CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical name:

3,4-dimethyl-1*H*-pyrazole

EC Number: **429-130-1**

CAS Number: **2820-37-3**

Index Number: **613-248-00-5**

Contact details for dossier submitter:

Belgian Federal Public Service Health, Food Chain Safety and Environment
DGEM/ Department of Product Policy and chemical Substances / Management of Chemical Substances
Avenue Galilée, 5/2
1020 Brussels
Belgium

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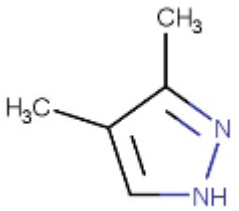
CONTENTS

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)	3,4-dimethyl-1*H*-pyrazole
Other names (usual name, trade name, abbreviation)	1H-Pyrazole, 3,4-dimethyl- 3,4-Dimethylpyrazol 3,4-Dimethylpyrazole DMP
ISO common name (if available and appropriate)	/
EC number (if available and appropriate)	429-130-1
EC name (if available and appropriate)	3,4-dimethyl-1*H*-pyrazole
CAS number (if available)	/
Other identity code (if available)	/
Molecular formula	C5H8N2
Structural formula	
SMILES notation (if available)	CC1=CNN=C1C
Molecular weight or molecular weight range	/
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)	/

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 (CLP)	Current self-classification and labelling (CLP)
3,4-dimethyl-1H-pyrazole		Acute Tox. 4, H302 Eye Dam. 1, H318 Aquatic Chronic 3, H412	Registration dossier: Acute Tox. 4, H302 Acute Tox. 4, H312 Acute Tox. 4, H332 Eye Dam. 1, H318 Carc. 2, H351 STOT RE 2, H373

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 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
There are no impurities relevant for classification				

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 (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: For substance with an existing entry in Annex VI of CLP

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	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	613-248-00-5	3,4-dimethyl-1*H*-pyrazole	429-130-1	2820-37-3	Acute Tox. 4* Eye Dam. 1 Aquatic Chronic 3	H302 H318 H412	GHS07 GHS05 Dgr	H302 H318 H412			
Dossier submitters proposal	613-248-00-5	3,4-dimethyl-1*H*-pyrazole	429-130-1	2820-37-3	**Retain** Eye Dam. 1 Aquatic Chronic 3 **Add** Carc. 2 Repr. 2 Acute Tox. 4 Acute Tox. 4 STOT RE 2 **Modify** Acute Tox. 4	**Retain** H318 H412 **Add** H351 H361f H332 H312 H373 (nasal cavity) **Modify** H302	**Add** GHS08	**Retain** H318 H412 **Add** H351 H361f H332 H312 H373 (nasal cavity) **Modify** H302		**Add** inhalation: ATE = 2.1 mg/L dermal: ATE = 1100 mg/kg bw oral: ATE = 500 mg/kg bw	
Resulting Annex VI entry if agreed by RAC and COM	613-248-00-5	3,4-dimethyl-1*H*-pyrazole	429-130-1	2820-37-3	Carc. 2 Repr. 2 Acute Tox. 4 Acute Tox. 4 Acute Tox. 4 STOT RE 2 Eye Dam. 1 Aquatic Chronic 3	H351 H361f H332 H312 H302 H373 (nasal cavity) H318 H412	GHS08 GHS07 GHS05 Dgr	H351 H361f H332 H312 H302 H373 (nasal cavity) H318 H412		inhalation: ATE = 2.1 mg/L dermal: ATE = 1100 mg/kg bw oral: ATE = 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** **ATE: 500 mg/kg bw**	Yes
Acute toxicity via dermal route	**Acute Tox. 4, H312** **ATE: 1100 mg/kg bw**	Yes
Acute toxicity via inhalation route	**Acute Tox. 4, H332** **ATE: 2.1 mg/L**	Yes
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	**Carc. 2, H351**	Yes
Reproductive toxicity	**Repr. 2, H361f**	Yes
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	**STOT RE 2, H373 (nasal cavity)**	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

3,4-dimethyl-1*H*-pyrazole has a harmonised classification and labelling (ATP 01[1]):

Acute Tox. 4*, H302

Eye Dam. 1, H318

Aquatic Chronic 3, H412

Classification and labelling included in the registration dossier:

Acute Tox. 4, H302

Acute Tox. 4, H312

Acute Tox. 4, H332

Eye Dam. 1, H318

Carc. 2, H351

STOT RE 2, H373

Several self-classifications are registered in the C&L inventory (22/11/2022). **It should be noted that although a harmonised classification is available for the environmental hazards, this classification and labelling is not applied by all notifiers**:

Classification		Labelling			Specific Concentration limits, M-Factors	Notes	Classification affected by Impurities / Additives	Additional Notified Information	Number of Notifiers	Joint Entries	
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code(s)							
Acute Tox. 4	H302			GHS08 GHS07 GHS05 Dgr			✔	State/Form	2	✔	View details
		H302+H312+H332									
Acute Tox. 4	H312										
Eye Dam. 1	H318	H318									
Acute Tox. 4	H332										
Carc. 2	H351	H351									
STOT RE 2	H373 (other:olfactory...)	H373									
Acute Tox. 4	H302	H302		GHS07 GHS05 Dgr			✔	State/Form	2	✔	View details
Eye Dam. 1	H318	H318									
Aquatic Chronic 3	H412	H412									
Acute Tox. 4	H302	H302		GHS08 GHS07 GHS05 Dgr				State/Form	87		View details
Eye Dam. 1	H318	H318									
Repr. 2	H361 (fertility, unbo...)	H361									
STOT RE 2	H373 (other:not avail...)	H373									
STOT RE 2	H373 (not available)	H373									
Acute Tox. 4	H302	H302		GHS07 GHS05 Dgr				State/Form	55		View details
Eye Dam. 1	H318	H318									
Aquatic Chronic 3	H412	H412									

Number of Aggregated Notifications: 4

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 self-classified as repro 2, H361 and Carc. 2, H351.

[1] ATP 01: adaptations to technical progress 01 is included in the consolidated version of the CLP regulation https://echa.europa.eu/regulations/clp/legislation

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

Acute toxicity: change in existing entry due to changes in the criteria
STOT: Disagreement by DS with current self-classification: other organs than olfactory affected as well.

5 IDENTIFIED USES

No public registered data are available indicating whether or in which chemical products the substance might be used (consumer, professional or industrial use). The substance is manufactured and/or imported into Europe.

6 DATA SOURCES

REACH Registration dossier: https://echa.europa.eu/substance-information/-/substanceinfo/100.102.749

Full study reports

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	Solid	Anonymous, 1998	/
Melting/freezing point	56.3 °C	Anonymous, 1998	OECD TG 102
Boiling point	223.2 °C (at 1013.3 hPa)	Anonymous, 1998	EU A.2
Relative density	1.077 (at 20 °C)	Anonymous, 1998	EU A.3 Pycnometer method
Vapour pressure	1.6 Pa (at 20 °C) 2.8 Pa (at 25 °C) 37 Pa (at 50 °C)	Anonymous, 1998	EU A.4 Effusion method
Surface tension	66.1 mN/m (at 20 °C, 1g/L)	Anonymous, 1998	EU A.5
Water solubility	656 g/L (at 25 °C, pH ≥6.9 - ≤7.7)	Anonymous, 1998	EU A.6
Partition coefficient n-octanol/water	Log Know= 1.26 (at 25 °C, pH ≥6.8 - ≤6.89)	Anonymous, 1998	EU A.8 (shake flask method)
Flash point	/	/	No data available
Flammability	Substance does not ignite and propagate combustion either by burning with flame or smouldering along 200 mm of the powder train within the 2 minutes test period.	Anonymous, 2016	UN Manual of Tests and Criteria: Test N.1
Explosive properties	/	/	No data available

Property	Value	Reference	Comment (e.g. measured or estimated)
Self-ignition temperature	/	/	No data available
Oxidising properties	/	/	No data available
Granulometry	/	/	No data available
Stability in organic solvents and identity of relevant degradation products	/	/	No data available
Dissociation constant	pKa= 4.05 (at 22 °C)	Anonymous, 1998	OECD TG 112
Viscosity	29.1 mPa s	Anonymous, 2009	DIN 51562 Part 1 Capillary viscometer (static)

8 EVALUATION OF PHYSICAL HAZARDS

Hazard class not assessed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not assessed in this CLH dossier.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Table 8: 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 Oral (gavage) OECD TG 423 GLP	Rat (Wistar) 3 females/group	3,4-dimethyl-1*H*-pyrazole Vehicle: corn oil	First Exp: 2000 mg/kg bw Second Exp: 500 mg/kg bw Third Exp: 500 mg/kg bw Single exposure	> 500 and < 2000 mg/kg bw	Anonymous, 2015

No human data or other data available

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

In acute oral toxicity study (Anonymous, 2015), following the OECD TG 423, a group of 3 females were initially exposed by gavage to the test substance at a concentration of 2000 mg/kg bw. Immediately after exposure, animals exhibited a poor general state, piloerection, atonia, abdominal position, shallow

breathing and within 2 hours, all animals died. Necropsy revealed dark red spot in all lung lobes, spotted liver, yellowish discoloration of the stomach contents, red discoloration of the small intestine.

A second experiment was performed, and 3 new females were exposed to the test substance at a concentration of 500 mg/kg bw. In this experiment, 1 female showed dyspnoea, piloerection, impaired general state followed by poor general state, cowering position, staggering, abdominal position, apathy, atonia and lack of defecation and was sacrificed on day 1, due to this moribund state. The necropsy showed dark red spot discoloration of all lung lobes and congestion of the kidneys. The 2 other females survived during the observation period of 14 days, however general state was disrupted (impaired general state (from 0 h to 4 h after administration) which increased to poor general state (at 5 h) and decreased to impaired general state (on day 1 after exposure)) and clinical signs were observed such as piloerection, dyspnea, cowering position, abdominal position, staggering, and reduced defecation.

A third experiment was also performed, as for the 2nd experiment, 3 females were exposed to the test substance at a concentration of 500 mg/kg bw. During this 3rd experiment, no mortality occurred, but all females exhibited impaired general state, piloerection, cowering position and dyspnea.

Based on the results, the LD_{50} was comprised between 500 and 2000 mg/kg bw.

10.1.2 Comparison with the CLP criteria

Table 9: comparison with the CLP criteria regarding acute toxicity via oral route

CLP criteria	Results of available studies
Acute toxicity category 4: oral LD_{50}: > 300 but ≤ 2000 mg/kg bw	LD_{50} of the key study was comprised between 500 and 2000 mg/kg bw This LD_{50} is comprised in the range of the category 4.
Regarding ATE: based on the table 3.1.2 in the CLP Regulation ("conversion from experimentally obtained acute toxicity range values to acute toxicity point estimates") For a substance in category 4 oral route: the converted acute toxicity point estimate = 500 mg/kg bw	In the key study, in the 2nd Exp which exposed animals to 500 mg/kg bw, 1 F out of 3 died and the 2 others had severe clinical signs, and in the 3rd Exp, the 3 F had severe clinical signs. Regarding the CLP Regulation and a precautionary approach, an ATE of 500 mg/kg is warranted.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the available results, a classification as **Acute Tox. Cat. 4, H302 (Harmful if swallowed)** is warranted. Based on CLP regulation, an **$ATE_{(oral)}$ of 500 mg/kg bw** is warranted.

10.2 Acute toxicity - dermal route

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

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Value LD_{50}**	**Reference**

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
Acute dermal toxicity study Semi-occlusive OECD TG 402 GLP	Rat (Wistar) 5/sex/group	3,4-dimethyl-1*H*-pyrazole Purity: 99.3 % Vehicle: corn oil	200, 2000 and 5000 mg/kg bw 24 h	> 200 and < 2000 mg/kg bw	Anonymous, 2015
Acute dermal toxicity study Semi-occlusive OECD TG 402 GLP	Rat (Wistar) 5/sex/group	3,4-dimethyl-1*H*-pyrazole Purity: 95.9 % Vehicle: corn oil	1000 mg/kg bw 24 h	> 1000 mg/kg bw	Anonymous, 2017

No human data or other data available

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

In an acute dermal toxicity study (Anonymous, 2015), following the OECD TG 402, groups of 5 male and 5 female Wistar rats were exposed to the test substance at a concentration of 200, 2000 or 5000 mg/kg bw. Animals were covered by semi-occlusive dressing for 24 hours, the application area was approx. 40 cm^2. After removal of the patch, a rinsing of the application site was performed with warm water.

Animals exposed to 5000 mg/kg bw exhibited, already 1 hour after application, poor general state, piloerection, abdominal position, atonia and shallow breathing. All animals died 2 hours after the beginning of exposure. Necropsy revealed absence of rigor mortis and a well-defined erythema of grade 2 at the application site in all animals. Furthermore, in few animals, red discoloration of the small intestine, congestion of the kidneys, spotted discoloration of liver, spotted discoloration of all lung lobes and bloody contents in the bladder were observed.

As observed at 5000 mg/kg bw, all animals exposed to 2000 mg/kg bw died (see Table 11). All animals exhibited impaired general state (1 h after application) followed by poor general state, piloerection, dyspnoea, abdominal position and atonia. At necropsy, findings were also observed such as absence of rigor mortis in 1 male and 1 female, discoloration of all lung lobes (dark red in 1 male and 2 females, red in 2 males and 2 females and red spotted in 2 males and 1 female), dark discoloration of the small intestine contents in 1 female, spotted discoloration of the liver in 3 males and in all females, unilateral congestion of the kidney in 3 males and 1 female. Furthermore, 1 female exhibited a well-defined erythema of grade 2 at the application site.

Table 11: Mortality

		Males	Females
5h after application	Found dead	1	1
D 1	Found dead	3	3
	Sacrificed in a moribund state	1	1

Animals exposed to 200 mg/kg bw did not exhibit clinical signs. Furthermore, all animals survived during the observation period (of 14 days) and the necropsy did not reveal treatment-related findings.

Based on the results, the LD_{50} was comprised between 200 and 2000 mg/kg bw.

In another acute dermal toxicity study (Anonymous, 2017), following the OECD TG 402, 5 male and 5 female Wistar rats were exposed to the test substance at a concentration of 1000 mg/kg bw. Animals

were covered by semi-occlusive patch (approx. 40 cm²). After an application period of 24 hours, the application site was rinsed, and animals were observed during 14 days.

On day 1 of the observation period, 2 females had poor general state, abdominal position, flat respiration and chromodacryorrhoea. Due to their moribund state, these females were sacrificed and necropsy revealed pale skin, muscles and organs. At the study day 1, slight erythema at the application site (grade 1) was observed in 2 males and in 4 females. No other clinical signs were observed.

Based on the results, the LD_{50} of this study was higher than 1000 mg/kg bw, the only tested dose.

10.2.2 Comparison with the CLP criteria

Table 12: comparison with the CLP criteria regarding acute toxicity via dermal route

CLP criteria	Results of available studies
Acute toxicity category 4: dermal LD_{50}: > 1000 but ≤ 2000 mg/kg bw	- All animals died at 2000 and 5000 mg/kg bw and no mortality occurred at 200 mg/kg bw (Anonymous, 2015) LD_{50} > 200 and < 2000 mg/kg bw - No mortality occurred in the 2nd study (LD_{50} > 1000 mg/kg bw) (Anonymous, 2017) The range of the available LD_{50} is comprised in the range of the category 4
Regarding ATE: based on the table 3.1.2 in the CLP Regulation ("conversion from experimentally obtained acute toxicity range values to acute toxicity point estimates") For a substance in category 4 dermal route: the converted acute toxicity point estimate = 1100 mg/kg bw	As no clear LD_{50} is available and based on the CLP Regulation table 3.1.2, an ATE of 1100 mg/kg bw is warranted

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Based on the available results, a classification as **Acute Tox. Cat. 4, H312 (Harmful in contact with skin)** is warranted. Based on CLP regulation, an **$ATE_{(dermal)}$ of 1100 mg/kg bw** is warranted.

10.3 Acute toxicity - inhalation route

Table 13: 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 Aerosol	Rat (Wistar) 5/sex/group	3,4-dimethyl-1*H*-pyrazole Purity: 99.3 %	2.1 and 5.1 mg/l 4h	> 2.1 and < 5.1 mg/l	Anonymous, 2015

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**
OECD TG 403 GLP					

No human data or other data available.

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

In an acute inhalation toxicity study (Anonymous, 2015), following the OECD TG 404, groups of 5 male and 5 female Wistar rats were exposed by aerosol to the test substance at a concentration of 2.1 or 5.1 mg/L during a 4 hours.

Animals exposed to 2.1 mg/L survived during the study period. At 5.1 mg/L, all animals died at day 1. At this highest dose, 1 male and 1 female were found death whereas the remaining animals were sacrificed in extremis on day 1 due to moribund condition (decreased activity, labored breathing and increased salivation, and at the end of exposure period, marked apathy was observed). Necropsy did not reveal treatment-related findings.

Based on the results, the LC_{50} of this study was comprised between 2.1 and 5.1 mg/L.

10.3.2 Comparison with the CLP criteria

Table 14: comparison with the CLP criteria regarding acute toxicity via inhalation route

CLP criteria	Results of available studies
Acute toxicity category 4: inhalation (dusts and mists) LC_{50}: > 1.0 but ≤ 5.0 mg/L	LC_{50} of the key study was comprised between 2.1 and 5.1 mg/L (Anonymous, 2015)
Regarding ATE: based on the table 3.1.2 in the CLP Regulation ("conversion from experimentally obtained acute toxicity range values to acute toxicity point estimates") For a substance in category 4 inhalation route (dust/mist): the converted acute toxicity point estimate = 1.5 mg/L	Regarding ATE: no clear LC_{50} is available and the range defined in the available study was higher than the ATE proposed in the CLP Regulation. Based on the available study, an ATE value of 2.1 mg/L which was the lower limit value of the LC_{50} is warranted as a precautionary approach.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on the available results, a classification as **Acute Tox. Cat. 4, H332 (Harmful if inhaled)** is warranted. Based on CLP regulation, an **$ATE_{(inhalation\text{-}dust/mist)}$ of 2.1 mg/L** is warranted.

10.4 Skin corrosion/irritation

Hazard class not assessed in this dossier.

10.5 Serious eye damage/eye irritation

Hazard class not assessed in this dossier.

10.6 Respiratory sensitisation

Hazard class not assessed in this dossier.

10.7 Skin sensitisation

Hazard class not assessed in this dossier.

10.8 Germ cell mutagenicity

Hazard class not assessed in this dossier.

10.9 Carcinogenicity

Table 15: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Combined chronic toxicity/carcinogenicity study Oral (diet) Wistar rat 50/sex/group for main groups and 10/sex/group for satellite groups OECD TG 453 GLP	3,4-dimethyl-1*H*-pyrazole Purity: 95.9 % Doses: 0, 1, 5, 30 and 60 mg/kg bw/d Duration of exposure: 12 months for satellite groups 24 months for main groups	Satellite groups: Mortality: 1 F exposed to 5 mg/kg bw/d was sacrificed in a moribund state BWG: dose-related increase in M, and decrease at 5 and 60 mg/kg bw/d in F Necropsy: no treatment-related macroscopic findings Neoplastic examination: One female exposed to 30 mg/kg bw/d: unilateral benign thecoma Main groups: Mortality rate: 22, 20, 16, 12 and 44 % in M and 20, 24, 26, 20 and 30 % in F, resp. at 0, 1, 5, 30 and 60 mg/kg bw/d BWG: sign. lower at the 2 highest doses in F and at the highest dose in M Necropsy: FBW sign. modified at the highest dose in both sexes and at 5 and 30 mg/kg bw/d	Anonymous, 2021

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		in F Neoplastic examination: Malignant epith tumors in the posterior part of the nasal cavity (level III) in 7 M of the highest dose. Locally invaded observed Malignant lymphoma in 6 M at the highest dose (vs in 1 M in control)	

No human data or other data available

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

In a combined chronic toxicity/carcinogenicity study (Anonymous, 2021), performed following OECD TG 453, groups of 50 male and 50 female Wistar rats (main groups) were exposed daily to the test substance at a concentration of 0, 1, 5, 30 or 60 mg/kg bw/d during 24 months. Additionally, groups of 10 male and 10 female Wistar rats (satellite groups) were given the test substance at a concentration of 0, 1, 5, 30 or 60 mg/kg bw/d during 12 months.

Satellite groups:

During the study period, one female exposed to 5 mg/kg bw/d was sacrificed in a moribund state. Necropsy revealed a mass in the axillary region correlated with a fibroadenoma. Furthermore, 3 males of the control group and 2 males of the low dose group exhibited palpable mass through skin while 1 male of the control group had skin lesions. Mass through skin was also observed in 1 female of the control group.

At necropsy, neoplastic examination revealed that one female exposed to 30 mg/kg bw/d had an extremely high ovarian weight and an unilateral benign thecoma.

Main groups:

During the study period, a lot of animals died in all groups. Few animals were found dead and few were sacrificed in a moribund state (see Table 82 in STOT RE section). Compared to the historical control data (value between 2007 and 2017), the mortality rate obtained for males exposed to the highest dose was above the historical control range (0 to 32 % for males (mean 15.2 %) and 16 to 34 % for females (mean 23.6 %)). No treatment-related clinical signs were observed during the study.

Regarding neoplastic examination, malignant epithelial tumors in the posterior part of the nasal cavity (level III) were observed in 7 males exposed to 60 mg/kg bw/d. Tumors locally invaded to the nasal cavity level II in 2 males, to the nasal cavity level IV in 6 males and to the brain in 3 males. 5 males out of these 7 affected males died during the study period. Furthermore, incidence of malignant lymphoma was higher at the highest dose in males (6 males vs 1 male in control group).

For more information regarding the study, see section 10.12.1.

Table 16: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Wistar rats	Nasal malignant epithelial tumors	Nasal cavity	Described as malignant and invading	/	Males		Oral	
	Malignant lymphoma	Hemolymphoreticular system	Described as malignant	/	Males		Oral	

10.9.2 Comparison with the CLP criteria

Table 17: Comparison with the CLP criteria regarding carcinogenicity

CLP criteria for Category 1	CLP criteria for Category 2
Known or presumed human carcinogens A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as: Category 1A: Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or Category 1B: Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence. The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from: — human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or — animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of	Suspected human carcinogens The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited (1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

carcinogenicity in experimental animals.	

Since no human information regarding carcinogenicity are available, a classification as Carc. 1A is not appropriate.

Regarding a classification as Carc. 1B, CLP Regulation indicated that "— sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites;"

As only one chronic toxicity study was available, point (a) and (b) are not fulfilled. Furthermore, in the available study, neoplastic findings are restricted to males and only at 2 sites. Based on these information, a classification as Carc. 1B is not appropriate.

To classify as Carc. 2, CLP Regulation mentions that "— limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs."

In the available combined chronic toxicity/carcinogenicity study (Anonymous, 2021), after 12 months of exposure, satellite groups were euthanized and examined. Only one female exposed to 30 mg/kg bw/d exhibited an ovarian unilateral benign thecoma. While, in the main groups, which were exposed to the test-substance during 24 months, malignant neoplasms were observed in all groups (control and treated). However, a significant increased incidence of malignant epithelial tumors in the posterior part of the nasal cavity was observed in males. These tumors locally invaded in the nasal cavity, level II in 2 males, level IV in 6 males and to the brain in 3 males. Moreover, 5 males out of these 7 affected males died prematurely.

Furthermore, an increased incidence of malignant lymphoma was also observed at the highest dose, as 6 males were affected vs only 1 in control group. 4 lymphoma were described as of T-cell type, one as of the B-cell type and one could not be classified.

