Committee for Risk Assessment

RAC

Annex 1

Background document

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

2-methylimidazole

EC Number: 211-765-7
CAS Number: 693-98-1

CLH-O-0000001412-86-178/F

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 2017

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance name: 2-methylimidazole

EC Number: **211-765-7**

CAS Number: **693-98-1**

Index Number:

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Version number: 2 **Date: October 21, 2016**

Note on confidential information

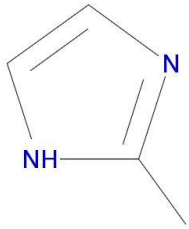
Please be aware that this report is intended to be made publicly available. Therefore it should not contain any confidential information. Such information should be provided in a separate confidential Annex to this report, clearly marked as such.

CONTENTS

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)	2-methyl-1H-imidazole
Other names (usual name, trade name, abbreviation)	Usual name: **2-methylimidazole**
ISO common name (if available and appropriate)	n/a
EC number (if available and appropriate)	211-765-7
EC name (if available and appropriate)	2-methylimidazole
CAS number (if available)	693-98-1
Other identity code (if available)	-
Molecular formula	$C_4H_6N_2$
Structural formula	
SMILES notation (if available)	c1([nH]ccn1)C
Molecular weight or molecular weight range	82.1038
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	n/a
Description of the manufacturing process and identity of the source (for UVCB substances only)	n/a
Degree of purity (%) (if relevant for the entry in Annex VI)	Not relevant

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self- classification and labelling (CLP)**

identifier)			
2-methylimidazole EC no.: 211-765-7 CAS no.: 693-98-1	98-100%	No current entry	Current self-classification in the full/lead registration: Repr. 1B - H360 Carc. 2 - H351 Acute Tox. 4 - H302 Skin Corr. 1C - H314 Eye Dam. 1 - H318 --- In addition the following hazard classes (with frequency of occurrence) are notified among the 20 other aggregated self-classifications in the C&L Inventory: 8/20: Skin Corr. 1B - H314 2/20: Repr. 2 - H361 2/20: STOT RE2 - H373 (thyroid) 2/20: STOT RE2 - H373 (endocrine system) 1/20: Skin Irrit. 2 - H315 1/20: Acute Tox. 4 - H312 1/20: Acute Tox. 4 - H332 1/20: Eye Irrit. 2 - H319 1/20: STOT SE3 - H335 (Lungs, inhalation)

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) for endpoints not specified in the Annex VI entry)**	**The impurity contributes to the classification and labelling**
Imidazole EC no.: 206-019-2 CAS no.: 288-32-4	Confidential information	Acute Tox. 4 – H302 Repr. 1B - H360D Skin Corr. 1C - H314. (AnnexVI entry number: 613-319-00-0)	Acute Tox. 3 - H301 STOT SE 3 - H336 Eye Dam 1 – H318	**Reproductive toxicity:** The impurity is not considered to contribute to the classification. See section 9.10.5 for further information

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
none					

Table 5: Test substances (non-confidential information)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information
2-methylimidazole EC no.: 211-765-7 CAS no.: 693-98-1	99.8%	-	Used in the reproductive toxicity studies (BASF 2013a and 2013b).
2-methylimidazole CAS no.: 693-98-1	99.1 – 100.8 %, depending on method of analysis.		14 days and 15 week repeated tox studies (NTP 2004a)
2-methylimidazole CAS no.: 693-98-1	> 95.5%		2-year repeated toxicity studies (NTP 2004b)

Note: The test substance is the same as the substance for which CLH is proposed.

2. PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	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 state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	-	-	-	-	-	-	-	-	-	-	-
Dossier submitters proposal	-	**2-methylimidazole**	**211-765-7**	**693-98-1**	**Repr. 1B**	**H360Df**	**GHS08** Danger	**H360Df**	-	-	-
Resulting Annex VI entry if agreed by RAC and COM	To be determined	**2-methylimidazole**	**211-765-7**	**693-98-1**	**Repr. 1B**	**H360Df**	**GHS08** **Danger**	**H360Df**	-	-	-

Table 7: 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 applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Hazard class not applicable	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 applicable	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 applicable	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity via oral route	Hazard class not assessed in this dossier	No
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	Hazard class not assessed in this dossier	No
Skin corrosion/ irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Hazard class not assessed in this dossier	No
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	**Harmonized classification proposed**	**Yes**
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No

Specific target organ toxicity-repeated exposure	Hazard class not assessed in this dossier	No
Aspiration hazard	Hazard class not applicable	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

2-methylimidazole has no prior harmonized classification, please see Table 2 for information on current self-classification. The substance was registered in 2013, and updated 2014, and was identified as a candidate for harmonised classification during ECHAs Manual Screening of substances for CLH in 2014.

4. JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

2-methylimidazole has a CMR property (developmental toxicity). Harmonised classification and labelling for CMR and respiratory sensitisation is a community-wide action under article 36 of the CLP regulation.

5. IDENTIFIED USES

2-Methylimidazole is used as a starting material, a chemical intermediate, and as a component in the manufacture of pharmaceuticals, photographic- and photothermographic chemicals, dyes and pigments, agricultural chemicals, and rubber. It is also widely used as a polymerization cross-linking accelerator and hardener for epoxy resin systems for semiconductor potting compounds and soldering masks. It is a component of numerous polymers including epoxy resin pastes, acrylic rubber-fluororubber laminates, films, adhesives, textile finishes and epoxy silane coatings. It is also used as a dyeing auxiliary for acrylic fibres and plastic foams (NTP, 2004b).

Usages registered in EU includes: industrial use of processing aids in processes and products, not becoming part of articles; industrial use resulting in manufacture of another substance (use of intermediates); and industrial use of process regulators for polymerisation processes in production of resins, rubbers, polymers (REACH registration, 2014).

6. PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties[1]

Property	Value	Reference [1]	Comment (e.g. measured or estimated)
Physical state at 20°C and 1013 hPa	Solid	REACH registration (2014)	.
Melting/freezing point	1) 144 – 145 °C at 1013 hPa 2) 142°C 3) 144 °C at 1013 hPa	1). GESTIS - Substance Database 2) Begg et al 1973, Australien Journal of Chemistry, 1973, vol. 26, p. 2435,246 3) Lide, D.R. CRC Handbook of Chemistry and Physics 88TH Edition 2007-2008. CRC Press, Taylor & Francis, Boca Raton, FL 2007, p. 3-356 as	1) Experimental result 2) Experimental result 3) Experimental result
Boiling point	1) 267 °C (No value for atmospheric pressure available) 2) 268 °C at 1013hPa 3) 267°C (No value for atmospheric pressure available) 4) 267 °C at 1013 hPa	1) Golovnya, R. V et al., 2000. Russian Chemical Bulletin, 2000, vol. 49, # 2 p. 319-324 as cited in Reaxys data base (Registry Number: 1368) 16 February 2011. 2). GESTIS - Substance Database (Information system on hazardous substances of the Berufsgenossenschaften) as cited 18.03.2011. 3) Yaws' Handbook of Physical Properties for Hydrocarbons and Chemicals As cited in Knovel e-books 18 February 2011 4) Lide, D.R. CRC Handbook of Chemistry and Physics 88TH Edition 2007-2008. CRC Press, Taylor & Francis, Boca Raton, FL 2007, p. 3-356 as cited in HSDB 18 March 2011 CSR	1-4: Experimental results
Relative density	1.096 g/ml at 20°C.	REACH registration (2014)	OECD Guideline 109 (Density of Liquids and Solids)
Vapour pressure	• 0.00043 hPa (extrapolated),at 20°C • 0.00078 hPa(extrapolated), at 25°C and • 0.011 hPa at 50°C.	REACH registration (2014)	OECD Guideline 104 (Vapour Pressure Curve)
Surface tension	Not surface active	REACH registration (2014)	Based on chemical structure, no surface activity is to be expected.
Water solubility	REACH registration	REACH registration (2014)	Experimental result - OECD Guideline 105 (Water Solubility)
Partition coefficient n-octanol/water	1) Log Pow = 0.24 (@25°C) 2) Log Pow = 0.22 @ 25°C 3) Log Pow = -0.17 @ 25°C 4) Log Pow = 0.24 @	1) Hansch, C., Leo, A., D. Hoekman. Exploring QSAR - Hydrophobic, Electronic, and Steric Constants. Washington, DC: American Chemical Society., 1995., p. 8 as cited in HSDB 18 March 2011 2)REACH registration (2014) 3)Domanska et al 2002, Journal of	1) Estimated by calculation 2) Experimental result, study similar to OECD TG 107. 3) Experimental result _guideline not reported 4) Experimental result

Property	Value	Reference [1]	Comment (e.g. measured or estimated)
	25°C	Chemical & Engeneering Data, 2002, vol. 47, #3 p456-466 as cited in Reaxys data base (Registry Number: 1368) 16 February 2011. 4) HANSCH, C ET AL. 195. As cited in SRC PhysProp Database, online query 16 Feb 2011.	
Flash point	Not relevant		The substance is a solid.
Flammability	Brief burning and rapid extinction was observed The test substance is not considered highly flammable	REACH registration (2014)	Experimental result (EU Method A.10 (Flammability (Solids))
Explosive properties	Data Waived in REACH registration	REACH registration (2014)	
Self-ignition temperature	Not relevant	REACH registration (2014)	The substance is a solid with a melting point < 160°C.
Oxidising properties	Data Waived in REACH registration	REACH registration (2014)	The Substance is incapable of reacting exothermically with combustible materials on the basis of the chemical structure.
Granulometry	The particle size distribution is: < 100 µm: 9.1 %; < 10µm: 0.0% < 4 µm: 0.0%	REACH registration (2014)	Experimental result (OECD Guideline 110)
Stability in organic solvents and identity of relevant degradation products	Data waived in REACH registration	REACH registration (2014)	The stability of the substance is not considered as critical.
Dissociation constant	1) pKa = 7.88 @ 25 °C. 2) pKa = 7.86 @ 25 °C. 3) pKa = 7.85 @ 25 °C.	1) REACH registration (2014) 2) Perrin, HH. Dissociation Constants of Organic Bases in Aqueous Solution: Supplement 1972. London: International Union of Pure and Applied Chemistry, 1972 as cited in HSDB 18 March 2011 3) PERRIN,DD 1965, as cited in SRC PhysProp Database, online query 16 February 2011	1) Model calculation using SPARC v4.5 2) Experimental result, guidline not reported. 3) Experimental result, guidline not reported.
Viscosity	not applicable	REACH registration (2014)	The substance is a solid.

