:**	**Supportive study** medium reliability (unconventional dosing regimen) *Changes in bwg were calculated by RAC using the reported body weights in Annex I to the CLH report* maternal NOAEL = 100 mg/kg bw foetal NOAEL = 50 mg/kg bw

Method, guideline, species, strain, sex, no/group Reference	Test substance, dose levels duration of exposure	Results	Remarks
		↓bw (-12.6 % compared to controls on GD20, BUT: -4.8 % on GD1) uncorrected bwg GD1-20: -43.9 % compared to controls BUT: bwg GD1-18: -9.1 % compared to controls GD1-20 *Foetuses* **No information** on viability indices, survival rates, sex ratio **400 mg/kg bw/d:** significantly ↓foetal bw significantly ↓placental weight Due to high maternal lethality, foetuses available from 2 dams only; all foetuses presented at least one malformation (syndactyly, retrodactyly, amelia, cleft palate, urogenital syndrome, horizontal cardiac apex) **200 mg/kg bw/d:** significantly ↓foetal bw malformation rate: 46 % (syndactyly, retrodactyly, amelia, anaemia, cleft palate, urogenital syndrome, hydronephrosis, ecchymosis, horizontal cardiac apex) **100 mg/kg bw/d:** ↓foetal bw malformation rate: 11 % (syndactyly, retrodactyly of forelimb, urogenital syndrome, hydronephrosis, ecchymosis, horizontal cardiac apex) **50 mg/kg bw/d:** malformation rate: 2 % (hydronephrosis)	*For detailed numbers on malformations, see table below.*
Developmental toxicity study **Rat** (Wistar) Nb. of animals: not specified **No guideline** **Non-GLP**	Purity unknown Vehicle: water Oral (no more information) Conc.: 0, 20, 40, 80 and 160 mg/kg bw/d	*Dams* **no information** on bwg, gravid uterus weights, clinical observations, reproductive parameters, organ weights, histopathological/necropsy findings no effects on bw reported	**low reliability** short exposure duration, poor reporting maternal NOAEL unknown

Method, guideline, species, strain, sex, no/group **Reference**	**Test substance, dose levels duration of exposure**	**Results**	**Remarks**
Bleyl, 1990	Duration of exposure: GD10-11	*Offspring* **no information** on pup weights, sex ratio, postnatal development **160 mg/kg bw/d:** significantly ↓viability (survival index at weaning 26 %), **offspring died at PND1** significantly ↓live birth index (77 %) urogenital syndrome at necropsy PND44 renal function in females: significantly ↓abs./rel. urine volume, creatinine clearance significantly ↑protein levels in urine **80 mg/kg bw/d:** **no information** on survival rate or viability index urogenital malformations (most cases uni-lateral kidney agenesis coupled with hydronephrosis in the remaining kidney; other pups exhibited bilateral kidney agenesis) in 15.6 % of living foetuses PND44 renal function in females: ↓abs. urine volume, creatinine clearance **40 mg/kg bw/d:** PND44 renal function in females: ↓abs./rel. urine volume, creatinine clearance	offspring NOAEL = 40 mg/kg bw/d *Note: During the renal function experiments offspring were treated with phenylmercury acetate on postnatal day 43 (males) or 44 (females) to stimulate diuresis*
Prenatal toxicity study **Rat** (strain unknown) 8 pregnant females/group **No guideline** **No GLP** Anon. 18, 1989	Purity unknown Vehicle unknown Gavage Conc.: 0, 25, 100, 175 and 225 mg/kg bw/d Duration of exposure: GD 6-15	*Dams* **no information** on exact bw, bwg, gravid uterus weights, organ weights, clinical observations, haematology, histopathological/necropsy findings, reproductive parameters, mating procedure **225 mg/kg bw/d:** **all animals died or had to be killed in extremis**	**low reliability** small number of animals per group, poor reporting maternal NOAEL = 25 mg/kg bw/d foetal NOAEL = 25 mg/kg bw/d

Method, guideline, species, strain, sex, no/group Reference	Test substance, dose levels duration of exposure	Results	Remarks
		175 mg/kg bw/d: **6/8 animals died or had to be killed in extremis** no live foetuses in surviving dams **100 mg/kg bw/d:** "moderate to severe" ↓bw higher resorption rate (no numbers reported) *Foetuses* **no information** on litter size, sex ratio, viability indices, survival rates **100 mg/kg bw/d:** "severe" ↓foetal bw 1 foetus with cleft palate (BUT: weighed only 1.2 g)	

RAC considers both of the OECD TG 414 studies to be key studies, which are sufficiently reported and reliable. In both studies rats were treated orally with 3-methylpyrazole from GD6 to GD15, either by gavage or via drinking water.

