Committee for Risk Assessment

RAC

Annex 1

Background document

to the 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

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

5 December 2019

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

International Chemical Identification:

3-methylpyrazole

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

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

Index Number: **NA**

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Version number: 2 **Date: 12 february 2019**

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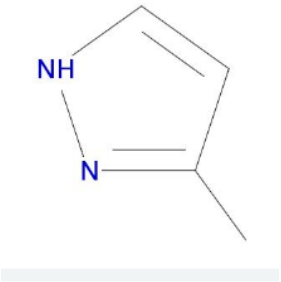
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 3-METHYLPYRAZOLE

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	3-methyl-1H-pyrazole
Other names (usual name, trade name, abbreviation)	3-methylpyrazole
ISO common name (if available and appropriate)	
EC number (if available and appropriate)	215-925-7
EC name (if available and appropriate)	3-methylpyrazole
CAS number (if available)	1453-58-3
Other identity code (if available)	
Molecular formula	C4H6N2
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	82.10
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	
Description of the manufacturing process and identity of the source (for UVCB	

substances only)	
Degree of purity (%) (if relevant for the entry in Annex VI)	>98.2%

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self- classification and labelling (CLP)**
3-methylpyrazole EC n° 215-925-7	>98.2 - <98.6%	NA	Acute Tox. 4, H302 Skin Corr. 1B, H314 Eye Dam. 1, H318 Repr. 2, H361

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self- classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
See confidential annex				The impurities are not considered relevant for the classification of the substance

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
NA					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	613-RST-VW-Y	3-methypyrazole	215-925-7	1453-58-3	Acute Tox. 4, Skin Corr. 1B, Eye Dam. 1, STOT RE 1, Repr.1B	H302 H314 H318 H372 (lung) H360D	GHS08 GHS07 GHS05 Dgr	H302 H314 H318 H372 (lung) H360D		ATE (oral) : 500 mg/kg bw	
Resulting Annex VI entry if agreed by RAC and COM	613-RST-VW-Y	3-methylpyrazole	215-925-7	1453-58-3	Acute Tox. 4, Skin Corr. 1B, Eye Dam. 1, STOT RE 1 Repr.1B	H302 H314 H318 H372 (lung) H360D	GHS08 GHS07 GHS05 Dgr	H302 H314 H318 H372 (lung) H360D		ATE (oral) : 500 mg/kg bw	

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	Acute Tox. 4, H302	Yes
Acute toxicity via dermal route	data lacking	No
Acute toxicity via inhalation route	data conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	Skin Corr. 1B, H314	Yes
Serious eye damage/eye irritation	Eye Dam. 1, H318	Yes
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	hazard class not assessed in this dossier	No
Germ cell mutagenicity	hazard class not assessed in this dossier	No
Carcinogenicity	hazard class not assessed in this dossier	No
Reproductive toxicity	Repro. 1B, H360D	Yes
Specific target organ toxicity-single exposure	hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	STOT RE 1, H372	Yes
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	hazard class not assessed in this dossier	No
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

3-methylpyrazole is a chemical substance which is registered under REACH (1907/2006/EC). The substance is not listed in annex VI of CLP and classification and labelling was not previously discussed by the TC C&L The substance is self-classified in the public registration dossier as :

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

The substance is also under substance evaluation (REACH).

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.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[A.] There is no requirement for justification that action is needed at Community level : the substance is toxic to reproduction. The substance is self-classified as Repr. 2, H361.

[B.] Justification that action is needed at Community level is required.

Justification for the hazard classes/differentiations other than reproductive toxicity and within the scope of this public consultation :

- Disagreement by DS with current self-classificationt (by the notifiers and/or registrants)
- Requirement for harmonised classification by other legislation or process : relevant for f.i. substance evaluation

5 IDENTIFIED USES

The substance is used in fertilisers.

6 DATA SOURCES

Registration dossier

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Physical state at 20°C and 101,3 kPa	Liquid at 20°C and 101.3 kPa	Anonymous 1 (2011)	EPA OPPTS 830.6303 GLP

Property	Value	Reference	Comment (e.g. measured or estimated)
			Rel.1
Melting/freezing point	> -53.9 - < -39.1 °C at 1 013 hPa	Anonymous 1 (2011)	OECD TG 102 (capillary method) GLP Rel.1 3-methylpyrazole showed a melting range, no melting point
Boiling point	204 °C at 1 013 hPa	-W.M. Haynes, 2011 CRC-Handbook of Chemistry and Physics, 91 st Edition, 2011, p. 3-372 -Syracuse Research Corporation (SRC), Physical Properties Database (PHYSPROP), 2012	No guideline available GLP : not specified Rel.2
Relative density	1.02 g/cm³ at 25°C	W.M. Haynes, 2011 CRC-Handbook of Chemistry and Physics, 91 st Edition, 2011, p. 3-372	No guideline available GLP : not specified Rel.2
Vapour pressure	182 Pa at 20 °C and 243 Pa at 25°C	Anonymous 2 (2009)	OECD TG 104 (static method) GLP Rel.1
Surface tension	63.26 mN/m at 20°C	Anonymous 3 (2009)	OECD TG 115 (plate method) GLP Rel.1
Water solubility	> 1 000 g/L at 20°C	Anonymous 4 (2011)	OECD TG 105 (flask method) Rel.1 Deviation from guideline : Due to the high water solubility of the substance, it was not possible to weigh the fivefold saturation concentration of the test item in water in order to perform a main study
Partition coefficient n-octanol/water	Log Pow= 0.475 at 25°C, pH6.9	Anonymous 5 (2008)	OECD TG 117 (HPLC method) GLP Rel.1
Flash point	103.5 °C at 1 013 hPa	Anonymous 6 (2011)	EU A.9 (equilibrium method closed cup) GLP Rel. 1
Flammability	Non-flammable		
Explosive properties	Non explosive	Anonymous 7 (2008)	EU A.14

Property	Value	Reference	Comment (e.g. measured or estimated)
			GLP Rel.1
Self-ignition temperature	532 °C at 1 013 hPa	Anonymous 8 (2011)	EU A.15 GLP Rel.1
Oxidising properties	Based on the molecular structure, 3-methylpyrazole is considered non oxidising		
Granulometry	Not relevant for a liquid		
Stability in organic solvents and identity of relevant degradation products	the stability of 3-methylpyrazole is stated to be uncritical		
Dissociation constant	pKa of 3.450 ± 0.012 at 20.0 ± 0.1°C pKb of 10.550 ± 0.012 at 20.0 ± 0.1°C	Anonymous 9 (2013)	OECD TG 112 GLP Rel.1
Viscosity	15.352 ± 0.001 mPa * s at 20.00 ± 0.02 °C and 5.968 ± 0.019 mPa * s at 40.00 ± 0.00 °C	Anonymous 10 (2013)	OECD TG 114 (Rolling Ball Viscosimeter) Rel.1

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this CLH dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Toxicokinetics *in vivo* study (ADME) Gavage In rats (Wistar) : 5/sex for ADE examination, 3/sex for M analysis and 3 animals for examination of passage of placental barrier Number of exposure : single, 5 or 7 times Doses : 5 mg/kg bw (for single and 5 times) and 50 mg/kg bw (for 7 times) 3-methylpyrazole Vehicle : water Non-guideline	Maximal organ burden : after 30-60min Excretion : quickly (93-96% after 24h) via urine Crosses the placental barrier No bioaccumulation	No analysis of possible metabolites was performed	Anonymous 11 (1982)

Method	Results	Remarks	Reference
Non-GLP			

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

In a toxicokinetic study (anonymous 11 (1982)), female and male rats were given by gavage 3-methylpyrazole. 5 males and 5 females received test item to examine ADE, 3 males and 3 females were exposed to examine M and 3 animals were gavaged to analyse the passage of the placental barrier. Animals were given 5 mg/kg bw (single exposure or 5 times) or 50 mg/kg bw (7 times).

After administration, the maximal organ burden was determined after 30-60min. Thereafter, the substance is excreted via urine (93-96% within 24h). Moreover, 3-methylpyrazole crosses the placental barrier.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Table 9: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral toxicity study Gavage OECD TG 423 GLP	Rat (CD/Crl:CD(SD)) 3 females/group	3-methylpyrazole Purity 97.9% Vehicle : 0.8% aqueous hydroxyl-methylcellulose	Conc. : 300 and 2000 mg/kg bw Observation period : 14D	LD50 : > 300 and < 2000 mg/kg bw 2000 mg/kg bw : reduced motility and muscle tone, ataxia, dyspnoea + dorsal position in all animals (+ lateral position in 2 animals) All animals died (2 within 24h and all after 7D) 300 mg/kg bw : no effects and no premature death Necropsy : no effects	Anonymous 12 (2012)

No human data or other information available

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

In an acute oral toxicity study (anonymous 12 (2012)), performed following OECD TG 423, groups of 3 females rats were exposed by gavage to 3-methylpyrazole. At the first step, 3 females received 2000 mg/bw. If no mortality was observed, no further testing was performed. However, if 2 out of 3 animals died, a second step was performed and 3 females were treated with 300 mg/kg bw. After this second step, if less than 2 animals died, the dose level was retested. However, if 2 out of 3 animals died, a third dose level was performed (50 mg/kg bw).

During the first step of the study, all females exhibited ataxia, dyspnoea and dorsal position, moreover, lower motility and muscle tone were observed. 2 animals died within 24h and all were dead after 7d. At necropsy, no findings were noted.

Due to the results of the first step, 3 females were exposed to 300 mg/kg bw. No mortality or clinical signs were noted. The dose level has been retested and confirmed the results.

Based on the results, the LD50 was beetwen 300 and 2000 mg/kg bw.

10.1.2 Comparison with the CLP criteria

Oral acute toxicity criteria	Results of the available study
Category 4 : LD50 between 300 and 2000 mg/kg bw	LD50 between 300 and 2000 mg/kg bw (300 mg/kg bw : no mortality ; 2000 mg/kg bw : all animals die)

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the available results (LD50 between 300 and 2000 mg/kg bw), a classification as **Acute Tox. Cat. 4 H302 (Harmful if swallowed)** is warranted. Furthermore, based on Table 3.1.2 of the CLP Regulation ((EC) No 1907/2006), an ATE of 500 mg/kg bw is warranted.

10.2 Acute toxicity - dermal route

No information available

10.3 Acute toxicity - inhalation route

Table 10: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, , form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Acute inhalation toxicity study Gas Similar to OECD TG 403 Reliability 2 (study parameters not described in detail : size of test chamber, conc. Of test substance in the test chamber) however this study was considered valide No-GLP	Rat (Wistar) 5/sex/dose	3-methylpyrazole Vehicle : air	Dose levels : 2065, 3380, 4180, 7930, 18750 and 28110 mg/m³ Duration of exposure : 4h	> 28110 mg/m³ Mortality, clinical signs and necropsy examination : no effects observed	Anonymous 13 (1988)

No human data or other information available

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

In an acute inhalation toxicity study (anonymous 13 (1988)), similar to OECD TG 403, groups of 5 male and 5 female rats were exposed to 3-methylpyrazole at a concentration of either 2065, 3380, 4180, 7930, 18750 or 28110 mg/m³. No mortality, clinical signs or necropsy findings were observed. The LC50 was higher than 28110 mg/m³.

10.3.2 Comparison with the CLP criteria

Inhalation acute toxicity criteria	Results of the available study
Category 4 (gas) : LC50 between 2500 and 20000 ppm	One available study during which no mortality was observed (LC50 higher than 28110 mg/m³)

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on the results of the acute inhalation toxicity study (no mortality observed, LC50 > 28110 mg/m³), no classification is warranted.

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**.

10.4 Skin corrosion/irritation

Table 11: Summary table of other studies relevant for skin corrosion/irritation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
In vitro/ ex vivo skin irritation study (EpiDerm)	3-methylpyrazole Purity : 98.10%	Conc. : 50 µl Duration of exposure : 3 min or	After 3 min of exposure : Relative absorbance value : 73.8 %	Anonymous 14 (2011)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Human skin model test OECD TG 431 (no deviations)	Vehicle : none	1h Nb. of tissues : 2	(threshold for corrosivity : 50 %) After 1 h of exposure : Relative absorbance value : 14.9 % (threshold for corrosivity : 15 %) Absorption value after 3min : 1.482 (negative control : 2.009; positive control : 0.586) Absorption value after 1h : 0.281 (negative control : 1.883; positive control : 0.456)	

No animal or human data available

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

An *in vitro* human skin model test (anonymous 14 (2011)) was performed following OECD TG 431 (no deviations). 2 tissues were treated with 50 µl of 3-methylpyrazole during 3 min or 1 hour.

After 3 minutes of treatment, the relative absorbance value was reduced to 73.8% (above the threshold value for corrosion potential (50%)). After 1 hour of treatment, the relative absorbance value was reduced to 14.9% (below the threshold value for corrosion potential (15%)).

10.4.2 Comparison with the CLP criteria

EpiDerm criteria for classification	Results of the available study
Viability measured after exposure time points (3min and 1h) : ▪ < 50% after 3min ➔ Corrosive (optional Sub-category 1A) ▪ ≥ 50% after 3min AND < 15% after 60min ➔ Corrosive (a combination of optional sub-categories 1B and 1C) ▪ ≥ 50% after 30 min AND ≥ 15% after 60min ➔ non-corrosive	In the avalaible appropriate validated *in vitro/ex vivo* human skin model test (anonymous 14 (2011)) After 3min : 73.8% After 1h : 14.9% ➔ Corrosive substance

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Based on the available information, a classification as **Skin corrosion Cat. 1 H314 (Causes severe skin burns and eye damage)** 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.

10.5 Serious eye damage/eye irritation

Table 12: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
In vitro/ex vivo eye irritation study BCOP test Bovine eye OECD TG 437	3-methylpyrazole Purity : 98.10% Vehicle : /	Conc. : 750 µl Duration of exposure : 10min Nb. of tissues : 3	IVIS : Test item : 85.73 Positive control : 215.79 Negative control : - 0.216	Anonymous 15 (2011)

No animal or human data available

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

An *in vitro* eye irritation study, BCOP test (anonymous 15 (2011)), was performed following OECD TG 437. 3 tissues were treated with 750 µl of 3-methylpyrazole during 10 min.

The *in vitro* irritancy score was 85.73 for the test substance (215.79 for the positive control and -0.216 for the negative control).

10.5.2 Comparison with the CLP criteria

OECD TG 437 criteria	Results of the available study
IVIS : ▪ ≤ 3 : no category ▪ > 3 and ≤ 55 : no prediction can be made ▪ > 55 : Category 1	The appropriate validated *in vitro* eye irritation study (anonymous 15 (2011)) revealed an IVIS of 85.73

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Based on the available results, a classification as **Eye damage Cat. 1 H318 (Causes serious eye damage)** is warranted.

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**.

10.6 Respiratory sensitisation

No information available

10.7 Skin sensitisation

Not evaluated in this CLH report.

10.8 Germ cell mutagenicity

Not evaluated in this CLH report.

10.9 Carcinogenicity

Not evaluated in this CLH report.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

No information available

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

No two-generation or extended one-generation toxicity study is available. Furthermore, the repeated dose toxicity studies (see chapter 10.12) do not indicate lesions in the reproductive organs.