[1]All information provided in this table was extracted from the REACH registration. If available, references as cited in the REACH registration has been included.

7. EVALUATION OF PHYSICAL HAZARDS

Not evaluated in the dossier

8. TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 9: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Male and female (F344/N) rats received a single gavage dose of 25, 50 or 100 mg/kg bw 2-methylimidazole. Sampling: 25 and 50 mg/kg bw groups: 5, 10, 15, 30, 60, 120, 240, 460 and 720 minutes after dosing. 100 mg/kg bw group: 5, 10, 15, 30, 60, 120, 240, 460, 720 and 1440 minutes after dosing. 3 rats were sampled at each time point. Each rat was sampled at two occasions. Plasma samples were analysed for 2-MI. Concentration versus time data were evaluated using nonlinear least-squares estimation in WinNONLIN (version 2.1). A one-compartment model with first order absorption and elimination was used to fit the data ($C(t)= D \cdot K_{01}/V/(K_{01}-K_{10}) \cdot [\exp(K_{10} \cdot t)-\exp(K_{01} \cdot t)]$). AUC values were calculated using the trapezoidal rule. Clearance was calculated as D/AUC_{inf} and the half-lives for the absorption and elimination phases were calculated as $0.693/K_{01}$ and $0.693/K_{10}$, respectively.	Peak 2-MI plasma concentrations were reached within 35 to 50 min for all groups. The absorption half-life values ranged from 10 to 18 minutes and were linear with dose. Elimination half-life values ($T_{½}$) ranged from 61 to 96 minutes. In the 100 mg/kg bw group, $T_{½}$ was higher compared to what would be expected from the increase in dose. Absolute bioavailability for 2-MI was estimated to approach 97%.	Publication Study report Reliability: 2 GLP-compliant study Name of test material (as cited in publication): 2-methylimidazole Analytical purity: 97.9% (Johnson, J.D., et al. 2002) >99.5 (NTP, 2004b)	Johnson, J.D. et al 2002. National Toxicology Program (2004b).
Male and female (B6C3F1) mice received a single gavage dose of 25, 50 or 100 mg/kg bw 2-methylimidazole. Sampling: 25 and 50 mg/kg bw groups: 5, 10, 15, 30, and 45 minutes after dosing. 100 mg/kg bw group: 60, 90, 180, 360 and 720 minutes after dosing. 3 mice were sampled at each time point. Each mice was sampled at one occasion. Plasma samples were analysed for 2-methylimidazole. Concentration versus time data were evaluated as	Peak 2-MI plasma concentrations were reached within 20 min for all groups. The absorption half-life values ranged from 2 to 4 minutes and were linear with dose. $T_{½}$ ranged from 15 to 20 minutes. In the 100 mg/kg bw group, $T_{½}$ was higher compared to what would be expected from the increase in dose.	Study report Reliability: 2 GLP-compliant study Name of test material (as cited in study report): 2-methylimidazole, CAS No. 693-98-1 Analytical purity: > 99.5%	National Toxicology Program (2004b).

Method	Results	Remarks	Reference
described above.			
Per oral administration of radiolabelled 2-methylimidazole as a single dose of 5, 50 or 150 mg/kg bw, or by intravenous administration of 5 mg/kg to male F344/N rats Sampling: Urine and faeces: 4, 8, 12, 24 and 48 hours. Expired air: 4, 8, 12 and 24 hours. Tissue examinations: Post oral dosing: 2 h (50 mg/kg bw) and 48 h (5, 50 or 150 mg/kg bw groups). Post i.v.-dosing: 0.25, 0.5, 1, 2, 4, 6, 8 and 12 hours Biliary excretion was investigated following i.v injections of 5 mg/kg bw to 3 rats.	Approximately 90% of the total dose was eliminated in urine within 24 h. Remaining ^{14}C was excreted in feces and as expired $^{14}CO2$. Excretion data were similar following iv administration. Biliary excretion of 2-MI-derived 14C was negligible. Approximately 70% of the ^{14}C excreted in urine was as parent compound. HPLC chromatograms for all treatment groups were similar, indicating that metabolism of 2-MI in rats was not affected by dose or route of administration. Skin, kidney, and liver contained the highest concentrations of 14-C following oral administration. The highest concentrations following iv administration was found in the kidney.	Publication Reliability: 1 GLP-compliant study Name of test material (as cited in study report): 2-methylimidazole Analytical purity: 99% Radiochemical purity: > 98%	Sanders, J.M. et al., 1998.
Male and female F344/N rats (15/sex/dose) recieved a single intravenous dose of 10 mg/kg bw. Blood samples were collected at 5, 10, 15, 30 and 45 min and 1, 1.5, 2, 4 and 8 h from 3 animals at each time point. Each rat was sampled twice	The iv profiles was best described by a two-compartment model with first-order elimination. 2-MI was rapidly distributed. The distribution half-life was 5 to 8 min. The volume of distribution (*V*ss) of 2-MI was determined to be 1 to 2 L.	Publication Reliability: 2 GLP-compliant study Name of test material (as cited in publication): 2-methylimidazole Analytical purity: 97.9% (Johnson, J.D., et al. 2002) >99.5 (NTP, 2004b)	Johnson, J. D. 2002. National Toxicology Program (2004b).

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

Male and female F344/N rats, and male and female $B6C3F_1$ mice was administered 2-methylimidazole (25, 50 or 100 mg/kg bw) by gavage (Johnson et al. 2002; NTP 2004b). The absorption half-life values ranged from 10 to 18 minutes in rats, and 2 to 4 minutes in mice and were generally linear with dose. Elimination half-life values ranged from 61 to 96 minutes in rats, and from 15 to 20 minutes in mice and were generally increased in the 100 mg/kg groups. The data indicate that the 100 mg/kg bw dose is approaching the upper limit of the linear dosing range. The oral bioavailability was approximately 97% (Johnson et al. 2002).

The distribution half-life following iv-administration of 10 mg/kg bw to male and female F344/N rats was 5 to 8 min, indicating that distribution of 2-MI from the blood to tissues was complete within about 30 min after entering the systemic circulation (Johnson et al. 2002). The volume of distribution (*V*ss) of 2-MI was determined to be 1 to 2 L. The highest peak concentration of ^{14}C was observed in the kidney. Following oral administration of 5, 50 or 150 mg/kg bw to male F344/N rats, only trace levels of 2-methylimidazole-derived radioactivity remained in tissues 48 hours post exposure (Sanders et al. 1998). The highest concentrations of ^{14}C was found in skin, kidney, and liver. Concentrations of ^{14}C in tissues increased proportionally with dose, and tissue/blood ratios of ^{14}C were relatively constant throughout the dose range. Hence, the distribution of 2-MI seems not to be affected by exposure level or route of exposure.

Following oral administration of radiolabelled 2-methylimidazole to male rats, the total dose excretion in urine approached 90% within 24 hours (Sanders et al. 1998). Hence, 2-methylimidazole is eliminated primarily via urine. The parent compound accounted for up to 78% of the total radioactivity excreted in urine, whereas radioactivity from metabolites made up only 5%. Fecal elimination accounted for most of the remaining radioactivity, however a small amount of the dose was eliminated via breath as CO_2. Elimination of radioactivity was somewhat more rapid following intravenous administration. In the iv-group, over 90% of the administered radioactivity was recovered in urine within 12 hours of injection.

Mice had slightly higher clearance rates than rats, and therefore lower internal exposure to 2-methyl imidazole following oral gavage administration at the same dose level.

Conclusion

The experimental toxicokinetic data shows that 2-methylimidazole is rapidly and extensively absorbed as well as rapidly distributed and eliminated in the body following oral and iv administration, indicating that there is no build-up of 2-methylimidazole for repeated exposure. The toxicokinetic processes are linear at doses below 100 mg/kg bw, however the elimination becomes saturated at higher dose levels.

9. EVALUATION OF HEALTH HAZARDS

9.1 Acute toxicity - oral route

Hazard class not evaluated in this dossier.

9.2 Acute toxicity - dermal route

Hazard class not evaluated in this dossier.

9.3 Acute toxicity - inhalation route

Hazard class not evaluated in this dossier.

9.4 Skin corrosion/irritation

Hazard class not evaluated in this dossier.

9.5 Serious eye damage/eye irritation

Hazard class not evaluated in this dossier.

9.6 Respiratory sensitisation

Hazard class not evaluated in this dossier.

9.7 Skin sensitisation

Hazard class not evaluated in this dossier.

9.8 Germ cell mutagenicity

Hazard class not evaluated in this dossier.

9.9 Carcinogenicity

Hazard class not evaluated in this dossier.