One prenatal toxicity study (Anonymous 17, 1984) with an unconventional dosing regimen (GDs 4, 10, 13, and 18) is used as supportive evidence in the assessment.

Two studies (Bleyl, 1990; Anon. 18, 1989) were assigned low reliability by RAC. The reporting of the developmental toxicity study by Bleyl (1990) lacks crucial information (e.g. number of rats used for evaluation of foetuses and offspring, purity of the test substance, any kind of clinical parameters). Furthermore, animals were exposed for two days (GD10 and 11) only. In the prenatal toxicity study (Anon. 18, 1989), only a small number of rats were exposed (8/group) during organogenesis from GD6 to GD15. No malformations were observed; however, the reporting for this study was poor.

In the study presented by industry during the public consultation (Dow Chemical Co., 1990) significantly reduced maternal body weight gains were observed in rats after exposure via drinking water in the mid and high dose groups. At 50 mg/kg bw/d (actual intake 44.9 mg/kg bw/d) maternal body weight gain from GD6 to GD16 was 15 % lower than in controls, and in the 100 mg/kg bw/d group (actual intake 77.3 mg/kg bw/d) maternal body weight gain during this time period was 31 % lower than in controls. The study authors connected these changes to reduced water consumption due to unpalatability of the test substance, which was accompanied by a reduced food consumption in both dose groups (up to 17 % less when compared to controls). However, RAC notes that in several repeated dose toxicity studies in mice and rats, the substance was also administered via drinking water but no or only slight reductions in water consumption were reported at similar or higher doses. Foetal body weights were also significantly reduced in these groups. The only developmental effect observed in this study was a delayed ossification of the cervical and thoracic vertebral centra in the mid and high dose group. This effect is commonly regarded as a variation and may be a secondary effect due to maternal toxicity.

Reduced food consumption and body weight gains were also observed in the first key study (Anonymous 16, 1992) in the mid and high dose groups. At 45 mg/kg bw/d, maternal body weight gain from GD6 to 15 was reduced by 14.25 % compared to controls. During the same period, maternal body weight gain in the 90 mg/kg bw/d group was reduced by 47.2 % compared to controls. When corrected for gravid uterus weight, maternal body weight gains from GD6 to 20 were 5.3 % and 17.5 % lower than in controls in the mid and high dose group, respectively. However, corrected body weights on GD20 were only 1 % (mid dose) and 3 % (high dose) lower than control body weights (see table below). Since no other clinical effects were observed, reduced body weight gains may be attributed to concurrent reduced food consumption. While in the mid dose group foetal weights were significantly reduced compared to controls, no malformations were observed. In the high dose group, foetal weights were significantly reduced by 0.6 g compared to controls. Malformations observed in this group are summarised in a table below.

Table: *Body weight changes in the Anonymous 16 (1992) developmental toxicity study (modified from table 2 of Annex I to the CLH report).*

Dose level in mg/kg bw/d	**0**	**15**	**45**	**90**
Body weight D0	225.0 ±10.18	222.4 ±11.96	223.7 ±10.38	224.9 ±12.69
Uncorrected bw GD20	373.3 ±25.28	368.4 ±23.24	364.2 ±22.95	352.6* ±19.84
Uncorrected bw gain GD0-20 in g	148.3 ±18.02	146.0 ±16.93	140.4 ±18.58	127.7* ±14.69
Uncorrected bw gain GD0-20 compared to control in g (%)	-	2.3 (-1.55)	7.9 (-5.33)	20.6 (-13.89)
Gravid uterus weight GD20 in g	81.0 ±11.11	79.7 ±10.46	75.2 ±9.75	69.1* ±8.99
Corrected bw GD20 in g	292.3 ±17.8	288.7 ±16.94	288.9 ±18.67	283.5 ±14.48
Corrected bw GD20 compared to control in g (%)	-	-3.6 (-1.2)	-3.4 (-1.1)	-8.8 (-3)
Corrected bw gain GD0-20 in g	67.3	66.3	65.2	58.6
Corrected bw gain GD0-20 compared to control in g (%)	-	-1 (-1.5)	-2.1 (-3.1)	-8.7 (-12.9)
Corrected bw gain GD6-20 in g	37.8	37.8	35.8	31.2*
Corrected bw gain GD6-20 compared to control in g (%)	-	0 (0)	-2.0 (-5.3)	-6.6 (-17.5)
Uncorrected bw gain GD6-15 in g	45.6 ±9.06	44.1 ±7.23	39.1 ±8.37	24.1* ±12.60
Uncorrected bw gain GD6-15 compared to control in g (%)	-	-1.5 (-1.2)	-6.5 (-14)	-21.5 (-47)

* $p < 0.05$

Table: *Soft tissue and skeletal malformations and variations observed in the Anonymous 16 (1992) developmental toxicity study (modified from tables 3 and 4 of Annex I to the CLH report and table 16 of the CLH report).*