10.10.3 Comparison with the CLP criteria

Criteria for Category 1	Criteria for Category 2	Results of the available studies
"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. Category 1A : the classification is largely based on evidence from humans Category 1B : the classification is largely based on data from animals studies. Such data shall provide clear evidence of an adverse effect on sexuakl 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 no 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."	"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 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."	No two-generation or extended one-generation toxicity study is available. However, the repeated dose toxicity studies do not indicate lesions in the reproductive organs. No classification warranted due to lack of data

10.10.4 Adverse effects on development

Table 13: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Developmental toxicity study Rat (Wistar) 25 pregnant females/group	3-methylpyrazole Purity : 99.9% Vehicle : water Gavage	Dams : 90 mg/kg bw/d : decrease food consumption + significant lower bw and uterus weight 45 mg/kg bw/d : decrease food consumption + ↓ bw Foetuses :	Anonymous 16 (1992)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD TG 414 GLP	Conc. : 0, 15, 45 and 90 mg/kg bw/d Duration of exposure : GD 6-15	90 mg/kg bw/d : lower foetal bw Delayed ossification + malformation of the urogenital tract, cardio-vascular system and thoracic vertebral bodies 45 mg/kg bw/d : significant decrease foetal bw No teratogenic effects	
Developmental toxicity study Rat (Wistar) Nb. of animals : not specified No guideline followed No info on the GLP status	3-methylpyrazole Purity : unknown Vehicle : water Oral (no more information) Conc. : 0, 20, 40, 80 and 160 mg/kg bw/d Duration of exposure : 10 and 11 dpc	Dams : No effects observed Fetuses : 160 mg/kg bw/d : significant lower viability and live birth indices. Necropsy revealed urogenital syndrome 80 mg/kg bw/d : urogenital malformation (uni and bi-lateral kidney agenesis, hydronephrosis)	Bleyl D.W.R (1990)
Prenatal toxicity study Rat (Wistar) Nb of animals : 13, 13, 12, 14 and 6 rats (respectively at 0, 50, 10, 200 and 400 mg/kg bw/d) No guideline followed No GLP information	3-methylpyrazole Purity : unknown Vehicle : water Oral (by stomach tube) Conc. : 0, 50, 100, 200 and 400 mg/kg bw/exposure Day of exposure : GD 1, 4, 10, 13, 18 and 20	Dams : 400 mg/kg bw/exposure : 4 rats died prematurely Significantly bw changes Significant increase of the post implantation loss (higher resorption rate 75% vs 12-15% in the other groups) 200 mg/kg bw/exposure : significantly bw changes NOAEL : 100 mg/kg bw/exposure Fetuses : 400 mg/kg bw/exposure : significantly lower fetal weight + placental weight modified 200 mg/kg bw/exposure : significantly lower fetal weight Dose dependent increased malformation rate : 11%, 46% and 100% respectively at 100, 200 and 400 mg/kg bw/exposure (severe alteration of the urogenital tract) NOAEL : 50 mg/kg bw/exposure	Anonymous 17 (1984)
Prenatal toxicity study Rat (strain unknown) 8 pregnant females/group No guideline	3-methylpyrazole Oral (stomach tube) Conc. : 0, 25, 100, 175 and 225 mg/kg	Dams : 225 mg/kg bw/d : all animals died or had to be killed prematurely 175 mg/kg bw/d : 6 out of 8 animals died or had to be killed prematurely. The 2 surviving animals had no live fetuses 100 mg/kg bw/d : bw changes Higher resorption rate	Anonymous 18 (1989)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
followed No GLP information	bw/d Duration of exposure : GD 6-18	Fetuses : 100 mg/kg bw/d : severe decrease of the fetal bw 1 fetus exhibited a cleft palate	

No human data or other information available

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

In a developmental prenatal toxicity study (anonymous 16 (1992)), performed following OECD TG 414, groups of 25 pregnant rats were given 3-methylpyrazole at a concentration of 0, 15, 45 or 90 mg/kg bw/d through gestation day 6 to 15. On day 20 post-coitum, all females were sacrified and necropsied.

No mortality or clinical signs were observed. Mean body weight was significantly lower at the highest doses. Furthermore, the corrected body weight (bw at GD20 minus uterus weight minus bw at GD6) was significantly reduced in the animals exposed to 90 mg/kg bw/d. At necropsy, the uterus weight was significantly decreased at the highest dose and only one dam of the lowest dose exhibited a hydrometra.

Table 14 : Body weight data in g (in g)(extent of the changes in % with respect to the controls)

Dose level (in mg/kg bw/d)	0	15	45	90
GD 0	225.0	222.4 (-1.16)	223.7 (-0.58)	224.9 (-0.04)
GD 6	254.5	250.8 (-1.45)	253.1 (-0.55)	252.3 (-0.86)
GD15	300.0	295.0 (-1.67)	292.2 (-2.6)	276.4** (-7.87)
GD20	373.3	368.4 (-1.31)	364.2 (-2.44)	352.6** (-5.55)
BWG GD 6-15	45.6	44.1 (-3.29)	39.1 (-14.25)	24.1** (-47.15)
BWG GD 0-20	148.3	146.0 (-1.55)	140.4 (-5.33)	127.7** (-13.89)
Gravid uterus weight	81.0	79.7 (-1.60)	75.2 (-7.16)	69.1** (-14.69)
Net weight change from D6	37.8	37.8	35.8 (-5.29)	31.2* (-17.46)

* $p < 0.05$; ** : $p < 0.01$

The reproductive data were unaffected (such as conception rate, mean number of corpora lutea, implantation sites, pre- and post-implantation loss, number of resorption and viable foetuses).

Table 15 : reproduction data

Dose level (in mg/kg bw/d)	0	15	45	90
Nb of females mated	25	25	25	25
Nb of females pregnant	24	22	25	25
Dams with viable fetuses	24	22	25	25
Mean nb of corpora lutea	15.7	15.5	15.5	15.8

Mean nb of implantation sites		15.3	14.9	14.5	14.7
Mean % of preimplantation loss		2.3	3.8	6.2	6.5
Mean % of postimplantation loss		8.1	5.8	5.6	7.8
Mean resorptions	Tot.	1.3	0.9	0.8	1.2
	Early	1.2	0.7	0.8	1.0
	Late	0.0	0.2	0.0	0.1
Nb of dead fetuses		0	0	0	0
Mean nb of live fetuses		14.1	14.0	13.7	13.6

Examination of the foetuses did not reveal a difference in the sex distribution (51.5/48.5, 48.7/51.3, 50.1/49.9 and 47.2/52.8% of females/males respectively at 0, 15, 45 and 90 mg/kg bw/d) or in the placental weight (0.45, 0.46, 0.46 and 0.43g respectively at 0, 15, 45 and 90 mg/kg bw/d). However, the mean fetal weight was significantly lower at the 2 highest dose levels (3.9, 3.8, 3.6** and 3.3**g respectively at 0, 15, 45 and 90 mg/kg bw/d). One foetus of one dam which was exposed to 45 mg/kg bw/d exhibited a cleft palate. Soft tissue examination revealed severe malformations in the urogenital tract and/or in the cardiovascular system in the foetuses of the highest dose. Various malformations of the sternum and/or the vertebral column were also observed in all groups.

Table 16 : Incidence of fetal soft tissue malformations

Dose level (in mg/kg bw/d)		0	15	45	90
Nb. of foetuses evaluated (Nb. of litters evaluated)		164 (24)	149 (22)	166 (25)	163 (25)
Fetal incidence (tot nb)		0	0	0	14**
Litter incidence (tot nb)		0	0	0	8**
Efferent urinary tract severely dilated (litter incidence)	Fetal incidence (%)	0	0	0	5* (3.1)
	Litter incidence (%)	0	0	0	5 (20)
Malformation of great vessels (displaced aortic arch) (litter incidence)	Fetal incidence (%)	0	0	0	6* (3.7)
	Litter incidence (%)	0	0	0	2 (8.0)
Agenesie of kidney(s) (litter incidence)	Fetal incidence (%)	0	0	0	2 (1.2)
	Litter incidence (%)	0	0	0	2 (8.0)
Agenesie of ureter(s) (litter incidence)	Fetal incidence (%)	0	0	0	2 1.2)
	Litter incidence (%)	0	0	0	2 (8.0)
Dilatation of both ventricles (globular shaped heart) (litter incidence)	Fetal incidence (%)	0	0	0	2 (1.2)
	Litter incidence (%)	0	0	0	2 (8.0)

* $p < 0.05$; ** : $p < 0.01$

Table 17 : Incidence of skeletal malformations

Dose level (in mg/kg bw/d)	0	15	45	90	Historical data in %
Nb. of foetuses evaluated (Nb. of litters evaluated)	174 (24)	159 (22)	177 (25)	176 (25)	
Fetal incidence	8	8	8	49**	
Litter incidence	6	6	5	20**	

Thoracic vertebral body/bodies dumbbell-shaped (%)	Fetal incidence (%)	6 (3.4)	5 (3.1)	3 (1.7)	39** (22)	0 – 8.8
	Litter incidence (%)	4 (17)	5 (23)	2 (8)	17** (68)	0 – 39.1
Thoracic vertebral body/bodies bipartite (%)	Fetal 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

** : $p < 0.01$; no more information on the historical control data

In another developmental toxicity study (article : Bleyl DWR, 1990), groups of pregant Wistar rats (number of animals not mentioned) were exposed on GD 10 and 11 only to 3-methylpyrazole at a concentration of 0, 20, 40, 80 or 160 mg/kg bw/d.

Regarding dams, no effects was observed on the body weight or the liver weight after 20 days (no more information available).

At the highest dose level, the rate of living pups at birth was significantly reduced (77%* compared to the control group). Moreover, most of these living pups died in the first day of live. The survival index (at PND4) was 26%**. 15.6% of the living fetuses in the 80 mg/kg bw/d dose level group exhibited urogenital syndrome. In most cases, an unilateral kidney agenesis was noted (no left kidney) coupled with a hydronephrosis in the remaining kidney. The other pups exhibited a bilateral kidney agenesis. In males, the genital tract was complete, however some cases of undescended testis were recorded. Whereas in females, the kidney agenesis was always coupled with an incomplete differentiation of the uterus. The necropsy of the fetuses of the highest dose level, which died in the first day of live, revealed also the urogenital syndrome. (no more information available)

On PND 43 for males and PND 44 for females, the renal function of the surviving pups has been investigated and revealed disturbances in females at the 2 highest dose levels only.

In another prenatal developmental toxicity study (anonymous 17, 1984), groups of pregnant Wistar rats were given by gavage 3-methylpyrazole at a concentration of 0, 50, 100, 200 and 400 mg/kg bw/exposure. Groups were composed of 13, 13, 12, 14 and 6 females rats respectively at 0, 50, 100, 200 and 400 mg/kg bw/exposure.

4 dams exposed to the highest dose level died during the exposure period. The necropsy of these animals revealed a catarrhal enteritis and/or nephrosis syndrome. Moreover, a significant decrease of the body weight was observed at this dose level. A trend to decrease was also observed at the 200 mg/kg bw/exposure level.

Table 18 : Body weight (in g)

Dose (mg/kg bw/d)	Body weight (g)					
	GD1	GD4	GD10	GD13	GD18	GD20
0	268 ± 8	276 ± 7	288 ± 8	298 ± 9	387 ± 9	334 ± 9
50	245 ± 7	257 ± 8	273 ± 9	279 ± 10	309 ± 18	328 ± 11
100	255 ± 7	262 ± 7	280 ± 8	283 ± 10	315 ± 7	292 ± 33
200	251 ± 5	260 ± 6	270 ± 6	275 ± 7	298 ± 7	306 ± 8*
400	253 ± 15	264 ± 15	263 ± 15	256 ± 16**	246 ± 15**	258 ± 15**

* = $p<0.05$, ** = $p<0.01$

The necropsy of dams did not reveal absolute liver weight modifications, only a slight dose related increase relative liver weight (absolute liver weight : 12.2, 12.5, 13.1, 12.1 and 11.3 g respectively at 0, 50, 100, 200 and 400 mg/kg bw/exposure; relative liver weight : 2.6, 3.8, 3.9, 4.0 and 4.2 g/100g bw respectively at 0, 50, 100, 200 and 400 mg/kg bw/exposure).

No effects on the pre-implantation loss was recorded. Nevertheless, percentage of post-implantation loss was significantly increased at the highest dose level (11.5, 13.8, 11.8, 14.9 and 74.9**%** respectively at 0, 50, 100, 200 and 400 mg/kg bw/exposure). Furthermore, in 4 out of 6 dams of the highest dose level, all progeny died by resorptions.

Table 19 : Resorptions and dead foetus (in %)

Dose level (in mg/kg bw/d)	0	50	100	200	400
Early	9.6	6.7	10.1	10.5	2.5
Middle	1.9	7.1	0	3.0	69.8**
Late	0	0	0	0.5	0
Dead foetus	0	0	0	0.5	2.6*
MS calculated summation	11.5	13.8	10.1	14.5	74.9

** : $p < 0.01$

The apparent discrepancies between the reported % of post-implantation loss and the MS calculated summation (resopriotns and dead foetus) come directly from the data specified in the full study report (anonymous 17, 1984, page 19), BE CA transcribes the same data in the current document.

Regarding the foetal examination, the body weight was significantly reduced at the 2 highest dose levels (1.80, 1.80, 1.70, 1.40** and 1.05**g respectively at 0, 50, 100, 200 and 400 mg/kg bw/d). The placental weight was also modified (0.43, 0.46, 0.46, 0.41 and 0.28g** respectively at 0, 50, 100, 200 and 400 mg/kg bw/exposure). All fetuses of the highest dose presented at least one malformation (46.8% at 200 mg/kg bw/exposure and 11.1% at 100 mg/kg bw/exposure).

Table 20 : Malformation data (in %)

Dose (mg/kg)	0	50	100	200	400
Syndactilie/Retrodactilie					
Total	0	0	1.2 ± 0.8	15.3 ± 6.3§	81.3 ± 18.8§
Forelimb	0	0	1.2 ± 0.8	14.0 ± 6.5§	81.3 ± 18.8§
Hind limb	0	0	0	4.6 ± 3.4	50.0 ± 37.5§
Amelia	0	0	0	1.2 ± 0.8	6.3 ± 6.3
Anemia	0	0	0	2.6 ± 1.5+	0
Cleft palate	0	0	0	0.5 ± 0.5	12.5 ± 12.5
Urogenital syndrome					
Total	0	0	4.4 ± 4.4	40.8 ± 8.0§	58.8 ± 31.3§
Symmetric	0	0	3.3 ± 3.3	27.6 ± 8.5§	50.4 ± 25.0§
Asymmetric	0	0	1.1 ± 1.1	13.2 ± 3.0	31.3 ± 6.3§
Hydronephrose	0.5 ± 0.5	2.0 ± 1.0	5.1 ± 2.4+	1.9 ± 1.0	0
Ecchymosis	0.5 ± 0.5	0	3.8 ± 2.6	1.2 ± 0.8	0
Horizonal cardiac apex	0	0	2.8 ± 1.5	4.2 ± 1.9	6.3 ± 6.3
Total (%)	0.5 ± 0.5	2.0 ± 1.0	11.1 ± 4.5	46.8 ± 6.8§	100 ± 0.1§

$^{+}$ = $p<0.05$, §= $p<0.01$

In another developmental toxicity study (anonymous 18, 1989), groups of 8 pregnant rats were received by gavage 3-methylpyrazole at a concentration of 0, 25, 100, 175 and 225 mg/kg bw/d. Animals were exposed from GD 6 to 18 and were sacrified at GD20.

6 out of 8 dams exposed to 175 mg/kg bw/d and all dams exposed to 225 mg/kg bw/d died or were sacrificed in extremis respectively. Regarding the animals receiving 100 mg/kg bw/d of 3-methylpyrazole, moderate to severe decrease body weight was noted during GD 6-12. Furthermore, an increase in resorptions was noted at this dose level and no fetus was produced by the 2 females of the 175 mg/kg bw/d group which survived.

Regarding the fetuses examination, the fetal weight was reduced in the 100 mg/kg bw/d group. Moreover, one fetus of this group exhibited external malformations such as cleft palate. However, this fetus weighted only 1.2 g.

10.10.6 Comparison with the CLP criteria

Criteria for Category 1	Criteria for Category 2	Results of the available studies
"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. Category 1A : the classification is largely based on evidence from humans Category 1B : the classification is largely based on data from animals 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 no 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."	"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 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."	See above

In the developmental prenatal toxicity study (anonymous 16 (1992)), dams exhibited a significant decrease of body weight only at the highest dose level (90 mg/kg bw/d). At this dose level, severe fetal malformations in the urogenital tract and/or in cardiovascular system were observed. The incidence of these malformations was significantly higher at the highest dose (14 foetus exhibited malformations vs 0 in the other groups). In 2 out of these animals, an agenesis of kidney was noted. Furthermore, skeletal malformations were also

observed and were outside the range of the historical control data. In addition to that a significant decrease of the fetal body weight was already observed at the mid dose level (45 mg/kg bw) which cannot be explained by any parental toxicity.