9.10 Reproductive toxicity

No human data is available **for 2-methylimidazole.**

9.10.1 Adverse effects on sexual function and fertility

Table 10a: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference** **Reliability**
OECD Guideline 421 (Reproduction/Developmental toxicity screening test GLP-compliant Rat (Wistar) 10 animals per sex per dose.	EC name: 2-methylimidazole (CAS No. 693-98-1) Analytical purity: 99.8% Oral: gavage, once daily (vehicle: 1% carboxymethylcellulose in drinking water) Dose levels: 0, 50, 150 and 500 mg/kg bw/day Males were dosed for 28 days (2 weeks prior to mating, during mating (max 2 weeks), and up to the day prior to scheduled necropsy). Females were dosed from 2 weeks prior to mating until day 4 of lactation (last dose on the day prior to scheduled necropsy).	**Parental generation**: Mortalities and clinical observations:Two high dose dams died during or shortly after parturition (post-natal day (PND) 2 and 3) both animals showed signs of complicated parturition proceeding death (undelivered pups, umbilical cords not cut, newborn pups not nursed). There were no pathological findings which could explain these premature deaths. No other adverse clinical observation was recorded in the study. <u>Body weights</u> 500 mg/kg: Mean body weight gain during gestation (day 0 –20) was statistically significantly reduced (-18% as compared to the controls). On postnatal day 0, the mean maternal body weight was statistically significantly deceased (-7% as compared to controls). No effects at 150 and 50 mg/kg bw/day. No adverse effects on male body weights or food consumption **Organ weights and Histopathology** No effects on absolute and relative mean organ weights for testes and epididymides (the only reproductive organs that were weighed). No adverse finding at the histopathological examination of testes, epididymides and ovaries (these were the reproductive organs that were examined). **Fertility, parturition and sexual function** Male and female mating and fertility[1] indices were 100% in all 2-methylimidazole treated groups. The gestation index[2] was 100% at all dose	BASF (2013 a) As cited in REACH registration (2014) Reliability 1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference Reliability
		levels A statistically significant increase in mean duration of pregnancy (22.5 days) was recorded in the high dose dams. The recorded gestation length was however still comparable between the test substance-treated groups and the control group (i.e. between 21.9 and 22.5 days. (According to the registrant, Historical control data within the lab for this strain and type of study is 21.6 – 22.4 days) Mean number of implantation sites was comparable between all test substance-treated groups and the controls (12.9, 12.8, 13.0 and 11.8 implants/dam at 0, 50, 150 and 500 mg/kg bw/day, respectively). No effects on post-implantation losses (8.2%/ 7.5%/12.0% / 9.2%) or on mean number of pups (live + dead) delivered per dam (11.8, 11.9, 11.5 and 11.1 pups/dam at 0, 50, 150 and 500 mg/kg bw/day, respectively). ↑ number of stillborn pups in the high dose group (11*[p<=0.01] as compared to 0, 0 and 4 in the control, low and intermediate dose group, respectively). This was mainly caused by high-dose dam No. 140 (found dead on PND 3), which had 7 stillborn pups in its litter (12 pups in total). Consequently the live birth index was reduced at the high dose level, 90%** [p<= 0.01] when compared to the other dose groups (100%, 100%, 97% and) in the control, low and intermediate dose groups, respectively). Historical control data for live birth index in the lab 93-100 %). The registrant considers that the increase in gestation length and the reduced live birth index are adverse findings and these findings are the basis for setting LOAELs for reproduction. See Table 35a for more information regarding fetal examination.	

[1]Female fertility index (%) = (number of females pregnant* / number of females mated**) x 100. * defined as the number of females with implants in utero. ** defined as the number of females with vaginal sperm or with implants in utero. [2]Gestation index (%) = (number of females with live pups on the day of birth / number of females pregnant*) x 100. * defined as the number of females with implants in utero. [3]Live birth index (%) = (number of live-born pups at birth / total number of pups born) x 100..

Table 10b: Summary table of other studies relevant for toxicity on sexual function and fertility.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure.**	**Results**	**Reference** **Reliability**
RAT studies			
Oral repeated dose toxicity study in rat Not in compliance with GLP Rat (Sprague-Dawley) 10 animals per sex and per group	2-Methylimidazol >99% purity Oral gavage, once daily, 5 days/week for 4 weeks **Dose levels:** 100, 200, 400, 800 mg/kg bw/day	**Clinical signs and mortalities**: no mortality observed, slightly ruffled fur at 200 mg/kg dose; 400 and 800 mg/kg: yellow urine, ruffled fur and increased salivation. **Body weight and weight gain:** normal body weight gain in all exposure groups, except for males at 800 mg/kg bw. **Organ weights and histopathology**: No effects on testicular weights (no info on other reproductive organs). No adverse finding at the histopathological examination of testicles/ovaries, prostate/uterus, seminal vesicles and epididymis up to and including the high dose level.	Study report (Report date 1975-12-29) entitled " Exp Key Repeated dose toxicity: oral.001" in REACH registration (2014) Reliability 3 (dosing only 5 days /week , thus difficult to assess what dosing period the result from this study cover.
Non-guideline GLP-compliant repeated dose toxicity study Rat (Fischer 344) 5/sex/dose	2-methylimidazole (CAS No. 693-98-1) Analytical purity: 99.1 – 100.8 %, depending on method of analysis Dietary, 15 days daily **Dose levels:** Nominal: 0, 1200, 3300 and 10 000 ppm. Actual: 0, 108, 297 and 900 mg/kg bw/day.	**Clinical signs and mortalities** All male and female rats survived till the end of the study with no exposure-related clinical signs. **Body weight and weight gain** ↓ mean absolute body weight in high dose males (162g** as compared to 198g in the control group). ↓ mean body weight gain in high (38g**) and intermediate (63g**) dose males as compared to controls (75g); and in high dose females (18**) as compared to controls (34g). **Food consumption** In the groups treated with 900 mg/kg food consumption was significantly reduced for males and females. **Gross pathology and histopathology** No effect on testis weight (the only reproductive organ that was weighed) and no abnormal finding at the histopathological examination of the ovary and the testes (the only reproductive organs that were examined) at doses up to and including 900 mg/kg bw/day.	NTP (2004a)– Chan, P. et al., 2006. Reliability 2 (reliable with restrictions)
Similar or equivalent	2-methylimidazole	**Clinical signs and mortalities**: No mortalities	NTP (2004 a

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure.</th><th>Results</th><th>Reference
Reliability</th></tr>
<tr>
<td>to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)
GLP compliant
Rat (Fischer 344)
10/sex/dose in core study groups
<u>Additional analysis at end of study in controls and at the 3 highest dose levels:</u>
Oestrous cycle monitoring (during the last 12 study days) and sperm analysis (number and motility).</td>
<td>(CAS No. 693-98-1)
Analytical purity: 99.1 – 100.8 %, depending on method of analysis
Dietary, 14 weeks daily
<u>Dose levels:</u>
Nominal: 0, 625, 1250, 2500, 5000 and 10000 ppm.
Actual ingested: 0, 40, 80, 160, 300 and 560 mg/kg bw/day</td>
<td>Body weights. Mean body weight were significantly reduced in high-dose males (78% of control) and high-dose females (89% of control).

Organ weights
The only reproductive organ that was included in the list of organs that were weighed at necropsy was the right testis. In addition the weight of the organs specified below (at these dose levels) were recorded before processing these tissues for sperm analysis.
<table>
<tr><th>Dose (mg/kg bw/day)</th><th>0</th><th>160</th><th>300</th><th>560</th></tr>
<tr><td>Testis (g) (absolute)
- Right
- Left</td><td>
1.388
1.498</td><td>
1.478
1.539</td><td>
1.471
1.518</td><td>
1.247*
1.289**</td></tr>
<tr><td>Testis (g)(Relative)
- right
- left</td><td>
3.78
-</td><td>
4.22**
-</td><td>
4.38**
-</td><td>
4.37**
-</td></tr>
<tr><td>L epididymis (g)</td><td>0.4987</td><td>0.4965</td><td>0.4852</td><td>0.4341**</td></tr>
<tr><td>L cauda epididymis (g)</td><td>0.1777</td><td>0.1798</td><td>0.16512</td><td>0.1250**</td></tr>
<tr><td>Necropsy weight (g)</td><td>366 ±7</td><td>350±2</td><td>336±5*</td><td>294**</td></tr>
</table>
Gross Pathology
Small uteri in 10 000ppm females (Uterus weights were not recorded).

Histopathology
The incidence of animals with testicular degeneration was significantly increased in the high-dose group (2, 2, 1, 2, 2 and 9** in the control, 40, 80, 160, 300 and 560 mg/kg dose group. respectively). The group mean severity score (grading 0-4, 1 = minimal, 2=mild, 3=moderate, “4” = marked severity) was, however, lower in 2-methylimidazole treated groups as compared to controls (2.5, 1, 1, 1, 1, 1.2, in the 0, 40, 80, 160, 300 and 560 mg/kg dose group. respectively). There was no recording of adverse histopathological findings in the epididymidis, seminal vesicle, prostate, ovary or in the uterus

Sperm analysis and esterous cycling
<table>
<tr><th>Dose (mg/kg bw/day)</th><th>0</th><th>160</th><th>300</th></tr>
<tr><td>Spermatid heads</td><td></td><td></td><td></td></tr>
</table>
</td>
<td>Chan. P. et al., 2006.