Dose level (in mg/kg bw/d)	**0**	**15**	**45**	**90**	**Historical control data in %**
Soft tissue malformations					
Nb. of foetuses evaluated **Nb. of litters evaluated**	164 24	149 22	166 25	163 25	
Total foetal incidence	0	0	0	14** (8.6 %)	
Total litter incidence	0	0	0	8** (32 %)	
Urinary tract severely dilated (renal pelvis, ureters) Foetal incidence	0	0	0	5* (3.1 %)	
Litter incidence	0	0	0	5 (20 %)	
Malformation of great vessels: displacement of aortic arch Foetal incidence	0	0	0	6* (3.7 %)	
Litter incidence	0	0	0	2 (8 %)	
Agenesis of kidney(s) Foetal incidence	0	0	0	2 (1.2 %)	
Litter incidence	0	0	0	2 (8 %)	
Agenesis of ureter Foetal incidence	0	0	0	2 (1.2 %)	
Litter incidence	0	0	0	2 (8 %)	
Dilatation of both ventricles (globular shaped heart) Foetal incidence	0	0	0	2 (1.2 %)	
Litter incidence	0	0	0	2 (8 %)	
Skeletal malformations					
Nb. of foetuses evaluated **Nb. of litters evaluated**	174 24	159 22	177 25	176 25	
Total foetal incidence	8	8	8	49** (28 %)	
Total litter incidence	6	6	5	20** (80 %)	
Thoracic vertebral body/ bodies dumbbell-shaped Foetal incidence	6 (3.4 %)	5 (3.1 %)	3 (1.7 %)	39** (22 %)	0 – 8.8 %
Litter incidence	4 (17 %)	5 (23 %)	2 (8.0 %)	17** (68 %)	0 – 39.1 %
Thoracic vertebral body/ bodies bipartite Foetal incidence	0	1 (0.6 %)	4 (2.3 %)	16** (9.1 %)	0 - 1.6 %
Litter incidence	0	1 (4.5 %)	2 (8.0 %)	10** (40 %)	0 – 9.5 %

*p < 0.05; **p < 0.01

Significantly increased incidences of foetal malformations were observed in the high dose group in association with impaired maternal weight gain and significantly reduced foetal body weights. Soft tissue malformations were seen in the highest dose group in 14 out of 163 foetuses and 8 out of 25 litters (no HCD available). Agenesis of the left kidney and the left ureter occurred in two foetuses of different litters. Foetal incidences for dilatation of the renal pelvis and ureters (n = 5) and displacement of the aortic arch (n = 6) in the high dose group were significantly different from controls. Skeletal malformations were also observed in the mid and low dose groups as well as controls. However, only incidences in the highest dose group reached statistical significance in comparison to controls. In this dose group, incidences for both reported malformations clearly exceeded the HCD range.

In the supportive prenatal toxicity study (Anonymous 17, 1984) severe maternal toxicity was observed in the highest dose group (400 mg/kg bw/exposure) leading to the death of 4 out of 6 dams. The other two dams had significantly lower body weights compared to controls (by 22.75 %) with a significantly reduced body weight gain (by 92.4 %) compared to controls. Resorption rate was as high as 75 % in this dose group (11.5 % in controls).

In the second highest dose group (200 mg/kg bw/exposure), body weights and body weight gain were also reduced (by 8.4 % and 16.7 % as compared to controls, respectively). The resorption rate was 14.9 %. Body weights in the lower groups were (most likely) not affected. Foetal body weights were significantly reduced in the three highest dose groups, and not significantly reduced in the 100 mg/kg bw/exposure group. In the lowest dose group, no effect on foetal body weights was observed. Malformations reported in this study are summarised in the table below. Since only two out of six dams survived in the highest dose group and these had severely reduced body weights, malformation data from this group are considered unreliable and were not investigated. The number of examined foetuses or litters was not reported.

Table: *Malformations in percent reported in Anonymous 17 (1984, modified from table 20 of the CLH report); top dose data (400 mg/kg bw/exposure) was not investigated due to pronounced maternal mortality.*