Moreover, in another developmental toxicity study (Bleyl DWR, 1990), dams did not show any maternal toxicity. Nevertheless, the rate of living pups at birth was significantly reduced and most of the living pups died during the first day of life. Pups exhibited severe urogenital malformations (kidney(s) agenesis and/or hydronephrosis).

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.

3 different studies revealed severe malformations of the urogenital tract already at dose levels where no maternal toxicity was observed. Those malformations vary from severe dilatation of the efferent urinay tract and malformations of great vessels to the complete absence of kidney. Therefore, the criteria for the category 1B is fulfilled.

10.10.7 Adverse effects on or via lactation

No information available

10.10.8 Conclusion on classification and labelling for reproductive toxicity

Based on the available information, a classification as **Repr. 1B H360D (May cause damage on unborn child)** is warranted.

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 (IND) commented. The MSCAs supported classification as Repr. 1B; H360D. The IND 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.

Additional key elements

In an IND comment during the public consultation, two additional reproductive toxicity studies for 3-methylpyrazole were summarised.

One three-generation study in Wistar rats was summarised but no reference or details were given. It was assigned Klimisch score 3 by the commenting IND. In this study, up to 10 mg/kg bw/d were administered to three generations of rats via feed. No effects on mortality, health status, or behaviour were reported. At the high dose, fertility index at the first mating of the P and F2 generations was significantly reduced. No external malformations in pups were observed.

The second study was identified as a teratology study in Fisher 344 rats by Dow Chemical Company (U.S. National Technical Information Service (NTIS) number OTS0537366; report date: 23.10.1990). The study report is publicly available on the NTIS website. This study was performed according to GLP and OECD TG 414. 3-methylpyrazole with a purity of 99.5 % was administered via drinking water at concentrations targeted to provide doses of 0, 10, 50, and 100 mg/kg bw/d, to groups of 30 female rats from gestation day (GD) 6 to GD 15. Concentrations in drinking water were analytically verified. Actual intake was calculated from water consumption and body weight data as 0, 10.3, 44.9, and 77.3 mg/kg bw/d.

Doses were chosen based on a range finding study and a teratology probe study in the same strain of rats, with a highest dose of 300 mg/kg bw/d. In these studies, decreases in water consumption were detected at all dose levels tested. Statistically significant, dose-dependent decreases in body weight, body weight gains and food consumption, and increases in relative kidney weights, were observed in the 200 and 300 mg/kg bw/d groups. At 300 mg/kg bw/d, perineal soiling was observed in all animals.

In the main study, decreases in maternal body weight gain were observed in the 50 mg/kg bw/d and the 100 mg/kg bw/d groups (15 % and 31 % on DG 6 to 16 in the mid and high dose group, respectively). These were consistent with decreased water and food

consumption in these groups. There were no significant differences in the pregnancy rates, number of corpora lutea and implantation sites, pre- or post-implantation losses or litter size in any of the treatment groups. Dose-related, statistically significant decreases in foetal body weights were noted in all treated groups when compared with controls. These differences ranged from a 4.3 % decrease at 10 mg/kg bw/d to an 11.7 % decrease at 100 mg/kg bw/d. However, the average litter size in the low dose group was larger than in the controls (9.4 vs. 7.8 foetuses), and the difference in foetal body weight in this group was considered secondary to the larger litter size. A low incidence of foetuses with ocular malformations, within the historical control data (HCD) in this strain, was detected in all groups, including controls (no details available). No other malformations were observed. Statistically significant increases in the incidence of delayed ossification of the cervical and thoracic vertebral centra were observed at 50 and 100 mg/kg bw/d. The decreases in foetal body weights and the incidences of delayed ossification observed in the mid and high dose groups were associated with decreases in maternal weight gain and considered secondary to unpalatability of the drinking water by the study authors.

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, IND 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, IND 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 IND 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** Anonymous 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) ↓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	**Key study 2** maternal NOAEL = 10 mg/kg bw/d foetal NOAEL = 10 mg/kg bw/d

		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** Anonymous 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:** ↓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	**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 *For detailed numbers on malformations, see table below.*

		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)	
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 Duration of exposure: GD10-11	*Dams* **no information** on bwg, gravid uterus weights, clinical observations, reproductive parameters, organ weights, histopathological/necropsy findings no effects on bw reported *Offspring*	**low reliability** short exposure duration, poor reporting maternal NOAEL unknown offspring NOAEL = 40 mg/kg bw/d

Bleyl, 1990		**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	*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	Purity unknown Vehicle unknown Gavage Conc.: 0, 25, 100,	*Dams* **no information** on exact bw, bwg, gravid uterus weights, organ weights, clinical observations, haematology,	**low reliability** small number of animals per group, poor reporting

females/group **No guideline** **No GLP** Anonymous 18, 1989	175 and 225 mg/kg bw/d Duration of exposure: GD 6-15	histopathological/necropsy findings, reproductive parameters, mating procedure **225 mg/kg bw/d:** **all animals died or had to be killed in extremis** **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)	maternal NOAEL = 25 mg/kg bw/d foetal NOAEL = 25 mg/kg bw/d

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; Anonymous 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 (Anonymous 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 IND 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.

Criteria

Substances should be classified into Category 1 when there is sufficient evidence from human data (1A) or animal testing (1B), possibly supplemented with other information, to provide "a strong presumption that the substance has the capacity to interfere with reproduction in humans".

Substances are placed in Category 2, when evidence from human data or animal testing are

not sufficiently convincing to warrant classification in Category 1. Category 2 is also more appropriate when deficiencies in available studies make the quality of evidence less convincing.

Conclusion on classification

In a weight of evidence approach, RAC primarily considers the two OECD TG 414 studies in rats, 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 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**.

10.11 Specific target organ toxicity-single exposure

Not evaluated in this CLH dossier

10.12 Specific target organ toxicity-repeated exposure

Table 21: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Short-term oral toxicity study Mouse (B6C3F1) 5/sex/dose OECD TG 407 GLP	3-methylpyrazole (purity : 99.7%) Via drinking water Conc. : 0, 900, 1125 and 1575 ppm (corresponding to 0/0, 135/173, 153/198 and 167/245 mg/kg bw/d respectively in males/females) Duration of exposure : 28d	Mortality and clinical signs : no effects Bwg : change in ♀ (bw : unaffected) ↑ lung weight (abs. and rela.) Histopathology : change in lungs in all animals (karyomegaly in the epithelium of the air ducts, loss of domes in the Clara cells, hypotrophy of the air duct epithelia) No NOAEL identified	Anonymous 19 (1996)
Short-term oral toxicity study Mouse (B6C3F1) 5/sex/dose for main groups + 5/sex/dose for recovery groups (14D of recovery) EU Method B.7 GLP	3-methylpyrazole (purity : 99.4%) Via drinking water Conc. : 300, 900 and 1575 ppm (± 0/0, 70/82, 151/193 and 223/252 mg/kg bw/d in males/females at 0, 300, 900 and 1575ppm) Duration of exposure : 28d Recovery period : 14d	300 ppm : slight ↓ food and water consumption in ♀ ↑ lung weight in ♀ Moderate Clara cell alteration in ♂/♀ 900 ppm : tremor and hunched posture in ♀ ↓ bwg in ♀ and ↓ food and water consumption in ♂/♀ ↑ lung weight in ♂/♀ Moderate Clara cell alteration in ♂/♀ Parenchymal lung changes in a few mice 1575 ppm : tremor and hunched posture in ♀ ↓ bw, food and water consumption in ♂/♀ ↑ lung weight in ♂/♀ Moderate to marked Clara cell alteration in ♂/♀ Parenchymal lung changes in a few mice Complete recovery not accomplished in a 14 D follow up period No NOAEL identified	Anonymous 20 (1997)

Short-term oral toxicity study Mouse (B6C3F1) 3/sex/dose Non-guideline Non-GLP	3-methylpyrazole (purity : 99.77%) Via drinking water Conc. : 0, 225 and 675 ppm (corresponding to 0/0, 47/61 and 140/173 mg/kg bw/d in males/females) Duration of exposure : 2w	Mortality, clinical signs, bw, organ weight, gross pathology, histopathology : no treatment-related effects NOAEL : > 675 ppm	Anonymous 21 (1996)
Subchronic oral toxicity study Rat (Wistar) 10/sex/dose for main groups (+ 10/sex/dose for recovery groups (28D of recovery) OECD TG 407 and 408 GLP	3-methylpyrazole (99.34%) Via drinking water Conc. : 0 and 40 mg/kg bw/d Duration of exposure : 90d Recovery period : 28d	Mortality, clinical signs and bw : no effects Organ weight examination (kidneys, liver and lungs) : ↑ kidney and liver weights (abs. + rela.) in ♀ but fully reversible at the end of the recovery period Histopathology examination (kidneys, liver and lungs) : no treatment-related effects NOAEL : 40 mg/kg bw/d	Anonymous 22 (1999)
Subchronic oral toxicity study Rat (Wistar) 24/sex/dose (exception : 36/sex for control group) Non-guideline Non-GLP	3-methylpyrazole Via gavage Conc. : 0, 0.2, 2, 20 and 200 mg/kg bw/d Duration of exposure : 90d	200 mg/kg bw/d : ↓ bw and food consumption in ♂/♀ Hematology and clinical biochemistry : ↑ nb. of neutrophilic lymphocytes, ASAT, ALP activity, ↓ tot. prot., albumin, glucose in both sexe and ↓ ChE activity in ♀ Organ weight : lower brain, spleen, thymus and testes weight and higher liver weight at the highest dose level Alteration thyroid glands Liver : nucleus anisomorphism, fatty degeneration and cell death NOAEL : 20 mg/kg bw/d	Anonymous 23 (1980)
Subchronic oral toxicity study Mouse (B6C3F1) 10/sexe/dose + 10/sex/groups for recovery groups OECD TG 408 GLP	3-methylpyrazole (purity : 98.38%) Via drinking water Conc. : 0, 5, 10, 20 and 40 mg/kg bw/d Duration of exposure : 13w Recovery period 4w	Mortality, clinical signs, hematology, clinical biochemistry, organ weight : no effects Sign. lower bw in males in all dose levels Histopathology : ≥ 10 mg/kg bw/d : Clara cell alteration (mix degenerative and regenerative process)	Anonymous 24 (2000)

Chronic oral toxicity study Rat (Wistar) 32/sex/group Non-guideline Non-GLP	3-methylpyrazole Via drinking water Conc. : 0, 10, 40 and 2000/1000 ppm (2000ppm during w1-4 thereafter 1000ppm w5-80) Duration of exposure : 18m	High mortality rate in all groups ≥ 10 ppm : dyspnea, cachexia, pneumonia 2000/1000 ppm : ↓ bw (♂ 82.3 % and ♀ 70.6 % of control group), food and water consumption, erythrocyte, Hb and Ht ↑ aminotransferase, lucine aminopeptidase, alkaline phosphatase, inhibition activity of cholinesterase (♀), cholesterol ↑ heart, liver, kidneys, brain and thyroid weight Histopathology : focal alteration in liver Ovaries : no effects on follicular maturation and evolution of Corpus luteum Testis : no effects on spermiogenesis	Anonymous 25 (1985)

No human data or other information available

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

In a short term toxicity study (anonymous 19 (1996)), performed following OECD TG 407, 3-methylpyrazole was given via drinking water to groups of 5 male and 5 female mice at a concentrations of 0, 900, 1125 or 1575 ppm during 4 weeks. The concentration in the drinking water correspond to a mean daily test substance intake of 0, 154, 176 and 206 mg/kg bw/d respectively at 0, 900, 1125 and 1575 ppm.

No mortality and no clinical signs were noted during the study. Body weight was unaffected in all dose levels (29.3/23.7, 28.6/23.7, 28.0/23.7 and 28.7/22.9g in males/females respectively at 0, 900, 1125 and 1575 ppm), however body weight gain was significantly lower in females of the highest dose level (BWG 0-28D : 4.3/4.6, 3.5/4.1, 3.0/4.2 and 3.8/3.4** g in males/females respectively at 0, 900, 1125 and 1575 ppm).

Necropsy revealed a significant higher lung weight in both sexes at the highest dose level and in males at the lowest dose level. Liver weight was significantly reduced at 1125 ppm in male. The relative organ weight observation showed only changes in lung. Histopathology examination was only performed on the lungs. Lesions of the mucus cells of the air ducts and of the Clara cells in the bronchi and bronchioles were recorded. Clara cells alteration consisted of moderate to severe disorganization of the luminal lining cell layer due to flattening of the cells and loss of the apical parts of the Clara cells and due to development of irregular shaped clara cell nuclei. Moreover, hypotrophy of the air duct epithelia (focal or diffuse) was recorded (see table 22).

Table 22 : Organ weight data

		Males				Females			
Dose level (in ppm)		0	900	1125	1575	0	900	1125	1575
Number of animals examined		5	5	5	5	5	5	5	5
Organ weights									
FBW (in g)		24.86	23.78	22.84	23.4	18.82	20.16	20.08	19.12
Kidneys weight (in mg and %)	abs	486.6	471	457.8	462.6	360.2	358.6	366.8	357.2
	Rela	1.958	1.978	2.004	1.975	1.915	1.779	1.825	1.869
Liver weight (in mg and %)	abs	1081	1012.8	958**	1043.4	894.8	975.4	989	989.6
	Rela	4.354	4.257	4.194	4.449	4.741	4.838	4.923	5.175

Lungs weight (in mg and %)	Abs	224	330*	418.6	353.6*	215	287.4	312.4	334.8**
	rela	0.898	1.392*	1.833*	1.53*	1.142	1.422	1.554	1.755
Gross lesions									
Erosion/ulcer in glandular stomach : incidence		0	3	4	4	3	4	4	5
Histopathology : changes in lungs									
Clara cell lesion	incidence	0	5	5	5	0	5	5	5
	Karyomegaly : incidence by grade 3/4	0/0	1/4	0/5	1/4	0/0	0/5	2/3	1/4
	Loss of domes : incidence	0	5	5	5	0	5	5	5
Hypotrophy focal		0	4	2	2	0	0	0	1
Hypotrophy diffuse		0	1	3	3	0	5	5	4

* : p < 0.05 ; ** : p < 0.01

In a short-term oral toxicity study (anonymous 20 (1997)), performed following EU method B.7, groups of 5 male and 5 female mice (B6C3F1) (main groups) were exposed via drinking water to 3-methylpyrazole at a concentration of 0, 300, 900 or 1575 ppm during 28 days, corresponding to 0/0, 70/82, 151/193 or 223/252 mg/kg bw/d respectively in males/females. Additionally, 5 males and 5 females (recovery groups) were exposed to the same dose levels during 28 days and were examined during a recovery period of 14 days.

No mortality occurred during the study period. Females of the mid and high dose level exhibited tremors and/or hunched posture. The body weight examination revealed a statistically significant lower value in females at the highest dose. The body weight gain was already decreased in females at 900 ppm and in males at 1575 ppm. However, during the recovery period, no differences in body weight were observed.