Reliability 1 (reliable without restriction)</td>
</tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure.</th><th>Results</th><th>Reference
Reliability</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>(10^7/ g testis)
10^7/ testis)</td><td>8.63 ± 0.32
13.02 ± 0.83</td><td>8.70± 0.29
13.22 ± 0.52</td><td>8.63 ± 0.30
11.13 ± 0.42*</td></tr>
<tr><td>Spermatid counts (mean/10^{-4} mL susoension)</td><td>65.09 ± 4.17</td><td>67.22 ± 2.59</td><td>55.66 ± 2.09*</td></tr>
<tr><td>Epididymal spermatozoal measurements (Motility (%)

Conc (10^6/ cauda epididymal tissue)</td><td>
87.67 ± 0.36

439 ± 25</td><td>
87.91 ± 0.51

399 ± 38</td><td>
87.46 ± 0.62

487 ± 72</td></tr>
</table>
In females, the mean length of estrous cycle of the high-dose females was significantly increased as compared to the controls (4.30 ±.15, 4.61 ± 0.14, 4.65 ± 0.15 and 5.56±0.41**days in the controls, 160, 300 and 560 mg/kg dose group, respectively).</td><td></td></tr>
<tr><td>Equivalent or similair to OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies)
GLP compliant
Rat (F344/N)
60/sex/dose in core study groups.</td><td>2-methylimidazole
(CAS No. 693-98-1) (Analytical purity: > 99.5%)
Dietary,2 year, with interim evaluation of 10 animals/dose group at 6 months)
<u>Dose levels</u>: Nominal: 0, 300, 1000 and 3000 ppm (males); 0, 1000, 2500 and 5000 ppm (females).
Actual: 0, 13, 40 and 130 mg/kg bw (males); 0, 50, 120 and 230 mg/kg bw (females)</td><td>Clinical signs and mortalities:
Survival of dosed females was significantly less than that of the controls. Clinical findings included a thin body condition in high dose males and females. This was attributed to poor palatability of the feed rather than a toxic effect of 2-methylimidazole.
Body weight and weight gain
Mean body weights of high dose males and intermediate and high dose females were generally less than those of the controls during most of the study.
Organ weights No recording were taken during the study for reproductive organs
Histopatology
No increase in non-neoplastic findings in reproductive organs was recorded at histopathological examination at the interim evaluation.
2yr: Hyperplasia of uterus endometrium 10/50, 14/50, 15/50 and 15/50. Periovarian tissue, cyst (4/50, 5/50, 4/50, 6/50) Similar incidences of germinal epithelium atrophy and sperm granuloma of the epididymidis in concurrent control and compound.</td><td>NTP, 2004b.
Chan. P.C. et al., 2008.
Tani Y, et al., 2005
Reliability 1 (reliable without restriction)</td></tr>
<tr><td colspan="4">Studies in Mouse</td></tr>
<tr><td>Repeated dose toxicity study
GLP compliant
Non-guideline</td><td>2-methylimidazole (CAS No. 693-98-1)
Purity: Depending on analytical method 99.1</td><td>Clinical signs and mortalities: All male and female mice survived till the end of the study with no exposure-related clinical signs.
Body weight and body weight gain: In the females treated with 1933 mg/kg body weight gains</td><td>NTP (2004a)
Chan, P. et al., 2006.</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure.**	**Results**	**Reference** **Reliability**
Mouse B6C3F1 5/sex/dose	– 100.8%) Dietary, 15 days daily- **Dose levels.** Nominal: 0, 1200, 3300 and 10000 ppm. Actual ingested: 0, 232, 638 and 1933 mg/kg bw/day	were significantly reduced compared to controls. No effects on the weight of the testis (only reproductive organ that was examined). No adverse finding at the histopathological examination of the testis and ovaries (only reproductive organs that were examined) at dose levels up to and including 1933 mg/kg :	Reliability 2 (reliable with restrictions)
Equivalent/similar to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents) GLP compliant Mouse (B6C3F1) 10/sex/dose in core study groups. Additional analysis at end of study in controls and at the 3 highest dose levels: Oestrous cycle monitoring during last 12 study days and sperm analysis (number and motility).	2-methylimidazole (CAS No. 693-98-1) Analytical purity: depending on method of analysis 99.1 - 100.8%), Dietary, 14 weeks daily **Dose levels:** Nominal: 0. 625, 1250, 2500, 5000 and 10000 ppm. Actual ingested :0, 100, 165, 360, 780 or 1740 mg/kg bw/day (males); 0, 90, 190, 400, 800 and 1860 mg/kg bw/day (females)	**Clinical signs and mortalities** All mice survived. No chemical-related clinical signs of toxicity were observed. **Body weight and weight changes** ↓ Terminal body weight was recoded at the 5000 and 10000 ppm dose level in both males (33.7 g** and 30.0 g**, respectively as compared to controls, 37.4g) and females (26.5 g** and 23.5 g**, respectively as compared to controls, 32.0 g). **Organ weights:** No reported effects on the weight of the testis (the only reproductive organ that was weighed) **Histopatology** No adverse effect recorded at the histopathological examination of the reproductive organs**.** **Spermanalysis and vaginal cytology** No significant differences between exposed and control mice were found on sperm motility or on the esterous cycle length.	NTP, 2004a Chan, P. et al., 2006. Reliability 1 (reliable without restriction)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure.**	**Results**	**Reference** **Reliability**
Equivalent or similar to OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies) GLP compliant Mouse (B6C3F1 60/sex/dose in core study groups	2-methylimidazole) (CAS No. 693-98-1) (Analytical purity: > 99.5%) Dietary,2 year, with interim evaluation of 10 animals/dose group at 6 months Nominal: 0, 625, 1250 and 2500 ppm in diet. Actual ingested 0, 75, 150 and 315 mg/kg bw/day (males); 0, 80, 150 and 325 mg/kg bw/day (females)	**Clinical signs and mortalities**: Survival of all exposed groups was similar to that of the control group. No clinical findings were attributed to 2-methylimidazole exposure **Body weights:** Mean body weights of mid- and high-dose males and high dose females were less than those of the controls during most of the study **Histopatholog** *Evaluation at 6 month* No dose dependent increase of adverse findings were recorded at the histopathological examination of the reproductive organs *Evaluation at end of study:* No dose-dependent increase in adverse effects was recorded in the female reproductive organs An increase in sperm granuloma of the epididymidis (0/0/6/12% in control, low, intermediate and high dose, respectively) and of germinal epithelium atrophy (2/8/16/28% in control/low/intermediate and high dose, respectively) was recorded at the intermediate and high dose level. However these effects were only found after 2 years and are therefore considered to be of less relevance for the evaluation of reproductive toxicity.	NTP 2004b Chan, P.C. et al., 2008. Tani Y, et al., 2005. Reliability 1 (reliable without restriction)

The information provided in the table only relates to endpoints that are of specific interest for the evaluation of reproductive toxicity.

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

No effects on male or female fertility or mating indices were recorded in the available reproductive screening study (OECD 421, GLP; oral gavage) at dose level up to and including 500 mg/mg bw/day. No adverse histopathological findings were recorded at the examination of the testis, ovaries and epididymides or on the weight of the testicles (no other reproductive organs were analysed in the study). However, at the highest dose level, an increase in mean duration of pregnancy (22.5 days, statistically significant) was observed. The recorded gestation length was however comparable between the test substance-treated groups and the control group (i.e. between 21.9 and 22.5 days, historical control data within the performing laboratory for this strain and type of study is 21.6 – 22.4 days). Two high dose dams died during or shortly after parturition (PND 2 and 3), both showing signs of complicated parturition proceeding death (undelivered pups, umbilical cords not cut, newborn not nursed). As a consequence their pups either died or had to be killed for humane reasons. There were no pathological findings that could explain these deaths. There were no adverse clinical findings in the high dose group and the recorded effects on body weight on PND 0 (↓ 7% as compared to controls)

were mild in nature. The effect on parturition at the high dose level is considered to be an adverse effect on female reproduction that is not considered to be secondary to unspecific toxicity.

Some indications of an adverse effect on fertility were recorded in the rat 14 week dietary repeated dose toxicity study (NTP 2004a, GLP study of high reliability). In this study, a lower but statistically significant decreased testis weight (absolute: ~10% less as compared to the controls; P≤0.05) and epididymidis weight (13% less than the control; p≤0.01) was recorded at the high dose level (560 mg/kg bw/day). Spermanalysis revealed a decrease in the spermatid head count (p≤0.05, 14.5% less as compared to controls), but no effect on the motility or on the concentration of epididymal spermatozoa was recorded at the high dose level. No adverse histopathological finding was recorded at the examination of the ovary, uterus, epididymidis, seminal vesicle and prostate. A higher incidence of testis degeneration was recorded in the high dose males, but the group mean severity-score of the finding was mild and therefore this finding is considered to be of no importance for classification purposes. The high dose females displayed an increased oesterous cycle length (5.38±0.24 days (p≤0.01) as compared to 4.70 ±0.15 in the controls). However, there was no significant difference between the high dose and controls females, when one compared the relation of time spent in in each stage of the oesterous cycle. At the high dose level the group mean necropsy weight of males were ~20% less and that of the females was ~11% less as compared to the controls. No similar effect on sperm count, oesterous cyling and testis and epididymal weights was recorded in the 14 week dietary repeated dose toxicity study in mice (NTP 2004a, GLP study of high reliability) at dose level up to and including ~1800 mg/kg bw/day.

In the rat 2 year dietary carcinogenicity study (NTP 2004b, GLP), no histopathological findings of relevance for the evaluation of reproductive toxicity was recorded at the interim evaluation after 6 months or at the end of the study at dose levels up to and including 130 (m)/230(f) mg/kg bw/day. In the mice dietary 2 year carcinogenicty study (NTP 2004b, GLP), there were no histopathological findings of relevance for the evaluation of female reproduction. In male mice, there was an increase in the incidence of germinal cell atrophy as well as in the incidence of spermgranuloma of the epididymidis. However since no similar findings was recorded at the 6 month interim evaluation, the recording after 2 year is considered to be of less importance for the evaluation of male reproductive toxicity.

In conclusion, the available data from repeated dose toxicity studies do not give a concern for effects on the integrity of the male and female reproductive organs. No adverse effect was recorded for female and male fertility, mating and gestation indices in the OECD 421 study. However it should be emphasised that this study is a screening study (covering a limit number of endpoints and having less statistical power then the more comprehensive reproductive toxicity studies (2-generation, one generation or extended one generation reproductive toxicity studies), consequently an absence of signal should be interpreted with caution. In addition, since there is no two-generation/one-generation or extended one generation study available for 2-methylimidazole, there is no available study where fertility is assessed after an exposure period that fully covers spermatogenesis or folliculogenesis, neither do the available studies allow for an assessment of possible effects on sexual maturation. The available data set do however indicate that female reproduction is affected by 2-methylimidazole, i.e. two dams out of 10 died during or shortly after parturition in the OECD 421 study.