Dose (mg/kg bw/exposure)	**0**	**50**	**100**	**200**
Total incidence in %	0.5 ± 0.5	2.0 ± 1.0	11.1 ± 4.5	46.8 ± 6.8**
Syndactyly/Retrodactyly				
Total	0	0	1.2 ± 0.8	15.3 ± 6.3**
Forelimb	0	0	1.2 ± 0.8	14.0 ± 6.5**
Hind limb	0	0	0	4.6 ± 3.4
Amelia	0	0	0	1.2 ± 0.8
Anaemia	0	0	0	2.6 ± 1.5*
Cleft palate	0	0	0	0.5 ± 0.5
Urogenital syndrome				
Total	0	0	4.4 ± 4.4	40.8 ± 8.0**
Symmetric	0	0	3.3 ± 3.3	27.6 ± 8.5**
Asymmetric	0	0	1.1 ± 1.1	13.2 ± 3.0
Hydronephrosis	0.5 ± 0.5	2.0 ± 1.0	5.1 ± 2.4*	1.9 ± 1.0
Ecchymosis	0.5 ± 0.5	0	3.8 ± 2.6	1.2 ± 0.8
Horizontal cardiac apex	0	0	2.8 ± 1.5	4.2 ± 1.9

*p < 0.05; **p < 0.01

Syndactyly and retrodactyly were observed in the 100 and 200 mg/kg bw/exposure groups; incidence was significantly different from controls in the higher dose group. Anaemia was observed only in the 200 mg/kg bw/exposure group. Urogenital syndrome was also observed in these dose groups, but statistical significance was again reached only in the higher dose group where 41 % of the examined foetuses (total numbers were not reported) showed this pattern of malformations. Small percentages of foetuses with hydronephrosis and ecchymosis were

reported in all dose groups without a clear dose dependence and statistical significance only for hydronephrosis in the 100 mg/kg bw/exposure group.

Urogenital malformations were also observed in the developmental toxicity study published by Bleyl (1990) which was deemed to be of low reliability by RAC due to a lack of reporting of crucial endpoints. No information is available on the effects in dams. Malformations were reported in the two highest dose groups (80 and 160 mg/kg bw/d). However, in the highest dose group most offspring died on the first postnatal day. In the 80 mg/kg bw group, 15.5 % of the surviving foetuses (no absolute numbers given) exhibited uni- or bilateral agenesis and/or hydronephrosis.

Lactation

No data are available showing effects of 3-methylpyrazole on or via lactation.

Conclusion on classification

Weighing the evidence, RAC primarily considers the two OECD TG 414 studies in rats, to be sufficiently reported and reliable. In both studies, rats were treated orally with 3-methylpyrazole from GD6 to GD15, either by gavage or via drinking water. One prenatal toxicity study (Anon. 17, 1984) with an unconventional dosing regimen on gestational days 4, 10, 13, and 18 serves as supportive evidence in the assessment.

Soft tissue malformations in 8.6 % (14/163) of foetuses were observed in one TG 414 study at the top dose of 90 mg/kg bw/d given by gavage, including uni- and bilateral kidney agenesis, hydronephrosis, and malformations of great vessels. Corrected body weight gain of dams from GD6 to GD20 was 17.5 % lower than in control dams. In a similarly designed study at slightly lower actual dose levels applied via drinking water, no such malformations were observed. Maternal body weight gains were also reduced in this study.

RAC considers the findings from a third study with intermittent dosing as supporting evidence. Under these conditions, the malformation rate was 46 % (200 mg/kg bw/exposure) and 11 % (100 mg/kg bw/exposure) including malformations of the urogenital tract, cardiovascular system and forelimbs. At a dose level of 200 mg/kg bw/exposure, maternal body weight gain from GD1 to GD20 was 16.7 % lower than in controls.

In rat offspring from another non-guideline study, malformations of the urogenital system was described with limited reporting after a two days of treatment on GD10 and GD11 with doses of 80 and 160 mg/kg bw/exposure.

RAC concurs with the DS that malformations observed in the urogenital tract and other organ systems of foetuses in three studies in rats are consistent and severe. RAC notes that pregnant rats seem to be specifically sensitive to the substance. However, RAC is of the opinion that malformations cannot be attributed solely to maternal toxicity documented as reduced maternal weight gain. The quality of the data is considered sufficient for classification purposes. Based on these studies, RAC concludes that **classification as Repr. 1B; H360D is warranted**.

RAC concurs with the DS that **no conclusion can be drawn on classification for fertility and lactation effects, due to a lack of data**.

Additional references

Pack *et al*. (1981) *The cells of the tracheobronchial epithelium of the mouse: a quantitative light and electron microscope study*, J Anat 132 (1)7:1-84

Plopper *et al*. (1994) *Dose-Dependent Tolerance to Ozone - I. Tracheobronchial Epithelial Reorganization in Rats After 20 Months' Exposure*, Am J Pathol 144:404-421

Boers *et al*. (1999) *Number and Proliferation of Clara Cells in Normal Human Airway Epithelium,* Am J Respir Crit Care Med 159:1585–1591.

Dow Chemicals Co. (1990) *DRINKING WATER TERATOLOGY STUDY IN FISCHER 344 RATS,* NTIS report number: OTS0537366, https://ntrl.ntis.gov/NTRL/ (accessed: 16.10.2019)

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).