Table 23 : Body weight (in g)

	Males				Females			
Dose level (in ppm)	0	300	900	1575	0	300	900	1575
Exposure period								
Number of animals examined	10	10	10	10	10	10	10	10
D1	23.7	23.7	24.2	24.0	19.1	19.5	19.4	19.6
D15	25.7	26.2	25.7	25.7	21.4	21.9	20.8	20.4
D29	26.8	27.6	26.5	25.5	23.4	23.8	22.3	21.7**
BWG D0 – D29	13	16	10	6*	23	22	15**	11**
Recovery period								
Number of animals examined	5	5	5	5	5	5	5	5
D8	27.8	26.8	27.2	27.0	23.0	24.4*	23.4	22.4
D16	29.0	27.8	28.6	28.0	24.0	25.2	24.0	24.2

* : p < 0.05 ; ** : p < 0.01

Animals of the main groups were killed after 28 days of exposure. Macroscopic observations did not reveal any treatment-related changes. However, the absolute lung weight was increased in females at the mid and

high dose level, whereas the relative lung weight was already increased in females at the low dose level and was also increased in males at the mid and high dose level. Whereas, the necropsy of animals of the recovery groups revealed that the absolute lung weight was increased in females at the mid dose level while the relative lung weight was increased in females at 900 and 1575 ppm.

The microscopic examination of the main groups revealed a Clara cell alteration (moderate at 300 and 900 ppm, and moderate to marked at 1575 ppm). This modification was characterized by a loss of the characteristic dome-shaped appearance and the apical "bled", by cytokaryomegaly, and basophilia. Furthermore, at 1575 ppm, mitotic figures and/or macrophages were noted in the altered epithelium of the bronchi and bronchioli. In a few mice of the mid and high dose levels, interstitial histiocytosis, alveolar macrophages, alveolar hemorrhage, alveolar edema and/or interstitial edema/congestion were observed. The animals of all recovery groups showed also a Clara cell alteration (slight to moderate at 300 and 900 ppm and moderate to marked at 1575 ppm). In addition, slight to moderate Clara cell proliferation (characterized by increased numbers cytokaryomegalic, basophilic and sometimes multinuclear cells) was observed in all treated mice of these groups. These cells were arranged in two cell layers instead of the normal one layer. In addition, mitotic figures were occasionally observed.

Table 24 : Organ weight and histopathological modifications

		Males				Females			
Dose level (in ppm)		0	300	900	1575	0	300	900	1575
Main groups (5 mice/sex/dose)									
Absolute lungs weight (in g)		0.208	0.207	0.234	0.239	0.177	0.226	0.263**	0.249**
Relative lungs weight (in %)		0.839	0.807	0.970*	0.983*	0.794	1.006*	1.255**	1.178**
Clara cell alteration	Incidence	0/5	5/5	5/5	5/5	0/5	5/5	5/5	5/5
	Grade 2							1/5	
	Grade 3		5/5	5/5	2/5		5/5	3/5	4/5
	Grade 4				3/5			1/5	1/5
Interstitial histiocytosis incidence (grade 2)		0/5	0/5	0/5	3/5	0/5	0/5	1/5	1/5
Alveolar macrophages (grade 2)	Incidence	0/5	0/5	1/5	3/5	0/5	0/5	0/5	2/5
Alveolar hemorrhage	Incidence	0/5	0/5	2/5	4/5	0/5	0/5	2/5	2/5
	Grade 2			2/5	4/5			1/5	2/5
	Grade 3							1/5	
Alveolar edema incidence (grade 1)		0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5
Interstitial edema/congestion	Incidence	0/5	0/5	1/5	3/5	0/5	0/5	0/5	2/5
	Grade 1								1/5
	Grade 2			1/5	3/5				1/5
Recovery groups (5 mice/sex/dose)									
Absolute lungs weight (in g)		0.187	0.203	0.207	0.203	0.185	0.196	0.237**	0.221
Relative lungs weight (in %)		0.664	0.761	0.762	0.749	0.818	0.852	1.030**	0.977*
Clara cell alteration	Incidence	0/5	5/5	5/5	5/5	0/5	5/5	5/5	5/5
	Grade 2		3/5					1/5	

	Grade 3		2/5	5/5	2/5		5/5	4/5	4/5
	Grade 4				3/5				1/5
Clara cell proliferation	Incidence	0/5	5/5	5/5	5/5	0/5	5/5	5/5	5/5
	Grade 2		2/5	2/5	3/5		1/5	5/5	
	Grade 3		3/5	3/5	2/5		4/5		5/5
Alveolar macrophages incidence (grade 2)		0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5
Alveolar hemorrhage	Incidence	0/5	0/5	1/5	1/5	0/5	0/5	0/5	0/5
	Grade 2				1/5				
	Grade 3			1/5					

* : $p < 0.05$; ** : $p < 0.01$
Grade 1 : minimal/very few/very small ; Grade 2 : slight/few/small ; grade 3 : moderate/moderate number/moderate size ; Grade 4 : marked/many/large

In a short-term repeated dose toxicity study (anonymous 21 (1996)), groups of 3 male and 3 female mice were exposed via drinking water to 3-methylpyrazole at a concentration of 0, 225 or 675 ppm during 2 weeks. The concentration in the drinking water corresponded to a test substance intake of 0/0, 47/61 and 140/173 mg/kg bw/d respectively in males/females.

No mortality and no clinical signs were recorded. Body weight examination did not reveal significant changes (at D14 : 25.0/21.0, 25.2/21.2 and 25.7/21.3 g respectively at 0, 225 and 675 ppm).

Erosions/ulcers in the glandular stomach and discoloration of contents of the jejunum were observed in all groups (in 0/2 , 0/2 and 3 males/3females respectively at 0, 225 and 675 ppm).

In a subchronic toxicity study (anonymous 22 (1999)), following OECD TG 407 and 408, groups of 10 male and 10 female rats (Wistar) (main groups) were exposed via drinking water to 3-methylpyrazole at a concentration of 0 or 40 mg/kg bw/d during 90 days. Additionally, 10 males and 10 females (recovery groups) were exposed to the same dose levels during 90 days and were examined during a recovery period of 28 days.

No mortality, no clinical signs and no body weight modification occurred during the study period.

The organ weight and histopathology examination were performed on kidneys, liver and lungs. Significant increase in absolute and relative kidneys weight was noted in females (slight trend in males). Moreover, significant increase of liver weight (abs. and rela.) was observed in females. These changes did not appear at the end of the recovery period. 2 males of the control group and 1 male exposed to 40 mg/kg bw/d showed intracellular vacuoles in hepatocytes (low grade).

Table 25 : Organ weight

		Main groups				Recovery groups			
		Males		Females		Males		Females	
Dose level (mg/kg bw/d)		0	40	0	40	0	40	0	40
Kidney (left) weight (in mg)	Abs	1572.0	1602.8	1046.9	1166.3*	1679.5	1628.1	1088.3	1025.5
	Rela	0.3174	0.3356	0.3591	0.4006*	0.3294	0.3177	0.3420	0.3445
Kidney (right) weight (in mg)	Abs	1562.8	1595.3	1046.7	1183.8*	1653.6	1622.5	1067.7	1021.7
	Rela	0.3150	0.3347	0.3588	0.4069*	0.3239	0.3154	0.3349	0.3432
Liver weight (in	Abs	20229.8	19642.3	11151.3	12594.8*	20788.0	18629.9	11149.9	9715.6*

mg)	Rela	4.080	4.107	3.799	4.293*	4.036	3.596	3.474	3.271
Lungs weight (in mg)	Abs	2369.1	2241.0	1763.6	1865.4	2294.0	2234.1	1763.8	1731.7
	rela	0.4817	0.4692	0.6011	0.6381	0.4492	0.4346	0.5556	0.5823

* $p < 0.05$

In a subchronic toxicity study (anonymous 23 (1980)), rats were exposed to 3-methylpyrazole at a concentration of 0, 0.2, 2, 20 or 200 mg/kg bw/d during 90 days. Treated groups were composed of 24 females and 24 males and control groups were composed of 36 females and 36 males.

Only 2 males died during the study (1 exposed to 20 mg and 1 exposed to 200 mg/kg bw/d). The body weight decreased at the highest dose level, the modification was more severe in males.

Table 26 : Body weight data

	Males					Females				
Dose level (in mg/kg bw/d)	0	0.2	2	20	200	0	0.2	2	20	200
W0	78	78	76	76	76	72	73	72	72	71
W6	270	283	276	274	198	201	200	202	200	168
W12	359	372	362	359	262	249	248	249	246	222

Changes were noted during enzyme activity examination. At the highest dose level, aspartate aminotransferase, leucine aminotransferase and alkaline phosphatase activity were increased. Cholinesterase activity was reduced only in females at the highest dose level. (See table 27)

Table 27 : Enzyme activity data after 12w

Dose level (mg/kg bw/d)	Males					Females				
	0	0.2	2	20	200	0	0.2	2	20	200
Aspartate aminotransferase activity	1.54	1.31	1.43	1.45	2.02	1.44	1.45	1.51	1.31	1.79
Leucine aminopeptidase activity	6.25	5.36	6.15	6.38	8.60	6.12	6.80	6.70	6.81	10.57
Alkaline phosphatase activity	0.94	1.20	1.07	1.20	1.92	0.94	0.93	1.07	1.26	1.49
Cholinesterase activity	0.46	0.43	0.45	0.42	0.45	1.20	1.03	1.25	1.17	0.59

At necropsy, organ weight was examined and revealed some changes. Lower brain, spleen, thymus and testes weights were observed at the highest dose level compared to the control group. Liver weight was increased at 200 mg/kg bw/d compared to the control group. Lung weight was not recorded. The microscopic examination revealed changes in heart (slight activation of the histiocytes), thyroid (single excretory ducts dilated and filled with granulocytes and cellular debris), lungs (massing clubbing of lymphocytes) and liver (nucleus anisomorphism, fatty degeneration and cell death).

Table 28 : Organ weight (in g and in %)

Dose level (in mg/kg bw/d)		Males					Females				
		0	0.2	2	20	200	0	0.2	2	20	200
Adrenal glands	Abs	0.063	0.071	0.072	0.072	0.065	0.066	0.068	0.075	0.069	0.068
	Rela	0.0179	0.0196	0.0205	0.0198	0.0271	0.0272	0.0281	0.0313	0.0294	0.0354
Brain	Abs	1.82	1.79	1.88	1.80	1.63	1.71	0.69	1.72	1.73	1.56
	Rela	0.516	0.495	0.537	0.496	0.673	0.704	0.703	0.720	0.735	0.822

Kidneys	Abs	2.80	2.91	2.93	2.93	2.48	1.97	2.06	2.04	1.86	2.14
	Rela	0.788	0.808	0.836	0.807	0.998	0.812	0.852	0.851	0.792	1.107
Liver	Abs	15.8	15.6	18.2	18.4	16.9	10.3	11.7	13.0	11.2	13.9
	Rela	4.43	4.31	5.19	5.02	6.76	4.23	4.79	5.42	4.74	7.22
Spleen	Abs	0.763	0.766	0.811	0.771	0.500	0.638	0.642	0.663	0.600	0.477
	Rela	0.215	0.212	0.231	0.212	0.198	0.262	0.264	0.276	0.254	0.248
Thymus	Abs	0.329	0.351	0.288	0.301	0.193	0.284	0.255	0.278	0.274	0.179
	Rela	0.093	0.097	0.082	0.081	0.070	0.117	0.105	0.115	0.117	0.093
Testes/ovary	Abs	3.03	3.07	3.04	3.13	2.17	0.106	0.108	0.106	0.121	0.097
	rela	0.84	0.85	0.86	0.86	0.86	0.0436	0.0444	0.0441	0.0514	0.0502

In a subchronic toxicity study (anonymous 24 (2000)), following OECD TG 408, 10 male and 10 female mice received 3-methylpyrazole via drinking water at a concentration of 0, 5, 10, 20 or 40 mg/kg bw/d during 13w. Additionally, 10 male and 10 female rats received 3-methylpyrazole, at the same concentration as the main groups, during 13w and were observed during 4w of recovery period.

No test article-related mortality or clinical signs were observed. Significant body weight changes were noted in males.

Table 29 : Body weight data (in g)

	Males					Females				
Dose level (in mg/kg bw/d)	0	5	10	20	40	0	5	10	20	40
No. animals examined	20	20	20	20	20	20	20	20	20	20
D1	24.3	23.4*	24.1	23.4*	23.4*	20.3	20.0	20.1	20.0	20.4
D40	30.3	28.4**	28.9*	28.3**	27.9**	24.9	24.7	24.5	24.4	24.8
D89	33.3	30.2**	30.8**	30.5**	29.5**	26.7	26.1	26.9	26.4	26.5
Recovery groups										
No. animals examined	10	10	10	10	10	10	10	10	10	10
D5	32.9	29.8*	30.9	31.0	29.2**	26.1	26.8	26.5	26.4	26.7
D26	35.0	32.5	34.1	33.6	31.4*	27.2	27.2	27.4	27.4	27.6

* $p < 0.05$; ** : $p < 0.01$

Necropsy revealed few changes. Significant organ weight modifications were observed (see table 30). Lung weight was not recorded.

Table 30 : Organ weight (in g or %)

	Males					Females				
Dose level (in mg/kg bw/d)	0	5	10	20	40	0	5	10	20	40

After 13w											
FBW		30.0	25.4**	24.6**	24.9**	25.0**	26.1	25.6	25.4	24.6	25.1
Adrenal glands	Abs	0.008	0.007	0.008	0.007	0.008	0.014	0.015	0.012	0.014	0.015
	Rela	0.026	0.028	0.031	0.030	0.031	0.053	0.058	0.049	0.056	0.060
Brain	Abs	0.488	0.483	0.494	0.483	0.486	0.488	0.501	0.495	0.496	0.500
	Rela	1.643	1.912**	2.017**	1.969**	1.948**	1.872	1.964	1.965	2.018*	2.000
Liver	Abs	1.25	1.08	1.13	1.11	1.17	1.63	1.59	1.48	1.51	1.62
	Rela	4.15	4.27	4.59**	4.48	4.68**	6.23	6.22	5.85	6.14	6.48
Spleen	Abs	0.069	0.056	0.059	0.051**	0.065	0.082	0.084	0.083	0.083	0.081
	Rela	0.229	0.223	0.240	0.204	0.260	0.315	0.329	0.328	0.336	0.323
Testes	Abs	0.235	0.234	0.232	0.235	0.232					
	Rela	0.792	0.927**	0.947**	0.954**	0.928**					
Thymus	Abs	0.035	0.030	0.024**	0.025**	0.029	0.027	0.029	0.025	0.025	0.023
	Rela	0.117	0.116	0.099	0.100	0.115	0.104	0.114	0.098	0.100	0.091

* $p < 0.05$; ** : $p < 0.01$

The histopathology examination revealed changes in lungs such as an increase incidence of Clara cell alteration and proliferation.

Table 31 : Clara cells modifications

		Males					Females				
Dose level (in mg/kg bw/d)		0	5	10	20	40	0	5	10	20	40
After 13w											
Clara cell alteration	incidence	0	0	7	10	10	0	0	4	10	10
	Grade 1			3	2				1		
	Grade 2			4	7				2	6	
	Grade 3				1	5			1	4	3
	Grade 4					5					7
After 17w											
Clara cell alteration	Incidence	0	0	2	9	10	0	0	4	10	10
	Grade 1			1					1	1	
	Grade 2			1	7	1			3	9	
	Grade 3				2	9					10
Clara cell proliferation	Incidence	0	0	2	10	10	0	0	4	9	10
	Grade 1			2	2	3			3	2	1
	Grade 2				7	5			1	6	7
	Grade 3				1	2				1	2

A chronic toxicity study (anonymous 25 (1985)) exposed Wistar rats to 3-methylpyrazole during 18 months. Groups of 32 males and 32 females received the substance via drinking water at a concentration of 0, 10, 40 or 2000/1000 ppm. Due to the high mortality rate observed after 4 weeks (5 males and 8 females died), the highest dose level (2000 ppm) was reduced to 1000 ppm for the end of the exposure period (weeks 5 to 80).

Mortality was noted in control group and in all dose levels .

