# CLH report

# Proposal for Harmonised Classification and Labelling

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

# Chemical name:

# 3,5-dimethylpyrazole

**EC Number: 200-657-5**

**CAS Number: 67-51-6**

**Index Number: /**

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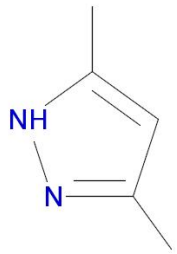
# CONTENTS

# 1 IDENTITY OF THE SUBSTANCE

## 1.1 Name and other identifiers of the substance

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

| | |
|---|---|
| **Name(s) in the IUPAC nomenclature or other international chemical name(s)** | 3,5-dimethyl-1H-pyrazole |
| **Other names (usual name, trade name, abbreviation)** | 3,5-Dimethylpyrazole<br>3,5-Dimethylpyrazol<br>3,5-Dimethyl pyrazole<br>1H-Pyrazole, 3,5-dimethyl-<br>67-51-6 |
| **ISO common name (if available and appropriate)** | 3,5-DMP |
| **EC number (if available and appropriate)** | 200-657-5 |
| **EC name (if available and appropriate)** | 3,5-dimethylpyrazole |
| **CAS number (if available)** | 67-51-6 |
| **Other identity code (if available)** | / |
| **Molecular formula** | C5H8N2 |
| **Structural formula** |  |
| **SMILES notation (if available)** | / |
| **Molecular weight or molecular weight range** | 96.13 |
| **Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)** | / |
| **Description of the manufacturing process and identity of the source (for UVCB substances only)** | / |
| **Degree of purity (%) (if relevant for the entry in Annex VI)** | / |

## 1.2 Composition of the substance

**Table 2: Constituents (non-confidential information)**

| Constituent (Name and numerical identifier) | Concentration range (% w/w minimum and maximum in multi-constituent substances) | Current CLH in Annex VI Table 3 (CLP) | Current self-classification and labelling (CLP) |
|---|---|---|---|
| 3,5-dimethylpyrazole<br><br>EC n° 200-657-5<br>CAS n° 67-51-6 | 100 % (w/w) | / | Acute tox. 4, H302<br>Repr. 2, H361d<br>STOT RE 2, H373 (liver) |

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

| Impurity (Name and numerical identifier) | Concentration range (% w/w minimum and maximum) | Current CLH in Annex VI Table 3 (CLP) | Current self-classification and labelling (CLP) | The impurity contributes to the classification and labelling |
|---|---|---|---|---|
| Unknown | < 0.1 % | / | / | No |

# 2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

## 2.1 Proposed harmonised classification and labelling according to the CLP criteria

### Table 4: For substance with no current entry in Annex VI of CLP

| | Index No | Chemical name | EC No | CAS No | Classification | | Labelling | | | Specific Conc. Limits, M-factors and ATEs | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Hazard Class and Category Code(s) | Hazard statement Code(s) | Pictogram, Signal Word Code(s) | Hazard statement Code(s) | Suppl. Hazard statement Code(s) | | |
| Current Annex VI entry | No current Annex VI entry | | | | | | | | | | |
| Dossier submitter's proposal | TBD | 3,5-dimethylpyrazole | 200-657-5 | 67-51-6 | Repr. 1B<br>Acute Tox. 4<br>STOT RE 2 | H360FD<br>H302<br>H373 (liver, blood) | GHS08<br>GHS07<br>Dgr | H360FD<br>H302<br>H373 (liver, blood) | | oral: ATE = 1700 mg/kg bw | |