These available information fulfilled the criteria for limited evidence restricted to a single experiment, and only males were affected.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Based on the available results, a classification as **Carc. 2, H351 (Suspected of causing cancer)** is warranted.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 18: 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
Two-generation reproductive toxicity study Oral (diet) Rat (Wistar) 25/sex/group OECD TG 416 GLP	3,4-dimethyl-1*H*-pyrazole Purity: 95.9 % Conc.: 0, 6, 25 and 100 mg/kg bw/d Duration of exposure: F0 and F1: at least 75 days prior mating, mating and until weaning of pups (males sacrificed shortly before weaning of pups and females sacrificed shortly after weaning)	F0 parental: No mortality and clinical signs Sperm parameters (motile, TS, abnormal): unaffected Oestrus cycle: unaffected Fertility index: 100.0, 96.0, 100.0 and 87.5 %, resp. at 0, 6, 25 and 100 mg/kg bw/d Mean duration of gestation: between 22.1 and 22.3 days Mean nb of implantation sites and % of PI loss: unaffected Mean nb of dams with stillborn pups: slightly higher at low and high doses Necropsy: macroscopic examination and FBW: unaffected Organ weight: few were modified in M at 100 mg/kg bw/d Histology: changes in adrenal cortex in M at 100 mg/kg bw/d + degeneration/regeneration of olf. epith. at the highest dose and also at the mid dose for the nasal cavity level III F1 pups: Tot nb of pups reduced at the highest dose but mean nb of pups similar Tot nb of stillborn pups slightly higher at low and high doses Viability index reduced at 100 mg/kg bw/d (93.9 % vs 99.0 % in control) Survival index, AGD and nipple retention: unaffected Preputial separation sign. higher at the highest dose (mean bw on the day unaffected) Macroscopic examination and organ weight (brain, spleen and thymus): not modified F1 parental: 1 M of the highest dose sacrificed	Anonymous, 2021

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		No treatment-related clinical signs observed Bw slightly lower at the highest dose Sperm parameters (motile, TS, abnormal): TS/gC slightly lower at the highest dose Precoital interval sign. modified at 25 mg/kg bw/d Oestrus cycle and fertility index: unaffected Mean nb of implantation sites lower at the highest dose (11 vs 12.3 in control) Mean % of PI loss higher at the highest dose (10.4 vs 5.1 % in control) Macroscopic examination and FBW: unaffected Few organ weight modified: abs and rela prostate and sem. ves. weight sign. lower at 100 mg/kg bw/d (also at 25 mg/kg bw/d for sem. ves.) + abs and rela ovaries weight sign. reduced at the highest dose Histology: changes in adrenal cortex in M and in vagina in F at the highest dose + degeneration/regeneration of the olf. epith. in both sexes at the highest dose (and also at the mid dose in nasal cavity level III) F2 pups: Tot nb of live pups slightly reduced at the highest dose as well as the mean nb of live pups (10.6 vs 11.6 in control) Survival index, AGD, pups bw and necropsy: unaffected	
Range-finding study Oral (diet) Wistar rat 4/sex/group No OECD guideline followed Not GLP	Test substance Purity unknown Doses: 0, 1500, 5000 and 10000 ppm Duration of exposure: 14 days	See results in summary Table 60	Anonymous, 2014
28-day repeated dose toxicity study	3,4-dimethyl-1*H*-pyrazole	See results in summary Table 60	Anonymous, 2021

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Oral Wistar rat 5/sex/dose OECD TG 407 GLP	Purity: 99.4 % Doses: 0, 1500, 3000 and 6500 ppm (corresp. to 0, 115.3, 235.2 and 526.0 mg/kg bw/d in M and 0, 134.2, 243.8 and 479.7 mg/kg bw/d in F) Duration of exposure: 28 days		
90-day repeated dose toxicity study Oral (diet) Wistar rat 10/sex/group OECD TG 408 GLP	3,4-dimethyl-1*H*-pyrazole Purity: 99.3 % Doses: 0, 150, 500, 2000 and 6000 ppm (corresp. to 0, 10.6, 33.7, 128.8 and 374.1 mg/kg bw/d in M and 0, 12.0, 36.3, 142.5 and 374.5 mg/kg bw/d in F) Duration of exposure: 90 days	See results in summary Table 60	Anonymous, 2017
Combined chronic toxicity/carcinogenicity study Oral (diet) Wistar rat 50/sex/group for main groups and 10/sex/group for satellite groups OECD TG 453 GLP	3,4-dimethyl-1*H*-pyrazole Purity: 95.9 % Doses: 0, 1, 5, 30 and 60 mg/kg bw/d Duration of exposure: 12 months for satellite groups and 24 months for main groups	See results in summary Table 60	Anonymous, 2021
14-day repeated dose toxicity study RF for the 28-day study Oral (diet) Mice 3/sex/group	3,4-dimethyl-1*H*-pyrazole Purity: not specified Doses: 0, 2000 and 5000 ppm (corresp. to 0, 408 and 776 mg/kg bw/d in M and 0, 610 and 956 mg/kg bw/d in F) Duration of exposure: 14 days	See results in summary Table 60	Anonymous, 2014
28 day repeated dose toxicity study Oral (diet) Mice 5/sex/group OECD TG 407	3,4-dimethyl-1*H*-pyrazole Purity: 99.4 % Doses: 0, 500, 1500 and 5000 ppm (corresp. to 0, 127, 328 and 885 mg/kg bw/d in M and 0, 113, 343 and 846 mg/kg bw/d in F) Duration of exposure: 4 weeks	See results in summary Table 60	Anonymous, 2015

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
GLP			
90-day repeated dose toxicity study Oral (diet) Mouse 10/sex/dose OECD TG 408 GLP	3,4-dimethyl-1*H*-pyrazole Purity: 99.3 % Doses: 0, 100, 300, 1750 and 5000 ppm (corresp. to 0, 22, 64, 375 and 944 mg/kg bw/d in M and 0, 30, 87, 529 and 1279 mg/kg bw/d in F) Duration of exposure: 3 months	See results in summary Table 60	Anonymous, 2017
RF 15-day repeated dose toxicity study Oral (capsule) Dog (Beagle) 4/sex/dose	3,4-dimethyl-1*H*-pyrazole Purity: not specified Doses: 50, 125 and 500 mg/kg bw/d Duration of exposure: 2 days for 1 M and 1 F exposed to 500 mg/kg bw/d and min 15 days for other animals	See results in summary Table 60	Anonymous, 2014
28-day repeated dose toxicity study Oral (capsule) Dog (Beagle) 4/sex/group OECD TG 409 GLP	3,4-dimethyl-1*H*-pyrazole Purity: 95.9 % Doses: 0, 10, 30 and 90 mg/kg bw/d Duration of exposure: 4 weeks	See results in summary Table 60	Anonymous, 2017
90-day repeated dose toxicity study Oral (capsule) Dog (Beagle) 5/sex/group OECD TG 409 GLP	3,4-dimethyl-1*H*-pyrazole Purity: 95.9 % Doses: 0, 10, 30 and 90 mg/kg bw/d Duration of exposure: at least 92 d	See results in summary Table 60	Anonymous, 2017
28-day repeated dose toxicity study Dermal: semi-occlusive dressing Rat (Wistar) 10/sex/group OECD TG 410 GLP	3,4-dimethyl-1*H*-pyrazole Purity: 95.9 % Doses: 0, 10, 30 and 100 mg/kg bw/d Duration of exposure: 4 weeks (6H/d on 5 day on a week)	See results in summary Table 60	Anonymous, 2018

No human data or other studies available.

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

In a two-generation reproductive toxicity study (Anonymous, 2021), groups of 25 male and 25 female Wistar rats were given, by diet, 3,4-dimethyl-1*H*-pyrazole at a concentration of 0, 6, 25 or 100 mg/kg bw/d. At least 75 days after the beginning of treatment, males and females of the same dose group were mated (during a max period of 2 weeks). The F0 males were sacrificed shortly before weaning of the F1 pups while the F0 females were sacrificed after weaning of the pups. After weaning, groups of 25 F1 males and 25 F1 females were exposed during 75 days. Afterwards, males and females were mated during a maximum period of 2 weeks. F1 males were sacrificed shortly before weaning of the F2 pups while F1 females were sacrificed just after weaning of the F2 pups.

F0 parental generation:

No mortality occurred during the study period and no treatment-related clinical signs were observed. Food consumption was unaffected in males while in females a slight reduction (dose-related) was observed at the beginning of the premating period (PMD 7 – 14) and during the lactation period. However, body weight was significantly higher in females exposed to the highest dose (see Table 19).

Table 19: Mean body weight data (in g)

		Males				Females			
Dose level (in mg/kg bw/d)		0	6	25	100	0	6	25	100
Pre-mating period	D 0	126.7	127.0	126.1	125.4	114.6	113.8	112.3	113.4
	D 7	174.5	174.5	173.7	173.6	136.3	135.5	134.9	138.2
	D 14	218.1	217.7	217.7	217.4	152.8	155.7	152.6	159.2*
	D 28	288.2	286.9	288.6	288.6	177.2	181.8	178.4	185.8*
	D 49	346.9	343.9	348.6	348.5	202.1	206.2	206.0	214.4**
	D 70	386.3	381.6	386.4	385.0	223.5	227.2	226.1	234.3*
Mating period	D 3	386.5	381.7	388.4	389.4	-	-	-	-
	D 10	399.9	394.6	397.4	397.4	-	-	-	-
Post-mating period	D 2	414.5	409.5	413.3	410.8	-	-	-	-
	D 16	431.3	425.5	430.4	428.0	-	-	-	-
Gestation period	D 0	-	-	-	-	225.9	229.9	228.6	238.8**
	D 20	-	-	-	-	345.5	346.4	340.8	356.6
Lactation period	D 1	-	-	-	-	253.5	258.0	257.3	268.1**
	D 12	-	-	-	-	292.3	294.4	290.5	298.0
	D 21	-	-	-	-	279.5	283.6	274.5	283.6

Regarding male fertility, sperm was examined at week 16, and did not reveal any treatment-related effects (see Table 20). One male exposed to the lowest dose and 3 males of the highest dose group did not generate pregnancy. The male fertility index was then reduced at the low and high dose groups (100, 96.0, 100 and 84.0 %, resp. at 0, 6, 25 and 100 mg/kg bw/d).

Table 20: Sperm analysis (at week 16)

Dose level (in mg/kg bw/d)	0	6	25	100
% of motile sperms	89	90	90	87
TS/gT (Mio/g)	113	NT	NT	112
TS/gC (Mio/g)	763	NT	NT	746
% of abnormal sperms	6.1	NT	NT	6.1

In females, oestrous cycle was unaffected by treatment as the mean number of oestrous cycle was of 4.16, 4.04, 3.96 and 4.20, resp. at 0, 6, 25 and 100 mg/kg bw/d and the mean duration was of 4.01, 4.20, 4.60 and 4.0 days, resp. at 0, 6, 25 and 100 mg/kg bw/d. One female of the lowest dose and 4 females of the highest dose did not become pregnant. The female fertility index was then of 100, 96.0, 100.0 and 87.5 %, resp. at 0, 6, 25 and 100 mg/kg bw/d. The mean duration of gestation was similar in all dose groups and was comprised between 22.1 and 22.3 days. Furthermore, the mean number of implantation sites as well as the mean percentage of post-implantation loss was unaffected by treatment (mean nb of implantation sites: 13.6, 13.0, 13.1 and 13.1, resp. at 0, 6, 25 and 100 mg/kg bw/d and % of post-implantation loss: 11.6, 4.8, 7.6 and 5.4 %, resp. at 0, 6, 25 and 100 mg/kg bw/d). At delivery, the mean number of dams with stillborn pups was slightly higher at the highest dose but also at the lowest dose, as it was of 2, 5, 1 and 6 dams, resp. at 0, 6, 25 and 100 mg/kg bw/d.

At necropsy, macroscopic examination did not reveal treatment-related effects. Organs were weighed and revealed modifications in males. Kidneys and liver weights were significantly higher at the highest dose while prostate weight was significantly lower at the highest dose. Furthermore, seminal vesicle weight was slightly reduced and this decreased was dose-related (see Table 21). Histopathology revealed treatment-related findings. Nasal cavity showed a minimal to marked degeneration/regeneration of the olfactory epithelium in all animals exposed to the highest dose (see Table 22).

Table 21: Organ weight (in g, mg or %)

		Males				Females			
Dose level (in mg/kg bw/d)		0	6	25	100	0	6	25	100
FBW		414.216	407.72	413.292	410.272	245.644	245.572	246.48	251.472
Kidneys (g)	abs	2.631	2.573	2.611	2.891**	1.738	1.709	1.715	1.759
	rela	0.635	0.633	0.633	0.705**	0.708	0.697	0.696	0.7
Liver (g)	abs	9.613	9.39	9.557	10.106	6.098	6.091	6.172	6.496
	rela	2.32	2.301	2.309	2.458**	2.484	2.481	2.502	2.58
Epididymides (g)	abs	1.217	1.183	1.198	1.188	-	-	-	-
	rela	0.295	0.293	0.292	0.291	-	-	-	-
Prostate (g)	abs	1.207	1.188	1.16	1.098*	-	-	-	-
	rela	0.291	0.292	0.281	0.27	-	-	-	-
Seminal vesicle (g)	abs	1.463	1.449	1.345	1.332	-	-	-	-
	rela	0.355	0.355	0.327	0.327	-	-	-	-
Testes (g)	abs	3.904	3.78	3.898	3.921	-	-	-	-
	rela	0.948	0.937	0.949	0.962	-	-	-	-

Ovaries (mg)	abs	-	-	-	-	132.8	134.24	134.6	129.2
	rela	-	-	-	-	0.054	0.055	0.055	0.051
Uterus (g)	abs	-	-	-	-	0.755	0.704	0.74	0.767
	rela	-	-	-	-	0.308	0.286	0.302	0.304

Table 22: Histological findings

	Grade	Males				Females			
Dose level (in mg/kg bw/)		0	6	25	100	0	6	25	100
Adrenal cortex									
Nb examined		25	25	25	25	25	1	1	25
vacuol., zona fasciculata	Inc.	14	11	10	21	0	0	0	0
Nasal cavity I									
Nb examined		25	0	0	25	25	0	0	25
Degeneration/regeneration,	Inc.	0	-	-	25	0	-	-	25
olf. epith	1	-	-	-	0	-	-	-	2
	2	-	-	-	8	-	-	-	15
	3	-	-	-	17	-	-	-	8
Nasal cavity II									
Nb examined		25	0	0	25	25	0	0	25
Degeneration/regeneration,	Inc.	1	-	-	25	1	-	-	25
olf. epith	1	1	-	-	1	1	-	-	4
	2	-	-	-	16	-	-	-	19
	3	-	-	-	8	-	-	-	2
Nasal cavity III									
Nb examined		25	25	25	25	25	25	25	25
Degeneration/regeneration,	Inc.	0	0	23	25	0	0	4	25
olf. epith	1	-	-	23	0	-	-	4	3
	2	-	-	-	6	-	-	-	21
	3	-	-	-	19	-	-	-	1
Nasal cavity IV									
Nb examined		25	0	0	25	25	0	0	24
Degeneration/regeneration,	Inc.	0	-	-	24	0	-	-	24
olf. epith	1	-	-	-	3	-	-	-	8
	2	-	-	-	10	-	-	-	14
	3	-	-	-	10	-	-	-	2
	4	-	-	-	1	-	-	-	0

Cavity I – IV: one level includes the nasopharyngeal duct; the 4 levels allow adequate examination of the squamous, transitional, respiratory and olfactory epithelium, and the draining lymphatic tissue

F1 pups generation:

At delivery, the total number of pups was reduced at the highest dose, as the number was of 304, 297, 304 and 260 pups, resp. at 0, 6, 25 and 100 mg/kg bw/d. However, the mean number of pups delivered was similar in all groups (12.7, 12.4, 12.7 and 12.4 pups, resp. at 0, 6, 25 and 100 mg/kg bw/d). As observed in Table 23, the number of stillborn pups was slightly higher at the low and high dose groups. Viability index calculated at day 4 was reduced at the highest dose, as it was of 99.0, 98.0, 99.5 and 93.9 %, resp. at 0, 6, 25 and 100 mg/kg bw/d. While the survival index calculated at day 21 was similar in all dose groups (100 % in all doses). Pups body weight examination did not reveal treatment-related modification (see Table 24), as well as anogenital distance and nipple development (see Table 25). Furthermore, vaginal opening was unaffected by treatment (30.5, 30.5, 31.4 and 31.0 days, resp. at 0, 6, 25 and 100 mg/kg bw/d), whereas preputial separation was significantly higher at the highest dose group (40.7, 41.0, 41.6, 42.1** days, resp. at 0, 6, 25 and 100 mg/kg bw/d (mean bw on the day: 173.8, 174.6, 177.1 and 173.7 g, resp. at 0, 6, 25 and 100 mg/kg bw/d)).

Table 23: Data on pups delivered

Dose level (in mg/kg bw/d)	0	6	25	100
Mean nb of liveborn pups	12.6	12.1	12.6	12.1
Nb of stillborn pups (%)	2 (0.7)	7 (2.4)	1 (0.3)	6 (2.3)
Mean % of perinatal loss	0.6	2.6	0.3	4.2
Sex ratio at D 0 (% M/F)	50.1/49.9	48.5/51.5	49.2/50.8	43.2/56.8

Table 24: Mean pup weight (in g)

Dose level (in mg/kg bw/d)		0	6	25	100
D 1	M	6.9	6.9	6.8	6.6
	F	6.5	6.6	6.5	6.2
	M+F	6.7	6.8	6.6	6.4
D 4	M	10.3	10.3	10.3	9.9
	F	9.9	10.1	10.0	9.5
	M+F	10.1	10.2	10.2	9.7
D 7	M	16.8	16.8	16.6	16.1
	F	16.1	16.5	16.2	15.6
	M+F	16.5	16.6	16.4	15.8
D 14	M	34.2	34.1	33.9	32.8
	F	33.3	33.6	33.2	31.9
	M+F	33.8	33.9	33.5	32.4
D 21	M	53.8	53.5	52.9	51.5
	F	52.2	52.3	51.2	50.3
	M+F	53.0	53.0	52.1	50.9

Table 25: Anogenital distance and nipple retention data

Dose level (in mg/kg bw/d)	0	6	25	100
Anogenital distance (in mm)				
In males	3.31	3.17	3.19	3.24
In females	1.57	1.56	1.55	1.52
Nipple development (%)				
At PND 13	64.7	65.1	67.7	64.2
At PND 20	0.0	0.0	0.0	0.0

At PND 21, 25 animals/sex/dose were randomly selected to become the F1 generation while other were sacrificed and necropsied. Necropsy did not reveal treatment-related effects, as no findings were observed at macroscopic examination and at the organ weight examination (brain, spleen and thymus) (see Table 26).

Table 26: Organ weight data (in g)

		Males				Females			
Dose level (in mg/kg bw/d)		0	6	25	100	0	6	25	100
Nb examined		24	24	24	20	24	24	24	20
Brain	Abs	1.561	1.537	1.557	1.584	1.508	1.496	1.505	1.501
	Rela	2.968	2.865	2.936	3.062	2.892	2.907	2.955	2.977
Spleen	Abs	0.254	0.250	0.259	0.248	0.256	0.242	0.242	0.249
	Rela	0.480	0.463	0.485	0.479	0.488	0.467	0.471	0.491
Thymus	Abs	0.237	0.253	0.241	0.241	0.258	0.254	0.240	0.244
	Rela	0.446	0.466	0.451	0.466	0.492	0.487	0.469	0.481

F1 parental generation:

During the study period, one male of the highest dose was sacrificed due to the paralysis of booth hindlimbs, no other clinical signs as observed. Body weight examination revealed a slight reduction at the highest dose, as observed in Table 27.

Table 27: Body weight data (in g)

		Males				Females			
Dose level (in mg/kg bw/d)		0	6	25	100	0	6	25	100
In-life	D 0	85.0	88.4	84.3	81.3	78.8	80.6	77.8	77.0
	D 35	296.6	292.3	291.7	280.1	189.0	188.1	191.0	182.9
	D 70	377.9	373.5	371.2	361.0	226.7	225.2	224.0	220.2
Mating	D 10	397.1	389.5	391.4	381.6	-	-	-	-
Post-mating	D 16	429.2	419.7	423.5	408.1	-	-	-	-
Gestation	D 0	-	-	-	-	230.8	229.1	229.8	224.0
	D 20	-	-	-	-	346.5	338.9	343.5	329.3
Lactation	D 1	-	-	-	-	262.2	261.1	260.1	254.4

	D 21	-	-	-	-	284.1	280.7	284.9	275.8

A sperm analysis was performed and did not reveal significant modification. However, total sperms per gram in cauda epididymis was slightly lower at the highest dose compared to the control group (see Table 28).

Table 28: Sperm analysis

Dose level (in mg/kg bw/d)	0	6	25	100
Motile (%)	88	89	84	86
TS/gT (Mio/g)	109	NT	NT	105
TS/gC (Mio/g)	688	NT	NT	650
% of abnormal	6.0	NT	NT	6.0

Males and females were mated and examined. The precoital interval was significantly higher at the mid dose group (2.3, 2.4, 3.0* and 2.6 days, resp. at 0, 6, 25 and 100 mg/kg bw/d). While, the mean number of oestrous cycle was unaffected by treatment (4.16, 4.36, 4.20 and 4.36, resp. at 0, 6, 25 and 100 mg/kg bw/d), as well as the mean duration of cycle (4.14, 4.04, 4.03 and 4.00 days, resp. at 0, 6, 25 and 100 mg/kg bw/d). Fertility index was unaffected by treatment (96 % in control group compared to 100 % in female treated group), as well as the duration of gestation which was of 22.0, 21.9, 22.1 and 22.1 days, resp. at 0, 6, 25 and 100 mg/kg bw/d. While, the mean number of implantation sites was slightly lower at the highest dose group (12.3, 11.9, 12.3 and 11.0, resp. at 0, 6, 25 and 100 mg/kg bw/d) and the mean percent of post-implantation loss was higher at this highest dose (5.1, 7.7, 6.4 and 10.4 %, resp. at 0, 6, 25 and 100 mg/kg bw/d). In consequence, the number of live births was lower at the highest dose (279, 278, 265 and 257 pups, resp. at 0, 6, 25 and 100 mg/kg bw/d).

Animals were necropsied and at the macroscopic examination, no treatment-related effect was observed. However, organ weight examination exhibited significant modification at the highest dose (see Table 29). Seminal vesicle weight was already disrupted at the mid dose group. As observed in parental generation, histopathology revealed degeneration/regeneration of the olfactive epithelial of the nasal cavity in all animals of the highest dose group.