Table 32 : Mortality and body weight (in g) data

Dose level (in ppm)		0		10		40		40		2000/1000	
		Number of animals examined	bw	Number of animals examined	bw	Number of animals examined	bw	Number of animals examined	bw	Number of animals examined	bw
♂	W0	32	88	32	78	32	85	32	77	32	84
	W4	32	264	32	261	32	266	32	262	27	121
	W40	31	530	32	511	32	508	32	513	26	434
	W60	22	605	23	583	23	580	22	601	14	499
	W80	19	500	13	544	21	547	17	516	8	416
♀	W0	32	77	32	77	32	75	32	77	32	74
	W4	32	188	32	195	32	199	32	193	22	103
	W40	32	342	31	333	32	333	32	335	21	268
	W60	23	379	22	380	24	386	24	374	9	299
	W80	19	385	16	372	22	369	17	343	4	272

Dyspnea, cachexia and pneumonia were observed in all dose levels. At 2000/1000 ppm, body weight was reduced. Furthermore, hematology and bioclinical examination revealed changes at this dose level (see table 33).

Table 33 : Hematology and enzyme activity after 18 months

	Males					Females				
Dose level (in ppm)	0	10	40	40	2000/1000	0	10	40	40	2000/1000
Hb	167.5	168.7	162.9	156.5	155.1	149.0	147.1	149.7	146.3	139.3
Ht	0.52	0.51	0.49	0.48	0.48	0.48	0.50	0.48	0.50	0.49
Alanine aminotrsanferase	1.29	1.16	1.29	1.19	1.24	1.11	1.18	1.43	0.98	1.77
Alkaline phosphatase	1.29	1.26	1.24	1.14	1.80	0.94	1.10	1.11	0.96	1.17
Aspartate aminotransferase	1.62	1.29	1.51	1.25	1.67	1.14	1.22	1.57	1.16	1.21
cholinesterase	0.48	0.51	0.50	0.53	0.47	1.71	1.54	1.34	1.61	0.83
Leucine aminopeptidase	5.52	4.79	5.37	3.94	5.92	5.67	6.29	6.17	5.34	8.43
Gamma globulin	13.56	13.10	12.05	11.57	10.87	11.79	13.11	12.17	11.95	10.03

Organ weight was examined and revealed few changes (see table 34). Lung weight was not recorded. The histopathological examination showed focal alteration in liver at 2000/1000 ppm.

Table 34 : Organ weight (in g or %)

		Males					Females				
Dose level (in ppm)		0	10	40	40	2000/1000	0	10	40	40	2000/1000
12 months											
Adrenal glands	Abs	0.064	0.063	0.060	0.056	0.052	0.083	0.084	0.093	0.091	0.083
	Rela	0.0110	0.0114	0.0110	0.0111	0.0115	0.0220	0.0246	0.0289	0.0236	0.0294
Brain	Abs	2.02	2.04	2.04	2.01	1.95	1.90	1.90	1.87	1.85	1.75
	Rela	0.350	0.368	0.375	0.399	0.432	0.512	0.557	0.581	0.480	0.629
Liver	Abs	23.8	24.1	19.5	17.1	21.8	13.3	15.4	13.1	13.8	15.8
	Rela	4.23	4.33	3.59	3.37	4.76	3.66	4.48	4.04	3.70	5.62
Spleen	Abs	0.825	0.907	0.801	0.805	0.779	0.690	0.747	0.621	0.665	0.544
	Rela	0.142	0.162	0.146	0.159	0.171	0.183	0.226	0.192	0.173	0.193
Thymus	Abs	0.162	0.169	0.139	0.152	0.137	0.138	0.109	0.126	0.107	0.126
	Rela	0.028	0.031	0.026	0.029	0.030	0.037	0.032	0.039	0.028	0.045
Testes ovary	Abs	3.86	3.89	3.93	3.87	3.67	0.102	0.102	0.118	0.097	0.082
	rela	0.67	0.70	0.72	0.76	0.81	0.0274	0.0302	0.0370	0.0253	0.0293
18 months											
Adrenal glands	Abs	0.084	0.068	0.071	0.100	0.057	0.098	0.113	0.094	0.110	0.070
	Rela	0.0192	0.0133	0.0140	0.0213	0.0146	0.0255	0.0325	0.0272	0.0331	0.0260
Brain	Abs	2.06	2.07	2.07	1.98	1.92	1.90	1.93	1.87	1.91	1.88
	Rela	0.459	0.399	0.396	0.412	0.474	0.504	0.563	0.538	0.578	0.704
Liver	Abs	22.5	22.9	21.7	20.3	20.0	16.0	15.5	16.4	14.5	13.8
	Rela	4.85	4.27	4.07	4.02	4.81	4.16	4.46	4.64	4.23	5.12
Spleen	Abs	0.866	0.788	0.975	0.767	0.794	0.715	0.790	0.701	0.695	0.609
	Rela	0.190	0.148	0.190	0.150	0.189	0.188	0.229	0.195	0.212	0.228
Thymus	Abs	0.121	0.103	0.131	0.139	0.105	0.085	0.095	0.073	0.106	0.131
	Rela	0.026	0.020	0.026	0.026	0.024	0.023	0.026	0.021	0.030	0.049
Testes ovary	Abs	3.46	3.79	3.95	3.65	3.25	0.107	0.107	0.100	0.112	0.090
	Rela	0.74	0.71	0.73	0.73	0.77	0.0280	0.0299	0.0291	0.0343	0.0333
Thyroid	Abs	0.046	0.041	0.044	0.075	0.048	0.038	0.042	0.036	0.050	0.049
	rela	0.0100	0.0077	0.0083	0.0159	0.0118	0.0100	0.0121	0.0102	0.0148	0.0183

Reproductive parameters were examined. In females, follicular maturation and evolution of corpus luteum were unaffected. Moreover, in males, no effects on spermiogenesis were observed.

Table 35: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Short-term oral toxicity study in mice (anonymous 19 (1996))	135/173 mg/kg bw/d in males/females (alteration in lungs : Clara cell lesion and hypotrophy)	4w	45/57.6 mg/kg in males/females	STOT RE 1
Short-term oral toxicity study in mice (anonymous 20 (1997))	223/252 mg/kg bw/d (alteration in lungs : Clara cell alteration (moderate at the low and mid dose and marked at the high dose, haemorrhage, macrophages, ..)	4w	74.33/84 mg/kg bw/d	STOT RE 1

10.12.2 Comparison with the CLP criteria

<table>
<tr><th>Category 1</th><th>Category 2</th><th>Results available</th></tr>
<tr>
<td>"Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals , can be presumed to have the potential to produce significant toxicity in humans following repeated exposure.

Substances are classified in category 1 for target organ toxicity (repeat exposure) on the basis of :
- Reliable and good quality evidence from human cases or epidemiological studies; or
- Observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations."

Classification in Category 1 is applicable, when significant toxic effects observed in a 90-day repeated-dose study conducted in experimental animals are seen to occur at or below the guidance values (C) as indicated in Table 3.9.2:

Table 3.9.2
Guidance values to assist in Category 1
<table>
<tr><th>Route of exposure</th><th>Units</th><th>Guidance values</th></tr>
</table>
</td>
<td>"Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure.

Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations."

Classification in Category 2 is applicable, when significant toxic effects observed in a 90-day repeated-dose study conducted in experimental animals are seen to occur within the guidance value ranges as indicated in Table 3.9.3:

Table 3.9.3
Guidance values to assist in Category 2 classification
<table>
<tr><th>Route of exposure</th><th>Units</th><th>Guidance values</th></tr>
<tr><td>Oral (rat)</td><td>Mg/kg bw/d</td><td>10<C≤100</td></tr>
<tr><td>Dermal (rat or rabbit)</td><td>Mg/kg bw/d</td><td>20<C≤200</td></tr>
<tr><td>Inhalation (rat) gas</td><td>ppV/6h/d</td><td>50<C≤250</td></tr>
</table>
</td>
<td>In 2 short term oral toxicity studies (anonymous 19 (1996) and anonymous 20 (1997)), alteration in lungs were observed already at 135/173 and 223/252 mg/kg bw/d respectively in males/females of the 2 studies. The dose levels extrapolated to 90-day exposure (see table 35) were of 45/57.6 and 74.33/84 mg/kg bw/d. These effects were confirmed in the subchronic toxicity study. In the subchronic toxicity study (anonymous 24 (2000)), clara cell alteration were observed. These modifications was of grade 4 at</td>
</tr>
</table>

Oral (rat)	Mg/kg bw/d	C≤10	Inhalation (rat) vapour	Mg/l/6h/d	0.2<C≤1.0	the highest dose tested (40 mg/kg bw/d) and was always seen at the end of the recovery period. However these effects were not confirmed by clinical signs.
Dermal (rat or rabbit)	Mg/kg bw/d	C≤20	Inhalation (rat) dust/mist/fume	Mg/l/6h/d	0.02<C≤0.2	
Inhalation (rat) gas	ppV/6h/d	C≤50				
Inhalation (rat) vapour	Mg/l/6h/d	C≤0.2				
Inhalation (rat) dust/mist/fume	Mg/l/6h/d	C≤0.02				

10.12.3 Conclusion on classification and labelling for STOT RE

Based on the effects observed in lungs (moderate to marked clara cell alteration), a classification as **STOT RE cat. 1 H372 Causes damage to organs (Lung)** is warranted.

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 1575 ppm: tremor and hunched posture in f	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

		↓ 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	**None** **NOAEL: 20 mg/kg bw/d**

		testes weight and ↑liver weight Alteration in thyroid glands 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	Incidence (out of 5 animals/sex)	0/0	5/5	5/5	5/5

alteration	m/f				
	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	Incidence (out of 10	0/0	0/0	2/4	9/10	10/10

alteration	animals/sex) m/f					
	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.

10.13 Aspiration hazard

Not evaluated in this CLH dossier

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in this CLH report.

12 ADDITIONAL LABELLING

NA

13 ABBREVIATIONS

* : p<0.05, statistically significant

** : p<0.01, statistically significant

♂ : male

♀ : female

↑ : increase

↓ : decrease

↓s : significantly decreased

Abs. : absolute

ADME : absorption, distribution, metabolism and excretion

ADE : absorption, distribution and excretion

ALP : alkaline phosphatase

ASAT : aspartate aminotransferase

BCOP : bovine corneal opacity/permeability test

Bw : body weight

Bwg : body weight gain

Cat. : category

chE : cholinesterase

Conc. : concentration

Corr. : corrosive

Dam. : damage

DMSO : dimethyl sulfoxide

Dpc : day post-coitum

E. Coli : Escherichia Coli

EC3 : estimated test substance concentration that will give a SI of 3

FBW : final body weight

GD : gestational day

GLP : good laboratory practive

Hb : haemoglobin

HPRT : hypoxanthine-guanine phosphoribosyl transferase

Ht : hematocrit

IVIS : *in vitro* irritancy score

LC50 : lethal concentration 50

LD50 : lethal dose 50

LLNA : local lymph node assay

M. : metabolism

Met. act. : metabolic activation

NA : not applicable

Nb.: number

NOAEL : no observed adverse effect level

OECD : Orgainisation for Economic Co-operation and development

PND : post natal day

Rela. : relative

S. Typh : Salmonella Typhimurium

SD : Sprague-Dawley

SI : simulation index

Sign. : significant

STOT RE : Specific target organ toxicity (repeated exposure)

STOT SE : Specific target organ toxicity (single exposure)

TG : test guideline

Tot. prot. : total protein

Tox. : toxicity

14 REFERENCES

Anonymous : see confidential annex chapter 15.2

Bleyl D.W.R (1990), 3-methylpyrazole-eine Modellsubstanz für Urogenitalschäden, Wiss. Z. Erns-Moritz. Arndt-Univ. Greifswald, 39(2), 21-22.

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)

15 ANNEXES

- Confidential annex

Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

3-methylpyrazole

EC Number: 215-925-7

CAS Number: 1453-58-3

Index Number: NA

Contact details for dossier submitter:

Belgian Federal Public Service Health, Food Chain Safety and Environment

Risk Management service

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Belgium

Version number: 2 **Date: 12 february 2019**

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 3-METHYLPYRAZOLE

CONTENTS

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 3-METHYLPYRAZOLE

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 3-METHYLPYRAZOLE

1 PHYSICAL HAZARDS

Not evaluated in this CLH dossier.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

2.1.1 Toxicokinetics *in vivo* study (anonymous 11 (1982))

Study reference:

Anonymous 11 (1982)

Test type

Non-guideline

Non-GLP

Test substance

- 3-methylpyrazole
- *Degree of purity :* no information available
- *Vehicle :* water

Test animals

- *Species/strain/sex :* rat / Wistar / both sexes
- *No. of animals per sex per dose :* 5/sex for ADE examination, 3/sex for M analysis and 3 animals to examine the passage of the placental barrier

Administration/exposure

- *Mode of administration* : gavage
- *Duration of test/exposure period :* single exposure, 5 or 7 times
- *Doses/concentration levels :* 5 mg/kg bw (for single and 5 times exposure) and 50 mg/kg bw (for 7 times exposure)
- *Control group and treatment :* /

Detailed study summary and results:

After administration, the maximal organ burden was determined after 30-60min. Quickly after, the substance is excreted via urine (93-96% within 24h. Moreover, 3-methylpyrazole crosses the placental barrier.

3 HEALTH HAZARDS

3.1 Acute toxicity - oral route

3.1.1 Animal data

3.1.1.1 Acute oral toxicity study (anonymous 12 (2012))

Study reference:

Anonymous 12 (2012)

Detailed study summary and results:

Test type

OECD guideline 423

GLP

Test substance

- 3-methylpyrazole
- *Degree of purity :* 97.9%

Test animals

- *Species/strain/sex :* rat / CD(Crl:CD(SD) / female
- *No. of animals per sex per dose :* 3 females/dose

Administration/exposure

- *Mode of administration* : gavage
- *Duration of test/exposure period :* single exposure
- *Doses/concentration levels :*
    - 3 females exposed to 2000 mg/kg bw. If 2/3 died -> testing to 300 mg/kg bw
    - 3 females exposed to 300 mg/kg bw. If 2/3 died -> testing to 50 mg/kg bw
    - 3 females exposed to 50 mg/kg bw. If 2/3 died -> testing to 5 mg/kg bw
    - 3 females exposed to 5 mg/kg bw.