## Table 5: Reason for not proposing harmonised classification and status under consultation

| Hazard class | Reason for no classification | Within the scope of consultation |
|---|---|---|
| **Explosives** | Hazard class not assessed in this dossier | No |
| **Flammable gases (including chemically unstable gases)** | Hazard class not assessed in this dossier | No |
| **Oxidising gases** | Hazard class not assessed in this dossier | No |
| **Gases under pressure** | Hazard class not assessed in this dossier | No |
| **Flammable liquids** | Hazard class not assessed in this dossier | No |
| **Flammable solids** | Hazard class not assessed in this dossier | No |
| **Self-reactive substances** | Hazard class not assessed in this dossier | No |
| **Pyrophoric liquids** | Hazard class not assessed in this dossier | No |
| **Pyrophoric solids** | Hazard class not assessed in this dossier | No |
| **Self-heating substances** | Hazard class not assessed in this dossier | No |
| **Substances which in contact with water emit flammable gases** | Hazard class not assessed in this dossier | No |
| **Oxidising liquids** | Hazard class not assessed in this dossier | No |
| **Oxidising solids** | Hazard class not assessed in this dossier | No |
| **Organic peroxides** | Hazard class not assessed in this dossier | No |
| **Corrosive to metals** | Hazard class not assessed in this dossier | No |
| **Acute toxicity via oral route** | **Acute Tox. 4, H302**<br>**ATE: 1700 mg/kg bw** | Yes |
| **Acute toxicity via dermal route** | Hazard class not assessed in this dossier | No |
| **Acute toxicity via inhalation route** | Data lacking | No |
| **Skin corrosion/irritation** | Hazard class not assessed in this dossier | No |
| **Serious eye damage/eye irritation** | Hazard class not assessed in this dossier | No |
| **Respiratory sensitisation** | Hazard class not assessed in this dossier | No |
| **Skin sensitisation** | Hazard class not assessed in this dossier | No |
| **Germ cell mutagenicity** | Hazard class not assessed in this dossier | No |
| **Carcinogenicity** | Hazard class not assessed in this dossier | No |
| **Reproductive toxicity** | **Repr. 1B, H360FD** | Yes |
| **Specific target organ toxicity-single exposure** | Hazard class not assessed in this dossier | No |
| **Specific target organ toxicity-repeated exposure** | **STOT RE 2, H373 (liver, blood)** | Yes |
| **Aspiration hazard** | Hazard class not assessed in this dossier | No |
| **Hazardous to the aquatic environment** | Hazard class not assessed in this dossier | No |
| **Hazardous to the ozone layer** | Hazard class not assessed in this dossier | No |

# 3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

3,5-dimethylpyrazole is a mono-constituent substance which is registered under REACH (1907/2006/EC) by means of a REACH full registration and a REACH intermediate registration.

The substance is currently not registered in annex VI of CLP.

The substance is self-classified in the full registration dossier as:

Acute tox. 4, H302

Repr. 2, H361d

STOT RE 2, H373 (liver)

Several different self-classifications are notified in the C&L inventory (29/09/2022):

NC

Acute Tox. 4, H312

Acute Tox. 4, H332

Skin Irrit. 2, H315

Eye Irrit. 2, H319

STOT SE 3, H335

STOT RE 2, H373 (liver, blood)

Repr. 2, H361fd

# 4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[A.] There is no requirement for justification that action is needed at Community level.

The substance is self-classified as Repr. 2, H361d

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

Reason for a need for action at Community level:

- Disagreement by DS with current self-classification for STOT RE as only liver is mentioned while DS supports a classification also for blood.
- Acute toxicity: addition of ATE to the self-classification

# 5 IDENTIFIED USES

This substance is used by professional workers (widespread uses) in formulation or re-packing of polymers, at industrial sites in polymers, coating products and processing aids and in manufacturing as an intermediate to manufacture another substance or to manufacture plastic products and machinery and vehicles.

# 6 DATA SOURCES

REACH registration dossier

Full study report (when available)

