Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

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

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

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

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

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Version number: 2 **Date: May 2023**

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1 PHYSICAL HAZARDS

Hazard class not assessed in this CLH dossier

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not evaluated in this CLH dossier

3 HEALTH HAZARDS

3.1 Acute toxicity - oral route

3.1.1 Animal data

3.1.1.1 Acute oral toxicity study (Anonymous, 2015)

Study reference

Anonymous, 2015

Detailed study summary and results

Test type

OECD TG 423

GLP

Test substance

- 3,4-dimethyl-1*H*-pyrazole
- *Degree of purity :* 99.3 %

Test animals

- *Species/strain/sex :* Rat / Wistar / female
- *Nb. of animals per sex per dose* : 3 females/group
- *Age and weight at the study initiation :* approx. 10 w

Administration/exposure

- *Mode of administration (gavage, in diet, other)* : oral, gavage
- *Duration of test/exposure period :* single exposure
- *Doses/concentration levels, rationale for dose level selection :* 2000 mg/kg bw as first experiment, 500 mg/kg bw as second experiment and 500 mg/kg bw as third experiment.
- *Post exposure observation period :* 14 d
- *Vehicle :* corn oil

Results and reliability

- *LD_{50} or LC_{50} value with confidence limits if calculated* : between 500 and 2000 mg/kg bw
- *Nb of deaths at each dose level and time of death :* 2000 mg/kg bw : all females died (within 2 h)

500 mg/kg bw (2nd exp) : 1 female sacrificed (on D 1, due to moribund state)

500 mg/kg bw (3rd exp) : no mortality occurred

- *Clinical signs :* 2000 mg/kg bw : poor general state, piloerection, atonia, abdominal position, shallow breathing (from 0 h until 1 h after exposure).

  500 mg/kg bw (2nd exp) : in the 2 surviving animals : impaired general state (from 0 h to 4 h after administration) which increase to poor general state (at 5 h) and decrease to impaired general state (on day 1 after exposure). Clinical signs, such as piloerection, dyspnea, cowering position, abdominal position, staggering, reduced defecation, were observed at few time observation period.

  The 3rd animal, which was sacrificed, showed dyspnea, piloerection, impaired general state followed by poor general state, cowering position, staggering, abdominal position, apathy, atonia, lack of defecation.

  500 mg/kg bw (3rd exp) : impaired general state and piloerection were observed in all females, cowering position and dyspnea were observed at few observation time period.

- *Body weight :*

Table 1 : Mean body weight (in g)

Dose level (in mg/kg bw)	2000 (exp 1)	500 (exp 2)	500 (exp 3)
D 0	187.7	174.0	179.7
D 7	-	198.5	194.3
D 14	-	207.0	198.3

- *Necropsy findings, including doses affected, severity and nb of animals affected :*

  At 2000 mg/kg bw : all females had dark red spot in all lung lobes, spotted liver, yellowish discoloration of the stomach contents, red discoloration of the gas-filled stomach and of the small intestine with partly red discoloured contents.

  At 500 mg/kg bw (2nd exp) : animal which was sacrificed exhibited dark red spot discoloration of all lung lobes, congestion of the kidneys.

  No macroscopic findings in animals sacrificed at the end of the observation period.

3.1.2 Human data

No human data available

3.1.3 Other data

No other data available

3.2 Acute toxicity - dermal route

3.2.1 Animal data

3.2.1.1 Acute dermal toxicity study (Anonymous, 2015)

Study reference

Anonymous, 2015

Detailed study summary and results

Test type

OECD TG 402

GLP

Test substance

- 3,4-dimethyl-1*H*-pyrazole
- *Degree of purity :* 99.3 %

Test animals

- *Species/strain/sex :* Rat / Wistar / both sexes
- *Nb. of animals per sex per dose :* 5/sex/group
- *Age and weight at the study initiation :* approx. 8 w for males and 12 w for females

Administration/exposure

- *Mode of administration :* semi-occlusive
- *Duration of test/exposure period* : 24 h
- *Doses/concentration levels, rationale for dose level selection* : 200, 2000 and 5000 mg/kg bw
- *Post exposure observation period* : 14 d
- *Vehicle :* corn oil
- *Area covered (e.g. x% of body surface) :* approx. 40 cm^2
- *Removal of test substance* : afterwards removal of patch, rinsing of the application site.

Results and reliability

- *LD_{50} or LC_{50} value with confidence limits if calculated :* between 200 and 2000 mg/kg bw
- *Nb of deaths at each dose level :* 5000 mg/kg bw : all animals died (2 h after application).

  2000 mg/kg bw : all animals died (1 male and 1 female at 5h after application, 3 males and 3 females were found dead on D 1, and 1 male and 1 female were sacrificed in a moribund state on D 1).

  200 mg/kg bw : no mortality occurred.

Additional information that may be needed to adequately assess data for reliability

- *Clinical signs :* 5000 mg/kg bw : 1 h after application : poor general state, piloerection, abdominal position, atonia and shallow breathing.

  2000 mg/kg bw : impaired general state 1 h after application. After, animals exhibited piloerection, dyspnoea, poor general state, abdominal position and atonia.

200 mg/kg bw : no clinical signs observed.

- *Body weight :*

Table 2 : Body weight (in g)

	Males			Females		
Dose level (in mg/kg bw)	200	2000	5000	200	2000	5000
D 0	232.0	232.4	225.0	208.4	206.6	209.6
D 1	-	225.8	-	-	202.5	-
D 7	257.4	-	-	217.2	-	-
D 14	288.6	-	-	229.4	-	-

- *Necropsy findings, including doses affected, severity and nb of animals affected :*

5000 mg/kg bw : absence of rigor mortis (all animals), well-defined erythema (grade 2) at the application site (all animals), red discoloration of the small intestine (4 M and 4 F), congestion of the kidneys (4 M and 3 F), spotted discoloration of the liver (4 M and 3 F), pale red discoloration of the liver (1 M and 3 F), spotted discoloration of all lung lobes (1 M and 3 F), bloody contents in the urine bladder (2 M and 1 F).

2000 mg/kg bw : absence of rigor mortis (1 M and 1 F), dark red discoloration of all lung lobes (1 M and 2 F), red discoloration of all lung lobes (2 M and 2 F), red spotted discoloration of all lung lobes (2 M and 1 F), red discoloration of the small intestine (4 M and 4 F), dark discoloration of the small intestine contents (1 F), spotted discoloration of the liver (3 M and 5 F), unilateral congestion of the kidney (3 M and 1 F), red discoloration of the glandular stomach and the contents (1 F), bloody contents of the urine bladder (1 F), well-defined erythema (grade 2) at the application site (1 F).

200 mg/kg bw : no macroscopic findings.

3.2.1.2 Acute dermal toxicity study (Anonymous, 2017)

Study reference

Anonymous, 2017

Detailed study summary and results

Test type

OECD TG 402

GLP

Test substance

- 3,4-dimethyl-1*H*-pyrazole
- *Degree of purity :* 95.9 %

Test animals

- *Species/strain/sex :* Rat / Wistar / both sexes
- *Nb. of animals per sex per dose :* 5/sex

- *Age and weight at the study initiation :* approx. 8w for males and 12w for females

Administration/exposure

- *Mode of administration :* semi-occlusive
- *Duration of test/exposure period* : single application of 24 h
- *Doses/concentration levels, rationale for dose level selection* : 1000 mg/kg bw
- *Post exposure observation period* : 14 d
- *Vehicle :* corn oil
- *Area covered (e.g. x % of body surface) :* approx. 40 cm²
- *Removal of test substance :* after removal, rinsing of the application site.

Results and reliability

- *LD_{50} or LC_{50} value with confidence limits if calculated :* > 1000 mg/kg bw
- *Nb of deaths at each dose level :* no mortality occurred. 2 females were sacrificed due to moribund condition on day 1 after application.

Additional information that may be needed to adequately assess data for reliability

- *Time of death (provide individual animal time if less than 24 hours after dosing) :* 2 females sacrificed on day 1.
- *Clinical signs :* no systemic clinical signs in males while 2 females had poor general state, abdominal position, flat respiration and chromodacryorrhoea on study day 1, these females were sacrificed.

  Slight erythema at the application site (grade 1) at study day 1 in 2 males and in 4 females (erythema persisted in 2 females until D 2).

- *Body weight :*

Table 3 : Body weight (in g)

	Males	Females
Dose level (in mg/kg bw)	1000	1000
D 0	229.0	207.8
D 1	-	202.0
D 7	260.8	209.7
D 14	290.8	219.0

- *Necropsy findings, including doses affected, severity and nb of animals affected :* pale skin, muscles and organs in 2 females which were sacrificed.

  No macroscopic findings observed in the remaining animals.

3.2.2 Human data

No human data available

3.2.3 Other data

No other data available

3.3 Acute toxicity - inhalation route

3.3.1 Animal data

3.3.1.1 Acute inhalation toxicity study (Anonymous, 2015)

Study reference

Anonymous, 2015

Detailed study summary and results

Test type

OECD TG 403

GLP

Test substance

- 3,4-dimethyl-1*H*-pyrazole
- *Degree of purity :* 99.3 %
- *Particle size of dust and mist given as mean mass aerodynamic diameter (MMAD) and geometric standard deviation (GSD)* : 1 to 4 µm for the MMAD and between 1.5 to 3 for the GSD

Test animals

- *Species/strain/sex :* Rat / Wistar / both sexes
- *Nb. of animals per sex per dose* : 5/sex/group
- *Age and weight at the study initiation :* 9-10 w, 262.3 to 327.2 g for M and 178.7 to 211.7 g for F

Administration/exposure

- *Type of inhalation exposure and test conditions :* aerosol, by nose-only, flow-past exposure
- *Duration of test/exposure period* : single exposition of 4 h
- *Doses/concentration levels, (ppmV (parts per million per volume) for gases, mg/l for vapours, mg/l for dusts and mists) and rationale for dose level selection* : 2.1 and 5.1 mg/L air
- *Post exposure observation period* : 14 d

Results and reliability

- *LD_{50} or LC_{50} value with confidence limits if calculated :* between 2.1 and 5.1 mg/L
- *Nb of deaths at each dose level :* 2.1 mg/L : all animals survived.

  5.1 mg/L : 1 male and 1 female were found death, the remaining animals were killed in extremis due to moribund condition.

Additional information that may be needed to adequately assess data for reliability

- *Time of death (provide individual animal time if less than 24 hours after dosing) :* all animals died at D 1.

- *Clinical signs :* 2.1 mg/L : after 2h of exposure, all animals showed decreased activity, laboured breathing and salivation. Recovered on D 2.

  5.1 mg/L : after 1 h of exposure, all animals exhibited decreased activity, laboured breathing and salivation. At the end of exposure period, marked apathy was observed.

- *Body weight :*

Table 4 : Body weight data (in g)

	Males		Females	
Dose level (in mg/L)	2.1	5.1	2.1	5.1
D 1	272.1	306.5	187.3	202.6
D 2	269.6	-	188.4	-
D 8	294.4	-	192.3	-
D 15	320.2	-	206.3	-

- *Necropsy findings, including doses affected, severity and nb of animals affected :* no substance-related findings.

  One male, of the low dose, had submandibular glands discoloration (red brown).

3.3.2 Human data

No human data available

3.3.3 Other data

No other data available

3.4 Skin corrosion/irritation

Hazard class not assessed in this dossier

3.5 Serious eye damage/eye irritation

Hazard class not assessed in this dossier

3.6 Respiratory sensitisation

Hazard class not assessed in this dossier

3.7 Skin sensitisation

Hazard class not assessed in this dossier

3.8 Germ cell mutagenicity

Hazard class not assessed in this dossier

3.9 Carcinogenicity

3.9.1 Animal data

3.9.1.1 Combined chronic toxicity/carcinogenicity study in Wistar rats (Anonymous, 2021)

See section 3.12.1.4

3.9.2 Human data

No human data available

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

No other data available

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Two-generation reproductive toxicity study in rats (Anonymous, 2021)

Study reference

Anonymous, 2021

Detailed study summary and results

Test type

OECD TG 416

GLP

Test substance

- 3,4-dimethyl-1*H*-pyrazole
- *Degree of purity :* 95.9 %

Test animals

- *Species/strain/sex :* Rat / Wistar / both sexes
- *Nb. of animals per sex per dose :*

  F0 generation : 25/sex/dose

  F1 generation : 25/sex/dose

- *Age and weight at the study initiation :* 36 D

Administration/exposure

- *Route of administration :* oral, diet
- *Duration and frequency of test/exposure period :* daily

  F0 : At least 75 D after the beginning of treatment, males and females of the same dose group were mated (max. 2 w). F0 generation were sacrificed shortly before weaning of the F1 pups for F0 males and after weaning of the F1 pups for the F0 females.

  F1 : after weaning, animals were exposed during at least 75 D. After that, males and females were mated (max. 2 w). F1 males were sacrificed shortly before weaning of the F2 pups while F1 females were sacrificed shortly after weaning of the F2 pups.
- *Doses/concentration levels, rationale for dose level selection :* 0, 6, 25 and 100 mg/kg bw/d

Table 5 : Mean test substance intake (in mg/kg bw/d)

		Low dose group	Mid dose group	High dose group
F0				
Males		5.8	24.0	95.9
Females	Premating period	6.1	25.7	102.2
	Gestation period	5.1	18.1	77.1
	Lactation period	5.6	21.0	84.5
F1				
Males		6.0	24.0	96.3
Females	Premating period	6.0	24.1	96.6
	Gestation period	6.5	27.7	108.0
	Lactation period	7.6	32.7	126.1

- *Vehicle :* diet

Description of test design

- *Details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy) :* ratio of 1:1 for a max. of 2 w.
- *Premating exposure period for males and females (P and F1) :* 75 D
- *Standardization of litters (yes/no and if yes, how and when) :* on PND 4, litter were standardized in such a way that each litter contained 4 males and 4 females.

Results and discussion

For P adults (per dose) :

- *Time of death during the study and whether animals survived to termination :* no mortality occurred.
- *Clinical observations :* no treatment-related effects.