9.10.3 Comparison with the CLP criteria

The available dataset indicate that there is some evidence for an adverse effect on the process of parturition. In the OECD 421 study two dams in the high dose group died during or shortly after parturition. Although no clear effect was seen on the group mean gestation time, the severity of the finding as such is considered to be high especially since the recorded level of toxicity in the high

dose group females was considered to be mild and no abnormal clinical findings were recorded for the other high dose dams during the study or for the period up until start of parturition for the two dams that were found dead. The observed mortalities are not considered to be secondary to non-specific toxicity but rather be specifically related to the process of parturition. Classification in Repr. 2 - H361f is therefore, warranted.

Classification in Repr. 1A – H360F is not justified since there is no human data that indicates that 2-methyl imidazole have an adverse effect on human fertility or sexual maturation.

Classification in Repr. 1B – H360F is not warranted since the data set available is limited and the signal strength is such that it is considered to provide “some” but not “clear” evidence for an adverse effect on fertility.

9.10.4 Adverse effects on development

Table 11: Summary table of animal studies on adverse effects on development

Method Guideline Deviation(s) from the guideline	Species Strain Sex no/group	Dose levels duration of exposure Test substance.	Results	Reference
GLP compliant OECD Guideline 421 (Reproduction / Developmental Toxicity Screening Test) All pups with scheduled sacrifice on PND 4, moribund/still born pups and pups that died before scheduled necropsy were examined externally and eviscerated; their organs were assessed macroscopically, paying particular attention to the heart and aortic vessels. All pups with findings and 10 control pups (5/sex) were further processed for histopathological examination	Rat (Wistar) 11-13 week at start of dosing male/female 10 animals per sex per dose:	EC name: 2-methylimidazole (CAS No. 693-98-1) Analytical purity: 99.8% Oral: gavage, once daily (vehicle: 1% carboxymethylcellulose in drinking water) 0, 50, 150 and 500 mg/kg bw/day (actual ingested) Males were dosed for 28 days (2 weeks prior to mating, during mating (max 2 weeks), and up to the day prior to scheduled necropsy). Females were dosed from 2 weeks prior to mating, until day 4 of lactation (last dose on the day prior to	Mortalities and clinical observations: see Table 34a. **Maternal toxicity** GD 0- 20 mean body weight gain stat sign reduced (-18% as compared to controls) on PND0 mean body weight stat sign deceased (-7%) as compared to controls. No effects at 150 and 50 mg/kg bw/day. **Developmental effects** No effects on post-implantation losses (8.2% / 7.5% / 12.0% / 9.2% in the control, low, intermediate and high dose group). ↑ number of stillborn pups in the high dose group (11*[p<=0.01] as compared to 0, 0 and 4 in the control, low and intermediate dose group, respectively). This was mainly caused by high-dose dam No. 140 (found dead on PND 3), which had 7 stillborn pups in its litter (12 pups in total). Consequently the live birth index[1] was decreased in the high dose group (90%** [p<=0.01] as compared to 100%, 100%, 97% in controls, low and intermediate dose groups, respectively. ↓ Viability index PND 0-4 (i.e. pups also died during lactation [28 pups died and 3 were cannibalized]) in the high dose group (59%** [p<= 0.01]) as compared to 99% / 98% / 97% in in the control (1 pup was cannibalized in the control), low and intermediate dose groups. No adverse clinical signs were observed in the F1 pups. Six runts[2] were born in the high dose group. Slightly, but not statistically significant, decreased pup mean body weight and body weigh changes were recorded during lactation. Gross pathological examination of pups identified aneurysms at different levels of the aorta, in the region of the ductus arteriosus and the pulmonary trunk. Frequently, aneurysms (balloon-like swellings) were observed simultaneously at different sites in the same pup. Number of affected pups were 0, 2, 14 and 42 in the control, low, intermediate and high dose group, respectively.	BASF 2013 a As cited in REACH registration (2014)

Method Guideline Deviation(s) from the guideline	**Species Strain Sex no/group**	**Dose levels duration of exposure Test substance.**	**Results**	**Reference**
of the basis of the heart and great vessels		scheduled necropsy).	In most cases, histopathology correlated with the aneurysm detected at gross pathology. Number of affected pups were 0, 2, 14 and 37 in the control, low, intermediate and high dose groups, respectively). No NOAEL for developmental effects was identified in the study.	
GLP-compliant modified reproduction/developmental screening study Examinations in adult females included clinical signs, parturition and lactation behaviour, body weight, food consumption and gross pathology Pup examinations included pup status, litter size and external examination at birth, viability, clinical signs, body weight, gross pathological examination, followed by histopathological examination of the great blood vessels of the heart of all	Rat (Wistar) 25 pregnant females/group	2-methylimidazole (CAS: 693-98-1) Purity: 99.8% Oral: gavage, once daily (vehicle: 1% carboxymethylcellulose in drinking water) **Dose levels**: 0, 2, 10 and 50 mg/kg bw/day **Duration of exposure** Once daily from gestation day 6 through post-natal day 3 (i.e. from implantation to one day prior to sacrifice).).	**Maternal toxicity** No test-substance related clinical signs or mortalities in any dose group. No effect on food consumption or on parental body weights of the parental females. **Development** No effects on Gestation index[3], live birth index[1] or mean litter-size at birth. The number of stillborn pups was comparable between the groups. No effects on viability index at PND4. No effect on mean pup body weight or body weight changes. The number of runts[2] was 1, 2, 1, 6 in the 0, 2, 10 and 50 mg/kg bw/day group, respectively. Histopathological examination of the base of the great vessels of the heart revealed dissection aneurysm in 0, 1, 3 and 3 pups in the controls, low intermediate and high dose group, respectively	BASF SE (2013b) As cited in REACH registration (2014)

Method Guideline Deviation(s) from the guideline	Species Strain Sex no/group	Dose levels duration of exposure Test substance.	Results	Reference
pups.				

[1]Live birth index (%) = (number of liveborn pups at birth / total number of pups born) x 100. [2] Runts defined as pups weighing less than 75% of the mean weight of concurrent control pups.

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

For examination of developmental effects two studies are available, a reproduction/developmental toxicity screening test in Wistar rats according to OECD 421 and GLP (BASF 2013a) and a follow-up study (GLP) according to a modified developmental toxicity protocol (BASF 2013b), with dosing of only females from gestation day 6 until postnatal day 3. The follow-up study was conducted because developmental toxicity (dissecting aneurysm of the great vessels of the heart) was observed at all dose levels (0, 2, 14 and 37 pups in the control, low, intermediate and high dose groups, respectively)) in this original study. In addition a decreased pup-viability index at PND 4 (53% as comp to 100 % in control) and a decreased live birth index (90%, although mainly due to one litter, as comp to 100% in controls) was recorded in the original study. No maternal toxicity were seen at the low and intermediate dose levels whereas a reduced gestational body weight gain (-18% as compared to controls) and a statistically significant decrease in mean body weight (7% less than the controls at PND 0) were recorded at the high dose level.

In the follow up study (BASF 2013b), no apparent maternal toxicity was recorded and no treatment-related effects on litter size, number of stillborn pups, on postnatal survival or on pup weight at birth and PND (see Table 35a for more details). Gross pathology examination of the pups revealed macroscopic dilations of the great vessels at the base of the heart (aorta, ductus arteriosus, pulmonary trunk and carotid artery) (0, 1, 4, 5 pups in the control, low, intermediate and high dose groups, respectively) mostly these effects were correlated microscopically with dissecting aneurysms, which occurred in 0, 1 (0.5%), 3 (1.2%) and 3 (1.3%) in the controls, low, intermediate and high dose level. Predominant locations were the ductus arteriosus and aorta. No aneurysms were observed in male and female control pups. The incidence in the low dose group was slightly higher as compared to the background incidence reported from the same lab (0.2% see Treumann et al., 2011).

Histopathology also revealed the presence of intramural haemorrhages that were not detected macroscopically. There was no dose –response relationship in the distribution of pups with haemorrhages and the incidence in 2-methylimidazole treated groups were similar to the incidence recorded in the control group i.e.in 2 (0.9%), 3 (1.4%), 1 (0.4%) and 2 (0.9%) in the controls, low, intermediate and high dose pups. Individual pups had either aneurysms or haemorrhages, but never both lesions together.

Imidazole, a substance that has a harmonised classification in Repr 1 B-360D (7^{th} ATP), is a known impurity in 2-methylimidazole (see table 3). However, considering that the stated purity of 2-methylimidazole used in the studies reported (BASF 2013a and 2013b, see REACH registration

2014) is 99.8%, the contribution from imidazole in the test substance used in the studies was at maximum 0.2% (i.e. below the GCL for imidazole). Furthermore, based on the available data, 2-methylimidazole is clearly more potent ($LOAEL_{developmental\ toxicity}$ = ≤2 mg/kg bw/day) than Imodazole ($NOAEL_{developmental\ toxicity}$ = 60 mg/kg bw/day and $LOAEL_{developmental\ toxicity}$ = 160 mg/kg bw/day, see ECHA 2013). It can therefore be concluded that it is highly unlikely that a possible impurity of imidazole (up to a maximum concentration of 0.2%) had any impact on the recorded developmental toxicity that was recorded in the two studies that examined the potential for 2-methylimidazole to cause developmental toxicity.

9.10.6 Comparison with the CLP criteria

Classification in Repr. 1A – H360D is not justified since there is no human data that indicates that 2-methyl imidazole have adverse effect on human fetal development.

Classification in Repr. 1B – H360D is warranted since the evidence for developmental toxicity is considered to be *clear*. Dissecting aneurysm of the vessel at the base of the heart was detected in rat pups in two separate studies (BASF 2013a; BASF 2013b). The adverse effect was seen at dose levels down to 10 mg/kg bw/day, and possibly at 2 mg/kg bw. In addition pup viability was decreased during the first days of lactation (viability index at PND 4 was 53% as compared to 100 % in controls) at 500 mg/kg bw/day. The recorded effects are relevant for humans, and are not considered to be secondary to non-specific maternal toxicity.