Table 29: Organ weight data

		Males				Females			
Dose level (in mg/kg bw/d)		0	6	25	100	0	6	25	100
FBW		405.576	399.164	404.36	390.471	241.06	244.848	242.384	236.244
Adrenal glands (mg)	Abs	70.92	67.8	70.56	78.083	82.833	86.12	83.4	87.76
	Rela	0.018	0.017	0.017	0.02**	0.034	0.035	0.034	0.037
Kidneys (g)	Abs	2.56	2.519	2.528	2.6	1.802	1.821	1.834	1.803
	Rela	0.633	0.633	0.629	0.667**	0.748	0.744	0.757	0.764
Liver (g)	Abs	9.734	9.59	9.819	9.888	7.02	7.197	7.159	7.46
	Rela	2.403	2.406	2.421	2.534**	2.91	2.938	2.95	3.156**
Epididymides (g)	Abs	1.233	1.217	1.166	1.168	-	-	-	-
	Rela	0.305	0.306	0.288	0.301	-	-	-	-
Prostate (g)	Abs	1.159	1.134	1.064	0.963**	-	-	-	-
	Rela	0.287	0.286	0.262	0.247**	-	-	-	-

Seminal vesicle (g)	Abs	1.321	1.272	1.176*	1.107**	-	-	-	-
	Rela	0.327	0.32	0.291*	0.284**	-	-	-	-
Testes (g)	Abs	3.909	3.895	3.852	3.859	-	-	-	-
	Rela	0.969	0.979	0.955	0.996	-	-	-	-
Ovaries (mg)	Abs	-	-	-	-	119.72	122.56	119.08	106.12*
	Rela	-	-	-	-	0.049	0.05	0.049	0.045**
Uterus (g)	Abs	-	-	-	-	0.7	0.833	0.634	0.574
	Rela	-	-	-	-	0.291	0.337	0.263	0.244

Table 30: Histopathology data

	Grade	Males				Females			
Dose level (in mg/kg bw/)		0	6	25	100	0	6	25	100
Adrenal cortex									
Nb examined		25	25	25	25	25	25	25	25
vacuol., zona fasciculata	Inc.	18	17	19	24	0	0	0	0
	1	15	14	17	7				
	2	3	3	1	10				
	3			1	7				
Nasal cavity, level I									
Nb examined		25	0	0	25	25	0	0	25
Degeneration/regeneration, olfactive epith	Inc.	0	-	-	25	0	-	-	25
	1		-	-	1		-	-	2
	2		-	-	5		-	-	14
	3		-	-	19		-	-	9
Nasal cavity, level II									
Nb examined		25	0	0	25	25	0	0	25
Degeneration/regeneration, olf epith	Inc.	0	-	-	25	0	-	-	25
	1		-	-	0		-	-	2
	2		-	-	18		-	-	19
	3		-	-	7		-	-	4
Nasal cavity, level III									
Nb examined		25	25	25	25	25	25	25	25
Degeneration/regeneration, olf epith	Inc.	0	0	25	25	0	0	24	25
	1			25	0			24	3
	2				19				16
	3				6				6

Nasal cavity, level IV									
Nb examined		25	0	0	25	25	0	0	24
Degeneration/regeneration, olf epith	Inc.	0	-	-	25	0	-	-	24
	1		-	-	1		-	-	5
	2		-	-	14		-	-	18
	3		-	-	10		-	-	1
	4		-	-	1		-	-	0
Vagina									
Nb examined		-	-	-	-	25	25	25	25
Diffuse atrophy	Inc	-	-	-	-	0	0	0	2

Cavity I – IV: one level includes the nasopharyngeal duct; the 4 levels allow adequate examination of the squamous, transitional, respiratory and olfactory epithelium, and the draining lymphatic tissue

F2 pups generation:

At delivery, the number of pups was lower at the highest dose and was of 279, 278, 265 and 257 pups, resp. at 0, 6, 25 and 100 mg/kg bw/d. The mean number of liveborn pups showed the same trend (11.6, 11.6, 11.4 and 10.6 pups, resp. at 0, 6, 25 and 100 mg/kg bw/d). Pups were examined until weaning and did not reveal significant weight modification (see Table 31). Survival index at weaning was of 100 % in all groups. Anogenital distance examination did not reveal any modifications, as it was of 3.18, 3.18, 3.16 and 3.18 mm in males and 1.60, 1.64, 1.59 and 1.59 in females, resp. at 0, 6, 25 and 100 mg/kg bw/d.

Table 31: Pups body weight (in g)

Dose level (in mg/kg bw/d)		0	6	25	100
D 1	M	7.1	7.0	7.1	6.9
	F	6.8	6.6	6.8	6.6
	M+F	7.0	6.8	7.0	6.7
D 7	M	17.1	17.1	17.1	16.5
	F	16.5	16.6	16.6	15.9
	M+F	16.8	16.9	16.8	16.2
D 21	M	53.1	54.0	53.7	51.8
	F	51.4	51.9	52.0	49.9
	M+F	52.2	53.0	52.8	50.7

At necropsy, no treatment-related macroscopic findings were observed. Furthermore, organ weight (brain, spleen and thymus) did not exhibit significant changes (see Table 32).

Table 32: Organ weight (in g)

		Males				Females			
Dose level (in mg/kg bw/d)		0	6	25	100	0	6	25	100
Brain	Abs	1.563	1.551	1.544	1.571	1.521	1.475	1.498	1.515
	Rela	2.937	2.913	2.873	3.034	2.934	2.815	2.890	3.043
Spleen	Abs	0.255	0.255	0.263	0.247	0.251	0.268	0.242	0.234

	Rela	0.478	0.475	0.486	0.475	0.482	0.506	0.467	0.468
Thymus	Abs	0.249	0.246	0.234	0.224	0.247	0.258	0.244	0.226
	Rela	0.466	0.458	0.433	0.432	0.474	0.490	0.470	0.452

Furthermore, repeated dose toxicity studies were performed in rats, mice and dogs. All these studies were described in details in section 3.12.1

10.10.3 Comparison with the CLP criteria

Table 33: Comparison with CLP criteria regarding fertility

CLP criteria for a classification as Repr. Cat. 1	CLP criteria for a classification as Repr. Cat. 2
Known or presumed human reproductive toxicant Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B). Category 1A: Known human reproductive toxicant The classification of a substance in this Category 1A is largely based on evidence from humans. Category 1B: Presumed human reproductive toxicant The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary nonspecific 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 the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Since no human studies are available for effects on fertility, a classification as Repr. 1A for fertility is not appropriate.

- **<u>Male fertility:</u>**

In the two-generation reproductive toxicity study (Anonymous, 2021), animals were exposed to the test substance at a concentration of 0, 6, 25 and 100 mg/kg bw/d. During the study period, examined sperm parameters did not show any modifications (see Table 34).

Table 34: Sperm parameters

	F0 Generation				F1 Generation			
Dose level (in mg/kg bw/d)	0	6	25	100	0	6	25	100
% of motile sperms	89	90	90	87	88	89	84	86
TS/gT (Mio/g)	113	NT	NT	112	109	NT	NT	105
TS/gC (Mio/g)	763	NT	NT	746	688	NT	NT	650
% of abnormal sperms	6.1	NT	NT	6.1	6.0	NT	NT	6.0

Regarding male reproductive organ weight, absolute prostate weight was significantly and dose-dependent lower at the highest dose in the F0 generation, while relative prostate weight was lower at this highest dose but the decrease was not significant. At the F1 generation, prostate was more affected, absolute as well as relative weights were significantly and dose-dependent decreased. Furthermore, at the F1 generation, absolute and relative seminal vesicle weights were significantly and dose-dependent decreased at 25 and 100 mg/kg bw/d.

Table 35: Male reproductive organ weights

		F0 Generation				F1 Generation			
Dose level (in mg/kg bw/d)		0	6	25	100	0	6	25	100
FBW (in g)		414.216	407.72	413.292	410.272	405.576	399.164	404.36	390.471 (-3.72 %)
Epididymides (g)	Abs	1.217	1.183	1.198	1.188	1.233	1.217	1.166	1.168
	Rela	0.295	0.293	0.292	0.291	0.305	0.306	0.288	0.301
Prostate (g)	Abs	1.207	1.188 (-1.6 %)	1.16 (-3.9 %)	**1.098*** (-9 %)	1.159	1.134 (-2.16 %)	1.064 (-8.2 %)	**0.963**** (-16.91 %)
	Rela	0.291	0.292	0.281	0.27 (-7.2 %)	0.287	0.286	0.262 (-8.71 %)	**0.247**** (-13.94 %)
Seminal vesicle (g)	Abs	1.463	1.449 (-1 %)	1.345 (-8.1 %)	1.332 (-8.9 %)	1.321	1.272 (-3.7 %)	**1.176*** (-10.1 %)	**1.107**** (-16.2 %)
	Rela	0.355	0.355	0.327	0.327	0.327	0.32	**0.291*** (-11.01 %)	**0.284**** (-13.15 %)
Testes (g)	Abs	3.904	3.78	3.898	3.921	3.909	3.895	3.852	3.859
	Rela	0.948	0.937	0.949	0.962	0.969	0.979	0.955	0.996

Even if examined sperm parameters were not modified, DS wants to highlight that the doses used in this study were relatively low. At the highest dose (100 mg/kg bw/d), no mortality, no clinical signs, no reduce body weight neither final body weight were observed.

In the repeated dose toxicity studies, sperm parameters were not examined. However, male reproductive organs were macroscopically and microscopically examined. In these studies, organ weight exhibited significant changes.

- In the 28-day repeated dose toxicity study performed in rats (Anonymous, 2021), at the highest dose (526.0 mg/kg bw/d), final body weight was significantly reduced (-16.29 %), and was slightly lower at the

mid dose (-6.07 %). However, prostate and seminal vesicle weights were dose-dependently decreased and were already significantly lower at the mid dose (235.2 mg/kg bw/d). Macroscopical examination showed that prostate and seminal vesicle size were reduced (in 3 males and 4 males, resp. at the mid and high doses for prostate and in 2 and 4 males resp. at the mid and high dose for seminal vesicle). Furthermore, histopathology demonstrated minimal to focal cribriform change in one male of the mid dose and in 3 males of the highest dose (out of 5 males/dose). Moreover, spermatogenic granuloma was observed in 2 males each of mid and high dose groups.

Table 36: Male reproductive organ weights

Dose level (in mg/kg bw/d)		0	115.3	235.2	526.0
FBW (g)		271.08	265.78	254.6	226.92 (-16.29 %)
Epididymides (g)	Abs	0.72	0.718	0.672 (-6.67 %)	0.632 (-12.22 %)
	Rela	0.265	0.27	0.265	0.278
Prostate (g)	Abs	0.606	0.504 (-16.83 %)	**0.416* (-31.35 %)**	**0.364** (-39.9 %)**
	Rela	0.223	0.19 (-14.8 %)	**0.163* (-26.9 %)**	**0.161* (-27.8 %)**
Seminal vesicle (g)	Abs	0.716	0.578 (-19.27 %)	**0.412** (-42.46 %)**	**0.36** (-49.72 %)**
	Rela	0.264	0.217 (-17.8 %)	**0.162** (-38.64 %)**	**0.159** (-39.77 %)**
Testes (g)	Abs	3.206	3.112 (-2.93 %)	3.074 (-4.12 %)	2.894 (-9.73 %)
	Rela	1.184	1.172	1.211 (+2.28 %)	1.269 (+7.18 %)

The full study report indicate that "the mean absolute and relative weights of prostate and seminal vesicles were significantly decreased in test groups 2 and 3".

- In the 90-day repeated dose toxicity study performed in rats (Anonymous, 2017), all animals survived and did not show clinical signs. However, at the highest dose (374.1 mg/kg bw/d), the body weight gain as well the final body weight were significantly decreased (-17.14 % for BWG and -10.44 % for FBW). Absolute prostate and epididymides weights were only significantly modified at the highest dose. However, even if the change was not dose-related, relative seminal weight was decreased at the mid and high dose groups.

Table 37: Male reproductive organ weights

Dose level (in mg/kg bw/d)		0	10.6	33.7	128.8	374.1
Epididymides (g)	Abs	1.152	1.115	1.134	1.109	**1.009* (-12.41 %)**
	Rela	0.314	0.289	0.297	0.284	0.306 (-2.55 %)
Prostate (g)	Abs	0.905	0.969	0.94	0.902	**0.729* (-19.45 %)**
	Rela	0.244	0.251	0.246	0.231	0.22
Seminal vesicle (g)	Abs	1.276	1.306	1.257	1.105 (-13.4 %)	**0.952* (-25.39 %)**
	Rela	0.343	0.338	0.328	**0.282** (-17.78 %)**	0.289 (-15.74 %)
Testes (g)	Abs	3.602	3.583	3.596	3.703 (+2.8 %)	3.73 (+3.55 %)
	Rela	0.981	0.928	0.941	0.949 (-3.26 %)	**1.127** (+14.88 %)**

- In the combined chronic toxicity/carcinogenicity study performed in rats (Anonymous, 2021), relative male reproductive organ weights tend to decrease in the highest dose satellite group while in the main group, these weights increased and the modification was significant for epididymides at 30 and 60 mg/kg bw/d. Furthermore, necropsy of the main groups revealed an increased incidence of (peri-)vasculitis in males exposed to 30 and 60 mg/kg bw/d. The incidence of this effect was significant at the highest dose and severity was of grade 3 and 4 in testes. Small arteries and arterioles were affected and the lesion was characterized by prominent perivascular accumulations of lymphocytes, plasma cells, and macrophages. Moreover, some vessels had necrosis of the tunica media and an accumulation of hyaline material within the intima. Furthermore, full study report indicate that "*The increased number of males with (peri-)vasculitis in*

different organs, especially in the testes (test groups 03 and 04) and pancreas (test group 04), was considered to be treatment-related."

Table 38: Male reproductive organ weights

Dose level (in mg/kg bw/d)		0	1	5	30	60
Satellite group						
Epididymides (g)	Abs	1.212	1.213	1.185	1.242	1.224
	Rela	0.264	0.26	0.242	0.253	0.241
Testes (g)	Abs	3.867	3.85	3.92	4.102	3.912
	Rela	0.839	0.826	0.8	0.833	0.797
Main group						
Epididymides (g)	Abs	1.166	1.129	1.189	1.201	1.169
	Rela	0.21	0.212	0.217	**0.225*** (+7.14 %)	**0.242**** (+15.24 %)
Testes (g)	Abs	4.426	4.252	4.316	4.118	4.07
	Rela	0.799	0.788	0.792	0.768	0.844

Table 39: Incidence and severity of (peri-)vasculitis

Dose level (in mg/kg bw/d)		0	1	5	30	60
Total inc. (with (peri-)vasculitis in any organ)		3	2	3	9	18
In testes	Inc	1	1	0	6	**17****
	Grade 1	1	-	-	3	4
	Grade 3	-	1	-	1	2
	Grade 4	-	-	-	2	11
In pancreas	Inc	1	0	2	2	**9****
	Grade 1	1	-	1	2	4
	Grade 2	-	-	1	-	5

- In the 28-day repeated dose toxicity study performed in mice (Anonymous, 2015), male reproductive organ weights exhibited also some modification even if these were not significant.

Table 40: Male reproductive organ weights

Dose level (in mg/kg bw/d)		0	127	328	885
Epididymides (mg)	Abs	52.2	52.4	56.0 (+7.28%)	47.0 (-9.61 %)
	Rela	0.249	0.256	0.274 (+10.04 %)	0.258 (+3.61 %)
Prostate (mg)	Abs	50.6	50.0	46.0 (-9.09 %)	36.6 (-27.67 %)
	Rela	0.241	0.241	0.225 (-6.64 %)	0.203 (-15.77 %)
Seminal vesicle (mg)	Abs	202.8	189.8	203.8	148.6 (-26.73 %)
	Rela	0.971	0.928	0.997	0.815 (-16.07 %)
Testes (mg)	Abs	186.4	174.8	186.6	137.8 (-26.07 %)
	Rela	0.893	0.85	0.911	0.753 (-15.68 %)

- **<u>Female fertility:</u>**

In the two-generation reproductive toxicity study (Anonymous, 2021), performed in rats, fertility parameters such as oestrous cycle and implantation were not modified in any generation. However, in the F0 generation, fertility index tended to decrease at the highest dose, even if the change was not dose-related. At this highest dose, absolute and relative ovaries weight were significantly lower in the F1 parental generation.

Table 41: Fertility parameters of the 2-generation reproductive toxicity study

	F0 Generation				F1 Generation			
Dose level (in mg/kg bw/d)	0	6	25	100	0	6	25	100
Mean nb of oestrous cycle	4.16	4.04	3.96	4.20	4.16	4.36	4.20	4.36

Mean duration of oestrous cycle (in days)	4.01	4.20	4.60	4.0	4.14	4.04	4.03	4.00
Mean nb of implantation sites	13.6	13.0	13.1	13.1	12.3	11.9	12.3	11.0
Mean % of post-implantation loss (in %)	11.6	4.8	7.6	5.4	5.1	7.7	6.4	10.4
Female fertility index (in %)	100	96.0	100	87.5	96.0	100	92.0	100

Table 42: Female reproductive organ weight

		F0 Generation				F1 Generation			
Dose level (in mg/kg bw/d)		0	6	25	100	0	6	25	100
FBW (in g)		245.644	245.572	246.48	251.472	241.06	244.848	242.384	236.244
Ovaries (mg)	Abs	132.8	134.24	134.6	129.2 (-2.71 %)	119.72	122.56	119.08	**106.12*** (-11.36 %)
	Rela	0.054	0.055	0.055	0.051 (-5.55 %)	0.049	0.05	0.049	**0.045**** (-8.16 %)
Uterus (g)	Abs	0.755	0.704	0.74	0.767	0.7	0.833	0.634	0.574 (-18.0 %)
	Rela	0.308	0.286	0.302	0.304	0.291	0.337	0.263	0.244 (-16.15 %)

Female reproductive organs were also examined in some repeated dose toxicity studies.

- In the 28-day repeated dose toxicity study performed in rats (Anonymous, 2021), absolute and relative ovaries weight were significantly reduced at the 2 highest doses (243.8 and 479.7 mg/kg bw/d). Furthermore, absolute and relative uterus weights were also significantly and severely reduced at the highest dose. Histology confirmed that these 2 organs were affected as in ovaries, 4 females of the highest dose exhibited a reduction in size and/or number of functional bodies (corpora lutea, tertiary follicles). In addition, changes in the interstitial glands occurred in 2 females of the mid dose group and in 4 females of the highest dose. Moreover, 2 females exposed to the highest dose showed an atrophy of uterus, cervix and vagina.

Table 43: Female reproductive organ weight

Dose level (in mg/kg bw/d)		0	134.2	243.8	479.7
Ovaries (mg)	Abs	94.2	90.4 (-4.0 %)	**73.6*** (-21.87 %)	**46.8*** (-50.32 %)
	Rela	0.054	0.051 (-5.55 %)	**0.044*** (-18.52 %)	**0.031**** (-42.59 %)
Uterus (g)	Abs	0.478	0.498 (+4.18 %)	0.516 (+7.95 %)	**0.176**** (-63.18 %)
	Rela	0.272	0.278 (+2.21 %)	0.303 (+11.4 %)	**0.117**** (-56.98 %)

- In the 90-day repeated dose toxicity study performed in rats (Anonymous, 2017), ovaries and uterus weights were reduced at the highest dose but changes were not significant. Microscopic effects were also observed. An increased interstitial vacuolation was observed in ovaries in 4 females exposed to 2000 ppm (142.(mg/kg bw/d) and in all females exposed to 6000 ppm (374.5 mg/kg bw/d). The severity was dose related increased as at 2000 ppm the lesion was of grade 1 while at 6000 ppm 4 females had lesion of grade 1, 4 females of grade 2 and 2 females of grade 3. Moreover, diffuse atrophy was noted in 2 females exposed to the highest dose.

Table 44: Female reproductive organ weight

Dose level in mg/kg bw/d (in ppm)		0	12.0 (150)	36.3 (500)	142.5 (2000)	374.5 (6000)
Ovaries (mg)	Abs	97.5	103.8 (+6.46 %)	104.4 (+7.08 %)	108.7 (+11.49 %)	84.1 (-13.74 %)
	Rela	0.045	0.047	0.048	0.051	0.045
Uterus (g)	Abs	0.663	0.746 (+12.52 %)	0.66	0.706	0.473 (-28.66 %)
	Rela	0.307	0.338 (+10.10 %)	0.307	0.34	0.256 (-16.61 %)

- In a combined chronic toxicity/carcinogenicity study (Anonymous, 2021), reproductive organ weights were also affected as uterus and ovaries weights were modified.

Table 45: Female reproductive organ weight

Dose level (in mg/kg bw/d)		0	1	5	30	60
Satellite group						
Ovaries (mg)	Abs	103.6	91.3 (-11.87 %)	87.89 (15.16 %)	294.8 (+184 %)	91.2 (-11.97 %)
	Rela	0.037	0.033	0.033	0.116	0.036
Uterus (g)	Abs	1.067	1.369 (+28.3 %)	1.158 (+8.53 %)	0.955 (-10.5 %)	0.925 (-13.31 %)
	Rela	0.408	0.498	0.432	0.374 (-8.33 %)	0.368 (-9.8 %)
Main group						
Ovaries (mg)	Abs	244.23	148.74 (-39.1 %)	243.27	141.03 (-42.26 %)	116.65 (-52.24 %)
	Rela	0.07	0.049 (-30 %)	0.066	0.043 (-38.6 %)	0.04 (-42.86 %)
Uterus (g)	Abs	2.794	1.181 (-57.7 %)	1.379 (-50.64 %)	1.154 (-58.7 %)	2.307 (-17.4 %)
	Rela	0.972	0.371 (-61.8 %)	0.439 (-54.84 %)	0.374 (-61.5 %)	0.798 (-17.9 %)

- In mice, female reproductive organs were also affected as in the 28-day repeated dose toxicity study (Anonymous, 2015), female reproductive organ weights were lower in all tested groups.

Table 46: Female reproductive organ weight

Dose level (in mg/kg bw/d)		0	113	343	846
Ovaries (mg)	Abs	13.6	11.0 (-19.12 %)	9.8 (-27.94 %)	10.8 (-20.59 %)
	Rela	0.078	0.067 (-14.1 %)	0.063 (-17.2 %)	0.071 (-8.97 %)
Uterus (mg)	Abs	131.4	115.0 (-12.48 %)	85.4 (-35.01 %)	87.4 (-33.48 %)
	Rela	0.761	0.694 (-8.8 %)	0.54 (-28.95 %)	0.57 (-25.1 %)

- The same trend was also observed in another species. Indeed in a 28-day repeated dose toxicity study (Anonymous, 2017) performed on dogs, absolute and relative uterus and ovaries weight were lowered even if modification was not significant.

➔ As mentioned in the CLP Guidance 3.7.1, "*Annex I: 3.7.1.3. Adverse effects on sexual function and fertility Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to,* ***alterations to the female and male reproductive system****, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems."*

In conclusion, the available studies show that male reproductive organs were affected by the test substance. Weights were modified and in different studies, the change was significant compared to the control group. Furthermore, necropsy revealed microscopic changes, including vasculitis and necrosis. These findings were considered as treatment-related and correspond to a male reproductive system alteration. Furthermore, in female, consistent results revealed that reproductive organ weights (ovaries and uterus) were reduced

compared to the control groups and in some repeated dose toxicity studies performed in rats, 28-day (Anonymous, 2021) and 90-day (Anonymous, 2017), microscopic modifications were observed. These effects were not considered to be a secondary non-specific consequence of other toxic effects.

Based on the alteration of the female and male reproductive system observed in both sexes and in different studies, a classification as **Repr. 2 for Fertility** is proposed.

10.10.4 Adverse effects on development

Table 47: 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**
Prenatal developmental toxicity study Gavage In rabbit (NZW) 25 females/group OECD TG 414 GLP	3,4-dimethyl-1*H*-pyrazole Purity: 95.9 % Doses: 0, 6, 20 and 60 mg/kg bw/d GD 6 to 28	Parental: 1 F in control and 1 of the mid dose were sacrificed after abortion (GD 21 and 19, resp.) + 1 of the mid dose was found dead (GD 24) + 1 of the highest dose died after gavage error Food cons. and bw: reduced at the 2 highest doses Resorption: early: sign. higher at 60 mg/kg bw/d late: sign. lower in all treated groups Mean gravid uterus weight: reduced in all dose groups Macroscopic examination: no treatment-related effects Pups: Mean nb of live pups: slightly reduced at low dose Fetal and placental weight: not sign. modified No treatment-related malformations or variations observed	Anonymous, 2021
Prenatal developmental toxicity study Gavage In rat (Wistar) 25 females/group OECD TG 414 GLP	3,4-dimethyl-1*H*-pyrazole Purity: 95.9 % Doses: 0, 15, 50 and 150 mg/kg bw/d GD 6 to 19	Parental: No mortality or clinical signs observed Food cons and bw not sign. modified 1 F of the highest dose had all resorptions % of PI loss increased at 150 mg/kg bw/d (11.5 vs 7.6 % in control) Necropsy: organ weight changes observed and degeneration/regenartion of the olf. epith in nasal cavity at the highest dose	Anonymous, 2021

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Pups: Mean nb of live pups + foetal and placental weights: unaffected No treatment-related malformations observed Increased incidence of variations observed	
Two-generation reproductive toxicity study Oral (diet) Rat (Wistar) 25/sex/group OECD TG 416 GLP	3,4-dimethyl-1*H*-pyrazole Purity: 95.9 % Conc.: 0, 6, 25 and 100 mg/kg bw/d Duration of exposure: F0 and F1: at least 75 days prior mating, mating and until weaning of pups (males sacrificed shortly before weaning of pups and females sacrificed shortly after weaning)	Results are described in details in Table 18	Anonymous, 2021

No human data or other studies available.