  Only one female of the low dose group exhibited protruding eyeball during premating, mating, gestation and lactation periods.
- *Food consumption :*

- *In males :* unaffected by treatment during premating period (D 0 to 70 : 23.7, 23.7, 23.9 and 23.7 g/animal/d, resp. at 0, 6, 25 and 100 mg/kg bw/d) and mating period (D 2 to 16 : 22.5, 21.9, 22.5 and 22.4 g/animal/d, resp. at 0, 6, 25 and 100 mg/kg bw/d).
- *In females :* reduced during premating period D 7 to 14 (17.5, 17.3, 16.5 and 16.1 g/animal/d, resp. at 0, 6, 25 and 100 mg/kg bw/d). Reduction was very slight regarding the whole premating period (D 0 to 70 : 20.3, 19.9, 20.7 and 19.8 g/animal/d, resp. at 0, 6, 25 and 100 mg/kg bw/d).

  During gestation period, no modifications observed (D 0 to 20 : 21.4, 21.3, 20.4 and 21.5 g/animal/d, resp. at 0, 6, 25 and 100 mg/kg bw/d).

  During lactation period : lower during the whole period (D 1 to 21 : 49.5, 48.9, 48.5 and 47.8 g/animal/d, resp. at 0, 6, 25 and 100 mg/kg bw/d).

- *Body weight data :*

Table 6 : Mean body weight (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

- *Haematological and clinical biochemistry findings :* not examined
- *Effects on sperm :*

Table 7 : 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

- *Nb of P females cycling normally and cycle length :*

- *Mean nb of oestrous cycle :* 4.16, 4.04, 3.96 and 4.20, resp. at 0, 6, 25 and 100 mg/kg bw/d.
- *Mean cycle length :* 4.01, 4.20, 4.60 and 4.0 days, resp. at 0, 6, 25and 100 mg/kg bw/d.

- *Mating data :*

Table 8 : Mating data

Dose level (in mg/kg bw/d)	0	6	25	100
Female mating index (in %)	100	100	100	96.0
Female fertility index (in %)	100	96.0	100	87.5
Male mating index (in %)	100	100	100	96.0
Male fertility index (in %)	100	96.0	100	84.0
Mean mating day until day 0 pc	2.4	2.2	2.9	2.3

- *Nb of pregnant and not-pregnant females :*
  - *Pregnant :* 25, 24, 25 and 21 females, resp. at 0, 6, 25 and 100 mg/kg bw/d.
  - *Not-pregnant :* 0, 1, 0 and 4 females, resp. at 0, 6, 25 and 100 mg/kg bw/d.
  - *Pregnant but not delivering :* 1, 0, 1 and 0 females, resp. at 0, 6, 25 and 100 mg/kg bw/d.
  - *Females delivering :* 24, 24, 24 and 21 females, resp. at 0, 6, 25 and 100 mg/kg bw/d.
- *Mean duration of gestation (calculated from day 0 of pregnancy) :* 22.3, 22.1, 22.1 and 22.1 days, resp. at 0, 6, 25 and 100 mg/kg bw/d.
- *Nb of implantations, corpora lutea, litter size :*
  - *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.
- *Nb of pre- and post-implantation loss :*
  - *Mean % of post-implantation loss :* 11.6, 4.8, 7.6 and 5.4 %, resp. at 0, 6, 25 and 100 mg/kg bw/d.
- *Nb of dams with abortions, early deliveries, stillbirths, resorptions and/or dead fetuses :*
  - *Nb of dams with stillborn pups :* 2, 5, 1 and 6 dams, resp. at 0, 6, 25 and 100 mg/kg bw/d (no females with all pups stillborn).
- *Total nb of pups delivered :* 304, 297, 304 and 260 pups, resp. at 0, 6, 25 and 100 mg/kg bw/d.
- *Data on functional observations :* not examined
- *Necropsy findings :* no treatment-related effects observed.
- *Organ weight :*

Table 9 : Organ weight (in mg, g 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
Adrenal glands (mg)	Abs	64.96	64.28	63.4	69.44	76.4	77.4	76.72	78.8
	Rela	0.016	0.016	0.015	0.017	0.031	0.032	0.031	0.031
Brain (g)	Abs	2.135	2.093	2.108	2.115	1.962	1.937	1.958	1.945
	Rela	0.519	0.519	0.513	0.519	0.8	0.791	0.797	0.776

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
Pituitary gland (mg)	Abs	9.64	9.0	9.16	9.16	12.68	12.24	12.16	12.68
	Rela	0.002	0.002	0.002	0.002	0.005	0.005	0.005	0.005
Spleen (g)	Abs	0.65	0.616	0.627	0.61	0.456	0.465	0.466	0.471
	Rela	0.157	0.151	0.151	0.149	0.185	0.189	0.189	0.187
Thyroid glands (mg)	Abs	20.76	19.68	21.56	21.0	18.24	18.52	19.04	17.48
	Rela	0.005	0.005	0.005	0.005	0.007	0.008	0.008	0.007
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

- *Histopathological findings :*

Table 10 : Incidence of histopathological 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, Olf. Epith.	Inc.	0	-	-	24	0	-	-	24
	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

For F1 pups/litters (per dose) :

- *Total nb of pups delivered :* 304, 297, 304 and 260 pups, resp. at 0, 6, 25 and 100 mg/kg bw/d.
- *Mean nb of pups delivered :* 12.7, 12.4, 12.7 and 12.4 pups, resp. at 0, 6, 25 and 100 mg/kg bw/d :
  - *Mean nb of liveborn pups :* 12.6, 12.1, 12.6 and 12.1, resp. at 0, 6, 25 and 100 mg/kg bw/d.
  - *Nb of stillborn pups (%) :* 2 (0.7 %), 7 (2.4 %), 1 (0.3 %) and 6 (2.3 %) , resp. at 0, 6, 25 and 100 mg/kg bw/d.
  - *Mean % of perinatal loss :* 0.6, 2.6, 0.3 and 4.2 %, resp. at 0, 6, 25 and 100 mg/kg bw/d.
- *Sex ratio (% M/F) :*
  - *At D 0 :* 50.1/49.9, 48.5/51.5, 49.2/50.8 and 43.2/56.8 % M/F, resp. at 0, 6, 25 and 100 mg/kg bw/d.
  - *At D 21 :* 51.6/48.4, 50.7/49.3, 50.3/49.7 and 50.0/50.0 % M/F, resp. at 0, 6, 25 and 100 mg/kg bw/d.
- *Mean nb of live pups :*

Table 11 : Mean nb of live pups

Dose level (in mg/kg bw/d)	0	6	25	100
D 0	12.6	12.1	12.6	12.1
D 4	12.5	11.8	12.5	12.4
D 7	8.0	7.9	8.0	8.0
D 14	8.0	7.9	8.0	8.0
D 21	8.0	7.9	8.0	8.0

- *Viability index (pups surviving 4 days/total births) :*
  - *Pups surviving days 0 to 4 :* 299, 284, 301 and 249, resp. at 0, 6, 25 and 100 mg/kg bw/d.
  - *Viability index :* 99.0, 98.0, 99.5 and 93.9 %, resp. at 0, 6, 25 and 100 mg/kg bw/d.
- *Nb of culled pups :* 107, 94, 110 and 89, resp. at 0, 6, 25 and 100 mg/kg bw/d.
- *Survival index at weaning :*
  - *Pups surviving days 4 to 21 :* 192, 190, 191 and 160, resp. at 0, 6, 25 and 100 mg/kg bw/d.
  - *Lactation index :* 100 % in all dose groups.
- *Mean litter or pup weight by sex and with sexes combined :*

Table 12 : 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

- *Anogenital distance :*
  - *Males :* 3.31, 3.17, 3.19 and 3.24 mm, resp. at 0, 6, 25 and 100 mg/kg bw/d (AG index cubic root : 1.74, 1.67*, 1.69 and 1.72, resp. at 0, 6, 25 and 100 mg/kg bw/d).
  - *Females :* 1.57, 1.56, 1.55 and 1.52 mm, resp. at 0, 6, 25 and 100 mg/kg bw/d (AG index cubic root : 0.85, 0.83, 0.83 and 0.83, resp. at 0, 6, 25 and 100 mg/kg bw/d).
- *Nipple development : % development :*
  - *At PND 13 :* 64.7, 65.1, 67.7 and 64.2 %, resp. at 0, 6, 25 and 100 mg/kg bw/d.
  - *At PND 20 :* 0.0 % in all dose groups.
- *Necropsy :* no treatment-related effects observed.
- *Organ weight at PND 21 :*

Table 13 : Organ weight (in g or %)

		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

- *Data on physical landmarks in pups and other postnatal developmental data :*
  - *Vaginal opening :* 30.5, 30.5, 31.4 and 31.0 days, resp. at 0, 6, 25 and 100 mg/kg bw/d (mean bw on the day : 96.2, 94.5, 98.2 and 94.4 g, resp. at 0, 6, 25 and 100 mg/kg bw/d).

- *Preputial separation :* 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).

For F1 adults (per dose) :

- *Time of death during the study and whether animals survived to termination :* one male of the highest dose was sacrificed due to the paralysis of booth hindlimbs.
- *Clinical observation :* no effects observed.
- *Food consumption :*
  - *Males* : in-life *:* mean D 0 to 70 : 20.9, 21.1, 20.7 and 19.9 g/animal/d, resp. at 0, 6, 25 and 100 mg/kg bw/d.

    Mating : D 3 to 10 : 21.3, 21.0, 19.1 and 20.0 g/animal/day, resp. at 0, 6, 25 and 100 mg/kg bw/d.

    Post-mating : D 2 to 16 : 23.2, 22.9, 22.4 and 21.6* g/animal/day, resp. at 0, 6, 25 and 100 mg/kg bw/d.
  - *Females :* in-life : mean D 0 to 70 : 15.0, 14.8, 14.6 and 14.4 g/animal/d, resp. at 0, 6, 25 and 100 mg/kg bw/d.

    Gestation : D 0 to 20 : 22.1, 21.4, 21.2 and 20.3** g/animal/d, resp. at 0, 6, 25 and 100 mg/kg bw/d.

    Lactation : D 1 to 21 : 51.6, 52.9, 51.9 and 49.7 g/animal/day, resp. at 0, 6, 25 and 100 mg/kg bw/d.
- *Body weight data :*

Table 14 : Body weight (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

- *Haematological and clinical biochemistry findings :* not examined
- *Effects on sperm (at week 16) :*

Table 15 : 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

- *Nb females cycling normally and cycle length :*
  - *Mean nb of cycles :* 4.16, 4.36, 4.20 and 4.36, resp. at 0, 6, 25 and 100 mg/kg bw/d.
  - *Mean cycle length :* 4.14, 4.04, 4.03 and 4.00 days, resp. at 0, 6, 25 and 100 mg/kg bw/d.
- *Mating data :*

Table 16 : Mating data

Dose level (in mg/kg bw/d)	0	6	25	100
Nb of females mated	25	25	25	25
Nb of females inseminated	25	25	23	25
Female mating index (%)	100.0	100.0	92.0	100.0
Nb of pregnant females	24	25	23	25
Female fertility index (%)	96.0	100.0	100.0	100.0
Nb of males mated	25	25	25	24
Male mating index (%)	100.0	100.0	92.0	100.0
Male fertility index (%)	96.0	100.0	92.0	100.0

- *Duration of gestation (calculated from day 0 of pregnancy) :* 22.0, 21.9, 22.1 and 22.1 days , resp. at 0, 6, 25 and 100 mg/kg bw/d.
- *Precoital interval (nb of days until mating and nb of estrous periods until mating)* : mean mating day until DPC 0 : 2.3, 2.4, 3.0* and 2.6 days, resp. at 0, 6, 25 and 100 mg/kg bw/d.
- *Mean nb of implantation sites :* 12.3, 11.9, 12.3 and 11.0, resp. at 0, 6, 25 and 100 mg/kg bw/d.
- *Mean percent of post-implantation loss :* 5.1, 7.7, 6.4 and 10.4 %, resp. at 0, 6, 25 and 100 mg/kg bw/d.
- *Nb of dams with abortions, early deliveries, stillbirths, resorptions and/or dead fetuses :*

Table 17 : Nb live and stillborn pups

Dose level (in mg/kg bw/d)	0	6	25	100
Nb of females at start	25	25	25	25
Nb of pregnant females	24	25	23	25
Nb of females without delivery	1	1	2	1
Nb of females with liveborn pups	24	24	23	24
Nb of females with stillborn pups	0	0	3	3

- *Nb of live births :* 279, 278, 265 and 257, resp. at 0, 6, 25 and 100 mg/kg bw/d.
- *Necropsy findings :* no treatment-related effects observed.
- *Organ weight :*

Table 18 : Organ weight data (in mg, g or %)

	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
Brain (g)	Abs	2.138	2.116	2.109	2.106	1.975	1.976	1.992	1.988
	Rela	0.531	0.533	0.526	0.543	0.822	0.809	0.824	0.844
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**
Pituitary gland (mg)	Abs	10.12	9.8	9.84	10.042	10.72	11.72**	11.36	10.56
	Rela	0.003	0.002	0.002	0.003	0.004	0.005	0.005	0.004
Spleen (g)	Abs	0.634	0.661	0.638	0.611	0.495	0.532	0.505	0.495
	Rela	0.157	0.165	0.158	0.157	0.205	0.217	0.208	0.21
Thyroid glands (mg)	Abs	22.24	24.48	22.88	21.875	19.36	19.16	19.8	18.04
	Rela	0.006	0.006	0.006	0.006	0.008	0.008	0.008	0..08
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

- *Histopathological findings :*

Table 19 : 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 I									
Nb examined		25	0	0	25	25	0	0	25
Degeneration/regeneration, Olf. Epith.	Inc.	0	-	-	25	0	-	-	25
	1	-	-	-	1	-	-	-	2
	2	-	-	-	5	-	-	-	14
	3	-	-	-	19	-	-	-	9
Nasal cavity II									
Nb examined		25	0	0	25	25	0	0	25
Degeneration/regeneration, Olf. Epith.	Inc.	0	-	-	25	0	-	-	25
	1	0	-	-	0	-	-	-	2

	2	-	-	-	18	-	-	-	19
	3	-	-	-	7	-	-	-	4
Nasal cavity 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 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

- *Differential ovarian follicle count :*

Table 20 : Differential ovarian follicle count

Dose level (in mg/kg bw/d)		0	100
Primordial	Abs	7281	6901
	Mean	291.24	276.04
Growing	Abs	367	356
	Mean	14.68	14.24
Primordial + growing	Abs	7648	7257
	mean	305.92	290.28

For F2 pups/litters (per dose) :

- *Nb of pups delivered :* 279, 278, 265 and 257, resp. at 0, 6, 25 and 100 mg/kg bw/d.
- *Mean nb of live pups (litter size) :*

Table 21 : Live pups data

Dose level (in mg/kg bw/d)	0	6	25	100
Nb of pups delivered	279	278	265	257
Mean nb of pups liveborn	11.6	11.6	11.4	10.6
Mean nb of pups stillborn (tot. nb)	0.0	0.0	0.1 (3)	0.1 (3)
Mean % of perinatal loss	0.0	0.0	1.2	1.0

- *Sex ratio :*
  - *At D 0 :* 53.0, 49,0, 53.7 and 43.1* % of live males, resp. at 0, 6, 25 and 100 mg/kg bw/d.
  - *At D 21 :* 50.5, 51.2, 52.3 and 46.2 % live males, resp. at 0, 6, 25 and 100 mg/kg bw/d.