# 7 PHYSICOCHEMICAL PROPERTIES

## Table 6: Summary of physicochemical properties

| Property | Value | Reference | Comment (e.g. measured or estimated) |
|---|---|---|---|
| **Physical state at 20°C and 101.3 kPa** | White crystalline powder | Registration dossier: Denton, 1994 Baltussen, 2012 | / |
| **Melting/freezing point** | 107 °C at 1012 ± 3 hPa | Registration dossier: Anonymous 1, 2012 | OECD TG 102/ EU A.1/ EPA OPPTS 830.7200 |
| **Boiling point** | 220 °C at 1012 ± 3 hPa | Registration dossier: Anonymous 1, 2012 | OECD TG 103/nEU A.2/ EPA OPPTS 830.7220 |
| **Relative density** | 1.14 g/cm$^3$ at 20 °C | Registration dossier: Anonymous 1, 2012 | OECD TG 109 /EU A.3/ EPA OPPTS 830.7300 |
| **Vapour pressure** | 0.37 Pa at 20 °C<br>0.7 Pa at 25 °C | Registration dossier: Anonymous 1, 2012 | OECD TG 104/ EU A.4/ EPA OPPTS 830.7950 |
| **Surface tension** | Based on structure surface activity: not expected | / | / |
| **Water solubility** | 28.9 g/L at 20 °C and pH 7.5 | Registration dossier: Anonymous 1, 2012 | OECD TG 105/ EU A.6/ EPA OPPTS 830.7840 |
| **Partition coefficient n-octanol/water** | Log Know = 2.1 at 35 °C | Registration dossier: Anonymous 1, 2012 | OECD TG 117/ EU A.8/ EPA OPPTS 830.7570 |
| **Flash point** | Not measured as the substance is a solid | / | / |
| **Flammability** | Non-flammable | Registration dossier: Anonymous 1, 2012 | EU A.10/UN N.1: “Test Method for Readily Combustible Solids” 2003 |
| **Explosive properties** | No chemical groups present that imply explosive properties | Registration dossier: Anonymous 2 2012 | Estimated by calculation |
| **Self-ignition temperature** | Not measured as the substance is a solid | / | / |
| **Oxidising properties** | No chemical groups present that imply oxidising properties | Registration dossier: Anonymous 2, 2012 | Estimated by calculation |
| **Granulometry** | MMAD: 268.990 µm<br>Median diameter: 251.932 µm | Registration dossier: Anonymous 3, 2012 | OECD TG 110/ CTL SOP No. 417/ ISO 13320:2009/ CIPAC MT 187 |
| **Stability in organic solvents and identity of relevant degradation products** | Stability in solvents considered not critical | / | / |
| **Dissociation constant** | pKa = 4.17 | Registration dossier: CompuDrug Chemistry Ltd, 2012 | QSAR prediction (Pallas, Compu Drug Chemistry v. 3.6.2.1) |

| Property | Value | Reference | Comment (e.g. measured or estimated) |
|---|---|---|---|
| **Viscosity** | Not measured as the substance is a solid | / | / |

# 8 EVALUATION OF PHYSICAL HAZARDS

Hazard class not assessed in this dossier.

# 9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not evaluated in this dossier.

# 10 EVALUATION OF HEALTH HAZARDS

## 10.1 Acute toxicity - oral route

**Table 7: Summary table of animal studies on acute oral toxicity**

| Method, guideline, deviations if any | Species, strain, sex, no/group | Test substance | Dose levels, duration of exposure | Value $LD_{50}$ | Reference |
|---|---|---|---|---|---|
| Acute oral toxicity study<br>Gavage<br>Similar to OECD TG 401<br>GLP<br>Rel. 1 (mentioned in the registration dossier) | Rat (SD)<br>5 F at low and mid doses<br>5 M and 5 F at the highest dose | 3,5-DMP<br>Purity: > 99 % | 1260, 1600 and 2000 mg/kg bw (no control group)<br>Single exposure | 1717 mg/kg bw in F<br>(2 F died at 1600 mg/kg bw and 2 M and 4 F died at 2000 mg/kg bw) | Anonymous, 1994 |
| Acute oral toxicity study<br>Gavage<br>No guideline followed<br>GLP: unspecified<br>Rel. 2 (mentioned in the registration dossier)<br>Full study report not available by DS | Rat (strain unspecified)<br>Both sexes<br>5 animals/dose (exact distribution of M and F not specified) | 3,5-DMP<br>Purity: unspecified | 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 g/kg bw (no control group)<br>Single exposure | 2140 mg/kg bw<br>(2/5 animals died at 2.0 g/kg bw and all animals died at 4.0, 8.0 and 16.0 g/kg bw) | Anonymous, 1976 |