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

In a prenatal developmental toxicity study (Anonymous, 2021), performed following OECD TG 414, groups of 25 mated female NZW rabbits were used at the beginning. Among these animals, 23, 25, 24 and 21 females were pregnant and exposed by gavage to 3,4-dimethyl-1*H*-pyrazole, resp. at a concentration of 0, 6, 20 or 60 mg/kg bw/d from GD 6 to 28.

Parental generation:

During the study period, one female of the control group and one of the mid dose group were sacrificed after abortion (at GD 21 and 19, resp.). Furthermore, one female exposed to 20 mg/kg bw/d was found dead on GD 24 and one of the highest dose died after a gavage error. Reduced defecation was observed in 0, 3, 4 and 2 females, resp. at 0, 6, 20 and 60 mg/kg bw/d, moreover, no defecation was noted in 1 female of the highest dose. Food consumption tends to decrease at the 2 highest doses during all the study period (142.5, 141.4, 131.3 and 124.5 g/animal/d). In the same way, body weight was also reduced at the 2 highest doses (see Table 48).

Table 48: Body weight (in g)

Dose level (in mg/kg bw/d)	0	6	20	60
GD 0	3588	3580	3575	3579
GD 6	3777	3751	3751	3769
GD 14	3943	3941	3870	3833
GD 21	3940	3981	3892	3850
GD 29	4107	4098	4010	3986

BWG 6 – 28	302.6	319.6	227.9	203.0

At the beginning, 25 females per group were mated, among these animals, 23, 25, 24 and 22 females were pregnant, resp. at 0, 6, 20 and 60 mg/kg bw/d. At the end of the study period, 22, 25, 22 and 21 females had viable foetuses. Early resorption was significantly higher at the highest dose while late resorption was significantly lower at this dose (see Table 49). No dams exhibited all resorptions.

Table 49: Resorption data

Dose level (in mg/kg bw/d)		0	6	20	60
Total	Mean	1.1	0.2*	0.4	1.0
	Mean %	9.7	2.1*	4.9	10.1
Early	Mean	0.2	0.1	0.3	0.9*
	Mean %	2.1	1.3	4.0	8.6*
Late	Mean	0.9	0.1**	0.1**	0.1**
	Mean %	7.5	0.8**	0.9**	1.5**

At necropsy, macroscopic examination did not reveal findings in 23, 23, 23 and 24 females, resp. at 0, 6, 20 and 60 mg/kg bw/d. Mean gravid uterus weight was reduced in all dose groups and net weight change from GD 6 showed also variation (see Table 50). Macroscopic examination did not reveal treatment-related findings.

Table 50: Mean gravid uterus weight and net maternal body weight change (in g)

Dose level (in mg/kg bw/d)	0	6	20	60
Nb animal examined	22	25	22	21
Gravid uterus weight	480.8	436.9	449.0	452.1
Carcass weight	3626.2	3661.0	3560.8	3534.1
Net weight change (from GD 6)	-148.3	-90.2	-195.8	-234.7

Pups:

Mean number of live birth was slightly reduced in the low dose group (8.8, 8.0, 8.7 and 8.5 pups, resp. at 0, 6, 20 and 60 mg/kg bw/d). Dead fetuses were only observed in the low dose group (6 fetuses). As observed in Table 51, foetal and placental weights did not exhibit significant modifications.

Table 51: Fetal and placental weight (in g)

Dose level (in mg/kg bw/d)		0	6	20	60
Foetal weight	All viable fetuses	37.1	38.4	36.8	36.3
	Male fetuses	37.5	39.2	37.3	36.9
	Female fetuses	37.0	36.8	36.3	35.9
Placental weights	All viable fetuses	4.9	5.2	4.9	5.2
	Male fetuses	5.0	5.4	5.0	5.4
	Female fetuses	4.8	5.0	4.8	5.1

Examination of malformations and variations did not reveal treatment effects as foetal incidence of all malformations was of 2, 2, 1 and 0 pups, resp. at 0, 6, 20 and 60 mg/kg bw/d and foetal incidence of all variations was of 184, 194, 183 and 169 pups, resp. at 0, 6, 20 and 60 mg/kg bw/d.

A second prenatal developmental toxicity study (Anonymous, 2021), following OECD TG 414, was performed in Wistar rats. Groups of 25 females were mated and among these animals, 24, 25, 25 and 24 were pregnant and were exposed by gavage to the test substance at a concentration of 0, 15, 50 and 150 mg/kg bw/d from GD 6 to 19.

Parental generation:

Among the 25 mated females per group, 24, 25, 25 and 24 females were pregnant, resp. at 0, 15, 50 and 150 mg/kg bw/d. During the study period, no mortality occurred and no treatment-related clinical signs were observed. Furthermore, maternal food consumption and mean body weight examination did not exhibit significant modification (see Table 52).

Table 52: Body weight (in g)

Dose level (in mg/kg bw/d)	0	15	50	150
GD 0	164.9	166.1	168.3	170.6
GD 6	198.4	197.8	200.4	201.9
GD 13	229.9	227.1	229.8	227.7
GD 20	294.2	289.7	295.2	295.4
BWG 6 to 19	84.6	81.2	82.7	81.7
BWG 0 to 20	129.3	123.7	126.9	124.8

No female aborted during the study period, and only one female of the highest dose exhibited all resorptions. However, post-implantation loss increased at 150 mg/kg bw/d, as the percentage was of 7.6, 6.4, 5.5 and 11.5 %, resp. at 0, 15, 50 and 150 mg/kg bw/d.

At necropsy, no abnormality was observed in 24, 25, 23 and 18 females, resp. at 0, 15, 50 and 150 mg/kg bw. 1 female of the control, the mid and the high doses were not pregnant. Furthermore, at the highest dose, 6 females had an enlarged adrenal cortex and in 1 of them an enlarged liver. Adrenal, kidneys, liver and spleen weights were examined and showed significant modifications (see Table 53). Histopathology revealed degeneration/regeneration of the olfactive nasal epithelium in all animals exposed to 150 mg/kg bw/d. Gravid uterus weight and net weight change were unaffected, as observed in Table 54.

Table 53: Organ weight (in g)

Dose level (in mg/kg bw/d)		0	15	50	150
FBW (g)		235.733	233.812	238.213	239.525
Adrenal glands (mg)	Abs	66.125	66.16	74.5**	92.542**
	Rela	0.028	0.028	0.031**	0.039**
Kidneys (g)	Abs	1.559	1.569	1.639	1.75**
	Rela	0.661	0.672	0.689*	0.73**
Liver (g)	Abs	10.267	10.305	10.803	11.471**
	Rela	4.354	4.405	4.532*	4.778**
Spleen (g)	Abs	0.535	0.512	0.531	0.521

	Rela	0.227	0.219	0.221	0.217

Table 54: Gravid uterus weight and net weight change (in g)

Dose level (in mg/kg bw/d)	0	15	50	150
Gravid uterus weight	58.4	55.9	56.6	55.9
Carcass weight	235.7	233.8	238.7	239.5
Net weight change from day 6	37.3	36.1	38.3	37.7

Pups:

At birth, the mean number of live pups was unaffected, as it was of 10.3, 9.8, 10.0 and 10.6 pups, resp. at 0, 15, 50 and 150 mg/kg bw/d. Placental and foetal weights were unaffected by treatment (see Table 55). No treatment-related malformation was observed. However, an increased incidence of variations was noted. Litter incidence of incomplete ossification of supraoccipital was significantly higher at the highest dose (14, 11, 12 and 21* litter, resp. at 0, 15, 50 and 150 mg/kg bw/d and the foetal incidence was of 28, 14, 31 and 66 pups, resp. at 0, 15, 50 and 150 mg/kg bw/d). Furthermore, litter incidence of incomplete ossification of the nasal bone was significantly increased at the highest dose (0, 0, 1 and 4* litter, resp. at 0, 15, 50 and 150 mg/kg bw/d, while the foetal incidence was of 0, 0, 1 and 6 pups, resp. at 0, 15, 50 and 150 mg/kg bw/d). Finally, a significantly higher litter incidence of misshapen sternebra was observed at the highest dose (19, 21, 20 and 23 litters, resp. at 0, 15, 50 and 150 mg/kg bw/d, while the foetal incidence was of 36, 44, 45 and 47 pups, resp. at 0, 15, 50 and 150 mg/kg bw/d).

Table 55: Mean placental and foetal weights (in g)

Dose level in mg/kg bw/d)		0	15	50	150
Placental weight	All viable fetuses	0.48	0.49	0.48	0.46
	M	0.49	0.50	0.48	0.48
	F	0.47	0.48	0.47	0.46
Foetal weight	All viable fetuses	3.8	3.8	3.7	3.6
	M	3.8	3.9	3.8	3.7
	F	3.7	3.7	3.7	3.5

A two-generation reproductive toxicity study (Anonymous, 2021), was performed in Wistar rats. Methods and results are described in details in section 10.10.2

10.10.6 Comparison with the CLP criteria

Table 56: Comparison with the CLP criteria regarding developmental toxicity

CLP criteria for a classification as Repr. Cat. 1	CLP criteria for a classification as Repr. Cat. 2
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	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

supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B). Category 1A: Known human reproductive toxicant The classification of a substance in this Category 1A is largely based on evidence from humans. Category 1B: Presumed human reproductive toxicant The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary nonspecific 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.	development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Since no human studies are available for effects on development, a classification as Repr. 1A for development is not appropriate.

Two prenatal toxicity studies were available, one in rabbits and the second in rats.

In the study performed in rabbits (Anonymous, 2021), a significant increased early resorption was observed, however, a significantly decreased late resorption was noted, and the mean number of live pups was not significantly modified. Furthermore, foetal weight examination did not exhibit significant modification and no treatment-related malformation or variation was observed.

In the prenatal toxicity study performed in rats (Anonymous, 2021), % of PI loss was higher at the highest dose (11.5 % vs 7.6 % in control group). However, mean number of live pups was similar in all groups. No treatment-related malformation was observed, but an increased incidence of incomplete ossification was noted at the highest dose.

Furthermore, a two-generation reproductive toxicity study was also available (Anonymous, 2021). In the F0 parental generation and F1 pups, duration of gestation, % of PI loss, mean number of pups were similar in all groups. In the F1 parental generation and F2 pups, % of PI loss showed variation, not dose-related, in treated groups, however the mean number of live pups was not significantly modified.

In conclusion, based on the available results, DS is of the opinion that a classification for development is not warranted as no coherence in effects was observed in the 3 available studies. Furthermore, the mean number of live pups was unaffected and no severe malformations was observed.

10.10.7 Adverse effects on or via lactation

Table 57: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Two-generation reproductive toxicity study Oral (diet) Rat (Wistar) 25/sex/group OECD TG 416 GLP	3,4-dimethyl-1*H*-pyrazole Purity: 95.9 % Conc.: 0, 6, 25 and 100 mg/kg bw/d Duration of exposure: F0 and F1: at least 75 days prior mating, mating and until weaning of pups (males sacrificed shortly before weaning of pups and females sacrificed shortly after weaning)	F0 parental: No mortality or clinical signs Mean duration of gestation: between 22.1 and 22.3 days Mean nb of dams with stillborn pups: slightly higher at low and high doses F1 pups: Tot nb of pups reduced at the highest dose but mean nb of pups similar Tot nb of stillborn pups slightly higher at low and high doses Viability index reduced at 100 mg/kg bw/d (93.9 % vs 99.0 % in control) Survival index, AGD and nipple retention: unaffected Preputial separation sign. higher at the highest dose (mean bw on the day unaffected) Macroscopic examination and organ weight (brain, spleen and thymus): not modified F1 parental: 1 M of the highest dose sacrificed No treatment-related clinical signs observed Bw slightly lower at the highest dose F2 pups: Tot nb of live pups slightly reduced at the highest dose as well as the mean nb of live pups (10.6 vs 11.6 in control) Survival index, AGD, pups bw and necropsy: unaffected	Anonymous, 2021

No human data or other data available.

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

See section 10.10.1

10.10.9 Comparison with the CLP criteria

Table 58: Comparison with the CLP criteria regarding lactation

CLP criteria
EFFECTS ON OR VIA LACTATION Effects on or via lactation are allocated to a separate single category. It is recognised that for many substances there is no information on the potential to cause adverse effects on the offspring via lactation. However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the: (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.

No human information is available to demonstrate toxicity after an exposure during lactation.

In the 2-generation reproductive toxicity study (Anonymous, 2021), survival index (days 4 to 21) did not exhibit change in the F1 as well as in the F2 pups (100 % in all dose groups and in both generation). Furthermore, as observed in Table 59, pups body weight did not show significant modification in any generation.

Table 59: Pups body weight during lactation period

		F1 pups				F2 pups			
Dose level (in mg/kg bw/d)		0	6	25	100	0	6	25	100
D 1	M	6.9	6.9	6.8	6.6	7.1	7.0	7.1	6.9
	F	6.5	6.6	6.5	6.2	6.8	6.6	6.8	6.6
	M+F	6.7	6.8	6.6	6.4	7.0	6.8	7.0	6.7
D 4	M	10.3	10.3	10.3	9.9	-	-	-	-
	F	9.9	10.1	10.0	9.5	-	-	-	-
	M+F	10.1	10.2	10.2	9.7	-	-	-	-
D 7	M	16.8	16.8	16.6	16.1	17.1	17.1	17.1	16.5
	F	16.1	16.5	16.2	15.6	16.5	16.6	16.6	15.9
	M+F	16.5	16.6	16.4	15.8	16.8	16.9	16.8	16.2
D 21	M	53.8	53.5	52.9	51.5	53.1	54.0	53.7	51.8
	F	52.2	52.3	51.2	50.3	51.4	51.9	52.0	49.9
	M+F	53.0	53.0	52.1	50.9	52.2	53.0	52.8	50.7

Based on the available information in the 2-generation reproductive toxicity study (Anonymous, 2021), a classification to lactation is not warranted.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Based on the available results, a classification as **Repr. 2, H361f (May damage fertility)** is warranted

10.11 Specific target organ toxicity-single exposure

Hazard class not assessed in this dossier.

10.12 Specific target organ toxicity-repeated exposure

Table 60: 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**
ORAL ROUTE			
In Rats			
Range-finding study Oral (diet) Wistar rat 4/sex/group No OECD guideline followed Not GLP	Test substance Purity unknown Doses: 0, 1500, 5000 and 10000 ppm Duration of exposure: 14 days	Mortality: all animals exposed to 10000 ppm sacrificed in moribund state Food cons and bw sign. lower at 5000 and 10000 ppm Haematology: sign. changes in F of the mid dose group (Hb, Ht and MCHC) Necropsy: FBW sign. lower at 5000 ppm in both sexes Histopathology: Adrenal hypertrophy/hyperplasia in all M of the mid dose Changes in liver in both sexes already at 1500 ppm	Anonymous, 2014

28-day repeated dose toxicity study Oral Wistar rat 5/sex/dose OECD TG 407 GLP	3,4-dimethyl-1*H*-pyrazole Purity: 99.4 % Doses: 0, 1500, 3000 and 6500 ppm (corresp. to 0, 115.3, 235.2 and 526.0 mg/kg bw/d in M and 0, 134.2, 243.8 and 479.7 mg/kg bw/d in F) Duration of exposure: 28 days	No mortality or clinical signs BWG sign. lower in both sexes at 6500 ppm Haematology: RBC sign. higher at 3000 and 6500 ppm Organ weight: few sign. modifications observed FBW sign. low at 6500 ppm Prostate, sem. ves. and ovaries weight sign. decreased at the 2 highest doses Uterus weight sign. reduced at highest dose Histopathology: ⇨ Min. to slight centrilobular hepatocellular hypertrophy in 1, 4 and 5 M and in 0, 4 and 5 F, resp. at 1500, 3000 and 6500 ppm ⇨ Nasal cavity: degeneration/regeneration of the olf. epith. in all treated animals. Severity dose-related ⇨ Mandibular atrophy in all treated M and in 3, 5 and 5 F resp. at 1500, 3000 and 6500 ppm. Severity dose-related ⇨ Coagulating glands lower in size in 2 and 3 males of the mid and high dose resp. ⇨ Epididymides: minimal focal cribriform changes at the 2 highest dose + spermatogenic granuloma ⇨ Prostate lower in size in 3 and 4 M, resp. at 3000 and 6500 ppm ⇨ Sem. ves. lowered in size in 2 and 4 M, resp. at 3000 and 6500 ppm ⇨ Ovaries lower in size in 4 F at 6500 ppm + interstitial changes ⇨ Atrophy of uterus + cervix and vagina in 2 F at 6500 ppm	Anonymous, 2021

90-day repeated dose toxicity study Oral (diet) Wistar rat 10/sex/group OECD TG 408 GLP	3,4-dimethyl-1*H*-pyrazole Purity: 99.3 % Doses: 0, 150, 500, 2000 and 6000 ppm (corresp. to 0, 10.6, 33.7, 128.8 and 374.1 mg/kg bw/d in M and 0, 12.0, 36.3, 142.5 and 374.5 mg/kg bw/d in F)	No mortality or clinical signs Bw sign. lower at the highest dose in both sexes Haematology: sign. changes observed (PLT and WBC in F) Clinical biochemistry: ALT sign increased at the highest dose in M and F, ALP sign. higher at 6000 ppm in F Necropsy: FBW sign. lower at 6000 ppm in both sexes + some organ weight sign. changed Histology: ⇨ Min. to moderate centrilobular hepatocellular hypertrophy at 2000 and 6000 ppm in both sexes. Severity was dose-related. Focal necrosis observed at the highest dose ⇨ Diffuse atrophy in mandibular glands was noted at 500, 2000 and 6000 ppm. Incidence and severity dose-related ⇨ Degeneration/regeneration of the olf epith at 500, 2000 and 6000 ppm. Incidence and severity dose-related ⇨ Skeletal muscle: min to slight (multi)focal degeneration in 2 M and 7 F at 6000 ppm ⇨ Ovaries: increased vacuolation in 4 F at 2000 ppm and in all F at 6000 ppm. Severity dose-related	Anonymous, 2017

Combined chronic toxicity/carcinogenicity study Oral (diet) Wistar rat 50/sex/group for main groups and 10/sex/group for satellite groups OECD TG 453 GLP	3,4-dimethyl-1*H*-pyrazole Purity: 95.9 % Doses: 0, 1, 5, 30 and 60 mg/kg bw/d Duration of exposure: 12 months for satellite groups and 24 months for main groups	Satellite groups: Mortality: 1 F exposed to 5 mg/kg bw/d was sacrificed in a moribund state BWG: dose-related increase in M, and decrease at 5 and 60 mg/kg bw/d in F Necropsy: no treatment-related macroscopic findings Histology: ⇨ Nasal cavity: degeneration/regeneration of the olf. epith. observed. Incidence and severity were dose-related ⇨ Mandibular glands: diffuse atrophy at the 2 highest doses in M and at the highest dose in F ⇨ Liver: 5 M at the highest dose had centrilobular hypertrophy Main groups: Mortality rate: 22, 20, 16, 12 and 44 % in M and 20, 24, 26, 20 and 30 % in F, resp. at 0, 1, 5, 30 and 60 mg/kg bw/d BWG: sign. lower at the 2 highest dose in F and at the highest dose in M Necropsy: FBW sign. modified at the highest dose in both sexes and at 5 and 30 mg/kg bw/d in F Histology: ⇨ Nasal cavity: degeneration/regeneration of the olf. epith. observed in both sexes. Dose-related incidence and severity + sign. increased incidence of animals with min. to severe infl. cells in the lumen at the highest dose ⇨ Mandibular glands: increased incidence of diffuse atrophy at 30 and 60 mg/kg bw/d. Incidence and severity dose-related ⇨ Skeletal muscle: (multi-)focal degeneration observed in 15 M at the highest dose vs 5 in control ⇨ (Peri-)vasculitis: observed at the 2 highest dose in M (in testes and pancreas) Neoplastic examination: ⇨ malignant epith. tumors in the posterior part of the nasal cavity (level III) in 7 M of the highest dose. Locally invaded observed ⇨ Malignant lymphoma in 6 M at the highest dose (vs in 1 M in control)	Anonymous, 2021
In Mice			

14-day repeated dose toxicity study RF for the 28-day study Oral (diet) Mice 3/sex/group	3,4-dimethyl-1*H*-pyrazole Purity: not specified Doses: 0, 2000 and 5000 ppm (corresp. to 0, 408 and 776 mg/kg bw/d in M and 0, 610 and 956 mg/kg bw/d in F) Duration of exposure: 14 days	No mortality nor clinical signs observed BWG: sign. lower at the highest dose in both sexes Necropsy: no macroscopic abnormalities observed Organ weight and microscopic examinations not performed	Anonymous, 2014
28-day repeated dose toxicity study Oral (diet) Mice 5/sex/group OECD TG 407 GLP	3,4-dimethyl-1*H*-pyrazole Purity: 99.4 % Doses: 0, 500, 1500 and 5000 ppm (corresp. to 0, 127, 328 and 885 mg/kg bw/d in M and 0, 113, 343 and 846 mg/kg bw/d in F) Duration of exposure: 4 w	No mortality nor clinical signs observed BW sign. reduced at the highest dose in both sexes (food consumption lower at this dose) Haematology and biochemistry parameters: sign. changes for ALP in M and MCH and % Ret in F Necropsy: FBW sign lower at the highest dose in both sexes and also at 1500 ppm in F Some organ weight changes Histopathology: ⇨ Liver: centrilobular hypertrophy at the highest dose ⇨ Nasal cavity: degeneration/regeneration of the olf. epith. in all treated group (dose-related incidence and severity)	Anonymous, 2015

90-day repeated dose toxicity study Oral (diet) Mouse 10/sex/dose OECD TG 408 GLP	3,4-dimethyl-1*H*-pyrazole Purity: 99.3 % Doses: 0, 100, 300, 1750 and 5000 ppm (corresp to 0, 22, 64, 375 and 944 mg/kg bw/d in M and 0, 30, 87, 529 and 1279 mg/kg bw/d in F) Duration of exposure: 3 m	Mortality: 1 M exposed to 5000 ppm died prematurely BWG: sign. higher in M at 100 ppm Sign. lower at 1750 ppm in F and at 5000 ppm in F and M Haematological findings: sign. increase % of Ret in F Clinical biochemistry: ALT, ALP and tot. prot. sign. modified Necropsy: no treatment-related macroscopic findings FBW sign. lower at the 2 highest doses in F and at the highest dose and the lowest dose in M Some organ weights sign. modified Histology: ⇨ Liver: centrilobular hypertrophy observed at the highest dose in both sexes ⇨ Nasal cavity (level III): degeneration/regeneration of the olf. epith. at the 3 highest doses (dose-related incidence and severity) ⇨ Harderian glands: vacuolation decreased in 7 M at 5000 ppm	Anonymous, 2017
In Dog			
RF 15-day repeated dose toxicity study Oral (capsule) Dog (Beagle) 4/sex/dose	3,4-dimethyl-1*H*-pyrazole Purity: not specified Doses: 50, 125 and 500 mg/kg bw/d Duration of exposure: 2 days for 1 M and 1 F exposed to 500 mg/kg bw/d and min 15 days for other animals (see Table 93)	Mortality: 1 M and 1 F were sacrificed on D1 in moribund state At 125 mg/kg bw/d: unsteady gait was observed and a decreased food consumption Other parameters not examined	Anonymous, 2014
28-day repeated dose toxicity study Oral (capsule) Dog (Beagle) 4/sex/group OECD TG 409 GLP	3,4-dimethyl-1*H*-pyrazole Purity: 95.9 % Doses: 0, 10, 30 and 90 mg/kg bw/d Duration of exposure: 4 w	No mortality nor treatment clinical findings Haematological and clinical biochemistry: at 90 mg/kg bw/d: sign changes HGD, AST in M and ALP in both sexes Necropsy: no macroscopic or microscopic treatment-related findings	Anonymous, 2017

90-day repeated dose toxicity study Oral (capsule) Dog (Beagle) 5/sex/group OECD TG 409 GLP	3,4-dimethyl-1*H*-pyrazole Purity: 95.9 % Doses: 0, 10, 30 and 90 mg/kg bw/d Duration of exposure: at least 92 d	No mortality nor treatment-related clinical signs observed Haematological and clinical biochemistry parameters: no consistent changes at D 45 and D 90 Necropsy: no treatment-related macroscopic findings Adrenal gland weight sign. higher in F exposed to 90 mg/kg bw/d Histology: no treatment-related abnormalities	Anonymous, 2017
DERMAL ROUTE			
In Rats			
28-day repeated dose toxicity study Dermal: semi-occlusive dressing Rat (Wistar) 10/sex/group OECD TG 410 GLP	3,4-dimethyl-1*H*-pyrazole Purity: 95.9 % Doses: 0, 10, 30 and 100 mg/kg bw/d Duration of exposure: 4 w (6H/d on 5 day on a week)	No mortality nor clinical signs observed BWG: trend of decrease in M Haematological and clinical biochemistry: not sign. modified at 100 mg/kg bw/d Necropsy: no treatment-related macroscopic findings Histology: degeneration olf. epith. in 5 M and 5 F at 100 mg/kg bw/d	Anonymous, 2018

No human data or other data available.