- *Viability index (pups surviving 4 days/total births) :* 100.0, 99.4, 100.0 and 98.4 %, resp. at 0, 6, 25 and 100 mg/kg bw/d .
- *Survival index at weaning :* 100.0 %, resp. at 0, 6, 25 and 100 mg/kg bw/d.
- *Mean litter or pup weight by sex and with sexes combined :*

Table 22 : Pups 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

- *Anogenital distance :*
  - *Males :* 3.18, 3.18, 3.16 and 3.18 mm, resp. at 0, 6, 25 and 100 mg/kg bw/d (AG index cubic root : 1.65, 1.66, 1.64 and 1.67, resp. at 0, 6, 25 and 100 mg/kg bw/d).
  - *Females :* 1.60, 1.64, 1.59 and 1.59 mm, resp. at 0, 6, 25 and 100 mg/kg bw/d (AG index cubic root : 0.85, 0.87, 0.84 and 0.85, resp. at 0, 6, 25 and 100 mg/kg bw/d).
- *Necropsy :* no treatment-related effects observed (incidence : 5, 3, 2 and 5 males and 3, 4, 2 and 3 females exhibited effects, resp. at 0, 6, 25 and 100 mg/kg bw/d).
- *Organ weight :*

Table 23 : 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

3.10.1.2 Prenatal developmental toxicity study in rabbits (Anonymous, 2021)

Study reference

Anonymous, 2021

Detailed study summary and results

Test type

OECD TG 414

GLP

Test substance

- 3,4-dimethyl-1*H*-pyrazole
- *Degree of purity :* 95.9 %

Test animals

- *Species/strain/sex :* Rabbit / NZW / female
- *Nb. of animals per sex per dose :* 25 females/group were mated
- *Age and weight at the study initiation :* 3019 - 4094 g at GD 0, 16 - 17 w

Administration/exposure

- *Route of administration :* oral, gavage
- *Duration and frequency of test/exposure period :* GD 6 to 28, daily (sacrificed at GD 29)
- *Doses/concentration levels, rationale for dose level selection :* 0, 6, 20 and 60 mg/kg bw/d
- *Vehicle :* 0,5 % sodium carboxymethyl cellulose

Results and discussion

For P adults (per dose) :

- *Nb of animals at mating :* 25 per group
- *Nb of pregnant females :* 23, 25, 24 and 22 females, resp. at 0, 6, 20 and 60 mg/kg bw/d
- *Time of death during the study and whether animals survived to termination :* 1 female of the control group and one of the mid dose group were sacrificed after abortion (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.
- *Clinical signs :* nb of females not pregnant : 2, 0, 1 and 3, resp. at 0, 6, 20 and 60 mg/kg bw/d.

  One female exposed to 0 mg/kg bw/d and 2 exposed to 60 mg/kg bw/d had blood in bedding (on GD 28, 26 and 27, resp.).

  Reduced defecation was noted in 0, 3, 4 and 2 females, resp. at 0, 6, 20 and 60 mg/kg bw/d and no defecation was observed in one female of the highest dose.
- *Mean maternal food consumption :*

Table 24 : Mean food consumption (g/animal/d)

Dose level (in mg/kg bw/d)	0	6	20	60
GD 0 – 6	179.0	173.9	172.0	177.0
GD 6 – 28	133.4	133.5	121.1	110.5
GD 0 – 29	142.5	141.4	131.3	124.5

- *Body weight data :*

Table 25 : Body weight data (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

- *Haematological and clinical biochemistry findings :* not examined
- *Reproduction data :*

Table 26 : Reproduction data

Dose level (in mg/kg bw/d)	0	6	20	60
Nb of females mated	25	25	25	25
Nb of pregnant females	23	25	24	22
Nb of females which aborted	1	0	1	0
Female mortality	1	0	2	1
Nb of dams with viable fetuses	22	25	22	21
Nb of dams with all resorptions	0	0	0	0
Nb of pregnant females at terminal sacrifice	22	25	22	21

- *Nb of implantations, corpora lutea, litter size :*
  - *Mean nb of corpora lutea :* 10.4, 9.6, 9.6 and 9.8, resp. at 0, 6, 20 and 60 mg/kg bw/d.
  - *Mean nb of implantation sites :* 9.9, 8.4, 9.1 and 9.6, resp. at 0, 6, 20 and 60 mg/kg bw/d.
- *Nb of pre- and post-implantation loss : (mean %)*
  - *Pre- :* 5.1, 12.4, 5.3 and 2.5 %, resp. at 0, 6, 20 and 60 mg/kg bw/d.
  - *Post- :* 9.7, 4.1, 4.9 and 10.1 %, resp. at 0, 6, 20 and 60 mg/kg bw/d.
- *Resorption :*

Table 27 : 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**

- *Nb of dead fetuses :* 0, 6, 0 and 0, resp. at 0, 6, 20 and 60 mg/kg bw/d.
- *Mean nb of live births :* 8.8, 8.0, 8.7 and 8.5, resp. at 0, 6, 20 and 60 mg/kg bw/d.
- *Necropsy findings :* 25 females per group were examined.

  Nothing abnormal detected in 23, 23, 23 and 24 females, resp. at 0, 6, 20 and 60 mg/kg bw/d.

  At highest dose : 1 female had findings after gavage error.

At mid dose : 2 females had particular findings on implants (1 dams sacrificed moribund and the other was dams which aborted).

At low dose : 1 female had absent lung lobe (lobe inferior medialis) and 1 had erosion in stomach.

In control : 1 female had bilobed gallbladder and 1 female had particular findings on implants (dams which aborted).

- *Histopathological findings :* not examined.
- *Body weight change and gravid uterine weight, including optionally, body weight change corrected for gravid uterine weight : (carcass weight : terminal bw minus uterus weight ; net weight change from GD 6 : carcass weight minus bw at GD 6)*

Table 28 : 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

For F1 pups/litters (per dose):

- *Total nb of fetuses :* 193, 206, 192 and 179, resp. at 0, 6, 20 and 60 mg/kg bw/d (6 fetuses of the low dose group were dead).
- *Mean nb of live pups (litter size) :*

Table 29 : Mean nb of live pups

Dose level (in mg/kg bw/d)		0	6	20	60
Live fetuse	Mean	8.8	8.0	8.7	8.5
	Mean %	90.3	95.9	95.1	89.9
Females	Mean	4.6	4.3	4.1	4.2
	Mean %	48.2	48.2	43.7	44.6
Males	Mean	4.1	3.7	4.6	4.3
	Mean %	42.1	47.8	51.4	45.4

- *Sex ratio :* 52.8/47.2, 53.5/46.5, 46.9/53.1 and 49.2/50.8 % of live female/male, resp. at 0, 6, 20 and 60 mg/kg bw/d.
- *Viability index (pups surviving 4 days/total births) :* /
- *Survival index at weaning :* /
- *Mean placental and fetal body weight :*

Table 30 : Fetal and placental weight (in g)

Dose level (in mg/kg bw/d)		0	6	20	60
Fetal 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 weight	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

- *Nb and percent of fetuses and litters with malformations (including runts) and/or variations as well as description and incidences of malformations and main variations (and/or retardations) :*
    - *Fetal incidence of all malformations :* 2, 2, 1 and 0 pups, resp. at 0, 6, 20 and 60 mg/kg bw/d.
    - *Fetal incidence of all variations :* 184, 194, 183 and 169 pups, resp. at 0, 6, 20 and 60 mg/kg bw/d.
    - *External observation :* no malformations or variations observed.
    - *External unclassified observation :* 6 pups of the low dose group exhibited placentae necrobiotic (litter incidence : 1) and 6 pups of the highest dose group had polyhydramnios (litter incidence : 1).
    - *Soft tissue observation :* malformation : 1 pups of the mid dose group had absent subclavian.

      Variation : fetal incidence : 1, 10, 1 and 6 pups, resp. at 0, 6, 20 and 60 mg/kg bw/d.

      Malpositioned carotid branch : 0, 7, 0 and 1 pups, resp. at 0, 6, 20 and 60 mg/kg bw/d (litter incidence : 0, 4, 0 and 1).

      Dilated aorta : 0, 1, 0 and 0 pups, resp. at 0, 6, 20 and 60 mg/kg bw/d.

      Narrowed pulmonary trunk : 0, 1, 0 and 1 pups, resp. at 0, 6, 20 and 60 mg/kg bw/d.

      Absent lung lobe (Lobe inferior medialis) : 1, 2, 1 and 4 pups, resp. at 0, 6, 20 and 60 mg/kg bw/d.

      Dilated renal pelvis : 0, 0, 0 and 1 pups, resp. at 0, 6, 20 and 60 mg/kg bw/d.
    - *Soft tissue unclassified observation :*

      Discoloured spleen : 1, 0, 0 and 0 pups, resp. at 0, 6, 20 and 60 mg/kg bw/d.

      Blood coagulum around urinary bladder : 2, 0, 0 and 0 pups, resp. at 0, 6, 20 and 60 mg/kg bw/d.
    - *Skeletal observation :* malformations : 2 pups of the control group had thoracic hemivertebra and branched rib (cartilage present), 1 pup of the low dose group had lumbar hemivertebra and another of the low dose had branched rib (cartilage present).

      Variations : fetal incidence : 184, 194, 183 and 169 pups, resp. at 0, 6, 20 and 60 mg/kg bw/d (however not dose-related effects).

3.10.1.3 Prenatal developmental toxicity study in rats (Anonymous, 2021)

Study reference

Anonymous, 2021

Detailed study summary and results

Test type

OECD TG 414

GLP

Test substance

- 3,4-dimethyl-1*H*-pyrazole
- *Degree of purity : 95.9 %*

Test animals

- *Species/strain/sex :* Rat / Wistar / females
- *Nb. of animals per sex per dose :* 25 mated females/group
- *Age and weight at the study initiation :* 10 – 12 weeks, 141.9 to 193.5 g

Administration/exposure

- *Route of administration :* oral, gavage
- *Duration and frequency of test/exposure period :* GD 6 to 19, daily (sacrificed on GD 20)
- *Doses/concentration levels, rationale for dose level selection :* 0, 15, 50 and 150 mg/kg bw/d
- *Vehicle :* 0.5 % of sodium carboxymethyl cellulose

Results and discussion

For P adults (per dose):

- *Nb of animals at mating :* 25 females per group
- *Nb of pregnant females :* 24, 25, 25 and 24 females, resp. at 0, 15, 50 and 150 mg/kg bw/d.
- *Time of death during the study and whether animals survived to termination :* no mortality occurred during the study period.
- *Clinical signs :* no treatment-related effect observed.
- *Mean maternal food consumption :*

Table 31 : Maternal food consumption (g/animal/d)

Dose level (in mg/kg bw/d)	0	15	50	150
GD 0 – 6	16.0	16.0	15.9	16.5
GD 6 – 19	21.1	20.9	21.0	20.3
GD 0 - 20	19.7	19.5	19.6	19.4

- *Body weight data :*

Table 32 : 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

- *Haematological and clinical biochemistry findings :* not examined
- *Duration of gestation (calculated from day 0 of pregnancy) :* no premature birth
- *Reproduction data :*

Table 33 : Reproduction data

Dose level (in mg/kg bw/d)	0	15	50	150
Nb of female mated	25	25	25	25
Nb of pregnant females	24	25	25	24
Nb of female which aborted	0	0	0	0
Nb of premature births	0	0	0	0
Nb of dams with viable fetuses	24	25	25	23
Nb of dams with all resorptions	0	0	0	1

- *Precoital interval (nb of days until mating and nb of estrous periods until mating)* : /
- *Mean nb of implantations, corpora lutea, litter size :*
  - *Corpora lutea :* 11.4, 11.0, 11.2 and 11.9, resp. at 0, 15, 50 and 150 mg/kg bw/d.
  - *Implantation sites :* 11.1, 10.4, 10.6 and 11.3, resp. at 0, 15, 50 and 150 mg/kg bw/d.
- *Mean % of pre- and post-implantation loss :*
  - *Pre- :* 2.5, 5.4, 5.4 and 4.7 %, resp. at 0, 15, 50 and 150 mg/kg bw/d.
  - *Post- :* 7.6, 6.4, 5.5 and 11.5 %, resp. at 0, 15, 50 and 150 mg/kg bw/d.
- *Mean nb of resorptions :*
  - *Total :* 0.8, 0.6, 0.6 and 1.2, resp. at 0, 15, 50 and 150 mg/kg bw/d.
  - *Early :* 0.8, 0.5, 0.6 and 1.1, resp. at 0, 15, 50 and 150 mg/kg bw/d.
  - *Late :* 0.0, 0.1, 0.0 and 0.1, resp. at 0, 15, 50 and 150 mg/kg bw/d.
- *Mean nb of live births :* 10.3, 9.8, 10.0 and 10.6 pups, resp. at 0, 15, 50 and 150 mg/kg bw/d.
- *Necropsy findings :* no abnormalities observed in 24, 25, 23 and 18 females, resp. at 0, 15, 50 and 150 mg/kg bw/d.

  Nb of females not pregnant : 1, 0, 1 and 1, resp. at 0, 15, 50 and 150 mg/kg bw/d.