| **Method, guideline, deviations if any** | **Species, strain, sex, no/group** | **Test substance** | **Dose levels, duration of exposure** | **Value $LD_{50}$** | **Reference** |
|---|---|---|---|---|---|
| Acute oral toxicity study<br>Oral (no more information available)<br>No information about guideline<br>No GLP<br>Rel. 4 (mentioned in the registration dossier) | Rat (strain and sex unspecified)<br>Nb of animals unspecified | 3,5-DMP<br>Purity: unspecified | Dose not specified | > 500 mg/kg bw<br>No more information about mortality, clinical signs and necropsy | Dewitt J.B. *et al.*, 1953 |
| Acute oral toxicity study<br>Oral (no more information available)<br>No information about guideline or GLP<br>Rel. 3 (mentioned in the registration dossier)<br>Full study report not available by DS, only short explanation available in the registration dossier | Mouse (strain and sex unspecified)<br>Nb of animals unspecified | 3,5-DMP<br>Purity: unspecified | Dose not specified | 1060 mg/kg bw<br>No more information about mortality, clinical signs and necropsy | Anonymous, 2009 |
| Acute oral toxicity study<br>Oral (no more information available)<br>No information about guideline or GLP<br>Rel. 4 (mentioned in the registration dossier)<br>Full study report not available by DS, only short explanation available in the registration dossier | Mouse and rat (strain and sex unspecified)<br>Nb of animals unspecified | 3,5-DMP<br>Purity: unspecified | Dose not specified | > 2140 mg/kg bw in rat<br>> 500 mg/kg bw in mouse<br>No more information about mortality, clinical signs and necropsy | Anonymous, 2007 |
| Acute oral toxicity study | Mouse (strain and sex unspecified) | 3,5-DMP<br>Purity: | Dose not specified | 1060 mg/kg bw<br>No more | Anonymous, 2008 |

| Method, guideline, deviations if any | Species, strain, sex, no/group | Test substance | Dose levels, duration of exposure | Value $LD_{50}$ | Reference |
|---|---|---|---|---|---|
| Oral (no more information available)<br>No information about guideline or GLP<br>Rel. 3 (mentioned in the registration dossier)<br>Full study report not available by DS, only short explanation available in the registration dossier<br>The study seems similar to the Acute oral toxicity study (Anonymous, 2009) | Nb of animals unspecified | unspecified | | information about mortality, clinical signs and necropsy | |

No human or other data available.

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

In an acute oral toxicity study (Anonymous, 1994), similar to OECD TG 401, SD rats were exposed by gavage to 3,5-dimethylpyrazole. Groups of 5 females received 1260, 1600 or 2000 mg/kg bw of the test substance, additionally 5 males were exposed to 2000 mg/kg bw of the test substance.

During the post-exposure observation period, 2 females of the mid dose group died (one the 1st day and the second the 2nd day). Furthermore, 2 males and 4 females, exposed to the highest dose, died (2 females died the 1st day (after 3 and 4 hours), while other animals died on day 2).

All animals exhibited palor of extremities and decreased respiratory rate. Furthermore, clinical signs were observed: abnormal carriage, abnormal gait, lethargy, increased salivation, prostration. Recovery was complete by either 3, 4 and 5 days, resp. at 1260, 1600 and 2000 mg/kg bw.

At necropsy, all dead animals exhibited thickening of the stomach walls and fluid contents in the stomach and intestines.

Based on the available results, a $LD_{50}$ of 1717 mg/kg bw in females was calculated.

In another acute oral toxicity study (Anonymous, 1976), groups of 5 rats were exposed by gavage to 3,5-dimethylpyrazole at a concentration of 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 g/kg bw.

All animals exposed to 4.0, 8.0 and 16.0 g/kg bw died on day 1. Furthermore, 2 animals on 5 exposed to 2.0 g/kg bw died (one on day 2 and one on day 5).

During the observation period, all animals had unkept coat. Moreover, additional clinical signs were observed already at 2.0 g/kg bw. At this dose, lethargy, hematuria, slight nasal haemorrhage, diarrhoea and swelling of the neck and cranium were observed. At the exposure dose of 4.0 g/kg bw and higher, laboured breathing and lethargy preceded coma and death.

In this study, a $LD_{50}$ of 2140 mg/kg bw was calculated.

Four other acute oral toxicity study with minimal description of methods and results reported a $LD_{50}$ in rats of > 500 mg/kg bw (Dewitt J.B. *et al.*, 1953) and > 2140 mg/kg bw (Anonymous, 2007) and in mouse of 1060 mg/kg bw (Anonymous, 2009), > 500 mg/kg bw (Anonymous, 2007) and 1060 mg/kg bw (Anonymous, 2008). These studies were not available to the DS and the results cannot be verified.