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

Oral route

In a range-finding study (Anonymous, 2014), groups of 4 male and 4 female Wistar rats were given the test substance via their diet during 14 days, at a concentration of either 0, 1500, 5000 or 10000 ppm (see Table 61).

Table 61: Substance intake (in mg/kg bw/d)

	Males			Females		
Dose level (in ppm)	1500	5000	10000	1500	5000	10000
D 0 – 3	161.0	292.1	289.6	137.2	257.7	408.3
D 3 – 7	153.6	424.0	-	135.5	383.6	-
D 7 – 10	147.8	476.0	-	145.7	482.2	-
D 10 – 14	141.7	469.2	-	142.1	447.3	-
Mean	151.0	415.3	289.6	140.1	392.7	408.3

During the study period, all animals exposed to 10000 ppm were sacrificed in a moribund state. At the mid dose group, 3 males and 3 females exhibited piloerection and all males and females had discoloured feces. At this dose, food consumption and body weights were significantly lowered (see Table 62).

Table 62: Body weight and food consumption

	Males				Females			
Dose level (in ppm)	0	1500	5000	10000	0	1500	5000	10000
Food consumption (g/animal/d)								
D 0 - 3	18.1	17.1	8.8**	4.0**	13.4	11.9	6.3**	4.7**
D 0 - 14	19.6	19.4	14.5**	-	14.4	13.4	10.5**	-
Body weight (g)								
D 0	151.3	150.0	152.2	149.2	125.9	126.6	125.2	120.6
D 3	170.8	168.0	149.3*	130.9**	132.0	133.3	120.5	110.3**
D 7	192.1	193.2	163.2**	-	141.0	141.0	125.1*	-
D 14	234.7	241.8	205.5*	-	165.8	162.2	150.0	-
BWG 0 - 3	19.5	18.0	-2.9**	-18.4**	6.1	6.7	-4.6**	-10.2
BWG 0 - 14	83.3	91.9	53.2**	-	39.9	35.6	24.9*	-

Haematological and biological parameters were examined. In females, haemoglobin was significantly higher at 5000 ppm as well as hematocrit and MCHC (see Table 63). As observed in Table 64, ALP was significantly modified in males exposed to 5000 ppm, while ALT and AST exhibited only slight change. However, creatinine exhibited a significantly lowered value in females exposed to 5000 ppm.

Table 63: Haematological data

	Males			Females		
Dose level (in ppm)	0	1500	5000	0	1500	5000
RBC (tera/L)	7.45	7.33	7.94	7.83	7.72	8.21
Hb (mmol/L)	8.4	8.2	8.9	8.5	8.4	9.2*
Ht (L/L)	0.410	0.403	0.430	0.408	0.399	0.428*
MCV (fL)	55.1	54.9	54.2	52.1	51.8	52.2
MCH (fmol)	1.13	1.12	1.12	1.08	1.08	1.12
MCHC (mmol/L)	20.54	20.43	20.62	20.80	20.94	21.52*
PLT (giga/L)	855	904	915	844	756	760
WBC (giga/L)	8.30	7.05	7.61	5.06	6.64	5.70

Table 64: Biological data

	Males			Females		
Dose level (in ppm)	0	1500	5000	0	1500	5000
ALT (µkat/L)	0.80	0.87	0.83	0.63	0.53	0.62
AST (µkat/L)	1.87	2.00	1.64	2.03	1.41	1.65
ALP (µkat/L)	3.33	2.69	2.46*	1.79	1.51	1.99
GGT_C (nkat/L)	0	0	0	0	0	0

Urea (mmol/L)	6.54	5.48	6.48	5.34	5.71	6.82
Crea (µmol/L)	21.8	20.1	15.9	21.8	22.5	17.5*
Tot. prot. (g/L)	62.01	60.68	60.57	64.78	65.77	64.67

At necropsy, one male of the highest dose had pelvic kidney's dilatation. Final body weight was significantly reduced in males and females exposed to 5000 ppm while relative kidney and liver weight were higher at this dose (see Table 65). Relative adrenal weight and absolute spleen weight were significantly modified only in males exposed to 5000 ppm. Microscopic examination revealed adrenal hypertrophy/hyperplasia in all males exposed to 5000 ppm. Furthermore, changes were also observed in liver, as observed in Table 66.

Table 65: Organ weight data

		Males			Females		
Dose level (in ppm)		0	1500	5000	0	1500	5000
FBW (g)		216.275	221.025	189.1*	150.175	148.325	135.35*
Adrenal glands (mg)	Abs	55.0	60.0	70.25	58.0	63.5	69.25
	Rela	0.025	0.027	0.037*	0.039	0.043	0.051
Heart (g)	Abs	0.8	0.81	0.718	0.573	0.57	0.518
	Rela	0.372	0.366	0.379	0.381	0.384	0.382
Kidneys (g)	Abs	1.68	1.9	1.78	1.265	1.298	1.308
	Rela	0.778	0.859	0.94*	0.842	0.875	0.966*
Liver (g)	Abs	6.14	6.685	7.218	4.428	4.385	4.725
	Rela	2.843	3.102	3.819*	2.948	2.956	3.488*
Spleen (g)	Abs	0.585	0.535	0.428*	0.3	0.33	0.28
	rela	0.27	0.241	0.227	0.2	0.223	0.206

Table 66: Microscopic findings in liver

		Males			Females		
Dose level (in ppm)		0	1500	5000	0	1500	5000
Nb examined		4	4	4	4	4	4
Centrilobular hypertrophy	Grade 1	0	2	0	0	1	0
	Grade 2	0	0	3	0	0	4
	Grade 3	0	0	1	0	0	0
Periportal fatty change (grade 1)		0	0	1	0	2	2
Single cell fatty change (grade 1)		0	0	2	0	3	3
(multi)focal necrosis		0	1	1	1	0	0
Lymphoid infiltration		4	4	4	4	3	4

In a 28-day repeated dose toxicity study (Anonymous, 2021), performed following OECD TG 407, groups of 5 male and 5 female Wistar rats were exposed to the test substance at a concentration of 0, 1500,

3000 and 6000 ppm, corresponding to 0, 115.3, 235.2 and 526.0 mg/kg bw/d in males and 0, 134.2, 243.8 and 479.7 mg/kg bw/d in females.

During the study period, no mortality occurred and no treatment-related clinical signs were noted. At the highest dose, body weight was significantly lower in both sexes (see Table 67). Haematological's examination revealed a treatment-related increase of RBC, and it was significantly modified at the mid and high dose groups (7.64, 7.93, 8.22* and 8.50* tera/L, resp. at 0, 1500, 3000 and 6500 ppm). Haemoglobin and hematocrit parameters were slightly increased but the modification was not significant.

Table 67: Body weight data (in g)

	Males				Females			
Dose level (in ppm)	0	1500	3000	6500	0	1500	3000	6500
D 0	160.0	159.1	159.4	160.2	128.9	132.0	130.1	129.7
D 7	204.0	201.1	186.2*	162.2**	151.0	155.8	146.4	125.2**
D 14	247.5	242.0	228.8*	202.4**	169.2	170.7	166.1	140.3**
D 21	277.3	270.7	261.0	216.6**	180.5	185.4	177.7	157.7**
D 28	294.0	288.7	277.3	247.3**	193.3	198.2	182.8	164.0**
BWG D 0 - 28	134.0	129.6	117.9	87.1**	64.4	66.2	52.7	34.3**

Table 68: Haematological data

	Males				Females			
Dose level (in ppm)	0	1500	3000	6500	0	1500	3000	6500
RBC (tera/L)	8.32	8.22	8.14	8.25	7.64	7.93	8.22*	8.50*
Hb (mmol/L)	9.0	9.0	8.9	9.1	8.6	8.8	9.0	9.1
Ht (L/L)	0.432	0.428	0.424	0.431	0.408	0.416	0.429	0.429
MCV (fL)	52.1	52.1	52.1	52.2	53.5	52.5	52.3	50.5
MCH (fmol)	1.08	1.09	1.10	1.10	1.12	1.10	1.10	1.07
MCHC (mmol/L)	20.77	21.02	21.08	21.10	20.90	21.01	20.93	21.22
PLT (giga/L)	780	699	695	769	757	730	657	661
HQT (sec)	40.2	39.1	37.7	37.9	36.1	34.7	36.0	37.6
WBC (giga/L)	7.75	6.72	7.29	5.82	4.87	4.76	6.14	6.13

At necropsy, macroscopic examination did not reveal any findings in 5, 5, 3 and 3 males and in 3, 5, 5 and 3 females, resp. at 0, 1500, 3000 and 6500 ppm. Two males exposed to 3000 ppm and 2 males exposed to 6500 ppm had yellow foci on epididymides. In females, one in the control group had cyst in kidneys and another had kidneys dilatation, while at the highest dose the size of the uterus was reduced in 2 females and one had an ovary size reduced. Regarding organ weight, significant modification was observed in some organ and in both sexes (see Table 69). In males, prostate and seminal vesicles weight (abs and rela) were significantly lowered at the mid and high dose groups, and furthermore these reduction were dose-related. Moreover, even if the modification was not significant, testes weight exhibited also a dose-related decrease (for absolute and relative weights). In females, ovaries and uterus weights showed also significant decrease

Table 69: organ weight (in mg, g or %)

		Males				Females			
Dose level (in ppm)		0	1500	3000	6500	0	1500	3000	6500
FBW (g)		271.08	265.78	254.6	226.92**	174.58	178.4	168.3	149.64**
Adrenal glands (mg)	Abs	61.0	67.4	72.6	73.4	66.2	80.0	81.4	67.8

	Rela	0.023	0.025	0.029*	0.033**	0.038	0.045	0.048	0.045
Brain	Abs	2.02	2.004	1.98	1.922	1.86	1.826	1.786*	1.734**
	Rela	0.746	0.754	0.779	0.849*	1.07	1.023	1.062	1.159**
Heart (g)	Abs	0.906	0.872	0.936	0.814	0.62	0.604	0.602	0.536
	Rela	0.334	0.328	0.368	0.359	0.355	0.339	0.358	0.357
Kidneys (g)	Abs	2.036	2.036	2.178	1.976	1.382	1.34	1.384	1.206
	Rela	0.751	0.766	0.858*	0.87**	0.795	0.75	0.823	0.805
Liver (g)	Abs	6.986	7.236	7.6	7.844	4.738	5.088	5.038	4.844
	Rela	2.577	2.723*	2.981**	3.446**	2.714	2.856	2.992*	3.24**
Spleen (g)	Abs	0.502	0.55	0.512	0.494	0.384	0.35	0.37	0.292*
	Rela	0.185	0.206	0.201	0.216	0.22	0.196	0.22	0.195
Thymus (mg)	Abs	537.0	531.4	485.6	500.8	446.2	557.8	489.8	445.4
	Rela	0.197	0.199	0.191	0.219	0.256	0.313	0.291	0.3
Thyroid glands (mg)	Abs	19.8	17.2	18.4	16.6	14.8	14.4	14.8	15.8
	Rela	0.007	0.006	0.007	0.007	0.009	0.008	0.009	0.011
Epididymides (g)	Abs	0.72	0.718	0.672	0.632	-	-	-	-
	Rela	0.265	0.27	0.265	0.278	-	-	-	-
Prostate (g)	Abs	0.606	0.504	0.416*	0.364**	-	-	-	-
	Rela	0.223	0.19	0.163*	0.161*	-	-	-	-
Seminal vesicle (g)	Abs	0.716	0.578	0.412**	0.36**	-	-	-	-
	Rela	0.264	0.217	0.162**	0.159**	-	-	-	-
Testes (g)	Abs	3.206	3.112	3.074	2.894	-	-	-	-
	Rela	1.184	1.172	1.211	1.269	-	-	-	-
Ovaries (mg)	Abs	-	-	-	-	94.2	90.4	73.6*	46.8*
	Rela	-	-	-	-	0.054	0.051	0.044*	0.031**
Uterus (g)	Abs	-	-	-	-	0.478	0.498	0.516	0.176**
	Rela	-	-	-	-	0.272	0.278	0.303	0.117**

Histopathology was performed and revealed findings in few organs:

- Liver: a minimal to slight centrilobular hepatocellular hypertrophy was noted in 1 male exposed to 1500 ppm, in 4 males and 4 females exposed to 3000 ppm and in all males and all females exposed to 6500 ppm.
- Nasal cavity, level I, II and III: a degeneration/regeneration of the olfactory epithelium was observed in all males and all females of all treated groups. The severity was dose-dependently increased.

Table 70: Incidence of degeneration/regeneration

		Males				Females			
Dose level (in ppm)		0	1500	3000	6500	0	1500	3000	6500
Level I	Inc	0	5	5	2	0	4	5	4
	Grade 1	-	2	1	-	-	-	-	-
	Grade 2	-	1	0	-	-	2	2	1

	Grade 3	-	1	3	1	-	2	3	1
	Grade 4	-	1	1	1	-	-	-	2
Level II	Inc	0	5	5	5	0	5	5	5
	Grade 1	-	-	-	-	-	-	2	-
	Grade 2	-	5	1	-	-	4	-	-
	Grade 3	-	-	4	1	-	1	2	2
	Grade 4	-	-	-	4	-	-	1	3
Level III	Inc	0	5	5	5	0	5	5	5
	Grade 1	-	-	-	-	-	-	-	-
	Grade 2	-	1	2	-	-	1	2	-
	Grade 3	-	4	-	-	-	4	2	1
	Grade 4	-	-	3	5	-	-	1	4

- Mandibular atrophy: a diffuse atrophy was observed in all males and in 3 females exposed to 1500 ppm, and in all animals exposed to 3000 and 6500 ppm. As in nasal cavity, the severity was dose-dependently increased. Atrophy was characterized by a reduced number and size of the secretory granular ducts and the number of eosinophilic granules within granular duct cells was reduced (and in the severe cases, these granules were absent).

Table 71: Incidence and severity of diffuse atrophy

	Males				Females			
Dose level (in ppm)	0	1500	3000	6500	0	1500	3000	6500
Inc	0	5	5	5	0	3	5	5
Grade 1	-	1	-	-	-	2	1	-
Grade 2	-	1	1	-	-	1	2	1
Grade 3	-	3	1	-	-	-	2	4
Grade 4	-	-	3	5	-	-	-	-

- Coagulating glands: the size was lower in 2 males of the mid dose group and in 3 males of the highest dose.
- Epididymides: a minimal focal cribriform change was noted in 1 male exposed to 3000 ppm and in 3 males of the highest dose. In addition, spermatogenic granuloma was observed in 2 males each of mid and high dose groups.
- Prostate: the size was lower in 3 males of the mid dose group and in 4 males of the highest dose.
- Seminal vesicles: the size was reduced in 2 males of the mid dose group and in 4 males exposed to 6500 ppm.
- Ovaries: in 4 females of the highest dose, a reduction in size and/or number of functional bodies were observed. In addition, changes of the interstitial glands occurred in 2 females of the mid dose group and in 4 females of the highest dose. In these females, the number of interstitial cells was slightly higher but seemed smaller, condensed.
- Uterus: 2 females exposed to 6500 ppm showed an atrophy of uterus, cervix and vagina.

In a repeated dose 90-day toxicity study (Anonymous, 2017), following OECD TG 408, groups of 10 male and 10 female Wistar rats were given via their diet the test substance at a concentration of 0, 150, 500, 2000 and 6000 ppm during 90 days (see Table 72, for the mean daily test substance intake in mg/kg bw/d).

Table 72: Mean daily test substance intake (in mg/kg bw/d)

Dose level (in ppm)	150	500	2000	6000
M	10.6	33.7	128.8	374.1
F	12.0	36.3	142.5	374.5

During the study period, all animals survived and did not show any clinical signs. At the highest dose, mean body weight was significantly reduced in both sexes (see Table 73). Haematological examination revealed significant modification of the platelet count and WBC in females. ALT was significantly higher at the highest dose in both sexes, furthermore, in females ALP was also significantly increased at the highest dose.

Table 73: Body weight (in g)

	Males					Females				
Dose level (in ppm)	0	150	500	2000	6000	0	150	500	2000	6000
D 0	153.4	154.6	155.2	153.5	152.7	130.0	130.7	130.1	127.6	129.3
D 6 -7	197.3	200.6	200.8	196.0	160.6	146.0	148.2	151.0	147.1	130.6**
D 28	291.9	299.6	298.8	301.6	266.6**	187.0	190.1	190.8	184.5	170.4**
D 56	352.5	366.0	361.8	370.6	315.3*	215.7	220.5	217.8	212.6	187.5**
D 91	393.5	413.6	406.2	414.6	353.2*	228.5	236.0	233.7	226.6	185.0**
BWG 0 - 91	242.1	259.0	251.0	261.1	200.6*	98.5	105.2	103.7	98.9	55.7**

Table 74: Haematological data

	Males					Females				
Dose level (in ppm)	0	150	500	2000	6000	0	150	500	2000	6000
RBC (tera/L)	8.60	8.57	8.63	8.58	8.60	7.74	7.61	7.65	7.82	8.07
Hb (mmol/L)	9.3	9.4	9.3	9.4	9.5	8.9	8.8	8.9	9.0	9.1
Ht (L/L)	0.424	0.427	0.423	0.423	0.425	0.404	0.403	0.400	0.406	0.410
MCV (fL)	49.3	49.8	49.1	49.3	49.5	52.2	52.9	52.3	52.0	50.9
MCH (fmol)	1.08	1.10	1.07	1.10	1.11	1.15	1.16	1.16	1.15	1.13
MCHC (mmol/L)	21.96	22.11	21.90	22.34	22.41	22.00	21.98	22.18	22.17	22.16
PLT (giga/L)	722	686	672	707	688	716	713	685	709	623*
HQT (sec)	38.2	38.6	37.2	37.7	36.9	34.8	35.1	35.7	35.6	38.1
WBC (giga/L)	5.52	5.14	5.48	6.29	6.04	3.30	3.24	3.33	4.40*	4.81*

Table 75: Biological data

	Males					Females				
Dose level (in ppm)	0	150	500	2000	6000	0	150	500	2000	6000
ALT (µkat/L)	0.67	0.65	0.66	0.81	1.11**	0.56	0.54	0.49	0.49	0.94**
AST (µkat/L)	1.53	1.50	1.66	1.70	1.89	1.72	1.65	1.42*	1.59	1.77
ALP (µkat/L)	1.23	1.32	1.20	1.03	1.11	0.62	0.53	0.61	0.61	0.91**
GGT_C (nkat/L)	2	4	5	5	4	10	13	14	11	18

Urea (mmol/L)	5.05	5.58	5.29	6.31**	5.98**	6.73	6.25	6.88	7.58	8.23**
Crea (mol/L)	24.9	25.9	27.7	26.3	19.7**	33.3	32.5	35.8	34.2	28.1**
Tot. prot. (g/L)	63.00	62.65	64.02	63.79	63.73	65.86	66.60	64.54	65.92	63.06

At necropsy, no abnormalities were observed in females and in 9, 8, 10, 10 and 9 males, resp. at 0, 150, 500, 2000 and 6000 ppm). 1 male of the control group had foci on epididymides, 2 of the low dose group showed foci on glandular stomach and 1 male exposed to 6000 ppm had foci on liver. As observed in Table 76, few organ's weights were significantly modified.

Table 76: Organ weight data

		Males					Females				
Dose level (in ppm)		0	150	500	2000	6000	0	150	500	2000	6000
FBW (g)		370.01	388.27	382.57	391.1	331.37*	214.9	221.15	217.03	212.62	184.91**
Adrenal glands (mg)	Abs	66.4	63.4	64.5	72.5	86.4**	73.1	70.5	70.6	73.5	73.2
	Rela	0.018	0.016	0.017	0.019	0.026**	0.034	0.032	0.032	0.035	0.04
Brain (g)	Abs	2.137	2.19	2.189	2.149	2.087	1.96	1.995	1.973	1.989	1.859**
	Rela	0.583	0.567	0.575	0.551	0.632*	0.913	0.903	0.913	0.94	1.007**
Heart (g)	Abs	1.138	1.143	1.073	1.132	1.095	0.726	0.728	0.702	0.734	0.685
	Rela	0.31	0.295	0.281	0.289	0.331	0.338	0.329	0.324	0.346	0.371*
Kidneys (g)	Abs	2.835	2.429	2.39	2.825**	2.834**	1.538	1.595	1.596	1.662	1.52
	Rela	0.649	0.628	0.625	0.722*	0.856**	0.716	0.722	0.736	0.782*	0.823**
Liver (g)	Abs	8.13	8.593	8.457	9.143	9.866**	4.824	5.354**	4.912	5.486**	5.483**
	Rela	2.191	2.215	2.206	2.337	2.977**	2.246	2.423*	2.265	2.578**	2.964**
Spleen (g)	Abs	0.575	0.61	0.561	0.59	0.642**	0.404	0.405	0.405	0.402	0.368
	Rela	0.156	0.158	0.146	0.151	0.194**	0.188	0.183	0.187	0.189	0.199
Thymus (mg)	Abs	339.0	362.7	290.7	299.0	364.7	284.3	278.0	264.2	272.5	317.9
	Rela	0.091	0.093	0.076	0.076	0.11	0.132	0.126	0.121	0.127	0.172*
Thyroid glands (mg)	Abs	21.5	22.6	20.1	20.4	21.1	16.5	17.1	15.6	16.3	16.3
	Rela	0.006	0.006	0.005	0.005	0.006	0.008	0.008	0.007	0.008	0.009
Epididy-mides (g)	Abs	1.152	1.115	1.134	1.109	1.009*	-	-	-	-	-
	Rela	0.314	0.289	0.297	0.284	0.306	-	-	-	-	-
Prostate (g)	Abs	0.905	0.969	0.94	0.902	0.729*	-	-	-	-	-
	Rela	0.244	0.251	0.246	0.231	0.22	-	-	-	-	-
Seminal vesicle (g)	Abs	1.276	1.306	1.257	1.105	0.952*	-	-	-	-	-
	Rela	0.343	0.338	0.328	0.282**	0.289	-	-	-	-	-
Testes (g)	Abs	3.602	3.583	3.596	3.703	3.73	-	-	-	-	-
	Rela	0.981	0.928	0.941	0.949	1.127**	-	-	-	-	-
Ovaries (mg)	Abs	-	-	-	-	-	97.5	103.8	104.4	108.7	84.1
	Rela	-	-	-	-	-	0.045	0.047	0.048	0.051	0.045

Uterus (g)	Abs	-	-	-	-	-	0.663	0.746	0.66	0.706	0.473
	Rela	-	-	-	-	-	0.307	0.338	0.307	0.34	0.256

Microscopic examination revealed findings in different organs:

- Liver: minimal to moderate centrilobular hypertrophy was observed at the mid and high dose groups and in both sexes. Severity was dose-dependently increased. Furthermore, focal necrosis was noted at the highest dose.