  At the highest dose, 6 females had an enlarged adrenal cortex and in 1 an enlarged liver.
- *Organ weight :*

Table 34 : Organ weight data (in mg, g or %)

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

- *Histopathological findings :*
  - Adrenal cortex : 6 females of the highest dose were examined and in 1 cortical hyperplasia was noted.
  - Nasal cavity : 25 females of the control and high doses were examined. In all female of the highest dose degeneration/regeneration of the olfactive epithelium of the nasal cavity (part II, III and IV) were observed.
- *Body weight change and gravid uterine weight, including optionally, body weight change corrected for gravid uterine weight :*

Table 35 : Mean 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 GD 6	37.3	36.1	38.3	37.7

For F1 pups/litters (per dose) :

- *Mean nb of live pups (litter size) :*

Table 36 : Mean nb of live pups

Dose level (in mg/kg bw/d)	0	15	50	150
Mean nb of live fetuses	10.3	9.8	10.0	10.6
Mean nb of females	5.3	4.8	4.8	5.7
Mean nb of males	5.0	5.1	5.2	4.9

- *Sex ratio :* 51.6/48.4, 48.4/51.6, 48.0/52.0 and 53.5/46.5 % of F/M, resp. at 0, 15, 50 and 150 mg/kg bw/d.
- *Viability index (pups surviving 4 days/total births) :* /
- *Survival index at weaning :* /
- *Mean placental and fetal weights by sex and with sexes combined :*

Table 37 : Mean placental and fetal 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
Fetal 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

- *External, soft tissue and skeletal malformations and other relevant alterations :*
  - *External observations :* no malformations or variations observed .
  - *Soft tissue observations :* 1 pups of the low dose group exhibited malformations (situs inversus) .

    Variations observed (dilated renal pelvis and dilated ureter) in 1, 3, 5 and 4 pups, resp. at 0, 15, 50 and 150 mg/kg bw/d.
  - *Skeletal observations :* malformations observed in 0, 1, 2 and 1 pups, resp. at 0, 15, 50 and 150 mg/kg bw/d. (absent thoracic vertebra in 1 pups of the low dose, cleft sternum in 1 pups of the mid dose and lumbar hemivertebra in 1 other pups of the mid dose and misshapen basisphenoid in 1 pups of the highest dose).

    Variations observed in 120, 122, 122 and 122 pups, resp. at 0, 15, 50 and 150 mg/kg bw/d.

    Significant increase litter incidence of incomplete ossification of supraoccipital (unchanged cartilage) (fetal incidence was of 28, 14, 31 and 66 pups affected and litter incidence was of 14, 11, 12 and 21* litter, resp. at 0, 15, 50 and 150 mg/kg bw/d).

    Significant increase litter incidence of incomplete ossification of nasal (unchanged cartilage) (fetal incidence was of 0, 0, 1 and 6 pups and litter incidence was of 0, 0, 1 and 4* litter, resp. at 0, 15, 50 and 150 mg/kg bw/d).

    Significant increase litter incidence of misshapen sternebra (unchanged cartilage) (fetal incidence was of 36, 44, 45 and 47 pups and litter incidence was of 19, 21, 20 and 23* litter, resp. at 0, 15, 50 and 150 mg/kg bw/d).

3.10.2 Human data

No human data available

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

No other data available

3.11 Specific target organ toxicity – single exposure

Hazard class not assessed in this dossier

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

3.12.1.1 Range finding (14-day) study in Wistar rats via oral route (Anonymous, 2014)

Study reference

Anonymous, 2014

Detailed study summary and results

Test type

Range finding study

GLP

Test substance

- 3,4-dimethyl-1*H*-pyrazole
- *Degree of purity :* 99.4 %

Test animals

- *Species/strain/sex :* Rat / Wistar / both sexes
- *Nb. of animals per sex per dose :* 4/sex/group
- *Age and weight at the study initiation :* no information available

Administration/exposure

- *Route of administration :* oral, diet
- *Duration and frequency of test/exposure period :* 14 days
- *Doses/concentration levels, rationale for dose level selection :* 0, 1500, 5000 and 10000 ppm

Table 38 : 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

- *Post exposure observation period :* /
- *Vehicle :* acetone

Results and discussion

- *Mortality :* all males and females of the highest dose were sacrificed moribund.
- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed) :* all males and females of the mid and high dose groups exhibited clinical signs.

  At the highest dose, all males and females had reduced nutritional condition and piloerection. And 1 female had semiclosed eyelid and ataxia.

At the mid dose, 3 males and 3 females exhibited piloerection and all males and females had discoloured feces. Furthermore, 2 females had reduced nutritional condition.

- *Food consumption :*

Table 39 : Food consumption (g/animal/d)

	Males				Females			
Dose level (in ppm)	0	1500	5000	10000	0	1500	5000	10000
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 and body weight changes :*

Table 40 : Body weight (in g)

	Males				Females			
Dose level (in ppm)	0	1500	5000	10000	0	1500	5000	10000
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*	-

- *Sensory activity, grip strength and motor activity assessments (when available) :* not examined
- *Haematological findings :*

Table 41 : 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
Hg (mmol/L)	8.4	8.2	8.9	8.5	8.4	9.2* (+ 8.2 %)
Ht (L/L)	0.410	0.403	0.430	0.408	0.399	0.428* (+ 4.9 %)
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* (+ 3.46 %)
PLT (giga/L)	855	904	915	844	756	760
WBC (giga/L)	8.30	7.05	7.61	5.06	6.64	5.70

- *Clinical biochemistry findings :*

Table 42 : 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

- *Gross pathology findings :* males : 1 of the control group exhibited skin lesion and 1 of the highest dose had pelvic kidneys dilatation.

  No effects observed in females.

- *Organ weight :*

Table 43 : Organ weight (in mg, g or %)

		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

- *Histopathology findings :*
  - *Adrenal cortex* : hypertrophy/hyperplasia observed in all males of the mid dose, fatty change and accessory cortical tissue were observed in 1 male of the low dose. In females, 1 of the control group and 3 of the low dose had lymphoid infiltration.

Table 44 : Incidence of microscopic findings in adrenal cortex

	Males			Females		
Dose level (in ppm)	0	1500	5000	0	1500	5000
Nb examined	4	4	4	4	4	4
Hypertrophy/hyperplasia (grade 1)	0	0	4	0	0	0
(multi)focal fatty change	0	1	0	0	0	0
Lymphoid infiltration	0	0	0	1	3	0

  - *Liver :* hypertrophy/hyperplasia was observed in 2 males and 1 female of the low dose group and in all males and all females of the mid dose group. Necrosis was noted in 1 male of the low dose, in 1 male of the mid dose and in 1 female of the control group. Lymphoid infiltration was observed in all animals (except in 1 female of the low dose group).

Table 45 : Incidence of 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

3.12.1.2 28-day repeated-dose toxicity study in Wistar rats (Anonymous, 2021)

Study reference

Anonymous, 2021

Detailed study summary and results

Test type

OECD TG 407

GLP

Test substance

- 3,4-dimethyl-1*H*-pyrazole
- *Degree of purity :* 99.4 %

Test animals

- *Species/strain/sex :* Rat / Wistar / both sexes
- *Nb. of animals per sex per dose :* 5/sex/dose
- *Age and weight at the study initiation :* 42 days (± 1 days)

Administration/exposure

- *Route of administration :* oral
- *Duration and frequency of test/exposure period :* 4 weeks, daily
- *Doses/concentration levels, rationale for dose level selection :* 0, 1500, 3000 and 6500 ppm

Table 46 : Mean intake of test substance (in mg/kg bw/d)

Dose level (in ppm)	1500	3000	6500
Males	115.3	235.2	526.0
Females	134.2	243.8	479.7

- *Post exposure observation period :* /
- *Vehicle :* Ground Kliba maintenance diet mouse/rat “GLP” meal

Results and discussion

- *Mortality :* no animal died during the study period.
- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed) :* no effects observed.

- *Body weight and body weight changes :*

Table 47 : Mean 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**

- *Food consumption :*

Table 48 : Mean food consumption (g/animal/day)

	Males				Females			
Dose level (in ppm)	0	1500	3000	6500	0	1500	3000	6500
Nb examined	1	1	1	1	1	1	1	1
D 6 - 7	18.4	18.2	17.0	12.2	12.7	15.9	13.0	7.8
D 10 - 11	-	-	-	-	11.2	14.3	17.8	11.1
D 13 – 14	20.3	18.9	17.4	19.1	24.6	13.5	9.9	12.1
D 20 – 21	21.3	19.4	18.8	-	17.2	-	16.7	11.3
D 27 - 28	19.3	19.3	20.5	18.2	15.9	17.3	15.1	12.3

- *Sensory activity, grip strength and motor activity assessments (when available) :*
  - *FOB (at day 24 in males and day 26 in females) :*

Table 49 : Functional observation battery data

	Males				Females			
Dose level (in ppm)	0	1500	3000	6500	0	1500	3000	6500
Rearing (N)	2	2	6	4	13	13	11	10
GS F (Newton)	9.7	10.3	9.8	9.9	8.4	9.0	9.2	8.6
GS H (newton)	7.2	6.5	6.7	6.0	4.8	4.6	4.3	3.2
FST (cm)	10.5	10.5	8.6	9.6	10.0	9.8	10.3	8.7

Furthermore, home cage observations (posture, tremors, convulsions, abnormal movements, gait), open field observations (behaviour, fur, skin, salivation, nose discharge, lacrimation, eyes/pupil size, posture, respiration, gait, abnormal movements, urine, feces) and sensorimotor tests (approach response, touch response, vision, pupillary reflex, audition, coordination of movements, vocalization, pain perception) did not revealed changes.

  - *Motor activity :* Interv. 1 – 12 sum interr. (day 24-25) : 2572.2, 2528.0, 2950.0 and 2937.8 in males and 2463.2, 2921.6, 2657.8 and 2923.2 in females, resp. at 0, 1500, 3000 and 6500 ppm.

- *Haematological findings :*

Table 50 : 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*
Hg (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

- *Clinical biochemistry findings :*

Table 51 : Biological data

	Males				Females			
Dose level (in ppm)	0	1500	3000	6500	0	1500	3000	6500
ALT (µkat/L)	0.69	0.79	0.91	1.23**	0.59	0.55	0.66	0.82*
AST (µkat/L)	1.86	2.00	1.84	2.46	1.56	1.61	1.81	1.81
ALP (µkat/L)	2.19	1.75	2.05	1.96	1.45	1.26	1.27	1.52
GGT_C (nkat/L)	0	0	0	0	0	0	1	3
Urea (mmol/L)	5.16	5.51	5.34	5.73	6.16	6.48	6.09	6.56
Crea (µmol/L)	23.4	23.5	20.4	19.7	27.3	27.7	24.3	19.9**
Tot. prot. (g/L)	60.33	59.05	58.30	58.60	61.48	60.75	59.66	59.10
Na (mmol/L)	140.7	139.9	139.2	139.8	139.8	140.4	141.4	141.5
K (mmol/L)	4.71	4.57	4.19**	4.17**	4.30	3.91**	3.92**	3.79
Cl (mmol/L)	97.0	95.9	94.3*	93.9**	98.1	97.2	96.8	96.3
INP (mmol/L)	2.60	2.47	2.31	2.10**	1.93	1.74	1.71	1.77

- *Gross pathology findings :*
  - *In males :* no abnormalities observed in 5, 5, 3 and 3 animals/5, resp. at 0, 1500, 3000 and 6500 ppm. 2 males of the mid dose and 2 of the high dose had foci epididymides and 1 male of the mid dose had foci liver.
  - *In females :* no abnormalities observed in 3, 5, 5 and 3 animals/5, resp. at 0, 1500, 3000 and 6500 ppm. In control, 1 female had cyst in kidneys and another had kidneys dilatation, while at the highest dose, 2 females had uterus size reduced, and one had liver deformation and 1 had ovaries reduce size.
- *Organ weight :*

Table 52 : 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 findings: incidence and severity :*

Table 53 : Histopathological findings

		Grade	Males				Females			
Dose level (in ppm)			0	1500	3000	6500	0	1500	3000	6500
Nb examined			5	5	5	5	5	5	5	5
Adrenal cortex	Accessory cortical tissue	Inc	0	2	1	0	0	1	0	0
	hypertrophy	Inc	1	0	0	0	0	0	0	0
	Vacuolation increased	Inc	0	1	1	0	0	0	0	1
Cervix	Diffuse atrophy	Inc	-	-	-	-	0	0	0	2
	dyscyclicity	Inc	-	-	-	-	0	0	2	2
Coagulating glands	Size reduced	Inc	0	0	2	3	-	-	-	-
Epididymides	Granuloma spermatogenic	Inc	0	0	2	2	-	-	-	-
	Cribriform change (grade 1)	Inc	0	0	1	3				
	Oligospermia	Inc	0	0	0	1	-	-	-	-

	Debris	Inc	0	0	0	1	-	-	-	-
Liver	Hypertrophy centrilobular	Inc	0	1	4	5	0	0	4	5
		1	-	1	3	3	-	-	3	1
		2	-	-	1	2	-	-	1	4
Mandibular glands	Diffuse atrophy	Inc	0	5	5	5	0	3	5	5
		1	-	1	-	-	-	2	1	-
		2	-	1	1	-	-	1	2	1
		3	-	3	1	-	-	-	2	4
		4	-	-	3	5	-	-	-	-
Nasal cavity, level I	Degen/regen olf epith	Inc	0	5	5	2	0	4	5	4
		1	-	2	1	-	-	-	-	-
		2	-	1	0	-	-	2	2	1
		3	-	1	3	1	-	2	3	1
		4	-	1	1	1	-	-	-	2
Nasal cavity, level II	Degen/regen olf epith	Inc	0	5	5	5	0	5	5	5
		1	-	-	-	-	-	-	2	-
		2	-	5	1	-	-	4	-	-
		3	-	-	4	1	-	1	2	2
		4	-	-	-	4	-	-	1	3
Nasal cavity, level III	Degen/regen olf epith	Inc	0	5	5	5	0	5	5	5
		1	-	-	-	-	-	-	-	-
		2	-	1	2	-	-	1	2	-
		3	-	4	-	-	-	4	2	1
		4	-	-	3	5	-	-	1	4
Ovaries	Reduction functional bodies	Inc	-	-	-	-	0	0	0	4
	Changes interstitial glands	Inc	-	-	-	-	0	0	2	4
Prostate	Size reduced	Inc	0	0	3	4	-	-	-	-
Seminal vesicle	Size reduced	Inc	0	0	2	4	-	-	-	-
Testes	Tubular degeneration	Inc	0	0	0	1	-	-	-	-
Uterus	Diffuse atrophy	Inc	-	-	-	-	0	0	0	2
	Dyscyclicity	Inc	-	-	-	-	0	0	1	2
Vagina	Diffuse atrophy	Inc	-	-	-	-	0	0	0	2