### 10.1.2 Comparison with the CLP criteria

**Table 8: Comparison with the CLP criteria regarding acute oral toxicity**

| CLP criteria | Results of available studies |
|---|---|
| Acute toxicity category 4: oral $LD_{50}$: > 300 but ≤ 2000 mg/kg bw | $LD_{50}$ of the key study was of 1717 mg/kg bw (Anonymous, 1994).<br>While another study (Anonymous, 1976) had a $LD_{50}$ of 2140 mg/kg bw/d.<br>However, the second study did not followed an OECD guideline, the purity was not reported and the distribution of males and females per group was not mentioned.<br>Another study(ies) (Anonymous, 2008 and 2009), which seems similar, revealed a $LD_{50}$ of 1060 mg/kg bw in mouse, however available information are very limited.<br>Regarding ATE: based on the $LD_{50}$ of the key study, a rounded ATE of 1700 mg/kg bw is warranted. |

### 10.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the available results, a classification as **Acute Tox. 4, H302 (Harmful if swallowed)** is warranted. Based on CLP regulation, an **$ATE_{(oral)}$ of 1700 mg/kg bw** is warranted.

## 10.2 Acute toxicity - dermal route

Hazard class not assessed in this dossier.

## 10.3 Acute toxicity - inhalation route

No study available.

## 10.4 Skin corrosion/irritation

Hazard class not assessed in this dossier.

## 10.5 Serious eye damage/eye irritation

Hazard class not assessed in this dossier.

## 10.6 Respiratory sensitisation

Hazard class not assessed in this dossier.

## 10.7 Skin sensitisation

Hazard class not assessed in this dossier.

## 10.8 Germ cell mutagenicity

Hazard class not assessed in this dossier.

## 10.9 Carcinogenicity

Hazard class not assessed in this dossier.

## 10.10 Reproductive toxicity

### 10.10.1 Adverse effects on sexual function and fertility

**Table 9: Summary table of animal studies on adverse effects on sexual function and fertility**

| Method, guideline, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| EOGRTS with DNT and DIT cohorts<br>Gavage<br>Rat (Wistar)<br>25 rats/sex/group in the F0 generation<br>20 rats/sex/group in the cohort 1A and 1B<br>10 rats/sex/group in the cohort 2A, 2B and 3<br>OECD TG 443<br>GLP<br>Rel. 1 (mentioned in the registration dossier) | 3,5-dimethylpyrazole<br>Purity: 99.98 %<br>Conc.: 0, 20, 50 and 100 mg/kg bw/d<br>Duration of exposure:<br>- F0 generation: up to 127 days in M (10 w prior mating, during pairing and until 42/43 days post-pairing) and up to 122 days in F (10 w prior pairing, during pairing and gestation and until LD 21)<br>- F1 pups: PND 14 to 21<br>- C1A: up to 73 days<br>- C1B: up 96 days for M and up to 75 days prior pairing, during pairing and gestation | F0:<br>Bw: not significantly changed (see Table 10 and Table 11).<br>Haematological effects already observed at the lowest dose in males and at the mid dose in females.<br>Male reproductive parameters: sperm count decreased.<br>VCL and VSL: slightly higher at 50 and 100 mg/kg bw/d.<br>Male fertility index not modified (96, 92, 96 and 92 %, resp. at 0, 20, 50 and 100 mg/kg bw/d).<br>Female reproductive parameters: nb of females with live born pups and mean oestrous cycle were similar in all dose groups.<br>Mean percentage of post- | Anonymous, 2020 |