Table 77: Microscopic liver findings

		Males					Females				
Dose level (in ppm)		0	150	500	2000	6000	0	150	500	2000	6000
Centrilobular hypertrophy	Inc	0	0	0	10	10	0	0	0	9	10
	Grade 1	-	-	-	6	-	-	-	-	7	-
	Grade 2	-	-	-	4	-	-	-	-	2	4
	Grade 3	-	-	-	-	10	-	-	-	-	6
Focal necrosis		1	0	0	0	5	0	0	0	0	2
Clear cell focus		0	0	0	0	5	0	0	0	0	2

- Mandibular glands: Diffuse atrophy, which was characterized by reduced number and size of the secretory granular ducts, was noted in the 3 highest dose groups in both sexes. Incidence increased in a dose-related manner as well as the severity (see Table 78).

Table 78: Incidence of diffuse atrophy

	Males					Females				
Dose level (in ppm)	0	150	500	2000	6000	0	150	500	2000	6000
Inc	0	0	6	10	10	0	0	2	10	10
Grade 1	-	-	6	-	-	-	-	-	-	-
Grade 2	-	-	-	-	-	-	-	2	6	-
Grade 3	-	-	-	10	-	-	-	-	4	-
Grade 4	-	-	-	-	10	-	-	-	-	10

- Nasal cavity, level III: degeneration/regeneration of the olfactory epithelium was observed at ≥ 500 ppm. As observed in Table 79, the increased incidence and severity were dose-related. Degeneration/regeneration was characterized by increased intercellular spaces, irregular epithelial architecture, dilation (ectasia) of nasal glands, necrotic epithelium and/or increased nuclear/cytoplasmic ratio.

Table 79: Incidence and severity of degeneration/regeneration of the olfactive epithelium

	Males					Females				
Dose level (in ppm)	0	150	500	2000	6000	0	150	500	2000	6000
Inc	0	0	9	10	10	0	0	2	10	10
Grade 1	-	-	6	-	-	-	-	-	-	-
Grade 2	-	-	3	-	-	-	-	2	6	-

Grade 3	-	-	-	10	-	-	-	-	4	-
Grade 4	-	-	-	-	10	-	-	-	-	10

- Skeletal muscle: a minimal to slight (multi)focal degeneration was noted in 2 males and 7 females exposed to the highest dose group. The finding was located at the insertion of the N. tibialis.
- Ovaries: an increased vacuolation was observed in 4 females exposed to 2000 ppm and in all females exposed to 6000 ppm. The severity was dose-dependently increased since at 2000 ppm the lesion was of grade 1 while at 6000 ppm 4 females had lesion of grade 1, 4 females of grade 2 and 2 females of grade 3.

In a combined chronic toxicity/carcinogenicity study (Anonymous, 2021), performed following OECD TG 453, groups of 50 male and 50 female Wistar rats (main groups) were daily exposed to the test substance at a concentration of 0, 1, 5, 30 or 60 mg/kg bw/d during 24 months. Additionally, groups of 10 male and 10 female Wistar rats (satellite groups) were given the test substance at a concentration of 0, 1, 5, 30 or 60 mg/kg bw/d during 12 months.

Satellite groups:

During the study period, one female exposed to 5 mg/kg bw/d was sacrificed in a moribund state. Necropsy revealed a mass in the axillary region correlated with a fibroadenoma. Furthermore, 3 males of the control group and 2 males of the low dose group exhibited palpable mass through skin while 1 male of the control group had skin lesions. Mass through skin was also observed in 1 female of the control group. As observed in Table 80, body weight gain (D 0 – 364) showed modifications but not significant. In males, the body weight gain was dose-dependently increased while, in females, it was reduced at 5 mg/kg bw/d and higher doses.

Table 80: Body weight data (in g)

	Males					Females				
Dose level (in mg/kg bw/d)	0	1	5	30	60	0	1	5	30	60
D 0	156.7	158.3	156.7	158.0	156.7	126.2	126.9	126.4	123.0	124.4
D 49	324.5	339.6	334.2	345.3	340.4	207.0	209.7	212.3	205.1	207.7
D 91	370.6	386.7	384.3	400.4	395.2	228.0	230.7	232.2	222.3	224.6
D 147	408.1	423.0	424.3	441.9	436.0	245.3	247.8	252.6	240.5	240.8
D 231	440.9	452.2	460.8	475.2	471.9	257.2	260.6	259.6	253.4	248.0
D 315	473.4	476.3	493.4	503.4	499.1	275.8	277.4	275.1	263.4	259.8
D 364	487.6	494.2	512.3	519.6	518.3	290.9	292.5	280.7	277.7	262.8
BWG 0 - 364	330.8	335.9	355.6	361.5	361.6	164.7	165.5	154.5	154.7	138.4

At necropsy, macroscopic examination did not reveal treatment-related effects. Final body weight was not significantly modified but exhibited variations. Absolute kidney's weight was significantly higher in males at 1, 30 and 60 mg/kg bw/d, while, relative kidney's weight was significantly higher at the highest dose in both sexes (see Table 81). One female exposed to 30 mg/kg bw/d had an extremely high ovarian weight and an unilateral benign thecoma was noted. Microscopic examination revealed degeneration/regeneration of the olfactive epithelium of the nasal cavity. This finding was observed at the highest dose and also at 30 mg/kg bw/d for the nasal cavity level III (see Table 82). Moreover, diffuse atrophy in mandibular glands was noted at the highest dose in both sexes and at 30 mg/kg bw/d in males.

Table 81: Organ weight (in mg, g or %)

		Males					Females				
Dose level (in mg/kg bw/d)		0	1	5	30	60	0	1	5	30	60
Nb examined		10	10	10	10	10	10	10	9	10	10
FBW (g)		463.46	470.35	490.09	493.84	494.64	276.93	277.07	265.845	265.42	251.88
Adrenal glands (mg)	Abs	55.8	53.9	56.3	57.8	55.9	64.3	67.7	65.111	66.0	62.4
	Rela	0.012	0.012	0.012	0.012	0.011	0.023	0.025	0.025	0.025	0.025
Brain (g)	Abs	2.257	2.255	2.227	2.324	2.251	2.063	2.041	2.091	2.077	2.098
	Rela	0.491	0.482	0.456	0.474	0.46	0.753	0.743	0.792	0.793	0.835**
Heart (g)	Abs	1.158	1.181	1.197	1.205	1.224	0.839	0.868	0.814	0.811	0.799
	Rela	0.251	0.252	0.244	0.244	0.247	0.304	0.315	0.307	0.309	0.318
Kidneys (g)	Abs	2.475	2.707*	2.584	2.721**	2.937**	1.679	1.725	1.714	1.751	1.806
	Rela	0.537	0.577	0.528	0.554	0.595*	0.61	0.626	0.646	0.666*	0.717**
Liver (g)	Abs	9.787	9.743	9.957	10.426	10.605	5.924	6.015	5.64	5.836	5.373
	Rela	2.11	2.072	2.029	2.112	2.138	2.139	2.177	2.126	2.213	2.136
Spleen (g)	Abs	0.745	0.768	0.699	0.721	0.744	0.494	0.491	0.466	0.505	0.451
	Rela	0.162	0.164	0.143	0.146	0.151	0.178	0.178	0.176	0.192	0.179
Thyroid glands (mg)	Abs	31.2	30.9	28.5	29.9	31.2	20.1	20.4	21.889	18.5	20.4
	Rela	0.007	0.007	0.006	0.006	0.006	0.007	0.007	0.008	0.007	0.008
Epididy-mides (g)	Abs	1.212	1.213	1.185	1.242	1.224	-	-	-	-	-
	Rela	0.264	0.26	0.242	0.253	0.241	-	-	-	-	-
Testes (g)	Abs	3.867	3.85	3.92	4.102	3.912	-	-	-	-	-
	Rela	0.839	0.826	0.8	0.833	0.797	-	-	-	-	-
Ovaries (mg)	Abs	-	-	-	-	-	103.6	91.3	87.889	294.8	91.2
	Rela	-	-	-	-	-	0.037	0.033	0.033	0.116	0.036
Uterus (g)	Abs	-	-	-	-	-	1.067	1.369	1.158	0.955	0.925
	Rela	-	-	-	-	-	0.408	0.498	0.432	0.374	0.368

Table 82: Histopathological findings

	Males					Females				
Dose level (in mg/kg bw/d)	0	1	5	30	60	0	1	5	30	60
Nasal cavity I, Degeneration/regeneration olfactive epithelium										
Nb examined	10	0	0	0	10	10	0	0	0	10
Inc	0	-	-	-	3	0	-	-	-	5
Grade 1	-	-	-	-	3	-	-	-	-	3
Grade 2	-	-	-	-	-	-	-	-	-	2
Nasal cavity II, Degeneration/regeneration olfactive epithelium										

Nb examined	10	0	0	0	10	10	0	0	0	10
Inc	0	-	-	-	10	0	-	-	-	10
Grade 1	-	-	-	-	-	-	-	-	-	1
Grade 2	-	-	-	-	1	-	-	-	-	4
Grade 3	-	-	-	-	9	-	-	-	-	5
Nasal cavity III, Degeneration/regeneration olfactive epithelium										
Nb examined	10	10	10	10	10	10	10	10	10	10
Inc	0	0	1	10	9	0	0	0	10	10
Grade 1	-	-	1	6	0	-	-	-	8	1
Grade 2	-	-	-	4	2	-	-	-	2	9
Grade 3	-	-	-	-	7	-	-	-	-	-
Nasal cavity IV, Degeneration/regeneration olfactive epithelium										
Nb examined	10	0	0	0	10	10	0	0	0	10
Inc	0	-	-	-	10	0	-	-	-	9
Grade 1	-	-	-	-	-	-	-	-	-	4
Grade 2	-	-	-	-	4	-	-	-	-	4
Grade 3	-	-	-	-	6	-	-	-	-	1
Mandibular glands, diffuse atrophy										
Nb examined	10	10	10	10	10	10	10	10	10	10
Inc	0	0	0	2	10	-	-	-	-	9
Grade 2	-	-	-	2	1	-	-	-	-	4
Grade 3	-	-	-	-	9	-	-	-	-	5
Liver, hypertrophy centrilobular										
Nb examined	10	10	10	10	10	10	2	0	0	10
Inc (all grade 1)	-	-	-	-	5	-	-	-	-	-

Main groups:

As observed in Table 83, during the study period, a lot of animals died in all groups. Some animals were found dead and some were sacrificed in a moribund state. Compared to the historical control data (value between 2007 and 2017), the mortality rate obtained for males exposed to the highest dose was above the historical control range (0 to 32 % for males (mean 15.2 %) and 16 to 34 % for females (mean 23.6 %)). No treatment clinical signs were observed during the study. Body weight was significantly reduced at the highest dose in males and at the 2 highest dose in females (see Table 84).

Table 83: Mortality

	Males					Females				
Dose level (in mg/kg bw/d)	0	1	5	30	60	0	1	5	30	60
Animals examined	50	50	50	50	50	50	50	50	50	50
Dead	50	50	50	50	50	30	30	32	29	34

Animals found dead	4	3	4	3	11	3	4	7	5	6
Animals sacrificed moribund	7	7	4	3	11	7	8	6	5	9
Mortality rate (in %)	22	20	16	12	44	20	24	26	20	30
Animals sacrificed at the end of the study period	39	40	42	44	28	20	18	19	19	19

Table 84: Body weight data (in g)

	Males					Females				
Dose level (in mg/kg bw/d)	0	1	5	30	60	0	1	5	30	60
D 0	162.4	161.4	161.0	159.6	158.1*	129.0	127.5	126.3	126.8	127.5
D 49	350.1	342.1	340.6	339.5	340.8	211.1	209.5	214.8	209.5	212.0
D 91	406.5	393.4	391.3	390.0	393.8	234.8	231.4	236.7	230.7	232.7
D 147	446.2	431.5	428.1*	425.3	431.2	248.4	246.5	254.6	247.3	248.0
D 231	480.9	465.9	461.9	459.1	465.4	262.8	258.8	267.3	259.2	260.0
D 315	510.3	494.1	492.9	490.3	494.4	277.9	271.5	277.4	271.4	270.8
D 399	540.2	522.0	521.1	519.6	520.6	296.1	289.0	293.4	291.4	288.9
D 483	562.3	547.6	546.2	542.9	540.1	317.4	302.8	307.7	303.4	297.7*
D 567	574.1	558.4	554.6	555.5	536.4*	337.5	319.3	321.5	312.7*	308.2**
D 651	575.8	563.6	565.4	564.9	541.6	352.6	334.5	334.1	325.2*	315.8**
D 728	583.1	559.4	569.4	561.3	513.3**	363.5	340.5	343.1	324.5**	315.6**
BWG 0 - 728	422.1	398.8	408.1	402.3	354.9**	235.0	211.7	216.7	197.8**	188.7**

At necropsy, macroscopic examination showed an increased number of foci in adrenal glands. This change was observed at the highest dose in both sexes (in 1, 2, 1, 4 and 6 males and in 5, 9, 4, 8 and 12 females, resp. at 0, 1, 5, 30 and 60 mg/kg bw/d). Furthermore, in males, an increased incidence of enlarged spleen was noted at the highest dose (2, 4, 2, 1 and 8 males, resp. at 0, 1, 5, 30 and 60 mg/kg bw/d). Final body weight was significantly modified at the highest dose in both sexes and also at 5 and 30 mg/kg bw/d in females. Furthermore, absolute and relative adrenal glands weights were significantly higher at the highest dose. Microscopic examination revealed an dose-related increased incidence and severity of degeneration/regeneration of the olfactive epithelium of the nasal cavity (see Table 85). Additionally, a significant increased incidence of animals with minimal to severe inflammation and/or inflammatory cells in the lumen of the nasal cavity was noted at the highest dose in both sexes. Furthermore, diffuse atrophy in mandibular glands was noted at the 2 highest dose groups in both sexes. This modification was dose-dependently increased in incidence and severity. (Multi-)focal degeneration in skeletal muscle was also noted in 15 males exposed to 60 mg/kg bw/d (vs in 5 males of the control group). As observed in Table 86, in males, an increased number of animals with (peri)-vasculitis was noted at the 2 highest doses. Small arteries and arterioles were affected and the lesion was characterized by prominent perivascular accumulations of lymphocytes, plasma cells, and macrophages. Moreover, some vessels had necrosis of the tunica media and an accumulation of hyaline material within the intima. The full study report indicates that "*The increased number of males with (peri-)vasculitis in different organs, especially in the testes (test groups 03 and 04) and pancreas (test group 04), was considered to be treatment-related*."

Table 85: Incidence and severity of microscopic findings

	Grade	Males					Females				
Dose level (in mg/kg bw/d)		0	1	5	30	60	0	1	5	30	60
Nasal cavity											

Nb examined			50	50	50	50	50	50	50	50	50	50
Nasal cavity, level I	Olf. epith degen/regen	Inc	0	0	0	3	10**	1	0	0	3	5
		1	-	-	-	3	6	1	-	-	3	4
		2	-	-	-	-	4	-	-	-	-	1
Nasal cavity, level II	Olf. epith degen/regen	Inc	1	0	0	46**	44**	0	0	0	15**	45**
		1	1	-	-	22	3	-	-	-	15	4
		2	-	-	-	24	36	-	-	-	-	33
		3	-	-	-	-	5	-	-	-	-	8
Nasal cavity, level III	Olf. hyperplasia	Inc	0	0	0	2	3	0	0	0	1	1
		1	-	-	-	1	1	-	-	-	1	-
		2	-	-	-	1	2	-	-	-	-	-
		5	-	-	-	-	-	-	-	-	-	1
	Olf. epith degen/regen	Inc	1	0	0	46**	47**	0	0	0	10**	47**
		1	1	-	-	29	3	-	-	-	8	5
		2	-	-	-	15	18	-	-	-	2	26
		3	-	-	-	2	26	-	-	-	-	16
Nasal cavity, level IV	Olf. hyperplasia	Inc	0	0	0	0	4	0	0	0	0	2
		1	-	-	-	-	1	-	-	-	-	-
		2	-	-	-	-	1	-	-	-	-	1
		3	-	-	-	-	-	-	-	-	-	1
		4	-	-	-	-	1	-	-	-	-	-
		5	-	-	-	-	1	-	-	-	-	-
	Olf. epith degen/regen	Inc	1	0	0	47**	41**	0	0	0	16**	47**
		1	1	-	-	26	2	-	-	-	-	7
		2	-	-	-	19	6	-	-	-	-	9
		3	-	-	-	2	25	-	-	-	-	28
		4	-	-	-	-	8	-	-	-	-	3
Mandibular glands												
Nb examined			50	50	50	50	49	49	49	49	48	46
Mandibular glands	Diffuse atrophy	Inc	1	0	0	35**	48**	0	0	0	30**	40**
		1	-	-	-	8	-	-	-	-	-	-
		2	1	-	-	9	-	-	-	-	19	11
		3	-	-	-	14	23	-	-	-	9	7
		4	-	-	-	4	25	-	-	-	2	22
Skeletal muscle												
Nb examined			50	50	50	50	50	-	-	-	-	-
Skeletal muscle	(multi-)focal degeneration	Inc	5	6	9	3	15**	-	-	-	-	-
		1	3	6	8	3	13	-	-	-	-	-
		2	2	-	1	-	1	-	-	-	-	-

		3	-	-	-	-	1	-	-	-	-	-

Table 86: Incidence of (peri-)vasculitis in males

Dose level (in mg/kg bw/d)		0	1	5	30	60
Total incidence (with (peri-)vasculitis in any organ)		3	2	3	9	18
In testes	Inc	1	1	0	6	17**
	Grade 1	1	-	-	3	4
	Grade 3	-	1	-	1	2
	Grade 4	-	-	-	2	11
In pancreas	Inc	1	0	2	2	9**
	Grade 1	1	-	1	2	4
	Grade 2	-	-	1	-	5

Regarding neoplastic examination, malignant epithelial tumors in the posterior part of the nasal cavity (level III) were observed in 7 males exposed to 60 mg/kg bw/d. Tumors locally invaded to the nasal cavity level II in 2 males , to the nasal cavity level IV in 6 males and to the brain in 3 males. 5 males out of these 7 affected males died during the study period. Furthermore, incidence of malignant lymphoma was higher at the highest dose in males (6 males vs 1 male in control group).

In a 14-day repeated dose toxicity study (Anonymous, 2014), performing as a range-finding study before a 28-day repeated dose toxicity study, groups of 3 male and 3 female mice were given via their diet, during 2 weeks, the test substance at a concentration of 0, 2000 or 5000 ppm, corresponding to 0, 408 and 776 mg/kg bw/d in males and 0, 610 and 956 mg/kg bw/d in females.

During the study period, no mortality occurred and no treatment-related clinical signs were observed. Body weight was significantly reduced in both sexes at the highest dose (see Table 87).

Table 87: Body weight data (in g)

	Males			Females		
Dose level (In ppm)	0	2000	5000	0	2000	5000
D 0	21.7	21.7	21.6	17.5	17.5	17.1
D 7	23.0	22.0	19.4**	18.1	18.0	15.8*
D 14	23.9	22.7	21.0	19.2	18.5	17.2**
BWG 0 - 14	2.2	1.0	-0.7*	1.7	1.0	0.1*

At necropsy, macroscopic examination did not reveal any abnormalities. Organ weight and histopathology were not examined in this study.

In a 28-day repeated dose toxicity study (Anonymous, 2015), groups of 5 male and 5 female mice were given during 4 weeks via their diet the test substance at a concentration of 0, 500, 1500 and 5000 ppm (corresponding approx. to 0, 127, 328 and 885 mg/kg bw/d in males and 0, 113, 343 and 846 mg/kg bw/d in females).

During the study period, no mortality occurred and no treatment-related clinical signs were observed. Food consumption was reduced at the highest dose in both sexes (the modification was significant in males only). At the end of the study period, body weight was significantly lowered at the highest dose in both sexes (see

Table 88). Haematological and clinical biochemistry parameters were examined and revealed significant changes at the highest dose for ALP in males and for MCH and reticulocytes in females.

Table 88: Body weight (in g)

	Males				Females			
Dose level (in ppm)	0	500	1500	5000	0	500	1500	5000
D 0	22.5	22.0	22.6	22.1	17.8	17.4	17.4	17.8
D 7	23.3	22.8	23.1	19.4**	18.6	18.4	18.2	17.7
D 14	23.7	23.1	23.2	20.9**	19.6	19.1	18.9	18.5*
D 21	24.6	24.4	24.3	21.6**	20.1	19.2	19.4	17.9**
D 28	25.1	24.8	24.8	22.4**	21.4	20.6	20.1	18.9**
BWG 0 - 28	2.7	2.8	2.2	0.2**	3.7	3.2	2.7	1.1**

Table 89: Haematological data

	Males				Females			
Dose level (in ppm)	0	500	1500	5000	0	500	1500	5000
RBC (tera/L)	9.30	9.82	9.77	10.28	9.76	9.46	9.50	9.47
Hb (mmol/L)	8.7	8.8	8.8	9.2	8.8	8.8	8.6	8.5
Ht (L/L)	0.438	0.458	0.458	0.473	0.456	0.446	0.444	0.435
MCV (fL)	47.1	46.7	46.9	46.0	46.7	47.1	46.8	45.9
MCH (fmol)	0.94	0.90	0.90	0.90	0.91	0.93	0.90	0.89*
MCHC (mmol/L)	20.02	19.31	19.21	19.51	19.49	19.82	19.22	19.45
Ret (%)	2.5	2.3	2.4	2.5	2.1	2.6	2.0	2.7*
PLT (giga/L)	1.302	1.372	1.294	1.568	1.130	1.036	1.213	1.219
WBC (giga/L)	4.61	3.02	4.47	4.32	3.60	3.41	1.50	5.46

Table 90: Clinical biochemistry data

	Males				Females			
Dose level (in ppm)	0	500	1500	5000	0	500	1500	5000
ALT (µkat/L)	0.99	0.88	0.85	1.24	1.13	1.41	1.43	1.76
AST (µkat/L)	3.70	2.95	3.63	4.31	3.88	3.76	5.14	5.29
ALP (µkat/L)	1.88	1.97	1.95	2.34**	2.17	2.01	2.75	2.52
GGT_C (nkat/L)	0	0	0	0	0	0	0	0
Crea (µmol/L)	57.2	48.8	54.5	46.9	55.3	54.8	52.1	46.1

At necropsy, one female of the mid dose group had focus on glandular stomach and discoloration of contents in jejunum. Final body weight was significantly lowered at the highest dose in males and at the 2 highest doses in females. Relative brain and liver weights exhibited significant changes as well as absolute and relative thymus weight. Absolute and relative prostate weight, seminal vesical weight and testes weight were decreased at the highest dose. In the same way, absolute and relative ovaries and uterus weights were reduced at the highest dose. Histopathology revealed centrilobular hypertrophy in liver at the highest dose. Furthermore, degeneration/regeneration of the olfactive epithelium in nasal cavity level III was observed in all treated group. Incidence and severity were dose-related.