  (If less than 2 animals died at 1 dose, the test item was retested)
- *Post exposure observation period :* 14d
- *Control group and treatment :* /
- *Vehicle :* 0.8% aqueous hydroxyl-methylcellulose

Results and reliability

- *LD50 or LC50 value :* > 300 - < 2000 mg/kg bw
- *Number of deaths at each dose level and time of death :*

  Table 1 : Mortality

Mortality	2000 mg/kg bw	300 mg/kg bw (first step)	300 mg/kg bw (second step)
Within 24h	2	0	0

Within 7d	3	0	0
Within 14d	3	0	0

- *Clinical signs:* animals exposed to 2000 mg/kg bw exhibited reduced motility and muscle tone, ataxia, dyspnoea and dorsal position. No effects were observed at 300 mg/kg bw.
- *Necropsy findings :* no effects observed

3.1.2 Human data

No information available

3.1.3 Other data

No information available

3.2 Acute toxicity - dermal route

No information available

3.3 Acute toxicity - inhalation route

3.3.1 Animal data

3.3.1.1 Acute inhalation toxicity study (anonymous 13 (1988))

Study reference:

Anonymous 13 (1988)

Detailed study summary and results:

Test type

Similar to OECD TG 403

No GLP

Test substance

- 3-methylpyrazole
- *Degree of purity :* no information available

Test animals

- *Species/strain/sex :* rat / Wistar / both sexes
- *No. of animals per sex per dose :* 5/sex/dose

Administration/exposure

- *Type of inhalation exposure and test conditions :* gas (no more information available)
- *Duration of test/exposure period :* 4h

- *Doses/concentration levels :* 2065, 3380, 4180, 7930, 18750 and 28110 mg/m³
- *Analytical verification of test atmosphere concentrations :* no

Results

- *LC50 :* > 28000 mg/m³
- *Mortality :* no animals died
- *Clinical signs :* no effects
- *Necrospy findings :* no effects

3.3.2 Human data

No information available

3.3.3 Other data

No information available

3.4 Skin corrosion/irritation

3.4.1 Animal data

No information available

3.4.2 Human data

No information available

3.4.3 Other data

3.4.3.1 *In vitro/ex vivo* eye irritation study (anonymous 14 (2011))

Study reference:

Anonymous 14 (2011)

Detailed study summary:

OECD TG 431, human skin model test (no deviations)

Test substance : 3-methylpyrazole (purity : 98.10%)

Conc. : 50 µl

Duration of exposure : 3 min and 1 h

Number of tissues : 2

Results :

After 3 min of exposure : relative absorbance value : 73.8 % (threshold for corrosivity : 50 %)

After 1 h of exposure : relative absorbance value : 14.9 % (threshold for corrosivity : 15 %)

Absorption value after 3min : 1.482 (negative control : 2.009; positive control : 0.586)

Absorption value after 1h : 0.281 (negative control : 1.883; positive control : 0.456)

3.5 Serious eye damage/eye irritation

3.5.1 Animal data

No information available

3.5.2 Human data

No information available

3.5.3 Other data

3.5.3.1 *In vitro/ex vivo* eye irritation study (anonymous 15 (2011))

Study reference:

Anonymous 15 (2011)

Detailed study summary:

BCOP test, bovine eye, OECD TG 437

3-methylpyrazole (purity : 98.10%)

Conc. : 750 µl

Duration of exposure : 10min

Results :

IVIS : 85.73 for test item (215.79 for positive control and -0.216 for negative control)

3.6 Respiratory sensitisation

No information available

3.7 Skin sensitisation

Not evaluated on this CLH report

3.8 Germ cell mutagenicity

Not evaluated on this CLH report

3.9 Carcinogenicity

Not evaluated on this CLH report

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Developmental toxicity study (anonymous 16 (1992))

Study reference:

Anonymous 16 (1992)

Detailed study summary and results:

Test type

OECD TG 414

GLP

Test substance

- 3-methylpyrazole
- *Degree of purity :* 99.9%

Test animals

- *Species/strain/sex :* rat / Wistar / pregnant females
- *No. of animals per sex per dose :* 25/group

Administration/exposure

- *Route of administration* : gavage
- *duration and frequency of test/exposure period :* GD 6-15
- *doses/concentration levels :* 0, 15, 45 and 90 mg/kg bw/d
- *vehicle :* water

Results and discussion

For dams :

- *time of death during the study and whether animals survived to termination :* no premature death observed
- *body weight data :* lower at the 2 highest dose levels.

Table 2 : Body weight data (in g)

Dose level (in mg/kg bw/d)	0	15	45	90
GD 0	225.0	222.4	223.7	224.9
GD 6	254.5	250.8	253.1	252.3
GD15	300.0	295.0	292.2	276.4**
GD20	373.3	368.4	364.2	352.6**
BWG GD 6-15	45.6	44.1	39.1	24.1**

BWG GD 0-20	148.3	146.0	140.4	127.7**
Gravid uterus weight	81.0	79.7	75.2	69.1**
Net weight change from D6	37.8	37.8	35.8	31.2*

* $p < 0.05$; ** : $p < 0.01$

- *clinical observations :* no effects
- *precoital interval (number of days until mating and number of estrous periods until mating)*
- *reproductive data :* unaffected (conception rate, mean number of corpora lutea, implantation sites, pre- and post-implantation loss, number of resorption and viable foetuses).
- *necropsy findings :* 1 female of the low dose exhibited hydrometra and did not become pregnant
- *gravid uterine weight, including optionally, body weight change corrected for gravid uterine weight :* Uterus weight was significantly lower at the highest dose. Furthermore, the corrected bw (bw at GD20 minus uterus weight minus bw at GD6) was also significantly decreased at this dose level. (see table 2)

For foetuses :

- *mean number of live pups (litter size) :* mean number of live foetuses : 14.1, 14.0, 13.7 and 13.6 respectively at 0, 15, 45 and 90 mg/kg bw/d
- *placental weight :* no effects (0.45, 0.46, 0.46 and 0.43 g respectively at 0, 15, 45 and 90 mg/kg bw/d)
- *sex ratio :* no effects (51.5/48.5, 48.7/51.3, 50.1/49.9 and 47.2/52.8 % of live female/male respectively at 0, 15, 45 and 90 mg/kg bw/d)
- *mean litter or pup weight :* the mean fetal weight was significantly lower at the 2 highest dose levels (3.9, 3.8, 3.6** and 3.3**g respectively at 0, 15, 45 and 90 mg/kg bw/d).
- *fetal external malformations/variations :* one foetus of the 45 mg/kg bw/d level exhibited a cleft palate.
- *Fetal soft tissue malformations/variations :* Soft tissue examination revealed severe malformations in the urogenital tract and/or in the cardiovascular system in the foetuses of the highest dose.

Table 3 : Malformation data

Dose level (in mg/kg bw/d)	0	15	45	90
Fetal incidence	0	0	0	14**
Litter incidence	0	0	0	8**
Efferent urinary tract severely dilated	0	0	0	5*
Malformation of great vessels	0	0	0	6*
Agenesie of kidney(s)	0	0	0	2
Agenesie of ureter(s)	0	0	0	2
Dilatation of both ventricles (globular shaped heart)	0	0	0	2

* $p < 0.05$; ** : $p < 0.01$

- *Fetal skeletal malformations/variations :* Various malformation of the sternum and/or the vertebral column were also observed in all groups. For the control, the low and the mid dose groups, these malformation were within the range of the historical fetal incidence.

Table 4 : Skeletal malformations data

Dose level (in mg/kg bw/d)		0	15	45	90	Historical data in %
Nb. of foetuses evaluated (Nb. of litters evaluated)		174 (24)	159 (22)	177 (25)	176 (25)	
Fetal incidence		8	8	8	49**	
Litter incidence		6	6	5	20**	
Thoracic vertebral body/bodies dumbbell-shaped (%)	Fetal incidence (%)	6 (3.4)	5 (3.1)	3 (1.7)	39** (22)	0 – 8.8
	Litter incidence (%)	4 (17)	5 (23)	2 (8/)	17** (68)	0 – 39.1
Thoracic vertebral body/bodies bipartite (%)	Fetal 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

* $p < 0.05$; ** : $p < 0.01$

3.10.1.2 Developmental toxicity study (Bleyl D.W.R., 1990)

Study reference:

Bleyl D.W.R. (1990)

Detailed study summary and results:

Test type

No guideline

No information about GLP compliance

The aim of this study was to reproduce the observation of urogenital syndrome after prenatal exposure.

Test substance

- 3-methylpyrazole
- *Degree of purity :* unknown

Test animals

- *Species/strain/sex :* rat / Wistar / female
- *No. of animals per sex per dose :* unknown

Administration/exposure

- *Route of administration* : oral (no more information)
- *duration and frequency of test/exposure period :* Pregnant rats were exposed only on GD 10 and GD 11
- *doses/concentration levels :* 0, 20, 40, 80 and 160 mg/kg bw/d
- *historical control data if available :* no information available
- *vehicle:* water

Description of test design:

- *details on mating procedure :* Mating, one male was left with two females for the night. Detection of sperm in the vaginal smear in the morning was monitored to determine the gestation day 1 (GD1). Fertilized females were then allocated to the different dose and control groups (6 dams per group).

Results and discussion

For dams:

- *body weight data for P :* no effects observed
- *clinical observations:* no information available
- *haematological and clinical biochemistry findings if available :* no information available
- *duration of gestation (calculated from day 0 of pregnancy) :* no information available
- *number of implantations, corpora lutea, litter size :* no information available
- *number of pre- and post-implantation loss :* no information available
- *number of dams with abortions, early deliveries, stillbirths, resorptions and/or dead fetuses :* no information available
- *necropsy findings :* no information available
- *histopathological findings: nature and severity :* no information available
- *body weight change and gravid uterine weight, including optionally, body weight change corrected for gravid uterine weight :* no information available
- *other organ weight changes* liver weight : no effects observed

For F1 pups/litters (per dose):

- *mean number of live pups (litter size) :* At the highest dose level, the rate of living pups at birth was of 77%**. No more information available
- *sex ratio :* no information available
- *viability index (pups surviving 4 days/total births) :* most animals exposed in utero to the highest dose died in the first days of life. No more information available
- *survival index at weaning :* 26%** at the highest dose. No more information available
- *mean litter or pup weight by sex and with sexes combined :* no information available
- *external, soft tissue and skeletal malformations and other relevant alterations :* 15.6% of the living fetuses of the 80 mg/kg bw/d dose level exhibited urogenital syndrome. In most cases, an unilateral kidney agenesis was noted (no left kidney) coupled with a hydronephrosis in the remaining kidney. The other pups exhibited a bilateral kidney agenesis. In males, the genital tract was complete however some cases of undescended testis were recorded. While in females, the kidney agenesis was always coupled with an incomplete differentiation of the uterus.

  The fetuses of the highest dose level died in the first day of live and the necropsy revealed urogenital syndrome.

- *data on physical landmarks in pups and other postnatal developmental data :* no information available
- *other information :* on PND 43 for males and PND 44 for females, the renal function of the surviving pups has been investigated and revealed disturbance only in females of the 2 highest dose levels.

Table 5 : Renal parameters data

Sex	Group	Dose (mg/kg)	Urine volume		Protein (g/L)	Creatinine (µmol/L)			Alk. Phosph.
			(mL)	(mL/kg)		Serum	Urine	Clearance (µL/sec)	
Male	0	0	3.7 ± 0.7	21.6 ± 3.9	0.48 ± 0.08	79.3 ± 2.9	2516 ± 524	2.57 ± 0.87	3.30 ± 20
	0_{HG}	0	4.5 ± 0.6	27.0 ± 3.3	0.36 ±0.05	76.2 ± 1.8	2340 ± 385	2.61 ± 0.62	269 ± 33
	2_{HG}	40	3.1 ± 0.4	18.1 ± 2.4	0.49 ± 0.05	74.9 ± 2.8	3420 ± 406	2.76 ± 0.48	249 ± 17
	3_{HG}	60	3.3 ± 0.5	19.6 ± 2.9	0.27 ± 0.04	77.0 ± 3.5	2092 ± 450	2.15 ± 0.85	217 ± 13
	4_{HG}	80	3.5 ± 0.4	21.2 ± 2.4	0.36 ± 0.06	79.6 ± 3.2	3140 ± 581	2.71 ± 0.68	216 ± 26
Female	0	0	2.7 ± 0.4	19.6 ± 2.9	0.23 ± 0.05	70.3 ± 3.7	3904 ± 241	2.03 ± 0.29	61 ± 11
	0_{HG}	0	2.5 ± 0.3	17.7 ± 2.0	0.24 ± 0.03	74.0 ± 3.1	3338 ± 634	2.22 ± 0.53	78 ± 13
	2_{HG}	40	2.2 ± 0.3	15.3 ± 2.2	0.21 ± 0.03	70.1 ± 3.0	2835 ± 752	1.77 ± 0.47	93 ± 23
	3_{HG}	60	2.3 ± 0.4	17.5 ± 3.3	0.23 ± 0.05	73.5 ± 3.7	3207 ± 646	1.96 ± 0.57	117 ± 55^{+}
	4_{HG}	80	1.6 ± 0.2^{+}	12.3 ± 1.2^{+}	0.33 ± 0.14^{+}	82.3 ± 5.6	1811 ± 500	0.67 ± $0.21^{\S}$	110 ± 31^{+}

$^{+}$ = p<0.05, § = p<0.01, $_{HG}$ = after addition of mercury

3.10.1.3 developmental toxicity study (Anonymous 17 (1984))

Study reference:

Anonymous 17 (1984)

Detailed study summary and results:

Test type

No guideline

No GLP compliance

Females were exposed to the test-substance on GD 4, 10, 13 and 18.

Test substance

- 3-methylpyrazole

- *Degree of purity :* not reported

Test animals

- *Species/strain/sex :* rat / Wistar / female
- *No. of animals per sex per dose :* 13, 13, 12, 14 and 6 female rats respectively at 0, 50, 100, 200 and 400 mg/kg bw/exposure

Administration/exposure

- *Route of administration :* gavage
- *duration and frequency of test/exposure period :* GD 4, 10, 13 and 18
- *doses/concentration levels :* 0, 50, 100, 200 and 400 mg/kg bw/exposure
- *vehicle:* water

Description of test design:

- For the mating, one male was left with two females for the night. Detection of sperm in the vaginal smear in the morning was monitored to determine the gestation day 1 (GD1). Fertilized females were then allocated to the different doses and control groups. Doses were given orally by gavage on GD4, GD10, GD13 and GD18. The test substance has been diluted in water, and the animals received 2.5 mL/kg bw. Body weight has been monitored on GD1, GD4, GD10, GD13, GD18 and GD20.The dams were killed by decapitation on GD20. After sacrifice, the liver has been weighted (absolute and relative).The numbers of corpora lutea, implantation, resorptions, live and dead fetuses have been recorded.
- Each living fetus was weighed, measured (crown-rump length) and examined to detect unexpected malformation. They were then fixed alternatively in a solution 96% ethanol (for skeletal examination) or in Bouin's solution (for soft tissue examination).

Results and discussion

For dams (per dose):

- *time of death during the study and whether animals survived to termination :* at the highest dose, 4 pregnant dams died (necropsy revealed a catarrhal enteritis and/or nephrosis)
- *body weight data for P animals :* significantly reduced at the highest dose level. The body weight of the 200 mg/kg bw/exposure dose level tend also to decline on GD20.

Table 6 : Body weight in g

Dose (mg/kg)	Body weight (g)					
	GD1	GD4	GD10	GD13	GD18	GD20
0	268 ± 8	276 ± 7	288 ± 8	298 ± 9	387 ± 9	334 ± 9
50	245 ± 7	257 ± 8	273 ± 9	279 ± 10	309 ± 18	328 ± 11
100	255 ± 7	262 ± 7	280 ± 8	283 ± 10	315 ± 7	292 ± 33
200	251 ± 5	260 ± 6	270 ± 6	275 ± 7	298 ± 7	306 ± 8^{+}
400	253 ± 15	264 ± 15	263 ± 15	256 ± 16§	246 ± 15§	258 ± 15§

$^{+}$ = $p<0.05$, §= $p<0.01$

- *body weight at sacrifice and absolute and relative organ weight data for the parental animals :* liver weight was recorded and did not reveal modification (absolute liver weight : 12.2, 12.5, 13.1, 12.1 and 11.3g respectively at 0, 50, 100, 200 and 400 mg/kg bw/exposure; relative liver weight : 2.6, 3.8, 3.9, 4.0 and 4.2 g/100g bw respectively at 0, 50, 100, 200 and 400 mg/kg bw/exposure). No more information available
- *clinical observations:* no information available
- *haematological and clinical biochemistry findings if available :* no information available
- *duration of gestation (calculated from day 0 of pregnancy) :* no information available
- *number of implantation, corpora lutea, pre- and post-implantation loss :* no effects on the pre-implantation loss. Nevertheless, the post-implantation loss was significantly reduced at the highest dose level.

Table 7 : Reproductive parameters

Dose (mg/kg)	Corpora lutea	Implantations	Preimplantation losses	
			Absolute	Relative (%)
0	13.1 ± 0.5	12.0 ± 0.6	1.2 ± 0.3	8.9 ± 2.2
50	11.9 ± 0.7	10.5 ± 0.9	1.4 ± 0.5	11.3 ± 4.9
100	18.7 ± 0.4	11.5 ± 0.6	1.2 ± 0.4	9.2 ± 3.5
200	18.8 ± .5	11.6 ± 0.7	1.3 ± 0.4	10.1 ± 3.8
400	10.3 ± 1.7^{+}	9.7 ± 1.5	0.7 ± 0.3	5.1 ± 2.4

$^{+}$ = $p<0.05$

- *number of dams with abortions, early deliveries, stillbirths, resorptions and/or dead fetuses :* in 4 out of 6 dams of the highest dose, all the progeny died by resorption.