Classification in Repr. 2 would be relevant if the data set only would provide “some evidence” of developmental toxicity, but as the present data base is considered to provide clear evidence classification in Repr. 1B is the proper classification.

9.10.7 Adverse effects on or via lactation

Pups were only followed until day 4 postnatally and this limits the assessment of possible effects on or via lactation. There is no information on whether the compound is transferred to the milk.

9.10.8 Comparison with the CLP criteria

The available database does not give support for a classification for effects on or via lactation.

9.10.9 Conclusion on classification and labelling for reproductive toxicity

Based on available data classification in Repr. 1B – H360Df is warranted.

9.11 Specific target organ toxicity-single exposure

Hazard class not evaluated in this dossier.

9.12 Specific target organ toxicity - repeated exposure

Hazard class not evaluated in this dossier.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

Sexual function and fertility

The Dossier Submitter (DS) noted that no effects on male or female fertility or mating indices were recorded in the available Reproduction/Developmental toxicity screening test (OECD TG 421, GLP; BASF 2013a) at dose levels up to and including 500 mg/kg bw/d by oral gavage. No adverse histopathological findings were recorded on examination of the testes, ovaries and epididymides or on the weight of the testicles (no other reproductive organs were analysed in the study). However, at the highest dose level, an increase in mean duration of pregnancy (to 22.5 days, statistically significant) was observed. The recorded duration was however comparable between the test substance-treated groups and the control group (i.e. between 21.9 and 22.5 days). Two top dose dams died during or shortly after parturition (postnatal days (PND) 2 and 3), both showing signs of complicated parturition, preceding death. As a consequence, their pups either died or had to be killed for humane reasons. There were no pathological findings that could explain these deaths.

The DS considered that the available data from repeated dose toxicity studies do not raise concerns for effects on the integrity of the male and female reproductive organs.

The available data indicated to the DS that there is some evidence for an adverse effect on the process of parturition. In the OECD TG 421 study, two dams in the top dose group died during or shortly after parturition. Although no clear effect was seen on the group mean duration of pregnancy, the severity of the finding as such is considered to be high, especially since the recorded level of general toxicity in the top dose group females was considered to be mild. No abnormal clinical findings were recorded for the other top dose dams during the study, or for the period up until start of parturition for the two dams that were found dead. The observed mortalities were not considered to be secondary to non-specific toxicity but were instead considered to be specifically related to the process of parturition. On this basis, the DS proposed classification as Repr. 2; H361f.

Development

For examination of developmental effects two studies were available, a Reproduction/ Developmental toxicity screening test in Wistar rats (OECD TG 421, GLP; BASF, 2013a) and a follow-up study (GLP; BASF 2013b) according to a modified developmental toxicity protocol, with dosing of only females from gestation day (GD) 6 until PND 3. The follow-up study was conducted because developmental toxicity (dissecting aneurysm of the great vessels of the heart) was observed at all dose levels in the original study. In addition, a decreased pup viability index at PND 4 and a decreased live birth index were recorded in the original study. No maternal toxicity was seen at the low and intermediate dose levels whereas a reduced gestational body weight gain (-18% as compared to controls) and a statistically significant decrease in mean body weight (7% less than the controls at PND 0) were recorded at the top dose level.

In the follow-up study, no apparent maternal toxicity was recorded and there were no treatment related effects on litter size, number of stillborn pups, on postnatal survival or on pup weight. Gross pathology examination of the pups revealed macroscopic dilations of the great vessels at the base of the heart (aorta, ductus arteriosus, pulmonary trunk and carotid artery). Mostly these effects were correlated microscopically with dissecting aneurysms. Predominant locations were the ductus arteriosus and aorta. No aneurysms were observed in male and female control pups. The incidence in the low dose group was slightly higher as compared to the background incidence reported from the same lab (0.2%; Treumann *et al*., 2011).

Histopathology also revealed the presence of intramural haemorrhages that were not detected macroscopically. There was no dose–response relationship in the distribution of pups with haemorrhages and the incidence in 2-methylimidazole treated groups were similar to the incidence recorded in the control group. Individual pups had either aneurysms or haemorrhages, but never both lesions together.

Imidazole, a substance that has a harmonised classification as Repr 1B; 360D (7^{th} ATP), is a known impurity in 2-methylimidazole. However, considering that the stated purity of 2-methylimidazole used in the studies reported is 99.8%, the contribution from imidazole in the test substance used in the studies was at maximum 0.2%. This is below the concentration limit for classification of imidazole as a developmental toxicant. Furthermore, based on the available data, 2-methylimidazole is clearly more potent ($LOAEL_{developmental\ toxicity}$ = ≤2 mg/kg bw/d) than imidazole ($NOAEL_{developmental\ toxicity}$ = 60 mg/kg bw/d and $LOAEL_{developmental\ toxicity}$ = 160 mg/kg bw/d; see ECHA, 2013). Therefore the DS concluded that it is highly unlikely that a possible impurity of imidazole (up to a maximum concentration of 0.2%) had any impact on the developmental toxicity that was recorded in the two studies with 2-methylimidazole.

The DS concluded that classification as Repr. 1B; H360D is warranted since the evidence for developmental toxicity is considered to be clear. Dissecting aneurysm of the vessel at the base of the heart was detected in rat pups in two separate studies. The adverse effect was seen at dose levels down to 10 mg/kg bw/d, and possibly at 2 mg/kg bw/d. In addition pup viability was decreased during the first days of lactation (viability index at PND 4 was 59% as compared to 100% in controls) at 500 mg/kg bw/d. The recorded effects are relevant for humans, and are not considered to be non-specific effects secondary to maternal toxicity.

Adverse effects on or via lactation

Pups were only followed until PND 4 and this limits the assessment of possible effects on or via lactation. There was no information on whether the compound is transferred to the milk. The available database did not support classification for effects on or via lactation.

Conclusion on classification

Based on available data the DS concluded that classification in Repr. 1B; H360Df is warranted.

Comments received during public consultation

Comments were submitted by three Member States Competent Authorities (MSCA) and one Company/Manufacturer.

Sexual function and fertility

One MSCA agreed with the DS's proposal to classify 2-methylimidazole in Category 2 for effects on sexual function and fertility.

One MSCA considered this to be a borderline case between Category 2 and no classification because the evidence supporting classification is limited to the deaths of two dams. This MSCA asked the DS to explain why the mortalities could not be due to a general maternal toxicity (statistically significant decrease in bodyweight gain).

The DS responded that: "The mortalities occurred at PND 2 and PND 3, where the mean body weight gain was reduced by 18% in the top dose group compared to control. During GD 0-20 the mean body weight gain was -18.1 % as compared to control. At lactation day 0 the mean body weight was statistically significantly reduced by 7.4 % as compared to control. The effects (mean values) on body weight and body weight changes are not considered severe. We do not have the full study report and can therefore not look into individual data of the two dams dying on PND 2 and 3. Nevertheless, in a study by Carney et al (2004) determining the effects of feed restriction in rat during in utero and postnatal life on standard reproductive toxicity and developmental immunotoxicity end points, reductions in maternal body weights down to 32% in feed restricted rats (as compared to control) during gestation and the lactation period did not cause any mortality or treatment-related clinical effects in the dams."

The same MSCA also suggested that the decrease in spermatid heads could be considered relevant for classification despite the fact that the number of spermatozoa was not affected. The DS noted that no effects on sperm count, testes or epididymis of relevance for the evaluation of reproductive toxicity were detected in the available mouse repeated dose toxicity studies (15 days, 14 weeks and 2 years) or in the rat 2-year combined chronic and carcinogenicity study. No effect on male functional fertility was recorded in the Reproduction/developmental toxicity screening test. Therefore the DS considered that the reduced sperm count in the 90-d study in rats was of low toxicological significance.

A third MSCA requested a more detailed justification for the proposal to classify for effects on fertility. This MSCA invited a consideration of the possible mechanism leading to abnormal parturition in Wistar rats. Since thyroid lesions were observed in F344 rats in a 90-d study, the MSCA asked whether there could be a link between disturbance of thyroid hormone levels and complications in parturition in rats. In response to the MSCA that raised this issue, the DS noted that in the screening study, the thyroid gland was not weighed and no hormonal analysis was performed. Moreover, as stated earlier, no individual data on the dams, including duration of pregnancy, were available to the DS. In the open literature, imbalances in thyroid hormone levels in humans are considered to be associated with complications during pregnancy and sequelea after delivery with

adverse maternal and fetal outcomes (e.g. Cignini *et al*., 2012). However, based on the available information in the current dossier, there were no indications of a possible mechanism of the abnormal parturitions and it would be solely speculative to discuss a potential mechanism. Therefore, it is not possible to convincingly link the observed deaths of the two dams (due to complications during parturition) to effects on the thyroid gland and hormonal imbalance.

A Company/manufacturer of 2-methylimidazole disagreed with the proposal for classification in category 2, commenting that general toxicity might have contributed to the observed problems during and shortly after parturition in the two dams which died at PND 1 and 2. Thus the specificity of this finding with regard to a fertility-impairing effect cannot be judged. The stakeholder noted that the duration of pregnancy at the top dose (22.5 days) was similar to historical control data for this rat strain from OECD screening studies from the same laboratory (21.6-22.4 days). The first female that died evidently showed insufficient maternal care, a non-consumed placenta and died at PND 2. The second female had undelivered pups palpable in the dam's abdomen, the umbilical cord was not cut and pups were not properly nursed at PND 0. The dam and all pups were found dead at PND 1. During clinical observation, there were no obvious severe findings in the top dose group and in the two animals that died (salivation after treatment and discoloured urine in all dams). There were also no particular macroscopic findings and no microscopic findings in ovaries, but no other inner organs were examined. However, dams in the top dose group showed statistically significant reduced food consumption during the first week of premating and lactation phase (-13.5% or -20.3%, respectively) compared to the control group. The maternal body weight gain during gestation (GD 0-20) and the body weight at lactation day 0 were also statistically significantly reduced: -18.1% or -7.4%, respectively, compared to controls. These findings in the top dose animals might be hints for systemic toxicity, which was observed in the available repeated dose toxicity studies.