- *Oestrus cycle :* at necropsy, nb of animals in the different stages :

Table 54 : Oestrous cycle data

Dose level (in ppm)	0	1500	3000	6500
Nb of animals in prooestrus	0	1	1	0
Nb of animals in oestrus	2	3	2	0
Nb of animals in metoestrus	0	0	0	1
Nb of animals in dioestrus	3	1	0	0
Nb of animals showing dyscyclicity	0	0	2	2
Nb of animals with atrophy	0	0	0	2

3.12.1.3 Repeated dose 90-dose oral toxicity study in rats (Anonymous, 2017)

Study reference

Anonymous, 2017

Detailed study summary and results

Test type

OECD TG 408

GLP

Test substance

- 3,4-dimethyl-1*H*-pyrazole
- *Degree of purity :* 99.3 %

Test animals

- *Species/strain/sex :* Rat / Wistar / both sexes
- *Nb. of animals per sex per dose :* 10/sex/dose
- *Age and weight at the study initiation :* 42 ± 1 days

Administration/exposure

- *Route of administration :* oral, diet
- *Duration and frequency of test/exposure period :* 90 days
- *Doses/concentration levels, rationale for dose level selection :* 0, 150, 500, 2000 and 6000 ppm

Table 55 : Mean daily test substance intake (mg/kg bw/d)

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

- *Post exposure observation period :* no
- *Vehicle :* /

Results and discussion

- *Mortality :* no animal died prematurely.
- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed :* no test substance effects.
- *Body weight and body weight changes :*

Table 56 : 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**

- *Sensory activity, grip strength and motor activity assessments (when available) :*
  - *Home cage observation :* no treatment-related effects.
  - *Open field observations :* no treatment-related effects.
  - *Sensorimotor tests/reflexes :* no treatment-related effects.
  - *FOB (at week 12) :*

Table 57 : FOB

	Males					Females				
Dose level (in ppm)	0	150	500	2000	6000	0	150	500	2000	6000
Rearing (N)	2	3	3	3	3	15	14	12	13	8**
GS F (newton)	11.1	11.8	13.1**	11.5	10.9	10.7	10.9	10.8	9.5	8.8*
GS H (newton)	7.2	8.5	7.4	7.3	6.8	6.2	5.8	6.1	6.2	4.6**
FST (cm)	10.7	11.8	11.5	11.2	11.1	10.6	10.4	10.6	9.9	9.1

  - *Motor activity :* sum Interv. 1 – 12 : 2448.4, 2502.0, 2151.6, 2640.1 and 2651.3 in males and 2669.7, 2494.8, 2619.8, 2835.1 and 2713.5 in females, resp. at 0, 150, 500, 2000 and 6000 ppm.

- *Haematological findings :*

Table 58 : Haematological findings

	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
Hg (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*

- *clinical biochemistry findings: incidence and severity :*

Table 59 : Biological findings

	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

- *Gross pathology findings : incidence and severity :*
  - *In males :* no abnormalities observed 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 had foci on glandular stomach and 1 male of the high dose had foci on liver).
  - *In females :* no abnormalities observed in all dose groups.
- *Organ weight :*

Table 60 : Organ weight (in mg, g or %)

		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

- *Histopathology findings :*
  - *In males :* adrenal glands : increased incidence of vacuolation of adrenal cortex (4 and 6 males, resp. at 2000 and 6000 ppm).

    Liver : increased of centrilobular hypertrophy (in all males exposed to 2000 and 6000 ppm), 5 males of the highest dose had (multi)focal necrosis (vs only 1 males of the control group).

    Mandibular glands : increased incidence of diffuse atrophy (in 6, 10 and 10 males, resp. at 500, 2000 and 6000 ppm).

    Nasal cavity (level III) : increased incidence of olfactive epith degeneration/regeneration (in 9, 10 and 10 males, resp. at 500, 2000 and 6000 ppm).

    Epididymides : one male of the highest dose had size reduced.
  - *In females :* adrenal glands : increased incidence of accessory cortical tissue (1, 1, 3, 4 and 4 males, resp. at 0, 150, 500, 2000 and 6000 ppm).

    Cervix : increased incidence of diffuse atrophy (in 2 females of the highest dose) and of dyscyclicity (in 1 and 2 females, resp. at 2000 and 6000 ppm).

    Liver : increased of centrilobular hypertrophy (in 9 and 10 females, resp. at 2000 and 6000 ppm) and (multi)focal necrosis in 2 females of the highest dose.

    Mandibular glands : diffuse atrophy observed in 2, 10 and 10 females, resp. at 500, 2000 and 6000 ppm.

    Nasal cavity (level III) : increased incidence of olfactive epith degeneration/regeneration (in 0, 0, 10, 10 and 10 females, resp. at 0, 150, 500, 2000 and 6000 ppm).

    Ovaries : interstitial glands vacuolation observed in 4 females exposed to 2000 ppm and in all females of the highest dose.

    Uterus and vagina : diffuse atrophy was noted in 2 females exposed to 6000 ppm.

Table 61 : Histopathological findings

	Grade	Males					Females				
Dose level (in ppm)		0	150	500	2000	60000	0	150	500	2000	6000
Adrenal cortex											
vacuolation	Inc	0	0	0	4	6	0	0	0	0	1
	1	-	-	-	4	1	-	-	-	-	1
	2	-	-	-	-	5	-	-	-	-	-

Harderian glands											
Degeneration/ Regeneration	Inc	2	4	1	3	4	1	0	0	2	6
	1	2	2	1	1	-	1	-	-	2	2
	2	-	2	-	-	-	-	-	-	-	1
	3	-	-	-	1	2	-	-	-	-	1
	4	-	-	-	1	2	-	-	-	-	2
Liver											
Centrilobular hypertrophy	Inc	0	0	0	10	10	0	0	0	9	10
	1	-	-	-	6	-	-	-	-	7	-
	2	-	-	-	4	-	-	-	-	2	4
	3	-	-	-	-	10	-	-	-	-	6
Nasal cavity, III											
Olf. epith. degen/ regen	Inc	0	0	9	10	10	0	0	2	10	10
	1	-	-	6	-	-	-	-	-	-	-
	2	-	-	3	-	-	-	-	2	6	-
	3	-	-	-	10	-	-	-	-	4	-
	4	-	-	-	-	10	-	-	-	-	10
Mandibular glands											
Diffuse atrophy	Inc	0	0	6	10	10	0	0	2	10	10
	1	-	-	6	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	2	6	-
	3	-	-	-	10	-	-	-	-	4	-
	4	-	-	-	-	10	-	-	-	-	10
Skeletal muscle											
(multi)focal degeneration	Inc	0	0	0	0	2	0	0	0	0	7
	1	-	-	-	-	2	-	-	-	-	5
	2	-	-	-	-	-	-	-	-	-	2
Ovaries											
Interstitial vacuolation	Inc	-	-	-	-	-	0	0	0	4	10
	1	-	-	-	-	-	-	-	-	4	4
	2	-	-	-	-	-	-	-	-	-	4
	3	-	-	-	-	-	-	-	-	-	2

3.12.1.4 Combined chronic toxicity/carcinogenicity study in Wistar rats (Anonymous, 2021)

Study reference

Anonymous, 2021

Detailed study summary and results

Test type

OECD TG 453

GLP

Groups of males and females were exposed to the test substance during 24 months (main groups) or 12 months (satellite groups).

Test substance

- 3,4-dimethyl-1*H*-pyrazole
- *Degree of purity :* 95.9 %

Test animals

- *Species/strain/sex :* Rat / Wistar / both sexes
- *Nb. of animals per sex per dose :* 50/sex/group for main groups
  + 10/sex/group for satellite groups
- *Age and weight at the study initiation :* 42 ± 1 days

Administration/exposure

- *Route of administration :* oral, diet
- *Duration and frequency of test/exposure period :*
  - *Satellite groups :* 12 months
  - *Main groups :* 24 months
- *Doses/concentration levels, rationale for dose level selection :* 0, 1, 5, 30 and 60 mg/kg bw/d
- *Post exposure observation period :* no
- *Vehicle :* /

Results and discussion

Satellite groups :

- *Mortality :* 1 female exposed to 5 mg/kg bw/d was sacrificed in a moribund state (necropsy : mass in the axillary region correlated with a fibroadenoma) .
- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed) :* 3 males of the control group and 2 of the low dose exhibited palpable mass through skin and 1 male of the control group had skin lesions. No findings observed in the other male groups.

  1 female of the control group exhibited poor general condition and pale skin, 1 female exposed to 5 mg/kg bw/d had palpable mass through skin and small eye.
- *Body weight and body weight changes :*

Table 62 : 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

- *Haematological findings :*

Table 63 : Haematological data

	Males					Females				
Dose level (in mg/kg bw/d)	0	1	5	30	60	0	1	5	30	60
	D 92					D 94				
RBC (tera/L)	8.74	8.42	8.75	8.60	8.72	7.59	7.80	7.74	7.62	7.91
Hg (mmol/L)	9.1	8.9	9.2	9.0	9.0	8.6	8.7	8.7	8.5	8.8
Ht (L/L)	0.426	0.416	0.426	0.418	0.422	0.390	0.396	0.395	0.386	0.397
MCV (fL)	48.8	49.4	48.8	48.6	48.4	51.4	50.8	51.1	50.7	50.3
MCH (fmol)	1.04	1.06	1.05	1.04	1.03	1.14	1.12	1.13	1.12	1.11
MCHC (mmol/L)	21.41	21.51	21.54	21.48	21.39	22.16	22.07	22.04	22.16	22.02
PLT (giga/L)	752	667	651	707	752	632	743	712	688	675
PTT (sec)	20.1	19.9	20.5	19.9	19.8	19.6	18.9	18.5	18.8	19.5
QT (sec)	20.0	19.8	19.1	19.4	19.0	18.4	18.3	17.7	18.6	19.0
WBC (giga/L)	5.48	6.50	5.81	6.04	5.31	2.96	3.78	3.61	3.73	4.00
	D 181					D 181				
RBC (tera/L)	8.88	8.62	8.90	8.86	8.83	7.71	7.82	7.84	7.82	8.03
Hg (mmol/L)	9.4	9.3	9.4	9.3	9.3	9.1	9.1	9.1	9.1	9.3
Ht (L/L)	0.431	0.423	0.432	0.426	0.424	0.401	0.402	0.404	0.399	0.407
MCV (fL)	48.6	49.1	48.6	48.1	48.0	52.1	51.4	51.6	51.1	50.7
MCH (fmol)	1.05	1.08	1.06	1.05	1.05	1.18	1.17	1.16	1.17	1.15
MCHC (mmol/L)	21.69	22.03	21.84	21.79	21.92	22.73	22.68	22.51	22.79	22.78
PLT (giga/L)	735	657	693	719	746	683	698	758*	619*	668
PTT (sec)	20.1	20.1	19.7	20.1	19.6	20.5	19.9	19.6	18.8*	19.9
QT (sec)	20.1	19.9	20.1	19.7	19.5	18.7	18.9	18.3	18.5	19.3
WBC (giga/L)	4.99	5.63	5.36	5.64	5.03	2.69	3.10	2.99	2.92	2.98
	D 365					D 367				
RBC (tera/L)	8.21	8.00	8.27	8.40*	8.41	7.46	7.44	7.44	7.51	7.82*
Hg (mmol/L)	8.6	8.6	8.7	8.7	8.7	8.6	8.6	8.6	8.6	8.8
Ht (L/L)	0.410	0.409	0.413	0.415	0.412	0.404	0.398	0.402	0.401	0.412
MCV (fL)	50.0	51.1	50.0	49.4	49.0*	54.2	53.5	54.1	53.5	52.8
MCH (fmol)	1.04	1.08*	1.06	1.03	1.03	1.16	1.16	1.15	1.15	1.13
MCHC (mmol/L)	20.92	21.17	21.12	20.96	21.09	21.38	21.60	21.29	21.43	21.38
PLT (giga/L)	681	624	657	663	688	635	601	647	582	580
PTT (sec)	18.7	18.7	18.8	18.9	18.6	19.7	18.8	19.2	19.1	19.3
QT (sec)	18.4	18.5	18.6	18.4	18.4	18.4	19.0	18.1	18.1	19.6
WBC (giga/L)	4.05	4.28	4.19	4.49	4.42	2.31	2.39	2.36	2.70	2.52

- *Clinical biochemistry findings :*

Table 64 : Clinical biochemistry parameters

	Males	Females

Dose level (in mg/kg bw/d)	0	1	5	30	60	0	1	5	30	60
	D 92					D 94				
ALT (µkat/L)	0.64	0.62	0.68	0.65	0.64	0.65	0.63	0.60	0.71	0.54
AST (µkat/L)	1.48	1.53	1.72	1.61	1.59	1.78	1.67	1.46	1.67	1.39
ALP (µkat/L)	1.27	1.15	1.22	1.17	1.19	0.64	0.54	0.59	0.64	0.57
GGT_C (nkat/L)	25	25	25	25	25	25	25	25	25	25
	D 181					D 181				
ALT (µkat/L)	0.64	0.67	0.72	0.67	0.80	0.69	0.66	0.62	0.70	0.62
AST (µkat/L)	1.67	1.64	1.73	1.69	1.86	1.82	1.68	1.64	2.04	1.72
ALP (µkat/L)	1.11	0.99	1.05	0.98	1.05	0.45	0.47	0.50	0.52	0.43
GGT_C (nkat/L)	25	25	25	25	25	25	25	25	25	25
	D 365					D 367				
ALT (µkat/L)	0.69	0.61	0.68	0.70	0.81	0.65	0.65	0.69	0.77	0.62
AST (µkat/L)	1.73	1.49	1.54	1.82	1.85	1.65	1.66	1.85	1.98	1.75
ALP (µkat/L)	1.05	0.89	0.98	0.96	1.03	0.52	0.38	0.46	0.50	0.40
GGT_C (nkat/L)	25	25	25	25	25	25	25	25	25	25

- *Gross pathology findings :* no treatment-related effects observed.
- *Organ weight :*

Table 65 : Organ weight data (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

- *Neoplastic findings :* one female exposed to 30 mg/kg bw/d exhibited an extremely high ovarian weight and an unilateral benign thecoma .
- *Histopathology findings :*

Table 66 : Histological findings

	Males					Females				
Dose level (in mg/kg bw/d)	0	1	5	30	60	0	1	5	30	60
Nasal cavity (level I), Degeneration/regeneration olf epith										
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 (level II), Degeneration/regeneration olf epith										
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 (level III), Degeneration/regeneration olf epith										
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 (level IV), Degeneration/regeneration olf epith										
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)	0	0	0	0	5	0	0	0	0	0

Main groups :

- *Mortality :*

Table 67 : 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

Nb of dead animals	50	50	50	50	50	30	30	32	29	34
Nb of animals found dead	4	3	4	3	11	3	4	7	5	6
Nb of 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
Nb of animals sacrificed at the end of the study period	39	40	42	44	28	20	18	19	19	19

All of the premature death were mainly caused by lymphoma or nasal tumors.

- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed) :* no treatment-related effects observed.
- *Body weight and body weight changes :*

Table 68 : 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**

- *Haematological findings :* not examined
- *Clinical biochemistry findings :* not examined
- *Gross pathology findings :* increase nb of foci in the adrenal glands in males and females of the highest dose (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).

  Males of the highest dose had also an increased incidence of enlarged spleen (2, 4, 2, 1 and 8 males, resp. at 0, 1, 5, 30 and 60 mg/kg bw/d (while in females : 2, 1, 1, 1 and 3 females, resp. at 0, 1, 5, 30 and 60 mg/kg bw/d).
- *Organ weight :*

Table 69 : 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		39	40	42	44	28	40	38	37	40	35
FBW (g)		559.759	537.203	551.393	537.28	487.132**	347.773	324.308*	326.984	312.79**	300.586**
Adrenal glands	Abs	57.974	58.9	56.976	61.409	69.214**	72.1	72.026	70.351	69.125	80.229*

(mg)	Rela	0.01	0.011	0.01	0.012*	0.014**	0.021	0.023	0.022	0.023	0.027**
Brain (g)	Abs	2.274	2.274	2.286	2.264	2.263	2.13	2.098	2.099	2.128	2.124
	Rela	0.411	0.428	0.419	0.425	0.472**	0.625	0.66	0.659	0.694**	0.717**
Heart (g)	Abs	1.34	1.323	1.345	1.369	1.44	0.972	0.962	0.953	0.957	0.998
	Rela	0.241	0.247	0.245	0.256**	0.297**	0.284	0.301	0.296	0.311**	0.334**
Kid-neys (g)	Abs	3.137	3.036	3.078	3.215	3.654	2.152	2.108	2.206	2.141	2.198
	Rela	0.564	0.569	0.56	0.602	0.755**	0.629	0.659*	0.691*	0.697**	0.739**
Liver (g)	Abs	11.723	10.971	11.533	11.334	10.905	7.731	6.927	7.074	6.85	7.063
	Rela	2.088	2.039	2.088	2.11	2.236	2.129	2.156	2.19	2.214	2.36**
Spleen (g)	Abs	0.974	0.994	1.001	1.006	1.077	0.706	0.602	0.646	0.653	0.677
	Rela	0.174	0.185	0.182	0.188*	0.218**	0.209	0.187	0.201	0.211	0.226*
Epidid-ymides (g)	Abs	1.166	1.129	1.189	1.201	1.169	-	-	-	-	-
	Rela	0.21	0.212	0.217	0.225*	0.242**	-	-	-	-	-
Testes (g)	Abs	4.426	4.252	4.316	4.118	4.07	-	-	-	-	-
	Rela	0.799	0.788	0.792	0.768	0.844	-	-	-	-	-
Ovaries (mg)	Abs	-	-	-	-	-	244.225	148.737	243.27	141.025	116.648
	rela	-	-	-	-	-	0.07	0.049	0.066	0.043	0.04
Uterus (g)	Abs	-	-	-	-	-	2.794	1.181	1.379	1.154	2.307
	rela	-	-	-	-	-	0.972	0.371	0.439	0.374	0.798

thyroid weight not mentioned

- *Neoplastic findings :*

Table 70 : Incidence of benign and malignant tumors

	Males					Females				
Dose level (in mg/kg bw/d)	0	1	5	30	60	0	1	5	30	60
Nb of animals examined	50	50	50	50	50	50	50	50	50	50
Nb of animals with benign neoplasms	27	27	33	30	32	40	32	31	37	35
Nb of animals with benign neoplasms Only	16	20	23	23	19	27	25	22	31	28
Nb of animals with malignant neoplasms	22	19	14	13	25	15	11	16	13	13
Nb of animals with malignant neoplasms Only	11	12	4	6	12	2	4	7	7	6
Nb of animals with systemic neoplasms	2	3	2	0	7	2	0	0	2	2
Nb of animals with metastasized neoplasms	8	5	3	4	12	4	4	7	2	5

- *Nasal cavity :* in 7** males exposed to 60 mg/kg bw/d, malignant epithelial tumors were observed in the posterior part of the nasal cavity (level III). Tumors locally invaded to the nasal cavity, level II in 2 males, level IV in 6 males and to the brain in 3 males. 5 of the affected males died (4 prematurely and 1 sacrificed in a moribund state).

  In 1 males exposed to the highest dose, malignant epithelial tumor was observed in the posterior part of the nasal cavity (level IV) (this tumor was unilateral in the lumen between ethmoid turbinates, characterized more glandular-like structure).
- *Hemolymphoreticular system :* increase incidence of malignant lymphoma at the highest dose in males (6 males at the highest dose vs 1 in control group). 4 lymphomas were of the T-cell type, one of the B-cell type and one could not be classified.

Table 71 : Incidence of the hemolymphoreticular system tumors

	Males					Females				
Dose level (in mg/kg bw/d)	0	1	5	30	60	0	1	5	30	60
Nb examined	50	50	50	50	50	50	12	13	11	49
Inc. malignant lymphoma	1	2	0	0	6	2	0	0	2	2
Inc. histiocytic sarcoma	1	1	2	0	2	0	0	0	0	0

- *Histopathology findings :*

Table 72 : Histopathological 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

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	-	-	-	-	-
(Multi-)focal degeneration	Inc	5	6	9	3	15**	-	-	-	-	-
	1	3	6	8	3	13	-	-	-	-	-
	2	2	-	1	-	1	-	-	-	-	-
	3	-	-	-	-	1	-	-	-	-	-

Nasal cavity : additionally, a sign increased incidence of animals with minimal to severe inflammation and/or inflammatory cells in the lumen was noted at the highest dose in both sexes.

Liver : minimal centrilobular hepatocellular hypertrophy was noted in 19** males exposed to 60 mg/kg bw/d (not observed in the other groups).

(Peri-)vasculitis : increased incidence in males exposed to 30 and 60 mg/kg bw/d. Lesions characterized by prominent perivascular accumulations of lymphocytes, plasma cells and macrophages + some vessels had necrosis of the tunica media and an accumulation of hyaline material with the intima.

Table 73 : Incidence 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

3.12.1.5 14-day repeated dose toxicity study in mice (Anonymous, 2014)

Study reference

Anonymous, 2014

Detailed study summary and results

Test type

Range-finding study before a 28-day study

Test substance

- 3,4-dimethyl-1*H*-pyrazole
- *Degree of purity :* not mentioned

Test animals

- *Species/strain/sex :* Mice / C57BL/6 RJ / both sexes
- *Nb. of animals per sex per dose :* 3/sex/group
- *Age and weight at the study initiation :* not mentioned

Administration/exposure

- *Route of administration* : oral, diet
- *Duration and frequency of test/exposure period :* 2 weeks
- *Doses/concentration levels, rationale for dose level selection :* 0, 2000 and 5000 ppm (corresp. resp. to 408 and 776 mg/kg bw/d in males and to 610 and 956 mg/kg bw/d in females)
- *Post exposure observation period :* /
- *Vehicle :* /

Results and discussion

- *Mortality and time to death (if occurring) :* no mortality occurred during the study period
- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed) :* no clinical signs observed.
- *Body weight and body weight changes :*

Table 74 : 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*

- *Sensory activity, grip strength and motor activity assessments (when available) :* not examined
- *Haematological findings:* not examined
- *Clinical biochemistry findings:* not examined
- *Gross pathology findings:* no abnormalities observed
- *Organ weight :* not examined
- *Histopathology findings:* not examined

  No more information available

3.12.1.6 28-day repeated dose toxicity study in mice (Anonymous, 2015)

Study reference

Anonymous, 2015

Detailed study summary and results

Test type

OECD TG 407

GLP

Test substance

- 3,4-dimethyl-1*H*-pyrazole
- *Degree of purity :* 99.4 %

Test animals

- *Species/strain/sex :* Mouse / C57BL/6 RJ / both sexes
- *Nb. of animals per sex per dose :* 5/sex/group
- *Age and weight at the study initiation :* 48 – 50 days

Administration/exposure

- *Route of administration :* oral, diet
- *Duration and frequency of test/exposure period :* 4 weeks
- *Doses/concentration levels, rationale for dose level selection :* 0, 500, 1500 and 5000 ppm (corresp to 0, 127, 328 and 885 mg/kg bw/d in males and 0, 113, 343 and 846 mg/kg bw/d in females, resp. at 0, 500, 1500 and 5000 ppm).
- *Post exposure observation period :* /
- *Vehicle :* /

Results and discussion

- *Mortality and time to death (if occurring) :* no mortality occurred during the study period.
- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed) :* no effects observed.
- *Body weight and body weight changes :*

Table 75 : 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**

- *Food/water consumption :* reduced at the highest dose (significantly in males).
- *Sensory activity, grip strength and motor activity assessments (when available) :* not examined
- *Haematological findings :*

Table 76 : Haematological findings

	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
Hg (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

- *Clinical biochemistry findings:*

Table 77 : Biochemistry parameters

	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

- *Gross pathology findings :* one female of the mid dose group exhibited focus on glandular stomach and discoloration of contents in jejunum.
- *Organ weight :*

Table 78 : Organ weight (in mg or %)

		Males				Females			
Dose level (in ppm)		0	500	1500	5000	0	500	1500	5000
FBW		20.88	20.54	20.48	18.14*	17.36	16.54	15.66*	15.16*
Adrenal glands (mg)	Abs	4.0	4.6	3.6	4.2	6.8	6.2	5.8	4.8
	Rela	0.019	0.022	0.018	0.023	0.039	0.037	0.037	0.032
Brain (mg)	Abs	454.4	449.6	456.4	443.0	444.4	442.8	442.4	428.4
	Rela	2.18	2.195	2.23	2.445**	2.574	2.685	2.826*	2.828*
Heart (mg)	Abs	130.6	133.4	126.0	109.4	109.0	103.6	107.6	91.4*
	Rela	0.621	0.651	0.616	0.603	0.629	0.627	0.686	0.604
Kidneys (mg)	Abs	301.2	288.2	298.0	258.2	232.4	232.8	215.2	222.2
	Rela	1.437	1.403	1.455	1.423	1.338	1.408	1.373	1.467
Liver (mg)	Abs	905.4	909.2	963.6	1014.8	841.8	844.0	776.6	923.2
	Rela	4.351	4.425	4.703	5.595**	4.827	5.099	4.955	6.091**
Spleen (mg)	Abs	40.8	41.2	40.4	34.2	54.4	44.2	43.6	41.8
	Rela	0.194	0.201	0.197	0.188	0.312	0.267	0.278	0.274
Thymus (mg)	Abs	27.0	25.2	30.2	39.4**	38.0	31.0	32.0	44.8
	Rela	0.13	0.123	0.148	0.217**	0.223	0.188	0.204	0.295

Epididymides (mg)	Abs	52.2	52.4	56.0	47.0	-	-	-	-
	Rela	0.249	0.256	0.274	0.258	-	-	-	-
Prostate (mg)	Abs	50.6	50.0	46.0	36.6	-	-	-	-
	Rela	0.241	0.241	0.225	0.203	-	-	-	-
Seminal vesicle (mg)	Abs	202.8	189.8	203.8	148.6	-	-	-	-
	Rela	0.971	0.928	0.997	0.815	-	-	-	-
Testes (mg)	Abs	186.4	174.8	186.6	137.8	-	-	-	-
	Rela	0.893	0.85	0.911	0.753	-	-	-	-
Ovaries (mg)	Abs	-	-	-	-	13.6	11.0	9.8	10.8
	Rela	-	-	-	-	0.078	0.067	0.063	0.071
Uterus (mg)	Abs	-	-	-	-	131.4	115.0	85.4	87.4
	Rela	-	-	-	-	0.761	0.694	0.54	0.57

- *Histopathology findings :*

Table 79 : Histopathological findings

	Grade	Males				Females			
Dose level (in ppm)		0	500	1500	5000	0	500	1500	5000
Liver									
Centrilobular hypertrophy	Inc	0	0	0	5	0	0	0	3
	1	-	-	-	5	-	-	-	3
Diffuse fatty change	Inc	5	3	5	0	4	4	5	3
Peripheral fatty change	Inc	0	0	0	5	0	0	0	2
Nasal cavity, level III									
Olf epith degen/regen	Inc	0	5	5	5	0	5	5	5
	1	-	4	-	-	-	2	-	-
	2	-	1	1	1	-	2	-	-
	3	-	-	3	2	-	-	4	-
	4	-	-	1	2	-	1	1	5
Eosinophilic globules	Inc	1	0	3	4	0	1	4	5
	1	1	-	3	-	-	-	-	-
	2	-	-	-	2	-	-	2	-
	3	-	-	-	1	-	-	2	1
	4	-	-	-	1	-	1	-	4

3.12.1.7 Repeated dose 90-day toxicity study in mice (Anonymous, 2017)

Study reference

Anonymous, 2017

Detailed study summary and results

Test type

OECD TG 408

GLP

Test substance

- 3,4-dimethyl-1*H*-pyrazole
- *Degree of purity :* 99.3 %

Test animals

- *Species/strain/sex :* Mouse / C57BL/6 J Rj / both sexes
- *Nb. of animals per sex per dose :* 10/sex/dose
- *Age and weight at the study initiation :* 49 ± 1 days

Administration/exposure

- *Route of administration :* oral, diet
- *Duration and frequency of test/exposure period :* 3 months
- *Doses/concentration levels, rationale for dose level selection :* 0, 100, 300, 1750 and 5000 ppm (corresp to 0, 22, 64, 375 and 944 mg/kg bw/d in males and 0, 30, 87, 529 and 1279 mg/kg bw/d in females, resp. at 0, 100, 300, 1750 and 5000 ppm).
- *Post exposure observation period :* /
- *Vehicle:* /