In a 90-day repeated dose toxicity study (Anonymous, 2017), groups of 10 male and 10 female mice were orally exposed to the test substance at a concentration of 0, 100, 300, 1750 and 5000 ppm (corresp to 0, 22, 64, 375 and 944 mg/kg bw/d in males and to 0, 30, 87, 529 and 1279 mg/kg bw/d in females). Animals were exposed during 3 months.

During the study period, one male exposed to 5000 ppm died prematurely. Body weight gain was significantly higher in the low dose group in male while it was significantly lowered at the highest dose in both sexes and also at 1750 ppm in females. Hematological examination revealed a significant increase of the % of reticulocytes in females exposed to 5000 ppm. In males, clinical biochemistry examination revealed few changes (see Table 91).

Table 91: Clinical biochemistry data

	Males					Females				
Dose level (in ppm)	0	100	300	1750	5000	0	100	300	1750	5000
ALT (µkat/L)	0.84	0.79*	0.83	0.98**	1.15**	1.30	1.49	1.40	1.29	1.34
AST (µkat/L)	5.98	5.43	5.61	6.74	7.02	8.66	9.86	7.59	8.25	8.60
ALP (µkat/L)	1.15	1.12	1.19	1.23	1.43**	2.05	2.08	1.93	2.08	2.20
GGT_C (nkat/L)	0	0	0	0	0	0	0	0	0	0
Tot. prot. (g/L)	51.54	52.41	52.25	50.12*	51.33	50.34	49.23	48.08*	48.66	46.25**

At necropsy, no treatment-related macroscopic findings were noted. Final body weight was significantly lowered at the highest dose in both sexes and also at 1750 ppm in females. As observed in Table 92, few significant organ weight modifications were observed. Histology revealed changes in liver as centrilobular hypertrophy was observed. Furthermore, an increased dose-related incidence and severity of degeneration/regeneration of the olfactive epithelium in nasal cavity level III were noted. Additionally, eosinophilic globules were observed in all animals at the 2 highest doses. In males, decreased vacuolation in harderian glands was observed in 7 males at 5000 ppm.

Table 92: Organ weight (in mg, g or %)

		Males					Females				
Dose level (in ppm)		0	100	300	1750	5000	0	100	300	1750	5000
FBW (g)		26.04	28.43*	26.9	25.09	22.133**	19.05	18.66	18.52	18.03**	17.0**
Adrenal glands (mg)	Abs	2.8	3.2	3.1	2.8	3.222	7.5	7.3	7.4	6.9	6.9
	Rela	0.011	0.011	0.011	0.011	0.015	0.04	0.039	0.04	0.038	0.04
Brain (mg)	Abs	473.5	471.9	460.6	456.6*	445.111**	460.3	465.1	457.1	451.4	441.0**
	Rela	1.833	1.667*	1.721	1.826	2.013*	2.418	2.496	2.472	2.504	2.598**
Heart (mg)	Abs	143.1	151.7	140.1	143.3	128.778*	115.8	122.4	118.2	115.4	98.2**
	Rela	0.553	0.536	0.522	0.573	0.581	0.608	0.657*	0.638	0.64	0.579
Kidneys (mg)	Abs	361.8	388.0	385.3	372.1	322.0**	277.1	276.8	274.2	261.4	238.7**
	Rela	1.395	1.368	1.436	1.492	1.454	1.454	1.483	1.479	1.449	1.406
Liver (mg)	Abs	1128.3	1191.1	1160.0	1189.6	1179.667	922.9	849.7	866.4	890.4	941.1
	Rela	4?346	4?194	4.322	4.738*	5.331**	4.839	4.552	4.682	4.938	5.533**
Spleen (mg)	Abs	54.4	57.9	52.2	50.8	44.444**	55.4	52.1	54.6	48.3	43.0**
	Rela	0.21	0.203	0.194	0.203	0.201	0.29	0.279	0.293	0.268	0.253
Thymus (mg)	Abs	32.5	37.0	33.7	28.6	32.778	31.0	29.9	28.5	32.0	35.3
	Rela	0.124	0.13	0.126	0.114	0.149*	0.163	0.16	0.153	0.177	0.207**

Epidid-ymides (mg)	Abs	70.6	73.4	71.4	69.9	63.222**	-	-	-	-	-
	Rela	0.273	0.259	0.267	0.28	0.286	-	-	-	-	-
Testes (mg)	Abs	208.9	199.5	201.6	213.4	205.667	-	-	-	-	-
	Rela	0.806	0.705*	0.751	0.853	0.928**	-	-	-	-	-
Ovaries (mg)	Abs	-	-	-	-	-	14.7	14.9	15.1	12.5	12.2
	Rela	-	-	-	-	-	0.077	0.08	0.082	0.069	0.072
Uterus (mg)	Abs	-	-	-	-	-	100.9	124.9*	102.6	107.0	89.9
	Rela	-	-	-	-	-	0.53	0.671*	0.553	0.594	0.528

Table 93: Incidence of the microscopic findings

	Grade	Males					Females				
Dose level (in ppm)		0	100	300	1750	5000	0	100	300	1750	5000
Liver											
Centrilobular hypertrophy	Inc	0	0	0	0	9	0	0	0	0	6
	1	-	-	-	-	-	-	-	-	-	5
	2	-	-	-	-	3	-	-	-	-	1
	3	-	-	-	-	6	-	-	-	-	-
Diffuse fatty change	Inc	10	10	10	10	5	10	9	10	10	6
	1	-	-	-	-	-	-	1	1	-	-
	2	3	1	-	1	-	1	4	2	4	3
	3	7	9	10	9	5	9	4	7	6	3
Peripheral fatty change	Inc	0	0	0	0	4	0	0	0	0	4
	2	-	-	-	-	3	-	-	-	-	1
	3	-	-	-	-	1	-	-	-	-	4
Nasal cavity, III											
Olf epith degen/regen	Inc	0	0	2	10	10	0	0	1	10	10
	1	-	-	2	-	-	-	-	1	-	-
	2	-	-	-	1	1	-	-	-	-	-
	3	-	-	-	7	2	-	-	-	6	5
	4	-	-	-	2	7	-	-	-	4	5
Eos. globules	Inc	0	1	1	10	10	2	2	2	10	10
	1	-	1	1	-	-	2	1	1	-	-
	2	-	-	-	1	2	-	1	-	-	-
	3	-	-	-	6	3	-	-	-	2	-
	4	-	-	-	3	5	-	-	1	8	10
Harderian glands											
Vacuolation decreased (grade 1)	Inc	0	0	0	0	7	0	-	-	-	0

In a range finding study performed before a 28-day repeated dose toxicity study (Anonymous, 2014), 4 male

and 4 female beagle dogs were exposed orally to 3,4-dimethyl-1*H*-pyrazole. Due to appearance or the absence of clinical findings, the dose setting was changed during the study (dosing schedule is explained in Table 94).

Table 94: Dosing schedule (in mg/kg bw/d)

Study day	Males				Females			
	1	2	3	4	1	2	3	4
1	500	-	-	-	500	-	-	-
2	500	-	-	-	500	-	-	-
3	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
5	-	50	-	-	-	50	-	-
6	-	50	-	-	-	50	-	-
7	-	50	-	-	-	50	-	-
8	-	50	50	50	-	50	50	50
9	-	50	50	50	-	50	50	50
10	-	50	50	50	-	50	50	50
11	-	50	50	50	-	50	50	50
12	-	125	50	50	-	125	50	50
13	-	125	50	50	-	125	50	50
14	-	125	50	50	-	125	50	50
15	-	125	125	125	-	125	125	125
16	-	125	125	125	-	125	125	125
17	-	125	125	125	-	125	125	125
18	-	125	125	125	-	125	-	125
19	-	125	125	125	-	125	125	125
20	-	125	125	125	-	125	125	125
21	-	125	125	125	-	125	125	125
22	-	125	125	125	-	125	125	125

Animals exposed to 500 mg/kg bw/d were sacrificed, already on day 1, in a moribund state (vomiting, lateral position, no food consumption, poor general condition). At 125 mg/kg bw/d, 1 male out of 3 and 2 females out of 3 exhibited unsteady gait. Furthermore, at this dose, food consumption was reduced. Haematological, clinical biochemistry and necropsy were not examined.

In a 28-day repeated dose toxicity study (Anonymous, 2017), groups of 4 male and 4 female beagle dogs were given by oral route the test substance at a concentration of 0, 10, 30 and 90 mg/kg bw/d. Animals were exposed during 4 weeks.

During the study period, no mortality and no clinical signs were observed. Body weight gain was dose-dependently decreased and the modification was significant at the highest dose in males. Haematological and clinical biochemistry parameters were examined and revealed significant changes in Hb, AST and ALP in males exposed to 90 mg/kg bw/d, while only significant higher ALP value were observed in females at 90 mg/kg bw/d.

At necropsy, all macroscopic findings occurred individually and final body weight was unaffected by treatment. As observed in Table 95, significant organ weight changes were noted in adrenal glands, liver and thyroid glands. Whereas no microscopic treatment-related findings were noted.

Table 95: Organ weight (in mg, g or %)

		Males				Females			
Dose level (in mg/kg bw/d)		0	10	30	90	0	10	30	90
FBW (g)		11950	11250	11350	11025	10125	10025	10025	10400
Adrenal glands (g)	Abs	1.148	1.145	1.185	1.47*	1.113	1.178	1.153	1.283
	Rela	0.01	0.01	0.011	0.013*	0.011	0.012	0.012	0.012
Brain (g)	Abs	87.345	90.04	86.458	83.375	79.965	84.178	78.365	81.008
	Rela	0.734	0.805	0.763	0.763	0.801	0.852	0.786	0.802
Heart (g)	Abs	77.355	82.625	85.06	86.32	81.27	70.835	86.295	82.073
	Rela	0.648	0.732	0.746	0.784	0.808	0.715	0.863	0.797
Kidneys (g)	Abs	51.185	55.313	52.473	50.15	43.42	41.488	44.218	45.76
	Rela	0.429	0.492	0.463	0.455	0.434	0.416	0.442	0.441
Liver (g)	Abs	372.86	344.978	361.768	388.053	292.068	280.305	292.14	336.425
	Rela	3.116	3.07	3.194	3.522*	2.88	2.795	2.926	3.249
Pituitary gland (mg)	Abs	71.5	68.5	69.0	71.75	73.25	62.0	69.25	75.5
	Rela	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Spleen (g)	Abs	29.015	27.428	27.47	27.583	25.498	37.195	25.653	31.225
	Rela	0.242	0.243	0.243	0.25	0.255	0.354	0.256	0.299
Thymus (g)	Abs	10.833	15.415	14.958	8.998	9.91	9.47	10.405	10.958
	Rela	0.067	0.102	0.118	0.112	0.097	0.094	0.103	0.104
Thyroid glands (g)	Abs	0.588	0.853	0.755	0.755	0.735	0.575	0.768	0.7
	Rela	0.005	0.008*	0.007*	0.007	0.007	0.006	0.008	0.007
Epidid-ymides	Abs	1.87	2.048	2.498	1.93	-	-	-	-
	Rela	0.016	0.018	0.022	0.017	-	-	-	-
Prostate (g)	Abs	2.65	2.333	3.378	2.025	-	-	-	-
	Rela	0.022	0.02	0.028	0.018	-	-	-	-
Testes (g)	Abs	8.018	11.788	13.643	12.365	-	-	-	-
	Rela	0.067	0.102	0.118	0.112	-	-	-	-
Ovaries (g)	Abs	-	-	-	-	0.793	0.725	0.893	0.643
	Rela	-	-	-	-	0.008	0.007	0.09	0.006
Uterus (g)	Abs	-	-	-	-	3.678	4.748	6.125	2.278
	Rela	-	-	-	-	0.037	0.051	0.061	0.022

In a 90-day repeated dose toxicity study (Anonymous, 2017), groups of 5 male and 5 female beagle dogs were given by capsule test substance at a concentration of 0, 10, 30 and 90 mg/kg bw/d during 3 months.

During the study period, no mortality nor treatment-related clinical signs were observed. As observed in Table 96, body weight examination did not show significant changes. Haematological and clinical biochemistry parameters were examined at study day 45 and 90 and did not exhibit consistent changes.

Table 96: Body weight data (in kg)

	Males				Females			
Dose level (in mg/kg bw/d)	0	10	30	90	0	10	30	90
D 0	11.0	11.2	10.8	11.1	9.9	9.9	10.0	9.7
D 14	11.5	11.7	11.4	11.9	10.3	10.3	10.4	9.5
D 28	12.0	12.2	11.6	12.3	10.4	10.4	10.6	9.8
D 42	12.2	12.3	11.9	12.7	10.5	10.6	10.7	10.0
D 63	12.6	12.8	12.2	13.0	10.7	10.7	10.9	10.2
D 77	13.1	13.1	12.6	13.4	11.0	11.0	11.0	10.4
D 91	13.0	13.2	12.6	13.6	11.1	11.1	11.1	10.4
BWG 0 - 91	2.0	2.1	1.8	2.5	1.2	1.2	1.1	0.7

At necropsy, no treatment-related macroscopic findings were observed. Regarding organ weight examination, absolute and relative adrenal glands weight was significantly higher in females exposed to 90 mg/kg bw/d. Histology did not reveal treatment-related abnormalities.

Dermal route

In a 28-day repeated dose toxicity study (Anonymous, 2018), groups of 10 male and 10 female beagle dogs were given 3,4-dimethyl-1*H*-pyrazole at a concentration of 0, 10, 30 or 100 mg/kg bw/d. Test substance was applied uniformly to the clipped dorsal skin for at least 6 hours using a semi-occlusive dressing. Exposure was repeated during 4 weeks (6h per day on 5 day on a week), corresponding to 20 applications for males and 21 applications for females.

During the study period, no mortality nor treatment-related clinical signs were observed. Body weight examination did not show significant changes, however, body weight gain (0 – 28) exhibited a trend to decrease in males. Haematological and clinical biochemistry parameters were not significantly affected at the highest dose in both sexes.

At necropsy, no treatment-related macroscopic findings were noted. Furthermore, final body weight was unaffected by treatment. Organ weight examination revealed only significant changes for thyroid glands in males. While, histology revealed degeneration of the olfactive epithelium in 5 males and 5 females exposed to 100 mg/kg bw/d (vs no animals affected in other groups).

Table 97: 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 90-day of exposure (mg/kg bw/d)**	**Classification supported by the study**
Nasal cavity (degeneration/regeneration olf. epith.)				
28 D oral study in rats	115.3 – 134.2	28 D	~ 38 - 45	STOT RE cat. 2

Study reference	**Effective dose (mg/kg/d)**	**Length of exposure**	**Extrapolated effective dose when 90-day of exposure (mg/kg bw/d)**	**Classification supported by the study**
(Anonymous, 2021)				
28 D oral study in mice (Anonymous, 2015)	113 – 127	28 D	~ 37 – 42	STOT RE cat. 2
28 D dermal study in rats (Anonymous, 2018)	100	28 D	~ 33	STOT RE cat. 2
90 D oral study in rats (Anonymous, 2017)	33.7 – 36.3	90 D	33.7 – 36.3	STOT RE cat. 2
90 D oral study in mice (Anonymous, 2017)	64 - 87	90 D	64 - 87	STOT RE cat. 2
Two-generation reproductive study (Anonymous, 2021)	25	115 – 130 D	~ 32 - 36	STOT RE cat. 2
Liver toxicity				
Range-finding study in rats (Anonymous, 2015)	140 – 151	14 D	21 – 23	STOT RE cat. 2
28 D oral study in rats (Anonymous, 2021)	115 – 134	28 D	38 – 45	STOT RE cat. 2
28 D oral study in mice (Anonymous, 2015)	113 – 127	28 D	37 - 42	STOT RE cat. 2
28 D oral study in dogs (Anonymous, 2017	90	28 D	30	STOT RE cat. 2
28 D dermal study in rats (Anonymous, 2018)	90	28 D	30	STOT RE cat. 2
90 D oral study in rats (Anonymous, 2017)	10	90 D	10	STOT RE cat. 2
90 oral study in mice (Anonymous, 2017)	22 – 30	90 D	22 – 30	STOT RE cat. 2
90 D oral study in dogs (Anonymous, 2017)	10	90 D	10	STOT RE cat. 2
Blood				
14 D oral study in rats (Anonymous, 2014)	392.7 – 415.3	14 D	± 61 – 64	STOT RE cat. 2
28 D oral study in rats (Anonymous, 2021)	115.3 – 134.2	28 D	± 38 – 45	STOT RE cat. 2
28 D oral study in dogs (Anonymous, 2017)	30	28 D	10	STOT RE cat. 1

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when 90-day of exposure (mg/kg bw/d)	Classification supported by the study
28 D dermal study in rats (Anonymous, 2018)	30	28 D	10	STOT RE cat. 1
90 D oral study in dogs (Anonymous, 2017)	10 mg (effects observed after 45 D)	45 D	5	STOT RE cat. 1

10.12.2 Comparison with the CLP criteria

Table 98: Comparison with the CLP criteria regarding STOT RE

<table>
<tr><th>Criteria for STOT RE 1</th><th>Criteria for STOT RE 2</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.

Substance 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 value (C) as indicated in table 3.9.2”
<table><tr><td>Route of exposure</td><td>Units</td><td>Guidance value</td></tr><tr><td>Oral (rat)</td><td>mg/kg bw/d</td><td>C≤10</td></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 toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in 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 range as indicated in table 3.9.3”
<table><tr><td>Route of exposure</td><td>Units</td><td>Guidance value range</td></tr><tr><td>Oral (rat)</td><td>mg/kg bw/d</td><td>10 < C ≤ 100</td></tr></table></td></tr>
</table>

- **Nasal cavity:**

Degeneration/regeneration of the olfactive epithelium was observed in few studies:

- In the three 14-day repeated dose studies (in rats, mice and dogs), performed as range finding studies, available information did not mention if the nasal cavity was microscopically examined.

- In the 28-day repeated dose studies, degeneration/regeneration of the olfactive epithelium was observed, see table below:

Table 99: Summary table of the microscopic findings in nasal cavity after an exposure period of 28 days

Dose level (in mg/kg bw/d)	0	10	30	90	100	115.3 – 134.2	113 - 127	235.2 – 243.8	328 - 343	479.7 – 526.0	846 - 885
Rats Oral Anonymous, 2021	No effect	NT	NT	NT	NT	Degen/regen. of the olf epith: In level I: in 5 M and 4 F In level II: in 5 M and 5 F In level III: in 5 M and 5 F Severity dose-related	NT	Degen/regen. of the olf epith: In level I: in 5 M and 5 F In level II: in 5 M and 5 F In level III: in 5 M and 5 F Severity dose-related	NT	Degen/regen. of the olf epith: In level I: in 5 M and 5 F In level II: in 5 M and 5 F In level III: in 5 M and 5 F Severity dose-related	NT
Mice Oral Anonymous, 2015	No effect	NT	NT	NT	NT	NT	All animals had degen/regen. of the olf epith (grade 1 or 2)	NT	All animals had degen/regen. (grade 1 to 4) Severity dose-related	NT	All animals had degen/regen. (grade 1 to 4) Severity dose-related
Dogs Oral Anonymous, 2017	No effect	No effect	No effect	No effect	NT	NT	NT	NT	NT	NT	NT
Rats	No	No	No	NT	5 M + 5 F	NT	NT	NT	NT	NT	NT

Dermal Anonymous, 2018	effect	effect	effect		degen olf epith in nasal cavity level III (minimal)						

In grey : range to classify in category 2

Classification in category 2 is warranted when significant toxic effects were occurred within the guidance value ranges of 30 to 300 mg/kg bw/d for oral route or of 60 to 600 mg/kg bw/d for dermal route for a 28-day of exposure period. While a classification in category 1 is applicable, when significant toxic effects were observed at or below 30 mg/kg bw/d for oral exposure and at or below 60 mg/kg bw/d for dermal route.

Regarding reliable available 28-day repeated dose toxicity studies, degeneration/regeneration of the olfactive epithelium was observed in the oral repeated dose toxicity study in rats and mice. In 28-day oral repeated dose toxicity study performed in rats (Anonymous, 2021), this microscopic abnormality was observed in nasal cavity level I, II and III, in all animals in all treated groups. Furthermore, the severity was dose-related. The low and mid dose groups (115.3 – 134.2 and 235.2 – 243.8 mg/kg bw/d, resp.) are comprised in the guidance value range of the classification in category 2. Additionally, in 28-day oral repeated dose toxicity study performed in mice (Anonymous, 2015), the same effect was observed in all animals exposed to the test substance (severity dose-related) and the lowest dose (113 – 127 mg/kg bw/d) was comprised in the guidance range value to classify in category 2.

Furthermore, regarding dermal route, degeneration of the olfactive epithelium was also observed in the available 28-day repeated dose toxicity study performed in rats (Anonymous, 2018). At the highest dose (100 mg/kg bw/d), 5 males and 5 females (out of 10 animals/sex) exhibited degeneration of the olfactive epithelium in the nasal cavity level III but at a minimal grade.

- Three 90-day repeated dose toxicity study are available.

Table 100: Summary table of the microscopic findings in nasal cavity after an exposure period of 90 days

Dose level (in mg/kg bw/d)	0	10	10.6 – 12.0	22 - 30	30	33.7 – 36.3	64 - 87	90	128.8 – 142.5	374.1-374.5	375 - 529	944 - 1279
Rats Oral Anonymous, 2017	No effect	NT	No effect	NT	NT	degen/regen olf epith in nasal cavity level III in 9 M + 2F (grade 1 and 2)	NT	NT	degen/regen olf epith in nasal cavity level III in 10 M + 10 F Severity dose-related	degen/regen olf epith in nasal cavity level III in 10 M + 10 F Severity dose-related	NT	NT
Mice Oral Anonymous, 2017	No effect	NT	NT	No effect	NT	NT	2 M 1 F degen/regen olf epith (grade 1)	NT	NT	NT	All animals degen/regen grade 1 to 4 Severity dose-	All animals degen/regen grade 1 to 4 Severity dose-

											related	related
Dogs Oral Anonymous, 2017	No effect	No effect	NT	NT	No effect	NT	NT	No effect	NT	NT	NT	NT

In grey : range to classify in category 2; NT: not tested

Classification in category 2 is warranted when significant toxic effects were occurred within the guidance range values of 10 to 100 mg/kg bw/d for oral route after a 90-day of exposure period. While a classification in category 1 is applicable, when significant toxic effects were observed at or below 10 mg/kg bw/d for oral exposure.