Development

Three MSCAs and one Company/manufacturer agreed with the proposal to classify 2-methylimidazole in Category 1B for developmental toxicity.

Two of the three MSCAs asked for clarification on the incidence at which aneurysms were observed in the screening study. The number of pups examined for each group was not available to the DS. However, based on the number of pups delivered and the assumed number of litters, the foetal incidences of this effect were calculated to be 0, 1.7, 3.5 and 33.3% at 0, 50, 150 and 500 mg/kg bw/d.

Additional key elements

14-week dietary study in the rat (NTP, 2004a)

In response to a comment made by an MSCA, the DS noted that diffuse thyroid follicular hyperplasia (≥ 160 mg/kg bw/d in females) and effects on the serum levels of T3, T4 and TSH (from day 8 of dosing) were recorded in the 14-week dietary study in the rat (NTP, 2004a). The DS provided the following information.

Table: Incidences of neoplasms and selected non-neoplastic lesions in rats in the 14-week feed study of 2-methylimidazole (NTP, 2004a)

Dose (ppm) **(mg/kg bw/d)**	0 **(0)**	625 **(40)**	1250 **(80)**	2500 **(160)**	5000 **(300)**	10000 **(560)**
Males						
Thyroid gland[a]	10	10	10	10	10	10
Follicular cell, hyperplasia, diffuse[b]	2 (1.5)[c]	0	8* (1.1)	10** (1.1)	10** (1.9)	10** (2.9)
Follicular cell cyst	0	0	0	0	0	1 (2.0)
Follicular cell adenoma	0	0	0	0	0	2
Testes	10	10	10	10	10	10
Degeneration	2(2.5)	2(1.0)	1(1.0)	2(1.0)	2(1.0)	9**(1.2)
Females						
Thyroid gland	10	9	10	10	10	10
Follicular cell, hyperplasia, diffuse	0	0	0	10** (1.0)	10** (2.0)	10** (3.0)

* Significantly different (p≤0.05) from the control group by the Fischer exact test

** p≤0.01

[a] Number of animals with organ examined microscopically

[b] Number of animals with lesions

[c] Average severity of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Table: Effects on serum levels of thyroid-stimulating hormone, triiodothyroxine and thyroxin in female rats in the 14-week feed study (NTP, 2004a)

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

Week 14	2.57±0.28	2.17±0.20	1.94±0.28	2.16±0.30	2.42±0.22	0.79±0.12**

[a] Female data only. Information extracted from Table 8 of the NTP 2004 reference.
* Significantly different (p≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (p≤0.01) from the control group by Shirley's test.

15-d dietary study in the rat

In addition, enlarged thyroid glands as well as an increased incidence (5/5 for both female and male) of mild to moderate diffuse hyperplasia of thyroid gland follicular cells was recorded at 297 and 900 mg/kg bw/d in the 15-d dietary repeated dose toxicity study in rats (see table below). No hormonal analysis was carried out in this study.

Table: Incidences of selected non-neoplastic lesions in rats in the 15-d feed study of 2-methylimidazole (NTP, 2004a)

Dose (ppm) **(mg/kg bw/d)**	0 **0**	1200 **115**	3300 **290**	10000 **770**
Males				
Thyroid gland[a]	5	5	5	5
Follicular cell, hyperplasia, diffuse[b]	0	0	5**	5**
Pituitary gland	5	5	5	5
Pars distalis, hypertrophy	0	0	5**	5**
Females				
Thyroid gland	5	5	5	5
Follicular cell, hyperplasia, diffuse	0	0	5**	5**
Pituitary gland	5	5	5	5
Pars distalis, hypertrophy	0	0	0	5**

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

Assessment and comparison with the classification criteria

Sexual Function and Fertility

1) GLP-compliant Reproduction/developmental toxicity screening test (OECD TG 421; BASF, 2013a)

Wistar rats (10/sex/dose) were exposed to 0, 50, 150 and 500 mg/kg bw/d of 2-methylimidazole, by gavage. Males were dosed for 28 days (two weeks prior to mating, during mating (maximum two weeks), and up to the day prior to scheduled necropsy). Females were dosed from two weeks prior to mating until day four of lactation (last dose on the day prior to scheduled necropsy).

Two top dose dams died during or shortly after parturition (on PND 2 and 3). Prior to their deaths, signs of complicated parturition were observed in both dams (undelivered pups, umbilical cords not cut, newborn pups not nursed).

Effects on parental generation bodyweight were observed in top dose females only. During gestation, mean bodyweight gain was 18% lower than in controls (statistically significant). The mean maternal bodyweight was statistically significantly reduced (by 7%) on PND 0.

The only reproductive organs that were weighed were the testes and epididymis. The reproductive organs that underwent histopathological examination were the testes, epididymis and the ovaries. No adverse effects on weight or histopathology were observed in these organs.

Exposure to 2-methylimidazole had no effect on gestation index or on male and female mating and fertility indices.

The DS reported that there was a statistically significant increase in mean duration of pregnancy at the top dose (22.5 days). This was just outside the historical control data range (21.6-22.4 days) in the same laboratory for this strain and type of study. However, the DS also commented that the recorded duration was "comparable between the test substance-treated groups and the control group (i.e. between 21.9 and 22.5 days)". Therefore the biological significance of this observation is unclear.

The number of implantation sites per dam was slightly lower at the top dose compared to the number in other dose groups (12.9, 12.8, 13.0 and 11.8 at 0, 50, 150 and 500 mg/kg bw/d, respectively). However, this effect was not statistically significant.

Under the conditions of this study, two top dose dams died during or shortly after (complicated) parturitions. In the absence of sufficient overt general toxicity in this dose group to account for these findings, RAC concurs with the DS and considers that these deaths may have been due to an adverse effect on female reproduction.

During the Public Consultation, one MSCA discussed the effects observed on sperm parameters. In addition to the screening test, information from repeated dose studies in rats and mice exposed to 2-methylimidazole is also available and is relevant for the discussion on male reproductive toxicity. Findings related to reproduction which were observed in repeated dose studies are summarised below.

1) 4-week gavage study, Sprague Dawley rats (10/sex/group; REACH registration dossier, study from 1975): doses up to 800 mg/kg bw/d. There were no effects on testicular weights. No information on the weights of other reproductive organs was provided. No adverse histopathological effects were observed on the testicles/ovaries, prostate/uterus, seminal vesicles or epididymis.

2) 15-d dietary study, Fischer 344 rats (5/sex/dose; NTP 2004a): doses up to 900 mg/kg bw/d. Testis weight was not affected by treatment. No other reproductive organs were weighed. No adverse histopathological effects on the ovary or testis were observed.

3) 90-d dietary study, Fischer 344 rats (10/sex/dose; NTP 2004a): doses of 0, 40, 80, 160, 300 or 560 mg/kg bw/d. 'Small uteri' were observed in top dose females. However, uterine weights were not recorded. In males, the changes in reproductive organ weights were as follows:

Table: Reproductive organ weight changes in male rats in a 90-d study (NTP, 2004a)

Dose (mg/kg bw/d)	0	160	300	560
Testis (g) (absolute) - Right - Left	 1.388 1.498	 1.478 1.539	 1.471 1.518	 1.247* 1.289**
Testis (g) (relative) - Right - Left	 3.78 -	 4.22** -	 4.38** -	 4.37** -
L epididymis (g)	0.4987	0.4965	0.4852	0.4341**
L cauda epididymis (g)	0.1777	0.1798	0.16512	0.1250**
Necropsy weight (g)	366 ± 7	350 ± 2	336 ± 5*	294**

* p≤0.05
** p≤0.01

As tabulated below, the incidence of testicular degeneration was significantly greater at the top dose. However, the severity of testicular degeneration was lower in top dose males than in controls and therefore this effect is not considered adverse.

Table: Testicular degeneration in male rats in a 90-day study (NTP, 2004a)

Dose (mg/kg bw/d)	0	40	80	160	300	560
Number of animals with testicular degeneration	2	2	1	2	2	9**
Group mean severity score[a]	2.5	1	1	1	1	1.2

[a] group mean severity score (grading 0-4, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked severity)
** p≤0.01

No adverse histopathological effects on the epididymis, seminal vesicle, prostate, ovary or uterus were reported.

A decrease in spermatid heads per testis and spermatid counts was observed in top dose males as shown below. However there were no notable changes on the motility or concentration of epididymal spermatozoa.

Table: Sperm parameters in male rats in a 90-day dietary study (NTP, 2004a)

Dose (mg/kg bw/d)	0	160	300	560
Spermatid heads (10^7/g testis) (10^7/testis)	 8.63 ± 0.32 13.02 ± 0.83	 8.74 ± 0.29 13.44 ± 0.52	 8.70 ± 0.29 13.22 ± 0.52	 8.63 ± 0.30 11.13 ± 0.42 [-14.5%]
Spermatid counts (mean/10^{-4} mL suspension)	65.09 ± 4.17	67.22 ± 2.59	66.09 ± 2.64	55.66 ± 2.09 [-14%]

Epididymal Spermatozoal measurements				
(Motility (%))	87.67 ± 0.36	86.88 ± 0.70	87.91 ± 0.51	87.46 ± 0.62
Conc (10^6/cauda epididymal tissue)	439 ± 25	378 ± 44	399 ± 38	487 ± 72 [+10%]
Total number of spermatozoa (10^6)/ cauda epididymis	78.01	67.96	65.88	60.88 [-20%]

In females, there was a dose-dependent increase in mean oestrous cycle length (4.30 ± 0.15, 4.61 ± 0.14, 4.65 ± 0.15 and 5.56 ± 0.41 ($p \leq 0.01$) days at 0, 160, 300 and 560 mg/kg bw/d, respectively).