Table 8 : Resorption and post implantation losses

Dose (mg/kg)	Resorption (%)			Dead fetuses (%)	Total postimplantation losses	
	Early	Middle	Late		Absolute	Relat. (%)
0	9.6 ± 3.8	1.9 ± 1.4	0	0	1.5 ± 0.5	11.5 ± 3.7
50	6.7 ± 2.5	7.1 ± 3.2	0	0	1.2 ± 0.3	13.8 ± 3.7
100	10.1 ± 2.6	0	0	0	1.2 ± 0.4	11.8 ± 3.3
200	10.5 ± 2.4	3.0 ± 1.1	0.5 ± 0.5	0.5 ± 0.5	1.7 ± 0.4	14.9 ± 3.8
400	2.5 ± 2.5	69.8 ± 19.1§	0	2.6 ± 2.6^{+}	7.0 ± 1.9§	74.9 ± 16.2§

§= $p<0.01$

Table 9 : Total losses

Dose (mg/kg)	Total loss	
	Absolute	Relative (%)
0	2.6 ± 0.6	19.6 ± 3.7
50	2.5 ± 0.6	23.5 ± 6.5
100	2.4 ± 0.6	19.2 ± 5.3

200	3.0 ± 0.6	23.0 ± 4.9
400	7.5 ± 1.8§	77.8 ± 14.5§

§= p<0.01

- *necropsy findings :* no information available
- *histopathological findings: nature and severity :* no information available
- *body weight change and gravid uterine weight, including optionally, body weight change corrected for gravid uterine weight :* no information available

For F1 (per dose):

- *mean number of live pups (litter size) :* see table 10
- *sex ratio :* no information available
- *viability index (pups surviving 4 days/total births) :* no information available
- *survival index at weaning :* no information available
- *mean litter or pup weight by sex and with sexes combined :* the weight of living fetuses was significantly decreased. The placental weight was severely reduced at this dose level.

Table 10 : Body weight and placental weight of the living fetuses

Dose (mg/kg)	Live fetuses			Weight of placenta (g)
	Total	Length (mm)	BW (g)	
0	10.5 ± 0.6	30.9 ± 0.3	1.80 ± 0.04	0.43 ± 0.02
50	9.3 ± 1.0	30.2 ± 0.2	1.80 ± 0.02	0.46 ± 0.05
100	10.2 ± 0.8	29.3 ± 0.5	1.70 ± 0.05	0.46 ± 0.03
200	9.9 ± 0.7	27.2 ± 0.7+	1.40 ± 0.05§	0.41 ± 0.03
400	8.7 ± 1.7§	23.7 ± 1.8§	1.05 ± 0.07§	0.28 ± 0.04§

+ = p<0.05, §= p<0.01

- *external, soft tissue and skeletal malformations and other relevant alterations :* all the fetuses of the highest dose presented at least one malformation (46.8% at 200 mg/kg bw/exposure and 11.1% at 100 mg/kg bw/exposure).

Table 11 : Malformation data

Dose (mg/kg)	0	50	100	200	400
Syndactilie/Retrodactilie					
Total	0	0	1.2 ± 0.8	15.3 ± 6.3§	81.3 ± 18.8§
Forelimb	0	0	1.2 ± 0.8	14.0 ± 6.5§	81.3 ± 18.8§
Hind limb	0	0	0	4.6 ± 3.4	50.0 ± 37.5§
Amelia	0	0	0	1.2 ± 0.8	6.3 ± 6.3
Anemia	0	0	0	2.6 ± 1.5+	0
Cleft palate	0	0	0	0.5 ± 0.5	12.5 ± 12.5
Urogenital syndrome					
Total	0	0	4.4 ± 4.4	40.8 ± 8.0§	58.8 ± 31.3§

Symmetric	0	0	3.3 ± 3.3	27.6 ± 8.5§	50.4 ± 25.0§
Asymmetric	0	0	1.1 ± 1.1	13.2 ± 3.0	31.3 ± 6.3§
Hydronephrose	0.5 ± 0.5	2.0 ± 1.0	5.1 ± 2.4+	1.9 ± 1.0	0
Ecchymosis	0.5 ± 0.5	0	3.8 ± 2.6	1.2 ± 0.8	0
Horizonal cardiac apex	0	0	2.8 ± 1.5	4.2 ± 1.9	6.3 ± 6.3
Total (%)	0.5 ± 0.5	2.0 ± 1.0	11.1 ± 4.5	46.8 ± 6.8§	100 ± 0.1§

+ = p<0.05, § = p<0.01

3.10.1.4 Developmental toxicity study (anonymous 18 (1989))

Study reference:

Anonymous 18, 1989

Detailed study summary and results:

Test type

No guideline followed

No GLP

Test substance

- 3-methylpyrazole
- *Degree of purity :* no information available

Test animals

- *Species/strain/sex :* rat / strain unknown / female
- *No. of animals per sex per dose :* 8 pregnant females / group

Administration/exposure

- *Route of administration :* gavage
- *duration and frequency of test/exposure period :* GD6-18
- *doses/concentration levels :* 0, 25, 100, 175 and 225 mg/kg bw/d
- *historical control data if available :* no information available
- *vehicle:* no information available

Description of test design:

- *details on mating procedure :* no information available
- *post exposure observation period :* sacrificed on GD20

Results and discussion

For dams (per dose):

- *time of death during the study and whether animals survived to termination :* 6 females exposed to 175 mg/kg bw/d and all females exposed to 225 mg/kg bw/d died or were sacrificed in extremis.
- *body weight data for P :* moderate to severe decrease was noted during GD 6 to 12 in animals exposed to 100 mg/kg bw/d.

- *body weight at sacrifice and absolute and relative organ weight data for the parental animals :* no information available
- *clinical observations: :* no information available
- *haematological and clinical biochemistry findings if available :* no information available
- *number of implantations, corpora lutea, litter size :* no information available
- *number of pre- and post-implantation loss :* no information available
- *number of dams with abortions, early deliveries, stillbirths, resorptions and/or dead fetuses :* an increase in resorptions was noted at 100 mg/kg bw/d. no fetuses were produced by the 2 survivor females exposed to 175 mg/kg bw/d.
- *number of live births :* no information available
- *necropsy findings :* no information available
- *histopathological findings: nature and severity :* no information available
- *body weight change and gravid uterine weight, including optionally, body weight change corrected for gravid uterine weight :* no information available
- *other organ weight changes if available :* no information available

For F1 pups/litters (per dose):

- *mean number of live pups (litter size) :* no information available
- *sex ratio :* no information available
- *viability index (pups surviving 4 days/total births) :* no information available
- *survival index at weaning :* no information available
- *mean litter or pup weight by sex and with sexes combined :* fetal weight was reduced at 100 mg/kg bw/d.
- *external, soft tissue and skeletal malformations and other relevant alterations:* One fetus in the 100 mg/kg bw/d dose level exhibited external malformations such as cleft palate. However this foetus weighted only 1.2g.

3.10.2 Human data

No information available

3.10.3 Other data (e.g. studies on mechanism of action)

No information available

3.11 Specific target organ toxicity – single exposure

Not evaluated in this CLH report

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

3.12.1.1 Short-term oral toxicity study (anonymous 19 (1996))

Study reference:

Anonymous 19 (1996)

Detailed study summary and results:

Test type

OECD TG 407

GLP

Test substance

- 3-methylpyrazole
- *Degree of purity :* 99.7%

Test animals

- *Species/strain/sex :* mouse / B6C3F1 / both sexes
- *No. of animals per sex per dose :* 5/sex/dose

Administration/exposure

- *route of administration :* via drinking water
- *duration and frequency of test/exposure period :* 28d
- *doses/concentration levels :* 0, 900, 1125 and 1575 ppm (corresponding to a mean daily test substance intake of 0, 154, 176 and 206 mg/kg bw/d respectively)

Results and discussion

- *body weight and body weight changes :* Body weight was unaffected in all dose levels (29.3/23.7, 28.6/23.7, 28.0/23.7 and 28.7/22.9g in males/females respectively at 0, 900, 1125 and 1575 ppm), however body weight gain was significantly lower in females of the highest dose level (BWG 0-28D : 4.3/4.6, 3.5/4.1, 3.0/4.2 and 3.8/3.4** g in males/females respectively at 0, 900, 1125 and 1575 ppm).
- *description, severity, time of onset and duration of clinical signs :* no effects observed
- *gross pathology findings:* Necropsy revealed a significant higher lung weight in both sexes at the highest dose level and in males at the lowest dose level. Liver weight was significantly reduced at 1125 ppm in male. The relative organ weight observation showed only changes in lung.
- *histopathology findings:* Histopathology examination was only performed on the lungs. Lesions of the mucus cells of the air ducts and of the Clara cells in the bronchi and bronchioles were recorded. Clara cells alteration consisted of disorganization of the luminal lining cell layer due to flattening of the cells and loss of the apical parts of the Clara cells and due to development of irregular shape

shaped clara cell nuclei. Moreover, hypotrophy of the air duct epithelia (focal or diffuse) was recorded

- *mortality and time to death :* no effects observed

3.12.1.2 Short-term oral toxicity study (anonymous 20 (1997))

Study reference:

Anonymous 20 (1997)

Detailed study summary and results:

Test type

EU Method B.7

GLP

Test substance

- 3-methylpyrazole
- *Degree of purity :* 99.4%

Test animals

- *Species/strain/sex :* mouse / B6C3F1 / both sexes
- *No. of animals per sex per dose :* 5/sex/group for main groups + 5/sex/group for recovery groups

Administration/exposure

- *route of administration :* via drinking water
- *duration and frequency of test/exposure period :* 28d
- *doses/concentration levels :* 300, 900 and 1575 ppm (corresponding to 0/0, 70/82, 151/193 or 223/252 mg/kg bw/d respectively in males/females)
- *post exposure observation period :* 14d for recovery groups

Results and discussion

- *body weight and body weight changes :* The body weight examination revealed a statistically significant lower value in females at the highest dose. The body weight gain was already decreased in females at 900 ppm and in males at 1575 ppm. However, during the recovery period, no differences in body weight were observed.
- *description, severity, time of onset and duration of clinical signs :* Females of the mid and high dose level exhibited tremors and/or hunched posture.
- *gross pathology findings:* Macroscopic observations did not reveal any treatment-related changes.
- *organ weight :* The absolute lung weight was increased in females at the mid and high dose level, whereas the relative lung weight was already increased in females at the low dose level and was also increased in males at the mid and high dose level. Whereas, the necropsy of animals of the recovery groups revealed that the absolute lung weight was increased in females at the mid dose level while the relative lung weight was increased in females at 900 and 1575 ppm.

- *histopathology findings :* The microscopic examination of the main groups revealed a Clara cell alteration (moderate at 300 and 900 ppm, and moderate to marked at 1575 ppm). This modification was characterized by a loss of the characteristic dome-shaped appearance and the apical "bled", by cytokaryomegaly, and basophilia. Furthermore, at 1575 ppm, mitotic figures and/or macrophages were noted in the altered epithelium of the bronchi and bronchioli. In a few mice of the mid and high dose levels, interstitial histiocytosis, alveolar macrophages, alveolar hemorrhage, alveolar edema and/or interstitial edema/congestion were observed. The animals of all recovery groups showed also a Clara cell alteration (slight to moderate at 300 and 900 ppm and moderate to marked at 1575 ppm). In addition, slight to moderate Clara cell proliferation (characterized by increased numbers cytokaryomegalic, basophilic and sometimes multinuclear cells) was observed in all treated mice of these groups. These cells were arranged in two cell layers instead of the normal one layer. In addition, mitotic figures were occasionally observed.
- *mortality and time to death :* no effects observed

3.12.1.3 Short-term oral toxicity study (anonymous 21 (1996))

Study reference:

Anonymous 21 (1996)

Detailed study summary and results:

Test type

No guideline followed

No GLP

Test substance

- 3-methylpyrazole
- *Degree of purity :* 99.77%

Test animals

- *Species/strain/sex :* Mouse / B6C3F1 / both sexes
- *No. of animals per sex per dose :* 3/sex/dose

Administration/exposure

- *route of administration :* via drinking water
- *duration and frequency of test/exposure period :* 2w
- *doses/concentration levels :* 0, 225 and 675 ppm (corresponding to a test substance intake of 0/0, 47/61 and 140/173 mg/kg bw/d respectively in males/females)

Results and discussion

- *body weight and body weight changes :* Body weight examination did not reveal significant changes (at D14 : 25.0/21.0, 25.2/21.2 and 25.7/21.3 g respectively at 0, 225 and 675 ppm).
- *description, severity, time of onset and duration of clinical signs :* no effects observed

- *gross pathology findings:* Erosions/ulcers in the glandular stomach and discoloration of contents of the jejunum were observed in control and in dose groups (in 0/2 , 0/2 and 3 males/3females respectively at 0, 225 and 675 ppm).
- *mortality and time to death :* no effects observed
-

3.12.1.4 Subchronic oral toxicity study (anonymous 22 (1999))

Study reference:

Anonymous 22 (1999)

Detailed study summary and results:

Test type

OECD TG 407 and 408

GLP

Test substance

- 3-methylpyrazole
- *Degree of purity :* 99.34%

Test animals

- *Species/strain/sex :* rat / Wistar / both sexes
- *No. of animals per sex per dose :* 10/sex/group for main groups + 10/sex/group for recovery groups

Administration/exposure

- *route of administration :* via drinking water
- *duration and frequency of test/exposure period :* 90d
- *doses/concentration levels :* 0 and 40 mg/kg bw/d
- *post exposure observation period :* 28d for recovery groups

Results and discussion

- *body weight and body weight changes :* no effects observed
- *description, severity, time of onset and duration of clinical signs* : no effects observed
- *gross pathology findings:* the organ weight and histopathological examination were only performed on kidneys, liver and lungs. Significant higher absolute and relative kidney weight was noted in females (slight trend in males). Moreover, significant increase of liver weight (abs. and rela.) was observed in females. These changes did not appear at the end of the recovery period.

Table 12 : Organ weight

		Main groups				Recovery groups			
		Males		Females		Males		Females	
Dose level (mg/kg bw/d)		0	40	0	40	0	40	0	40
Kidney (left)	Abs	1572.0	1602.8	1046.9	1166.3*	1679.5	1628.1	1088.3	1025.5

weight (in mg)	Rela	0.3174	0.3356	0.3591	0.4006*	0.3294	0.3177	0.3420	0.3445
Kidney (right) weight (in mg)	Abs	1562.8	1595.3	1046.7	1183.8*	1653.6	1622.5	1067.7	1021.7
	Rela	0.3150	0.3347	0.3588	0.4069*	0.3239	0.3154	0.3349	0.3432
Liver weight (in mg)	Abs	20229.8	19642.3	11151.3	12594.8*	20788.0	18629.9	11149.9	9715.6*
	Rela	4.080	4.107	3.799	4.293*	4.036	3.596	3.474	3.271
Lungs weight (in mg)	Abs	2369.1	2241.0	1763.6	1865.4	2294.0	2234.1	1763.8	1731.7
	rela	0.4817	0.4692	0.6011	0.6381	0.4492	0.4346	0.5556	0.5823

- *histopathology findings:* 2 males of the control group and 1 male exposed to 40 mg/kg bw/d showed intracellular vacuoles in hepatocytes (low grade).
- *mortality and time to death :* no effects observed

3.12.1.5 Subchronic oral toxicity study (anonymous 23 (1980))

Study reference:

Anonymous 23 (1980)

Detailed study summary and results:

Test type

No guideline followed

No GLP

Test substance

- 3-methylpyrazole
- *Degree of purity :* no information available

Test animals

- *Species/strain/sex :* rat / Wistar / both sexes
- *No. of animals per sex per dose :* 24/sex/group (except for control group : 36/sex)

Administration/exposure

- *route of administration :* via gavage
- *duration and frequency of test/exposure period :* 90d
- *doses/concentration levels :* 0, 0.2, 2, 20 and 200 mg/kg bw/d

Results and discussion

- *body weight and body weight changes :* the body weight decreased at the highest dose level, the modification was more severe in males.