Results and discussion

- *Mortality and time to death (if occurring) :* one male of the highest dose group died prematurely.
- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed) :* alopecia was observed in 1 male and 2 females in control group and in 1 male of the highest dose.
- *Body weight and body weight changes :*

Table 80 : Body weight (in g)

	Males					Females				
Dose level (in ppm)	0	100	300	1750	5000	0	100	300	1750	5000
D 0	22.5	22.6	22.3	22.6	23.0	18.1	17.8	18.2	18.3	17.7
D 7	23.1	24.4	23.4	23.0	21.0**	18.6	18.5	18.5	19.3	16.7**
D 21	24.8	25.9	25.3	24.2	23.4	19.9	20.2	20.2	19.7	18.8**
D 42	26.6	28.2	27.1	26.2	24.8*	21.1	21.0	21.3	20.4	19.2**
D 63	28.4	30.7*	29.3	28.2	26.2*	22.2	22.0	21.7	21.2*	20.3**
D 84	29.6	32.1*	30.6	28.8	26.4**	22.8	22.0	21.9	21.4*	20.1**
D 91	30.4	33.0*	31.7	29.1	26.8**	22.4	22.1	22.3	21.6	20.1**
BWG 0 - 91	7.9	10.4**	9.5	6.5	3.8**	4.4	4.3	4.1	3.3*	2.4**

- *Sensory activity, grip strength and motor activity assessments (when available) :* not examined
- *Haematological findings :*

Table 81 : Haematological data

	Males					Females				
Dose level (in ppm)	0	100	300	1750	5000	0	100	300	1750	5000
RBC (tera/L)	9.76	10.24	10.28	9.87	10.17	10.16	9.65	9.67	9.90	9.30
Hg (mmol/L)	8.6	9.1	9.1	8.7	9.0	9.1	8.7	8.7	8.8	8.4

Ht (L/L)	0.448	0.470	0.470	0.449	0.464	0.463	0.444	0.446	0.452	0.425
MCV (fL)	45.9	45.9	45.7	45.5	45.6	45.6	46.1	46.1	45.7	45.7
MCH (fmol)	0.89	0.89	0.88	0.89	0.89	0.90	0.91	0.90	0.89	0.91
MCHC (mmol/L)	19.25	19.40	19.24	19.51	19.39	19.67	19.66	19.45	19.55	19.82
RET (%)	2.5	2.6	2.5	2.7	2.7	2.0	2.1	2.1	2.2	2.6**
PLT (giga/L)	1.346	1.376	1.385	1.398	1.377	1.158	1.134	1.111	1.160	1.150
WBC (giga/L)	5.90	6.90	6.45	6.08	5.79	2.69	2.37	2.45	2.44	4.12

- *Clinical biochemistry findings :*

Table 82 : 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**
Crea (µmol/L)	7.7	8.8	8.4	8.6	8.0	12.7	12.0	10.9	12.1	12.2

- *Gross pathology findings :* no treatment-related effects observed
- *Organ weight :*

Table 83 : 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

- *Histopathology findings :*

Table 84 : 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, level 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

3.12.1.8 Range-finding study in dogs, 15 days (Anonymous, 2014)

Study reference

Anonymous, 2014

Detailed study summary and results

Test type

Range-finding

Aim : define the dose levels for the subsequent 28-day study

GLP

Test substance

- 3,4-dimethyl-1*H*-pyrazole
- *Degree of purity :* not mentioned

Test animals

- *Species/strain/sex :* Dog / Beagle / both sexes
- *Nb. of animals per sex per dose :* 4/sex/dose
- *Age and weight at the study initiation :* not specified

Administration/exposure

- *Route of administration :* oral, capsule
- *Duration and frequency of test/exposure period :* min. 15 days
- *Doses/concentration levels, rationale for dose level selection :* 50, 125 and 500 mg/kg bw/d

  Due to clinical findings or no clinical findings the dose setting was changed during the study.

Table 85 : Dosing schedule (in mg/kg bw/d)

Study day	Males				Females			
Animal nb	1	2	3	4	1	2	3	4
0	500	-	-	-	500	-	-	-
1	500	-	-	-	500	-	-	-
2	-	-	-	-	-	-	-	-
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

- *Post exposure observation period :* /
- *Vehicle :* /

Results and discussion

- *Mortality and time to death (if occurring) :* 500 mg/kg bw/d : 1 male and 1 female sacrificed on day 1 in moribund state (vomiting, lateral position, no food consumption, poor general condition).
- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed) :* 500 mg/kg bw/d : severe clinical findings on day 1.

  125 mg/kg bw/d : unsteady gait in 1 male (out of 3) and in 2 females (out of 3).

  50 mg/kg bw/d : no effects observed.
- *Body weight and body weight changes :*

Table 86 : Body weight (in kg)

	Males				Females			
	1	2	3	4	1	2	3	4
D -7	15.5	14.1	15.3	-	14.3	14.7	12.6	-
D 0	15.2	13.6	14.7	-	13.9	14.1	12.2	-
D 5	-	13.9	15.0	11.3	-	14.6	12.5	11.0
D 8	-	13.8	14.8	11.2	-	14.3	12.5	10.7
D 12	-	13.8	14.8	11.1	-	14.0	12.4	10.5
D 15	-	13.8	14.8	11.1	-	14.5	12.3	10.4
D 19	-	13.3	15.0	11.1	-	14.0	12.6	10.1
D 22	-	12.8	14.8	10.8	-	13.9	12.7	10.1

- *Food/water consumption :* reduced food consumption in 2/3 males and 3/3 females exposed to 125 mg/kg bw/d
- *Sensory activity, grip strength and motor activity assessments (when available) :* not examined
- *Ophthalmologic findings :* not examined
- *Haematological findings :* not examined
- *Clinical biochemistry findings :* not examined
- *Gross pathology findings :* not examined
- *Histopathology findings :* not examined

3.12.1.9 Repeated dose 28-day oral toxicity study in dogs (Anonymous, 2017)

Study reference

Anonymous, 2017

Detailed study summary and results

Test type

OECD TG 409

GLP

Test substance

- 3,4-dimethyl-1*H*-pyrazole
- *Degree of purity :* 95.9 %

Test animals

- *Species/strain/sex :* Dog / Beagle / both sexes
- *Nb. of animals per sex per dose :* 4/sex/group
- *Age and weight at the study initiation :* 5.1 – 6.3 m and 8.7 – 12.4 kg in M, and 6.9 – 7.4 m and 8.0 – 11.7 kg in F

Administration/exposure

- *Route of administration :* oral, capsule
- *Duration and frequency of test/exposure period :* 4 weeks
- *Doses/concentration levels, rationale for dose level selection :* 0, 10, 30 and 90 mg/kg bw/d
- *Post exposure observation period :* /
- *Vehicle :* /

Results and discussion

- *Mortality and time to death (if occurring) :* no mortality during the study period.
- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed) :* no treatment-related effects observed.
- *Body weight and body weight changes :*

Table 87 : Body weight (in kg)

	Males				Females			
Dose level (in mg/kg bw/d)	0	10	30	90	0	10	30	90
D 0	10.8	10.3	10.6	10.5	9.7	9.7	9.7	10.1
D 7	11.1	10.5	10.9	10.7	9.8	9.8	9.8	10.2
D 14	11.3	10.8	11.1	10.8	10.0	9.9	9.8	10.2
D 21	11.5	10.9	11.2	10.9	10.1	9.9	9.9	10.3
D 28	11.9	11.2	11.4	11.1	10.1	10.1	10.0	10.3
BWG 0 - 28	1.1	1.0	0.8	0.6*	0.4	0.4	0.3	0.2

- *Sensory activity, grip strength and motor activity assessments (when available) :*
  - *FOB :* no treatment-related effects observed.
- *Haematological findings (at D 26) :*

Table 88 : Haematological examination

	Males				Females			
Dose level (in mg/kg bw/d)	0	10	30	90	0	10	30	90
RBC (tera/L)	6.51	6.73	6.96	7.49	7.50	8.04	7.98	8.01

Hg (mmol/L)	9.1	9.3	9.6	10.2*	10.5	11.2	11.1	11.1
Ht (L/L)	0.449	0.456	0.468	0.500	0.509	0.546	0.537	0.542
MCV (fL)	68.8	67.6	67.3	66.8	67.9	68.0	67.03	67.7
MCH (fmol)	1.40	1.38	1.38	1.37	1.40	1.40	1.39	1.39
MCHC (mmol/L)	20.23	20.40	20.48	20.49	20.57	20.55	20.62	20.45
RET (%)	0.7	0.5	0.6	0.8	0.7	0.6	0.6	0.5
PLT (giga/L)	302	278	305	305	253	302	353	325
PTT (sec)	11.7	11.8	11.2	11.5	11.9	11.7	12.1	11.8
QT (sec)	8.4	7.5	7.5	7.3	7.4	7.4	7.5	7.7
WBC (giga/L)	10.18	10.29	10.89	9.64	10.43	9.29	10.54	9.30

- *Clinical biochemistry findings (at D 26) :*

Table 89 : Clinical biochemistry findings

	Males				Females			
Dose level (in mg/kg bw/d)	0	10	30	90	0	10	30	90
ALT (µkat/L)	0.45	0.45	0.39	0.49	0.72	0.53	0.57	0.51
AST (µkat/L)	0.51	0.44	0.48	0.63*	0.44	0.46	0.46	0.42
ALP (µkat/L)	1.95	1.83	1.72	3.78*	1.33	1.56	1.74	2.42*
GGT_C (nkat/L)	57	51	56	45	50	46	52	44
Tot. prot. (g/L)	52.45	56.24	56.31	55.82	56.23	55.64	56.59	57.91

- *Gross pathology findings :* all findings occurred individually.
- *Organ weight :*

Table 90 : Organ weight (in g or mg 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
Epididymides	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

- *Histopathology findings:* all findings occurred individually or were also observed in control.

  Nasal cavity (level III) : epithelial mineralization observed in one male of control group and infiltration of inflammation cells was noted in one male of the highest dose.

3.12.1.10 Repeated dose 90-day oral toxicity study in dogs (Anonymous, 2017)

Study reference

Anonymous, 2017

Detailed study summary and results

Test type

OECD TG 409

GLP

Test substance

- 3,4-dimethyl-1*H*-pyrazole
- *Degree of purity :* 95.9 %

Test animals

- *Species/strain/sex :* Dog / Beagle / both sexes
- *Nb. of animals per sex per dose :* 5/sex/group
- *Age and weight at the study initiation :* 6.4 – 7.6 m and 9.2 – 13.4 kg in M, and 6.6 – 7.7 m and 8.4 – 11.4 kg in F.

Administration/exposure

- *Route of administration :* oral, capsule
- *Duration and frequency of test/exposure period :* 3 months (at least 92 days)
- *Doses/concentration levels, rationale for dose level selection :* 0, 10, 30 and 90 mg/kg bw/d
- *Post exposure observation period :* /

- *Vehicle :* /

Results and discussion

- *Mortality and time to death (if occurring) :* no mortality occurred during the study period.
- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed) :* no treatment-related effects.

  Swollen nictitating membrane was noted in 1 female of the control and 1 female of the low dose group.

  Swelling of the body was observed in 1 female of the highest dose.
- *Body weight and body weight changes :*

Table 91 : Body weight data (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

- *Sensory activity, grip strength and motor activity assessments (when available) :* not examined
- *Haematological findings :*

Table 92 : Haematological data

	Males				Females			
Dose level (in mg/kg bw/d)	0	10	30	90	0	10	30	90
D 45								
RBC (tera/L)	6.29	6.85	6.60	6.62	6.88	6.91	7.03	7.26
Hg (mmol/L)	8.5	9.3	9.2	9.2	9.3	9.5	9.7	10.0
Ht (L/L)	0.411	0.452	0.448	0.444	0.447	0.458	0.471	0.482
MCV (fL)	65.3	66.0	68.0*	67.1	65.0	66.2	67.0	66.5
MCH (fmol)	1.36	1.36	1.39	1.38	1.35	1.37	1.39	1.38
MCHC (mmol/L)	20.80	20.61	20.45	20.61	20.83	20.72	20.70	20.70
RET (%)	0.7	1.1	1.0	0.9	0.6	0.9	0.9*	1.0**
PLT (giga/L)	359	407	342	353	309	354	354	322
PTT (sec)	11.6	11.6	11.4	11.8	11.5	11.8	11.5	11.7
QT (sec)	7.5	7.6	7.7	8.9	7.7	7.9	7.5	7.9
WBC (giga/L)	9.49	11.19	9.95	11.16	11.06	11.17	10.69	13.07
D 90								
RBC (tera/L)	6.70	6.39	6.53	6.71	6.70	6.78	6.84	6.93
Hg (mmol/L)	8.8	8.5	9.0	9.2	8.9	9.2	9.3	9.4
Ht (L/L)	0.436	0.424	0.447	0.458	0.437	0.454	0.461	0.467

MCV (fL)	65.2	66.4	68.4**	68.2	65.4	67.0	67.4	67.5
MCH (fmol)	1.32	1.34	1.38	1.37**	1.32	1.35	1.36	1.36
MCHC (mmol/L)	20.29	20.16	20.19	20.07	20.29	20.16	20.20	20.12
RET (%)	0.7	0.7	0.9	0.6	0.4	0.6	0.5	0.6
PLT (giga/L)	314	327	342	334	288	320	307	329
PTT (sec)	10.6	11.4	10.6	11.6*	11.1	11.4	11.0	11.8
QT (sec)	7.4	7.8	7.5	8.9	7.6	7.9	7.6	7.9
WBC (giga/L)	10.70	10.81	11.96	11.83	10.85	10.86	10.01	13.10