4) 2-year combined dietary chronic toxicity/carcinogenicity study, F344 rats (60/sex/dose; NTP 2004b): doses up to 130 and 230 mg/kg bw/d in males and females, respectively. Reproductive organs were not weighed. After 2 years, there were similar incidences of germinal epithelium atrophy, sperm granuloma of the epididymis and cysts of the periovarian tissue in concurrent controls and treated groups. Hyperplasia of uterine endometrium was observed in 10/50, 14/50, 15/50 and 15/50 females at 0, 50, 120 or 230mg/kg bw/d, respectively.

5) 15-d study (GLP), B6C3F1 mice (5/sex/dose; NTP 2004a): doses up to 1933 mg/kg bw/d. After limited investigation, no adverse histopathological or weight changes of reproductive organs were reported.

6) 90-d study (GLP), B6C3F1 mice (10/sex/dose; NTP 2004a): doses up to 1740 and 1860 mg/kg bw/d, respectively. Although investigation of the reproductive organs was limited, no adverse histopathological or weight changes of reproductive organs were reported. There were no significant differences in sperm motility or on oestrous cycle length in treated animals in comparison to controls.

7) 2-year combined dietary chronic toxicity/carcinogenicity study (GLP), B6C3F1 mice (60/sex/dose; NTP 2004b): doses of up to 315 and 325 mg/kg bw/d in males and females, respectively. No dose-dependent adverse effects were observed in female reproductive organs. In males, the following effects were observed after 2 years, but not at the 6 months interim evaluation.

Table: Effects on the reproductive organs of male mice in a chronic toxicity/carcinogenicity study (NTP, 2004b)

Dose (mg/kg bw/d)	0	75	150	315
Incidence of sperm granuloma of the epididymis (%)	0	0	6	12
Incidence of germinal epithelium atrophy (%)	2	8	16	28

Summary and conclusion of the findings from the repeated dose studies

Overall, the small changes in male fertility parameters observed in repeated dose studies are considered to be a possible indication of an adverse effect of 2-methylimidazole on fertility. However, RAC concurs with the DS's conclusion that the reduced sperm count in the 90-d study in rats was of low toxicological significance. The limited findings in the repeated dose studies are not considered sufficient to support classification for effects on sexual function and fertility.

Summary and Conclusion on sexual function and fertility

In the absence of evidence of overt general toxicity, RAC concurs with the conclusion of the DS, i.e. that the deaths of two dams during, or shortly after, parturition in the screening study was considered to be a specific adverse effect on female fertility that is not secondary to general toxicity. Signs of complicated parturition were observed in both dams preceding death. Therefore RAC considers that classification for effects on sexual function and fertility is warranted. It is noted that the adverse effects were seen in a single screening study with only 10 rats/sex/dose and that the evidence of an adverse effect on fertility is limited to that observed in two dams only. There is no mechanistic explanation for the findings. Overall, RAC agrees that there is some evidence for an adverse effect of 2-methylimidazole on female fertility, but that the evidence is not clear enough to support classification in Category 1B. Therefore RAC supports classification of 2-methylimidazole in **Category 2; H361f** for effects on sexual function and fertility.

Development

Two studies in rats are available.

1) GLP-compliant Reproduction/developmental toxicity screening study (OECD TG 421; BASF 2013a)

In the screening study, Wistar rats were exposed to 2-methylimidazole at 0, 50, 150 or 500 mg/kg bw/d, as described above in the section "Sexual Function and Fertility".

Maternal toxicity was limited to bodyweight changes at the top dose only: during gestation, mean bodyweight gain was 18% lower than in controls (statistically significant). The mean maternal bodyweight was statistically significantly reduced (by 7%) on PND 0.

As tabulated below, post-implantation losses and mean number of pups (live + dead) were unaffected by treatment. However, there was a significant increase in the number of stillborn pups, and consequently a statistically significant reduction in live birth index, at the top dose (90% compared to 100% in controls). The live birth index was outside the historical control data range in the laboratory (93-100%). The reduced live birth index in this study was mainly attributable to a single top dose dam, who gave birth to 12 pups. Seven of these pups were stillborn and this dam died on PND 3. The litter incidence of this effect reduces concern for developmental toxicity. In addition, there was a statistically significant reduction in the viability index at the top dose (59% compared to 99% in controls). In the controls, one pup was cannibalised, whereas at the top dose 28 pups died and three were cannibalised during PND 0-4. Five of these 31 pups were born to the dam that died on PND 3.

Table: Births and pup survival in the Reproduction/developmental toxicity screening study in rats (BASF, 2013a)

	Dose (mg/kg bw/d)			
	0	50	150	500
Post-implantation losses (%)	8.2	7.5	12.0	9.2
Mean number of pups (live + dead) delivered per dam	11.8	11.9	11.5	11.1
Number of stillborn pups	0	0	4	11*
Live birth index (%)	100	100	97	90**
Viability index (PND 0-4) [a] (%)	99	98	97	59**

[a] Pup survival from postnatal day 0-4
* p≤0.05
** p≤0.01

No adverse clinical signs were observed in the pups, but 2, 2 and 6 runts were born at the low, mid and top doses, respectively. Runts were defined as pups weighing less than 75% of the mean weight of concurrent control pups. During lactation, slight non-statistically significant decreases in pup mean body weight and body weight changes were recorded at the top dose.

As shown below, a dose-related increase in the incidence of aneurysms was observed at gross pathological examination and confirmed via histopathology. The aneurysms were observed at different levels of the aorta, in the region of the ductus arteriosus and the pulmonary trunk.

Table: Incidences of aneurysms in the screening study in rats (BASF, 2013a)

	Dose (mg/kg bw/d)			
	0	50	150	500
Aneurysms (gross pathology)	0	2	14	42
Aneurysms (histopathology)	0	2	14	37
(% foetal incidence)	0	1.7	3.5	33.3

In conclusion, the study shows that 2-methylimidazole is a developmental toxicant. The observation of increased incidences of aneurysms is a clear indication of a developmental effect.

2) <u>GLP-compliant modified Reproduction/developmental screening study (BASF, 2013b)</u>

In a second modified screening study, 25 pregnant Wistar rats per group were exposed to lower doses of 2-methylimidazole (0, 2, 10 or 50 mg/kg bw/d) by gavage from GD 6 to PND 3. No signs of maternal toxicity were observed. This follow-up study was conducted to define a NOEL for this endpoint because developmental toxicity was observed at all doses in the original study, as described above.

There were no substance-related effects on gestation index, live birth index, mean litter size at birth, number of stillborn pups, viability index on PND 4, pup bodyweight or pup bodyweight changes.

Adverse developmental effects are summarised in the table below.

Table: Developmental effects in the Reproduction/developmental screening study in rats (BASF, 2013b)

	Dose (mg/kg bw/d)			
	0	2	10	50
Number of runts	1	2	1	6
Macroscopic dilations of the great vessels at the base of the heart (aorta, ductus arteriosus, pulmonary trunk and carotid artery)	0	1	4	5
Aneurysms (number, (%)) (histopathology)	0	1 (0.5%)	3 (1.2%)	3 (1.3%)
Intramural haemorrhages (number, (%)) (histopathology)	2 (0.9%)	3 (1.4%)	1 (0.4%)	2 (0.9%)

Thus, the findings in this second study were consistent with those from the first. The macroscopic dilations of the great vessels at the base of the heart generally correlated with dissecting aneurysms identified upon histopathological investigation. There was a clear dose-dependent increase in the incidence of aneurysms in pups exposed to 2-methylimidazole during development. The incidence of aneurysms at the lowest dose (0.5%) was slightly higher than the incidence in historical controls from the same laboratory (0.2%).

Intramural haemorrhages were also observed histopathologically. However the incidences were not dose-dependent and were similar to the incidence in controls. It was reported that individual pups had either aneurysms or haemorrhages, but never both lesions together. According to the registrant, only single pups in each litter were affected, with one exception each in the mid and top dose groups. At each of these doses, there was a litter with two affected pups. Where two pups were affected in a single litter, one pup had an aneurysm and the other had a haemorrhage.

Summary and Conclusion on developmental toxicity

Since there is no evidence of 2-methylimidazole-induced reproductive toxicity in humans, classification in Category 1A is not appropriate.

Dose-related increases in the incidence of aneurysms were seen in pups in two studies, with exposure to 2-methylimidazole from doses as low as 2 mg/kg bw/d. In addition, there was a decrease in the viability index at 500 mg/kg bw/d. The observed developmental effects are not considered to be secondary to maternal toxicity because maternal toxicity was limited to bodyweight changes at 500 mg/kg bw/d. Therefore the criteria[1] for classification in Category 1B; H360D for developmental toxicity are met.

[1] Classification in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects.

Effects on or via lactation

Classification for effects on or via lactation can be assigned based on:

a) human evidence indicating a hazard to babies during the lactation period; and/or
b) results of one- or two-generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or
c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

There is no human evidence indicating a hazard to babies during the lactation period. During lactation in the original study, there was a statistically significant decrease in viability index (PND 0-4) at 500 mg/kg bw/d. At the top dose, 28 pups died and three were cannibalised during PND 0-4, in contrast to controls where one pup was cannibalised. Since the 28 deaths occurred during lactation, it is possible that 2-methylimidazole caused adverse effects on lactation. However, the pups may already have been compromised when they were born. Since it is not clear whether the postnatal pup deaths were due to effects on or via lactation and since there are no data available to inform on whether the substance is present in breast milk, the criteria for classification are not considered to be met.

Conclusion on classification for reproductive toxicity

RAC considers that 2-methylimidazole warrants **classification as Repr. 1B; H360Df**.

9.13 Aspiration hazard

Hazard class not evaluated in this dossier.

10. EVALUATION OF ENVIRONMENTAL HAZARDS

Hazard class not evaluated in this dossier.

11. REFERENCES

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12. ANNEXES

There are no Annexes.