No nasal cavity effects were observed in the study performed in dogs. While, in mice and rats, degeneration/regeneration of the olfactive epithelium was observed. In 90-day oral repeated dose toxicity study performed in rats (Anonymous, 2017), this finding was noted at the 3 highest doses (33.7 – 36.3, 128.8 – 142.4 and 374.1 – 374.4 mg/kg bw/d in M/F, resp.). The occurrence and severity were dose-related. At 33.7 – 36.3 mg/kg bw/d, 9 males and 2 females out of 10 animals/sex were affected. This dose is comprised between the guidance range value to classify in category 2. At the followed dose (128.8 – 142.4 mg/kg bw/d), which is just outside the guidance range value, all animals exhibited the finding at a grade of 2 or 3. Additionally, in the 90-day oral repeated dose toxicity study performed in mice (Anonymous, 2017), 2 males and 1 female (out of 10 animals/sex) exposed to 64 – 87 mg/kg bw/d (in M/F resp.) exhibited the degeneration/regeneration of the olfactive epithelium at a minimal grade.

- One combined chronic/carcinogenicity study performed in rats (Anonymous, 2021) is available (12 or 24 months of exposure).

Table 101: Summary table of the microscopic findings in nasal cavity after an exposure period of 12 or 24 months

Dose level (in mg/kg bw/d)	0	1	5	30	60
12 months Rats Oral Anonymous, 2021	No effect	No effect	1 M degen/regen olf epith grade 1	All animals degen/regen olf epith nasal cavity level III Severity dose-related	All animals degen/regen olf epith nasal cavity level I to IV Severity dose-related
24 months Rats Oral Anonymous, 2021	No effect	No effect	No effect	↗s inc degen/regen olf epith Severity + inc dose-related	↗s inc degen/regen olf epith Severity + inc dose-related

In grey : range to classify in category 2

Based on an exposure period of 12 months, the calculated guidance range value is comprise to 2.5 and 25 mg/kg bw/d. In the available combined chronic/carcinogenicity study (Anonymous, 2021), the lowest tested dose (5 mg/kg bw/d) was the only dose within the guidance range value. At this dose, 1 male out of 10 exhibited degeneration/regeneration of the olfactive epithelium (grade 1). At the next dose level, which is slightly

greater than the cut-off value, all animals had degeneration/regeneration of olfactive epithelium in nasal cavity level III (grade 1 and 2). The full study report indicate that *"The occurrence of degeneration/ regeneration of the olfactory epithelium was regarded to be treatment-related and adverse"*.

Based on an exposure period of 24 months, the calculated guidance range value is between 1.25 to 12.5 mg/kg bw/d. In the available study, the lowest treated dose, 5 mg/kg bw/d, is the only dose within the guidance range. At this dose, no microscopic effect was noted in nasal cavity.

- Furthermore, in the two generation reproductive toxicity study (Anonymous, 2021), detailed in the section 10.10.1, degeneration/regeneration of the olfactive epithelium was observed in the parental F0 and the parental F1 generations.

Table 102: Summary table of microscopic findings in nasal cavity in the reproductive study

Dose level (in mg/kg bw/d)		0	6	25	100
Rat Oral 75 D premating + max 2 w mating until weaning of pups (approx. 115-130 D) Anonymous, 2021	F0 parental generation	1 M and 1 F had degen/regen olf epith in the nasal cavity level II (grade 1)	No effect	4 F and 23 M had degen/regen olf epith in nasal cavity level III (grade 1)	All animals exhibited degen/regen olf epith in nasal cavity level I to IV (grade 1 to 3)
	F1 parental generation	No effect	No effect	24 F and 25 M had degen/regen olf epith in nasal cavity level III (grade 1)	All animals exhibited degen/regen olf epith in nasal cavity level I to IV (grade 1 to 3)

In grey : range to classify in category 2

Based on the duration of exposure during the two-generation toxicity study (Anonymous, 2021), which was approximately of 115 to 130 days (depending of duration of mating period), the calculated guidance value range is between approximately 7-8 and 70-80 mg/kg bw/d. In this study, the mid dose group was within the range to classify in category 2. At this dose level (25 mg/kg bw/d), almost all males of the P0 and F1 generation exhibited degeneration/regeneration of the olfactive epithelium in the nasal cavity level III. While in females, effect was more pronounced in the F1 parental generation, as 24 females out of 25 exhibited degeneration/regeneration of the olfactive epithelium in nasal cavity level III. For all these animals, effect was of grade 1. Higher grade was observed at the next dose level which was outside the guidance range value.

As mentioned in the CLP Guidance, section 3.9.1 Definition and General considerations for STOT RE, *"Specific target organ toxicity (repeated exposure) means specific, target organ toxicity arising from a repeated exposure to a substance or mixture. **All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included**."*.

➔ **In conclusion**, regarding the microscopic effects observed in nasal cavity in different studies, in 2 species (rats and mice), in 2 route of exposure and after different time of exposure period, a classification as **STOT RE Cat. 2** is warranted for the **nasal cavity**.

- **Liver:**

Different repeated dose toxicity studies performed in 3 species examined liver.

- In the three 14-day repeated dose studies (in rats, mice and dogs), performed as range finding studies, hepatic enzymes and microscopic examination were only performed in the rat's study (Anonymous, 2014).

Table 103: Summary table of the effects observed in liver after an exposure period of 14 days

Dose level (in mg/kg bw/d)	0	140.1-151.0	392.7-415.3
RF 14 D Oral In rats Anonymous, 2014	No effect	↘ ALP in M (dose-related) ↘ crea in M (dose-related) ↗ rela liver weight (dose-related) Hypertrophy/hyperplasia in 2 M and 1 F (grade 1)	↘* ALP in M (dose-related) ↘* crea in F (not sign in M but dose-related) ↗* rela liver weight (dose-related) Hypertrophy/hyperplasia in all animals (4 M + 4 F) (grade 2 and 3)

In grey : range to classify in category 2

Classification in category 2 is warranted when significant toxic effects occurred within the guidance value ranges of 60 to 600 mg/kg bw/d for oral route.

In the study performed in rats (Anonymous, 2014), ALP and creatinine were significantly modified. However, in this study, ALP level was higher in the control group due to 2 animals which had an ALP level of 4.55 and 3.22. Liver weight exhibited significant changes. And the microscopic examination revealed hypertrophy/hyperplasia in the 2 tested doses and the incidence and severity were dose-related. These effects were observed at doses comprised in the range to classify in category 2.

- In the three 28-day repeated dose toxicity studies, effects observed in liver were noted:

Table 104: Summary table of the effects observed in liver after an exposure period of 28 days

Dose level (in mg/kg bw/d)	0	10	30	90	113-127	115.3-134.2	235.2-243.8	328-343	479.7-526.0	846-885
28 D Oral In rats Anonymous, 2021	No effect	NT	NT	NT	NT	↗* rela liver weight in M (not sign in F) (dose-related) Centrilobular hypertrophy in 1M/5 (grade 1)	↗ ALT (not sign, but corresp. to + 31.88 % in M and + 11.86 % in F compared to control)) ↘ crea (not sign) ↗* rela liver weight (dose-related) Hypertrophy centrilobular	NT	↗* ALT in both sexes (dose-related in M) ↗ AST in both sexes ↘* crea in F (not sign in M) ↗* rela liver weight	NT

							in 4 M and 4 F (out of 5) (grade 1 and 2)		(dose-related) Hypertrophy centrilobular in all animals (grade 1 and 2)	
28 D Oral In mice Anonymous, 2015	No effect	NT	NT	NT	↗ ALT in F ↗ rela liver weight in M (dose-related)	NT	NT	↗ ALT + AST + ALP in F ↗ rela liver weight in M (dose-related)	NT	↗ ALT in both sexes (dose-related in F) ↗ AST in both sexes ↗* ALP in M (not sign in F) ↗* rela liver weight (dose-related in M) Centrilobular hypertrophy in all M and 3 F
28 D Oral In dogs Anonymous, 2017	No effect	No effect	No effect	↗* AST in M ↗* ALP in both sexes ↘ ALT in F ↗* rela liver weight in M (not sign in F)	NT	NT	NT	NT	NT	NT
28 D Dermal In rats Anonymous, 2018	No effect	No effect	No effect	↘ AST in F	NT	NT	NT	NT	NT	NT

In grey : range to classify in category 2

Classification in category 2 is warranted when significant toxic effects were occurred within the guidance value ranges of 30 to 300 mg/kg bw/d for oral route or of 60 to 600 mg/kg bw/d for dermal route for a 28-day of exposure period. While a classification in category 1 is applicable, when significant toxic effects were observed at or below 30 mg/kg bw/d for oral exposure and at or below 60 mg/kg bw/d for dermal route.

The three oral studies revealed modification in the hepatic enzymes as well as change in the liver weight. Microscopic modification (centrilobular hypertrophy) was only observed in the study performed in rats (Anonymous, 2021).

As observed in Table 104, in the 28-day repeated dose oral toxicity study, performed in rats, ALT level was only significantly modified at the highest dose which is outside the range to classify in category 2. In mid dose group, which is within the range to classify in category 2, ALT level was increased but not significantly.

- In the three 90-day repeated dose toxicity studies, effects were also noted in liver:

Table 105: Summary table of the effects observed in liver after an exposure period of 90 days

Dose level (in mg/kg bw/d)	0	± 10	22-30	± 30	64-87	90	128.8-142.5	374	375-529
90 D Oral In rats Anonymous, 2017	No effect	↗** abs + rela liver weight in F	NT	↘* AST in F	NT	NT	↗ ALT and AST in M ↘ ALP in M ↗** abs + rela liver weight in F (not sign in M) Centrilobular hypertrophy in all M and 9 F (grade 1 and 2)	↗* ALT In M and F ↗ AST in M ↗** abs + rela liver weight Centrilobular hypertrophy in all animals (grade 2 or 3)	NT
90 D Oral In mice Anonymous, 2017	No effect	NT	↘* ALT in M ↗ ALT and AST in F	NT	↗ ALT in F	NT	NT		↗** ALT in M ↗ AST and ALP in M
90 D Oral In dogs Anonymous, 2017	No effect	↘ ALT in M	NT	↘ ALT in M	NT	↘ ALT in M ↗* ALP in M (not sign in F) ↗ rela liver weight (not sign)	NT		NT

In grey : range to classify in category 2

Classification in category 2 is warranted when significant toxic effects were occurred within the guidance range values of 10 to 100 mg/kg bw/d for oral route after a 90-day of exposure period. While a classification in category 1 is applicable, when significant toxic effects were observed at or below 10 mg/kg bw/d for oral exposure.

As observed in Table 105, in the three available 90-day repeated dose toxicity studies, liver weight and enzyme were modified at dose within the range to classify in category 2. However, enzymes change was not coherent in all studies and microscopic modification was not observed in the range to classify.

- One combined chronic toxicity study is available:

Table 106: Summary table of the effects observed in liver after a chronic exposure

Dose level (in mg/kg bw/d)		0	1	5	30	60
Combined chronic study Oral In rats Anonymous, 2021	12 months	No effect	No effect	At D 92: AST ↗ in M and ↘ in F	At D 92: AST ↗ in M	At D 92: AST slightly ↗ in M and ↘ in F At D 181: ALT and AST ↗ in M At D 365: ALT and AST ↗ in M Centrilobular hypertrophy in 5M/10 (grade 1)
	24 months	No effect	No effect		Rela liver weight ↗ in F (not sign)	Rela liver weight ↗** in F (not sign in M)

Based on an exposure period of 12 months, the calculated guidance range value is comprised between 2.5 and 25 mg/kg bw/d. After 12 months of exposure, no liver modification was noted at 5 mg/kg bw/d.

However, during the 12 month exposure period, enzyme were examined at different time points. After 92 days of exposure, AST exhibited modification. Based on an exposure period of 92 days, all the tested dose were comprised in the range of the guidance value (10 - 100 mg/kg bw/d) to classify in category 2.

Based on an exposure period of 24 months, the calculated guidance range value is comprised between 1.25 and 12.5 mg/kg bw/d. The only tested dose which is within the range is 5 mg/kg bw/d and at this dose no liver effect was observed.

➔ **In conclusion,** modification was observed in liver (enzyme, liver weight as well as microscopic change). However, histopathological modification was not observed at doses which are within the range to classify in category 2. Moreover, enzyme modification was not coherent in the different studies. Although, effects were noted in several repeated dose toxicity studies performed in different species (rat, mouse and dog) and after different duration of exposure, DS is of the opinion that effects were not enough coherent and DS consider that a **classification as STOT RE for liver is not warranted**.

Blood – Hematological system:

Hematological system was examined in different repeated dose toxicity studies:

- In the 3 range finding studies which exposed animals during a period of 2 weeks, hematology was only examined in the rat study (Anonymous, 2014). As observed in Table 107, Hb, Ht and MCHC were significantly increased as well as RBC which increased at the highest tested dose but not significantly, which is within the range of a classification in category 2.

Table 107: Summary table of haematological effects after an exposure period of 2 w

Dose level (in mg/kg bw/d)	0	50	125	140.1-151.0	392.7-415.3	408-610	500	776-956
14 D oral In rats Anonymous, 2014	No effect	NT	NT	No effect	↗* Hb (+ 8.2 %), Ht (+ 4.9 %) and MCHC (+ 3.46 %) in F (not sign in M) RBC ↗ but not sign Plt ↘ in F and ↗ in M Abs spleen w ↘* in M (rela not sign)	NT	NT	NT
14 D oral In rats Anonymous, 2014	NE	NT	NT	NT	NT	NE	NT	NE
15 D oral In dogs Anonymous, 2014	NE	NE	NE	NT	NT	NT	NE	NT

- After an exposure period of 28 days, hematological effects were observed in the range to classify in category 2. As for the 14-day toxicity study performed in rats, Hb, Ht and RBC were increased in different studies. Changes were observed in rats and dogs after an oral exposure as well as in rats after a dermal exposure.

Table 108: Summary table of the haematological effects after an exposure period of 28 days

Dose level	0	10	30	90	100	113-127	115.3-134.2	235.2-243.8	328-343	479.7-526.0	846-885
28 D oral In rats Anonymous, 2021	No effect	NT	NT	NT	NT	NT	↗ RBC in F (+ 3.8 %) (dose-related)	↗* RBC in F (+ 7.59 %) (dose-related) ↗ Hb in F (+ 4.65 %) (dose-related)	NT	↗* RBC in F **(+ 11.26 %)** (dose-related) ↗ Hb in F (+ 5.81 %) (dose-related)	NT

								↘ **Plt in F (- 13.21 %)**		↗ Ht in F (+ 5.15 %) ↘ Plt in F (- 12.68 %) Abs spleen w ↘* in F (rela not sign)	
28 D oral In mice Anonymous, 2015	No effect	NT	NT	NT	NT	No effect	NT	NT	No effect	NT	↘* MCH in F ↗ RBC, Hb and Hb in M ↗ Plt ↘ spleen w (not sign)
28 D oral In dogs Anonymous, 2017	No effect	↗ Hb, Ht and RBC (dose related) in M ↘ QT (dose related) in M	↗ Hb (+ 5.49 %), Ht (+ 4.23 %) and RBC (+ 6.91 %) (dose-related) in M ↘ **QT (- 12.79 %)** (dose-related) in M	↗* **Hb (+ 12.09 %)** (dose related) in M (not sign and not dose-related in F) ↗ **RBC (+ 15.05 %) and Ht (+ 11.36 %)** (dose-related) in M ↘ **QT (- 13.1 %)** (dose-related) in M	NT	NT	NT	NT	NT	NT	NT
28 D dermal In rats Anonymous, 2018	No effect	No effect	↗* Hb in F (+ 3.45 %) ↘ Plt in F (- 8.21 %) ↗ HQT in F (dose-related) (+	NT	↘ Plt in F (- 4.24 %) ↗ HQT in F (dose-related) (+ 2.96 %)	NT	NT	NT	NT	NT	NT

			1.18 %)								

In grey: range to classify in category 2

- In the subchronic studies, hematological changes were only observed in study performed in dogs. The modifications were the same as for the shorter exposure, as Hb and Ht were increased at dose within the range to classify in category 2.

Table 109: Summary table of the effects observed in liver after subchronic exposure

Dose level	0	±10	22-30	30	33.7-36.3	64-87	90	128.8-142.5	±374	375-529	944-1279
90 D oral In rats Anonymous, 2017	No effect	No effect	NT	NT	No effect	NT	NT	WBC ↗* in F	Plt ↘* in F (↘ in M but not sign) WBC ↗* in F HQT ↗ in F, ↘ in M Abs + rela spleen w ↗**	NT	NT
90 D oral In mice Anonymous, 2017	No effect	NT	No effect	NT	NT	No effect	NT	NT	NT	No effect	↘ RBC, Hb and Ht in F (↗ in M) Abs spleen w ↘** (not rela)
90 D oral In dogs Anonymous, 2017	No effect	After 45 D: ↗ **Hb (+ 9.4 %) and Ht (+ 9.98 %)** dose related in M (not dose-related in F)	NT	After 45 D: ↗ Hb and Ht dose related in M (not dose-related in F) ↗* MCV in M After 90 D: ↗* MCV in M	NT	NT	After 45 D: ↗ Hb and Ht dose related in M (not dose-related in F) ↗ MCV in M (not sign) ↗ QT dose-related in M After 90 D:	NT	NT	NT	NT

							↗ dose-related Hb and Ht in F ↗** MCH in M ↗ QT in M				

In grey: range to classify in category 2

- One chronic toxicity study is available and was performed in rats (Anonymous, 2021). Hematology were not examined in the main groups, which were exposed during 24 months. In the satellite groups, which were given test substance during 12 months, RBC was significantly higher but only at the highest dose.

Table 110: Summary table of haematological effects after chronic exposure

Dose level (in mg/kg bw/d)		0	1	5	30	60
Combined chronic study Oral In rats Anonymous, 2021	12 months	No effect	No effect	No effect	No effect	At D 367: ↗* RBC in F ↘* MCV in M (not sign in F) ↘ Plt in F not sign ↗ QT in F not sign
	24 months (hemato not examined)	No effect	No effect	No effect	↗* rela spleen w in M	↗ inc enlarged spleen in M ↗* rela spleen w in M and F

➔ **In conclusion,** few studies exhibited hematological changes. These effects are all going in the same direction which was an increase of Hb, Ht and RBC and sometimes a decrease of QT. However, DS is of the opinion that effects observed in the range to classify in category 2 are not severe enough and consider that a **classification as STOT RE for blood is not warranted.**

Mandibular glands:

Diffuse atrophy was observed in 3 oral rat studies (see Table 111). Atrophy was characterized by a reduced number and size of the secretory glandular ducts and reduced number of eosinophilic granules within granular duct cells.

Incidence and severity are dose-dependently increased. In the 28-day oral repeated dose toxicity study (Anonymous, 2021), the low and mid dose groups are within the guidance range value to classify as STOT RE Cat. 2. In the low dose group, 3 females out of 5 and all males had mandibular atrophy, while at the mid dose, all animals were affected. Among these animals exposed to the mid dose, 3 males exhibited atrophy at a grade 4. Furthermore, in the 90-day oral repeated dose toxicity study (Anonymous, 2017), only one dose was within the range of the guidance to classify in cat. 2. At this dose, 2 females and 6 males (out of 10/sex) exhibited mandibular atrophy at a grade 1 or 2. At the following dose (128.8-142.4 mg/kg bw/d), which was just higher than the guidance range value (100 mg/kg bw/d), all animals had mandibular atrophy at a grade of 2 or 3. In the combined chronic/carcinogenicity study (Anonymous, 2021), mandibular atrophy was observed but at doses higher than the guidance range value.

Table 111: Summary table of the microscopic findings in mandibular glands

Exposure period of 28 days						
Dose level (in mg/kg bw/d)		0	115.3-134.2	235.2-243.8	479.7-526.0	
28 days, oral Anonymous 2021		No effect	3 F and 5 M (grade 1 to 3)	5 F and 5 M (grade 1 to 4)	5 F (grade 2 and 3) and 5 M (all grade 4)	
Exposure period of 90 days						
Dose level (in mg/kg bw/d)		0	10.6-12.0	33.7-36.3	128.8-142.5	374.1-374.5
90 days, oral Anonymous, 2017		No effect	No effect	2 F (grade 2) and 6 M (grade 1)	10 F (grade 2 and 3) and 10 M (grade 3)	10 M and 10 F (all grade 4)
Chronic exposure						
Dose level (in mg/kg bw/d)		0	1	5	30	60
Combined chronic, oral Anonymous, 2021	12 months	No effect	No effect	No effect	2 M (grade 2) 0 F	10 M and 9 F (grade 2 and 3)
	24 months	1 M (grade 2)	No effect	No effect	30 F (grade 2 to 4) and 35 M (grade 1 to 4)	40 F (grade 2 to 4) and 48 M (grade 3 and 4)

In grey: range to classify in category 2

Amano *et al.*, 2012, describe anatomy and histology of rodent and human major salivary glands. The paper concludes that "Rodent salivary glands used in animal experiments show a similar but different histology compared with the human glands. Especially, rodent submandibular glands develop GCTs producing a variety of cell growth factors." GCT is the granular convoluted tubule, which is located between the ID (intercalated) and SD (striated) in the rodent submandibular gland and the principal cell type of the GCT is a high-columnar secretory cell containing many secretory granules. Human salivary glands also secretes growth factors from portions within the glands other than the GCTs (which are not present in humans).

➔ **In conclusion,** as the adverse effects on mandibular glands are only observed in rat studies and as the histology and growth factors between rat and human are different, DS consider that a **classification as STOT RE for mandibular glands is not warranted.**

10.12.3 Conclusion on classification and labelling for STOT RE

Based on the available results, a classification as **STOT RE Cat. 2 H353 (nasal cavity)** is warranted.

10.13 Aspiration hazard

Hazard class not assessed in this CLH dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in this CLH dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this CLH dossier.

13 ADDITIONAL LABELLING

NA

14 REFERENCES

Amano *et al.*, 2012, Anatomy and histology of rodent and human major salivary glands, Overview of the Japan salivary gland society sponsored workshop, Acta histochem. Cytochem., 45 (5), 241-250.

Full study report

Registration dossier

15 ABBREVIATIONS

*: $p < 0.05$

**: $p < 0.01$

Abs: absolute

AGD: ano-genital distance

ALT: alanine aminotransferase

ALP: alkaline phosphatase

Approx.: approximately

AST: aspartate aminotransferase

ATE: acute toxicity estimate

Bw: body weight

BWG: body weight gain

Cat: category

Conc.: concentration

Cons.: consumption

Corresp.: corresponding

Creat: creatinin

Degen: degeneration

DMP: dimethylpyrazole

DS: dossier submitter

Eos: eosinophilic

Epith: epithelium

Exp: experiment

F: female

FBW: final body weight

FST: landing foot-splay test

GD: gestation day

GGT_C: serum-gamma-glutamyltransferase

GLP: good laboratory practice

GS F: grip strength forelimbs

GS H: grip strength hindlimbs

Hb: hemoglobin

HQT: prothrombine time (hepato quick's test)

Ht: hematocrit

Inc: incidence

Infl.: inflammatory

Interv: interval

LC50: lethal conc 50%

LD50: lethal dose 50%

M: male

MCH: mean corpuscular hemoglobin

MCHC: mean corpuscular hemoglobin concentration

MCV: mean corpuscular volume

Min: minimum

MMAD: mean mass aerodynamic diameter

NA: not applicable

Nb: number

NE: not examined

NT: not tested

NZW: New-Zealand white

Olf: olfactive

PI: post-implantation

PLT: platelets

PMD: post-mating day

PND: post-natal day

PTT: activated partial thromboplastin time

QT: prothrombin time (Quick's test)

RBC: red blood cell

Regen: regeneration

Rela: relative

Resp.: respectively

Ret: reticulocyte

RF: range-finding

Sem ves: seminal vesicle

Sign: significant

TG: test guideline

Tot: total

Tot prot: total protein

Tox: toxicity

TS: total spermatids

TS/gC: total spermatids/gram cauda epididymis

TS/gT: total spermatids/gram testis

Vacuol.: vacuolisation

WBC: white blood cell

16 ANNEXES

Annex I to the CLH report