Table 13 : Body weight data

	Males					Females				
Dose level (in mg/kg bw/d)	0	0.2	2	20	200	0	0.2	2	20	200
W0	78	78	76	76	76	72	73	72	72	71

W6	270	283	276	274	198	201	200	202	200	168
W12	359	372	362	359	262	249	248	249	246	222

- *clinical biochemistry findings:* changes were noted during enzyme activity examination. At the highest dose level, aspartate aminotransferase, leucine aminotransferase and alkaline phosphatase activity were increased. Cholinesterase activity was reduced only in females at the highest dose level. (See table 14)

Table 14 : Enzyme activity data after 12w

Dose level (mg/kg bw/d)	Males					Females				
	0	0.2	2	20	200	0	0.2	2	20	200
Aspartate aminotransferase activity	1.54	1.31	1.43	1.45	2.02	1.44	1.45	1.51	1.31	1.79
Leucine aminopeptidase activity	6.25	5.36	6.15	6.38	8.60	6.12	6.80	6.70	6.81	10.57
Alkaline phosphatase activity	0.94	1.20	1.07	1.20	1.92	0.94	0.93	1.07	1.26	1.49
Cholinesterase activity	0.46	0.43	0.45	0.42	0.45	1.20	1.03	1.25	1.17	0.59

- *organ weight :* at necropsy, organ weight was examined and revealed some changes. Lower brain, spleen, thymus and testes weights were observed at the highest dose level compared to the control group. Liver weight was increased at 200 mg/kg bw/d compared to the control group.

Table 15 : Organ weight (in g and in %)

Dose level (in mg/kg bw/d)		Males					Females				
		0	0.2	2	20	200	0	0.2	2	20	200
Adrenal glands	Abs	0.063	0.071	0.072	0.072	0.065	0.066	0.068	0.075	0.069	0.068
	Rela	0.0179	0.0196	0.0205	0.0198	0.0271	0.0272	0.0281	0.0313	0.0294	0.0354
Brain	Abs	1.82	1.79	1.88	1.80	1.63	1.71	0.69	1.72	1.73	1.56
	Rela	0.516	0.495	0.537	0.496	0.673	0.704	0.703	0.720	0.735	0.822
Kidneys	Abs	2.80	2.91	2.93	2.93	2.48	1.97	2.06	2.04	1.86	2.14
	Rela	0.788	0.808	0.836	0.807	0.998	0.812	0.852	0.851	0.792	1.107
Liver	Abs	15.8	15.6	18.2	18.4	16.9	10.3	11.7	13.0	11.2	13.9
	Rela	4.43	4.31	5.19	5.02	6.76	4.23	4.79	5.42	4.74	7.22
Spleen	Abs	0.763	0.766	0.811	0.771	0.500	0.638	0.642	0.663	0.600	0.477
	Rela	0.215	0.212	0.231	0.212	0.198	0.262	0.264	0.276	0.254	0.248
Thymus	Abs	0.329	0.351	0.288	0.301	0.193	0.284	0.255	0.278	0.274	0.179
	Rela	0.093	0.097	0.082	0.081	0.070	0.117	0.105	0.115	0.117	0.093
Testes/ovary	Abs	3.03	3.07	3.04	3.13	2.17	0.106	0.108	0.106	0.121	0.097

	rela	0.84	0.85	0.86	0.86	0.86	0.0436	0.0444	0.0441	0.0514	0.0502

- *histopathology findings:*
    - Heart : no inflammatory or degenerative change has been observed. A slight activation of the histiocytes has been occasionally seen but was not treatment-related. Moreover one rat exposed to the highest dose developed a non-ulcerous epicardite (incidental findings).
    - Trachea/thyroid : cross-sections at the thyroid height revealed an intact ciliated epithelium, rarely artificially separated from its underlay. The glands found in the submucosa were surrounded by lymph tissue more or less dense, which is partly characteristic of lymph nodes. In some cases single excretory ducts were dilated and filled with granulocytes and cellular debris. Few animals (independently of the dose given) showed some chronic peritubular inflammation in the form of development of callous-fibrocytic sleeves.
    - Lungs : sections of the lungs revealed frequently massive clubbing of lymphocytes and to a lesser extent of histiocytes. But these were only rarely indicative of an inflammatory response. These effects were not treatment-related. One rat exposed to the highest dose showed a subacute ulcerous bronchitis and peribronchitis. Moreover one rat of the lowest dose group developed an ulcerous pneumonia in one lobules.
    - Thyroid : alteration thyroid gland (no more information available)
    - Liver : nucleus anisomorphism, fatty degeneration and cell death (no more information available)
- *mortality and time to death:* 2 males died during study (1 exposed to 20 mg and 1 exposed to 200 mg/kg bw/d).

3.12.1.6 Subchronic oral toxicity study (anonymous 24 (2000))

Study reference:

Anonymous 24 (2000)

Detailed study summary and results:

Test type

OECD TG 408

GLP

Test substance

- 3-methylpyrazole
- *Degree of purity :* 98.38%

Test animals

- *Species/strain/sex :* Mouse / B6C3F1 / both sexes
- *No. of animals per sex per dose :* 10/sex/group for main groups + 10/sex/group for recovery groups

Administration/exposure

- *route of administration :* via drinking water
- *duration and frequency of test/exposure period :* 13w
- *doses/concentration levels :* 0, 5, 10, 20 and 40 mg/kg bw/d
- *post exposure observation period :* 4w for observation groups

Results and discussion

- *body weight and body weight changes :* significant body weight changes were noted in males.

Table 16 : Body weight data

	Males					Females				
Dose level (in mg/kg bw/d)	0	5	10	20	40	0	5	10	20	40
No. animals examined	20	20	20	20	20	20	20	20	20	20
D1	24.3	23.4*	24.1	23.4*	23.4*	20.3	20.0	20.1	20.0	20.4
D40	30.3	28.4**	28.9*	28.3**	27.9**	24.9	24.7	24.5	24.4	24.8
D89	33.3	30.2**	30.8**	30.5**	29.5**	26.7	26.1	26.9	26.4	26.5
Recovery groups										
No. animals examined	10	10	10	10	10	10	10	10	10	10
D5	32.9	29.8*	30.9	31.0	29.2**	26.1	26.8	26.5	26.4	26.7
D26	35.0	32.5	34.1	33.6	31.4*	27.2	27.2	27.4	27.4	27.6

* : $p < 0.05$; ** : $p < 0.01$

- *description, severity, time of onset and duration of clinical signs :* no effects observed
- *gross pathology findings:* significant organ weight modifications were observed. Lung weight was not recorded.

Table 17 : Organ weight (in g or %)

		Males					Females				
Dose level (in mg/kg bw/d)		0	5	10	20	40	0	5	10	20	40
After 13w											
FBW		30.0	25.4**	24.6**	24.9**	25.0**	26.1	25.6	25.4	24.6	25.1
Adrenal glands	Abs	0.008	0.007	0.008	0.007	0.008	0.014	0.015	0.012	0.014	0.015
	Rela	0.026	0.028	0.031	0.030	0.031	0.053	0.058	0.049	0.056	0.060
Brain	Abs	0.488	0.483	0.494	0.483	0.486	0.488	0.501	0.495	0.496	0.500
	Rela	1.643	1.912**	2.017**	1.969**	1.948**	1.872	1.964	1.965	2.018*	2.000
Liver	Abs	1.25	1.08	1.13	1.11	1.17	1.63	1.59	1.48	1.51	1.62
	Rela	4.15	4.27	4.59**	4.48	4.68**	6.23	6.22	5.85	6.14	6.48
Spleen	Abs	0.069	0.056	0.059	0.051**	0.065	0.082	0.084	0.083	0.083	0.081
	Rela	0.229	0.223	0.240	0.204	0.260	0.315	0.329	0.328	0.336	0.323

Testes	Abs	0.235	0.234	0.232	0.235	0.232					
	Rela	0.792	0.927**	0.947**	0.954**	0.928**					
Thymus	Abs	0.035	0.030	0.024**	0.025**	0.029	0.027	0.029	0.025	0.025	0.023
	Rela	0.117	0.116	0.099	0.100	0.115	0.104	0.114	0.098	0.100	0.091

* : p < 0.05 ; ** : p < 0.01

- *histopathology findings:* the histopathological examination revealed changes in lungs such as an increase incidence of Clara cell alteration and proliferation.

Table 18 : Clara cells modifications

		Males					Females				
Dose level (in mg/kg bw/d)		0	5	10	20	40	0	5	10	20	40
After 13w											
Clara cell alteration	incidence	0	0	7	10	10	0	0	4	10	10
	Grade 1			3	2				1		
	Grade 2			4	7				2	6	
	Grade 3				1	5			1	4	3
	Grade 4					5					7
After 17w											
Clara cell alteration	Incidence	0	0	2	9	10	0	0	4	10	10
	Grade 1			1					1	1	
	Grade 2			1	7	1			3	9	
	Grade 3				2	9					10
Clara cell proliferation	Incidence	0	0	2	10	10	0	0	4	9	10
	Grade 1			2	2	3			3	2	1
	Grade 2				7	5			1	6	7
	Grade 3				1	2				1	2

- *mortality and time to death :* no effects observed

3.12.1.7 Chronic oral toxicity study (anonymous 25 (1985))

Study reference:

Anonymous 25 (1985)

Detailed study summary and results:

Test type

No guideline followed

No GLP

Test substance

- 3-methylpyrazole
- *Degree of purity :* no information available

Test animals

- *Species/strain/sex :* rat / Wistar / both sexes
- *No. of animals per sex per dose :* 32/sex/group

Administration/exposure

- *route of administration :* via drinking water
- *duration and frequency of test/exposure period :* 18m
- *doses/concentration levels :* 0, 10, 40 and 2000/1000 ppm (2000 ppm during w 1-4 thereafter 1000ppm during w 5-80)
- *vehicle:*

Results and discussion

- *body weight and body weight changes :* lower bw was observed at the highest dose

Table 19 : mortality and body weight (in g) observation

Dose level (in ppm)		0		10		40		40		2000/1000	
		Number of animals examined	bw	Number of animals examined	bw	Number of animals examined	bw	Number of animals examined	bw	Number of animals examined	bw
♂	W0	32	88	32	78	32	85	32	77	32	84
	W4	32	264	32	261	32	266	32	262	27	121
	W40	31	530	32	511	32	508	32	513	26	434
	W60	22	605	23	583	23	580	22	601	14	499
	W80	19	500	13	544	21	547	17	516	8	416
♀	W0	32	77	32	77	32	75	32	77	32	74
	W4	32	188	32	195	32	199	32	193	22	103
	W40	32	342	31	333	32	333	32	335	21	268
	W60	23	379	22	380	24	386	24	374	9	299
	W80	19	385	16	372	22	369	17	343	4	272

- *description, severity, time of onset and duration of clinical signs:* dyspnea, cachexia and pneumonia were observed in all dose levels.
- *haematological and clinical biochemistry findings:* hematological and bioclinical examination revealed changes at the highest dose level (see table 20).

Table 20 : Hematology and enzyme activity after 18 months

	Males					Females				
Dose level (in ppm)	0	10	40	40	2000/1000	0	10	40	40	2000/1000
Hb	167.5	168.7	162.9	156.5	155.1	149.0	147.1	149.7	146.3	139.3
Ht	0.52	0.51	0.49	0.48	0.48	0.48	0.50	0.48	0.50	0.49
Alanine	1.29	1.16	1.29	1.19	1.24	1.11	1.18	1.43	0.98	1.77

aminotrsanferase										
Alkaline phosphatase	1.29	1.26	1.24	1.14	1.80	0.94	1.10	1.11	0.96	1.17
Aspartate aminotransferase	1.62	1.29	1.51	1.25	1.67	1.14	1.22	1.57	1.16	1.21
cholinesterase	0.48	0.51	0.50	0.53	0.47	1.71	1.54	1.34	1.61	0.83
Leucine aminopeptidase	5.52	4.79	5.37	3.94	5.92	5.67	6.29	6.17	5.34	8.43
Gamma globulin	13.56	13.10	12.05	11.57	10.87	11.79	13.11	12.17	11.95	10.03

- *gross pathology findings:* Organ weight was examined and revealed few changes

Table 21 : Organ weight (in g or %)

		Males					Females				
Dose level (in ppm)		0	10	40	40	2000/1000	0	10	40	40	2000/1000
12 months											
Adrenal glands	Abs	0.064	0.063	0.060	0.056	0.052	0.083	0.084	0.093	0.091	0.083
	Rela	0.0110	0.0114	0.0110	0.0111	0.0115	0.0220	0.0246	0.0289	0.0236	0.0294
Brain	Abs	2.02	2.04	2.04	2.01	1.95	1.90	1.90	1.87	1.85	1.75
	Rela	0.350	0.368	0.375	0.399	0.432	0.512	0.557	0.581	0.480	0.629
Liver	Abs	23.8	24.1	19.5	17.1	21.8	13.3	15.4	13.1	13.8	15.8
	Rela	4.23	4.33	3.59	3.37	4.76	3.66	4.48	4.04	3.70	5.62
Spleen	Abs	0.825	0.907	0.801	0.805	0.779	0.690	0.747	0.621	0.665	0.544
	Rela	0.142	0.162	0.146	0.159	0.171	0.183	0.226	0.192	0.173	0.193
Thymus	Abs	0.162	0.169	0.139	0.152	0.137	0.138	0.109	0.126	0.107	0.126
	Rela	0.028	0.031	0.026	0.029	0.030	0.037	0.032	0.039	0.028	0.045
Testes ovary	Abs	3.86	3.89	3.93	3.87	3.67	0.102	0.102	0.118	0.097	0.082
	rela	0.67	0.70	0.72	0.76	0.81	0.0274	0.0302	0.0370	0.0253	0.0293
18 months											
Adrenal glands	Abs	0.084	0.068	0.071	0.100	0.057	0.098	0.113	0.094	0.110	0.070
	Rela	0.0192	0.0133	0.0140	0.0213	0.0146	0.0255	0.0325	0.0272	0.0331	0.0260
Brain	Abs	2.06	2.07	2.07	1.98	1.92	1.90	1.93	1.87	1.91	1.88
	Rela	0.459	0.399	0.396	0.412	0.474	0.504	0.563	0.538	0.578	0.704
Liver	Abs	22.5	22.9	21.7	20.3	20.0	16.0	15.5	16.4	14.5	13.8
	Rela	4.85	4.27	4.07	4.02	4.81	4.16	4.46	4.64	4.23	5.12
Spleen	Abs	0.866	0.788	0.975	0.767	0.794	0.715	0.790	0.701	0.695	0.609
	Rela	0.190	0.148	0.190	0.150	0.189	0.188	0.229	0.195	0.212	0.228
Thymus	Abs	0.121	0.103	0.131	0.139	0.105	0.085	0.095	0.073	0.106	0.131

	Rela	0.026	0.020	0.026	0.026	0.024	0.023	0.026	0.021	0.030	0.049
Testes ovary	Abs	3.46	3.79	3.95	3.65	3.25	0.107	0.107	0.100	0.112	0.090
	Rela	0.74	0.71	0.73	0.73	0.77	0.0280	0.0299	0.0291	0.0343	0.0333
Thyroid	Abs	0.046	0.041	0.044	0.075	0.048	0.038	0.042	0.036	0.050	0.049
	rela	0.0100	0.0077	0.0083	0.0159	0.0118	0.0100	0.0121	0.0102	0.0148	0.0183

- *histopathology findings:* the histopathological examination showed focal alteration in liver at 2000/1000 ppm.
- *mortality and time to death :* mortality was observed in all groups (see table 19)
- *reproductive parameters :* Reproductive parameters were examined. In females, follicular maturation and evolution of corpus luteum were unaffected. Moreover, in males, no effects on spermiogenesis were observed.

3.12.2 Human data

No information available

3.12.3 Other data

No information available

3.13 Aspiration hazard

Not evaluated in this CLH dossier.

4 ENVIRONMENTAL HAZARDS

Not evaluated in this CLH dossier.