- *Clinical biochemistry findings :*

Table 93 : Clinical biochemistry data

	Males				Females			
Dose level (in mg/kg bw/d)	0	10	30	90	0	10	30	90
D 45								
ALT (µkat/L)	0.70	0.63	0.62	0.62	0.56	0.69	0.75	0.62
AST (µkat/L)	0.50	0.53	0.49	0.47	0.44	0.55	0.45	0.51
ALP (µkat/L)	1.32	1.35	1.42	2.26	1.73	1.20	1.81	2.73
GGT_C (nkat/L)	48	38	35	46	30	29	34	39
Tot. prot. (g/L)	51.51	54.43	52.95	53.06	53.13	52.24	52.65	54.85
D 90								
ALT (µkat/L)	0.93	0.66	0.65	0.68	0.58	0.74	0.63	0.62
AST (µkat/L)	0.51	0.52	0.54	0.52	0.42	0.54	0.42	0.51
ALP (µkat/L)	1.17	1.20	1.33	2.32*	1.66	1.14	1.62	2.35
GGT_C (nkat/L)	43	42	33	35	37	35	37	35
Tot. prot. (g/L)	54.03	54.38	54.87	54.69	53.42	53.60	54.19	56.28
Crea (µmol/L)	66.2	69.1	65.5	60.1	64.9	65.5	67.7	58.7

- *Gross pathology findings :* findings occurred individually or also observed in control group.
- *Organ weight :*

Table 94 : FBW and organ weight data (in mg, g or %)

		Males				Females			
Dose level (in mg/kg bw/d)		0	10	30	90	0	10	30	90
FBW (g)		13020	13300	12680	13660	11300	11260	11340	10580
Adrenal glands (g)	Abs	1.21	1.276	1.256	1.49	1.18	1.178	1.306	1.496*
	Rela	0.009	0.01	0.01	0.011	0.01	0.011	0.012	0.014*
Brain (g)	Abs	81.026	81.998	83.674	81.252	77.334	75.272	76.518	78.634
	Rela	0.623	0.619	0.662	0.596	0.687	0.672	0.676	0.746
Heart (g)	Abs	100.698	100.606	98.454	107.236	95.4	95.914	95.714	88.884
	Rela	0.772	0.755	0.778	0.787	0.846	0.86	0.848	0.847
Kidneys (g)	Abs	59.004	54.428	56.356	57.926	45.774	46.942	46.056	46.536
	Rela	0.452	0.406	0.443	0.422	0.405	0.419	0.406	0.441
Liver (g)	Abs	372.418	375.968	369.652	424.008	325.456	314.992	310.968	341.1
	Rela	2.861	2.818	2.927	3.101	2.878	2.805	2.747	3.242

Pituitary gland (mg)	Abs	81.2	75.4	76.8	79.8	72.8	69.4	72.8	75.4
	Rela	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Spleen (g)	Abs	28.098	26.064	23.862	31.164	31.59	29.96	29.296	28.326
	Rela	0.216	0.196	0.188	0.227	0.282	0.244	0.258	0.272
Thymus (g)	Abs	8.51	11.516	7.402	11.78	7.605	6.802	9.354	7.948
	Rela	0.066	0.087	0.059	0.086	0.067	0.059	0.081	0.075
Thyroid glands (g)	Abs	0.972	0.832	0.72	0.95	0.828	0.85	0.856	0.98
	Rela	0.007	0.006	0.006	0.007	0.007	0.008	0.008	0.009
Epididymides	Abs	3.672	3.506	3.336	3.344	-	-	-	-
	Rela	0.029	0.026	0.026	0.024	-	-	-	-
Prostate (g)	Abs	6.042	8.766	6.122	4.284	-	-	-	-
	Rela	0.046	0.065	0.048	0.031	-	-	-	-
Testes (g)	Abs	18.77	18.052	16.634	19.974	-	-	-	-
	Rela	0.144	0.134	0.131	0.145	-	-	-	-
Ovaries (g)	Abs	-	-	-	-	1.434	1.422	1.342	1.382
	Rela	-	-	-	-	0.012	0.012	0.012	0.013
Uterus (g)	Abs	-	-	-	-	8.538	9.158	10.368	9.726
	Rela	-	-	-	-	0.074	0.078	0.09	0.091

- *Histopathology findings:* findings occurred individually or also observed in control group.

  Nasal cavity (level III) : inflammatory cellular infiltration observed in 1 female of the mid dose and 1 female of the highest dose.

3.12.1.11 Repeated dose 28-day dermal toxicity study in rats (Anonymous, 2018)

Study reference

Anonymous, 2018

Detailed study summary and results

Test type

OECD TG 410

GLP

Test substance

- 3,4-dimethyl-1*H*-pyrazole
- *Degree of purity :* 95.9 %

Test animals

- *Species/strain/sex :* Rat / Wistar / both sexes
- *Nb. of animals per sex per dose :* 10/sex/group
- *Age and weight at the study initiation :* 63 ± 1 days

Administration/exposure

- *Route of administration :* dermal

  Test substance applied uniformly to the clipped dorsal skin.

- *Duration and frequency of test/exposure period :* 6 h per day on 5 day on a week for 4 weeks (male : 20 applications ; females : 21 applications).
- *Doses/concentration levels, rationale for dose level selection :* 0, 10, 30 and 100 mg/kg bw/d
- *Post exposure observation period :* /
- *Vehicle :* test substance with water containing 1 % of sodium carboxymethylcellulose + Tween 80

For dermal studies :

- *Area covered (e.g. 10% of body surface) :* at least 10 % of the body surface.
- *Occlusion (e.g. semi-occlusive) :* skin covered for at least 6 h using a semi-occlusive dressing.
- *Total volume applied :* 4 mL/kg
- *Removal of test substance (e.g. water or solvent) :* after removal of the dressing, treated skin was washed with lukewarm water.

Results and discussion

- *Mortality and time to death (if occurring) :* no mortality occurred during the study period.
- *Description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed) :* no clinical signs observed.

  Focal skin scales were observed in 2 males and 1 female exposed to 10 mg/kg bw/d and in 1 male exposed to 100 mg/kg bw/d.

  Crust formation was noted in 1 female of the low dose group and in 1 male and 1 female of the mid dose group.
- *Body weight and body weight changes :*

Table 95 : Body weight (in g)

	Males				Females			
Dose level (in mg/kg bw/d)	0	10	30	100	0	10	30	100
D 0	279.9	278.2	278.7	278.0	185.5	188.3	184.0	182.1
D 7	303.4	299.2	296.3	296.1	197.8	196.0	193.7	195.2
D 14	318.3	316.7	312.5	312.3	202.4	205.1	202.8	207.9
D 21	334.4	330.2	330.3	326.7	212.3	213.8	207.0	214.6
D 28	344.5	342.0	338.8	336.7	219.2	218.5	214.5	219.0
BWG 0 - 28	64.6	63.8	60.1	58.7	33.7	30.2	30.5	37.0

- *Food/water consumption :* no effects
- *Sensory activity, grip strength and motor activity assessments (when available)*
  - *Home cage observations :* no treatment-related effects.
  - *Open field observations :* no treatment-related effects.
  - *Sensorimotor tests/reflexes :* no treatment-related effects.
  - *Motor activity measurement :* no treatment-related effects.

Table 96 : FOB data (at D 25 in males and D 26 in females)

	Males	Females

Dose level (in mg/kg bw/d)	0	10	30	100	0	10	30	100
Rearing (N)	8	9	10	9	10	11	10	11
GS F (Newton)	11.2	11.0	11.0	11.1	10.4	10.1	10.7	10.6
GS H (Newton)	6.2	6.0	6.3	6.0	4.8	4.4	4.6	4.7
FST (cm)	12.1	11.8	10.8**	11.0	10.5	9.8	10.5	10.1
Motor activity Interv. 1 – 12 Sum	3159.7	3079.4	3434.1	3459.2	3073.7	2752.5	3542.8	3043.6

- *Haematological findings :*

Table 97 : Haematological data (at D 29 in males and D 30 in females)

	Males				Females			
Dose level (in mg/kg bw/d)	0	10	30	100	0	10	30	100
RBC (tera/L)	8.26	8.18	8.36	8.17	7.77	7.76	7.80	7.58
Hg (mmol/L)	9.0	8.9	9.1	9.1	8.6	8.7	8.9*	8.6
Ht (L/L)	0.420	0.414	0.430	0.419	0.398	0.402	0.409	0.395
MCV (fL)	50.8	50.6	51.5	51.3	51.2	51.9	52.5	52.1
MCH (fmol)	1.09	1.09	1.09	1.11	1.11	1.12	1.14	1.13
MCHC (mmol/L)	21.40	21.49	21.24	21.64	21.57	21.61	21.70	21.77
Ret (abs) (giga/L)	145.9	153.3	136.4	139.2	156.3	124.8**	137.2*	140.6
PLT (giga/L)	640	648	653	611	731	715	671	700
HQT (sec)	38.8	38.0	39.8	38.0	33.8	34.0	34.2	34.8
WBC (giga/L)	7.22	7.36	7.26	6.39	4.33	3.95	4.71	4.82

- *Clinical biochemistry findings :*

Table 98 : Biological data (at D 29 in males and D 30 in females)

	Males				Females			
Dose level (in mg/kg bw/d)	0	10	30	100	0	10	30	100
ALT (µkat/L)	0.68	0.64	0.73	0.70	0.58	0.56	0.60	0.59
AST (µkat/L)	1.68	1.82	1.80	1.64	1.73	1.63	1.67	1.49
ALP (µkat/L)	1.70	1.63	1.94	1.63	1.05	0.91	0.92	0.94
GGT_C (nkat/L)	25	25	25	25	25	25	25	25
Tot. prot. (g/L)	62.24	62.24	62.74	62.06	66.15	66.84	65.37	67.04

- *Gross pathology findings :* no treatment-related effects observed.
- *Organ weight :*

Table 99 :Organ weight data (in mg, g or %)

		Males				Females			
Dose level (in mg/kg bw/d)		0	10	30	100	0	10	30	100
FBW (g)		320.68	320.61	315.81	314.82	201.3	202.55	200.63	204.54
Adrenal glands (mg)	Abs	75.0	69.9	68.3	67.9	83.9	81.0	83.1	79.4
	Rela	0.023	0.022	0.022	0.022	0.042	0.04	0.041	0.039
Brain (g)	Abs	2.087	2.027	2.046	2.081	1.905	1.947	1.926	1.919
	Rela	0.653	0.633	0.65	0.663	0.949	0.964	0.961	0.94
Heart (g)	Abs	0.953	0.949	0.915	0.937	0.698	0.68	0.689	0.69
	Rela	0.297	0.296	0.29	0.298	0.347	0.336	0.344	0.337

Kidneys (g)	Abs	2.167	2.154	2.168	2.164	1.567	1.534	1.535	1.51
	Rela	0.676	0.673	0.687	0.688	0.78	0.76	0.765	0.739
Liver (g)	Abs	7.704	7.702	7.421	7.519	5.144	5.112	5.124	5.251
	Rela	2.4	2.402	2.345	2.388	2.56	2.525	2.553	2.568
Spleen (g)	Abs	0.552	0.604	0.507	0.536	0.4	0.398	0.432	0.421
	Rela	0.171	0.188	0.16	0.171	0.199	0.197	0.215	0.206
Thymus (mg)	Abs	421.3	367.0	422.6	372.0	381.2	380.5	397.2	411.2
	Rela	0.131	0.114	0.133	0.118	0.19	0.189	0.198	0.2
Thyroid glands (mg)	Abs	19.5	17.4*	19.1	17.0*	17.7	16.9	16.5	18.6
	Rela	0.006	0.005	0.006	0.005	0.009	0.008	0.008	0.009
Epididymides	Abs	0.964	0.949	0.915	0.937	-	-	-	-
	Rela	0.301	0.305	0.309	0.301	-	-	-	-
Testes (g)	Abs	3.334	3.365	3.304	3.267	-	-	-	-
	Rela	1.043	1.052	1.049	1.04	-	-	-	-
Ovaries (g)	Abs	-	-	-	-	100.6	102.3	95.6	106.4
	Rela	-	-	-	-	0.05	0.051	0.048	0.052
Uterus (g)	Abs	-	-	-	-	0.575	0.643	0.519	0.719
	Rela	-	-	-	-	0.286	0.316	0.259	0.35

- *Histopathology findings :*

Table 100 : Incidence of histopathological findings

	Males				Females			
Dose level (in mg/kg bw/d)	0	10	30	100	0	10	30	100
Epididymides								
Diffuse atrophy	0	NE	0	1	-	-	-	-
Spermatogenic granuloma	1	NE	1	3	-	-	-	-
Mandibular glands								
(multi)focal degeneration	0	NE	NE	1	-	-	-	-
Nasal cavity, level III								
Olf epith degeneration	0	0	0	5	0	0	0	5

3.12.2 Human data

No human data available

3.12.3 Other data

No other data available

3.13 Aspiration hazard

Hazard class not assessed in this CLH dossier

4 ENVIRONMENTAL HAZARDS

Not evaluated in this CLH dossier.

5 ABBREVIATIONS

* : $p < 0.05$

** : $p < 0.01$

Abs : absolute

AG : ano-genital

ALT : alanine aminotransferase

ALP : alkaline phosphatase

Approx : approximately

AST : aspartate aminotransferase

Bw : body weight

Bwg : body weight

Crea : creatine

Corresp. : corresponding

Degen. : degenerative

DMP : dimethylpyrazole

DPC : day post-coitum

DS : dossier submitter

Eos : eosinophilic

Epith : epithelium

Exp : experiment

F : female

FBW : final body weight

FOB : functional observational battery

FST : landing foot-splay test

GD : gestational day

GGT_C : serum-gamma-glutamyltransferase

GLP : good laboratory practice

GS F : grip strength forelimbs

GS H : grip strength hindlimbs

GSD : geometric standardisation

Hg : hemoglobin

HQT : prothrombine time (hepato quick’s test)

Ht : hematocrit

Inc : incidence

Interr. : beam interrupts

Interv. : interval

LC_{50} : lethal conc 50%

LD_{50} : lethal dose 50%

M : male

Max : maximum

MCH : mean corpuscular hemoglobin

MCHC : mean corpuscular hemoglobin concentration

MCV : mean corpuscular volume

Min : minimum

MMAD : mean mass aerodynamic diameter

NE : not examined

No or nb : number

NT : not tested

NZW : New-Zealand white

Olf : olfactive

P : parental

Pc : post-coitum

Plt : platelets

PND : post-natal day

PTT : activated partial thromboplastin time

QT : prothrombin time (Quick's test)

RBC : red blood cell

Regen. : regenerative

Rela : relative

Resp. : respectively

Ret : reticulocytes

TG : test guideline

Tot prot : total protein

TS/gC : total spermatids/gram cauda epididymis

TS/gT : total spermatids/gram testis

Vacuol. : vacuolisation

WBC : white blood cell