| Method, guideline, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| | and until LD 4 for F<br>- C2A: up to 56 days<br>- C2B: 1 day<br>- C3: up to 62 days | implantation loss slightly higher at 50 and 100 mg/kg bw/d (0.54, 0.61, 1.29 and 1.09 % resp. at 0, 20, 50 and 100 mg/kg bw/d).<br>Necropsy: adrenal, spleen and prostate weight were sign. modified (see Table 14).<br>Histology: higher incidence of degeneration/regeneration centrilobular of hepatocytes in all M treated groups and at the mid and high doses in F.<br>F1 pups:<br>Mean nb of pups delivered was similar in all groups (11.71, 11.26, 11.17 and 11.09, resp. at 0, 20, 50 and 100 mg/kg bw/d).<br>Viability and survival index slightly lowered at the highest dose.<br>Pups bw: not modified (see Table 16).<br>AGD: similar in all dose groups.<br>IMT4: sign. higher in M at the highest dose (see Table 17).<br>Necropsy: abs and rela thymus weight sign. lower in both sexes at 100 mg/kg bw/d.<br>Cohort 1A:<br>Bw: not modified (see Table 19).<br>Vaginal opening similar in all dose groups.<br>Balano-preputial separation slightly higher at the mid and high doses (48, 48, 50 and 51 days, resp. at 0, 20, 50 and 100 mg/kg bw/d) correlated to a slightly higher mean bw at completion.<br>Haematological effects observed (see Table 20).<br>Male reproductive parameters: sperm count lower at the low and high doses (835.9, 796.3, 831.0 and 812.6 Mio/g, resp. at 0, 20, 50 and 100 mg/kg bw/d).<br>VCL and VSL: increased in all treated groups (see Table 21).<br>Female reproductive parameters: sign. higher nb of primordial follicles | |

| Method, guideline, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| | | at the highest dose.<br><br>Oestrous cycle not modified (mean cycle length of 4.5 days in all groups).<br><br>Necropsy: Kidneys, liver, pituitary and seminal vesical weight sign. modified (see Table 22).<br><br>Histology: increased incidence of degeneration centrilobular of hepatocytes in all treated groups.<br><br>Immunophenotyping (see Table 24): % of spleen CD161+ sign. Lower.<br><br>Cohort 1B:<br><br>Bw: sign. higher in F at the mid dose group during gestation and lactation periods (see Table 25).<br><br>Vaginal opening and balano-preputial separation similar in all groups.<br><br>Female reproductive parameters: mean oestrous cycle length slightly increased (4.3, 4.2, 4.3 and 4.6 days, resp. at 0, 20, 50 and 100 mg/kg bw/d).<br><br>Lower mean nb of implantation sites (12.26, 11.35, 12.28 and 9.79, resp. at 0, 20, 50 and 100 mg/kg bw/d).<br><br>Fertility index not sign. modified (100, 100, 90 and 95 %, resp. at 0, 20, 50 and 100 mg/kg bw/d).<br><br>Necropsy: seminal vesicle and ovary weight sign. affected (see Table 26).<br><br>Pups: mean nb of live pups, viability index, mean pup weight and AGD not modified.<br><br>Cohort 2A:<br><br>Bw: not sign. affected (see Table 28).<br><br>Balano-preputial separation: slightly higher at the mid and high doses (48, 48, 49 and 50 days, resp. at 0, 20, 50 and 100 mg/kg bw/d).<br><br>Vaginal opening: sign. increased at 20 mg/kg bw/d (34, 32*, 33 and 34 days, resp. at 0, 20, 50 and 100 mg/kg bw/d).<br><br>Cohort 2B:<br><br>Clinical examination: no treatment- | |

| Method, guideline, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| | | related effects.<br>Bw: unaffected.<br>Necropsy: no effects observed.<br>Cohort 3:<br>Bw: unaffected.<br>Vaginal opening: increased dose-dependently.<br>Changes in the secondary IgM/IgG response to KLH.<br>FBW and spleen weight: unaffected. | |
| Combined repeated dose toxicity with the reproduction/developmental toxicity screening test<br>Gavage<br>Rat (Wistar)<br>10 animals/sex/group<br>OECD TG 422<br>GLP<br>Rel. 1 in registration dossier. However, full study report not made available to DS | 3,5-dimethylpyrazole<br>Purity: unspecified<br>Conc.: 0, 20, 60 and 200 mg/kg bw/d<br>Duration of exposure: 29 to 31 days in M (2 w prior mating, during mating and up to the day prior scheduled necropsy) and 45 to 56 days in F (2 w prior mating, during pairing, gestation and up to LD 4) | Parental generation:<br>Mortality: 1 F euthanized (due to total litter loss)<br>Bw: in M: slightly lower at the highest dose on PMD 8 and during the entire mating period.<br>In F: Bwg sign. reduced at the highest dose on PMD 8, PCD 11 and slightly lower on PCD 7, 14 to 17 and 20.<br>Fertility index: 60 % at 200 mg/kg bw/d vs 80 % in control group.<br>Necropsy: in M, lower FBW, testes, seminal vesicles, prostate and epididymides weight + findings at histopathology.<br>Pups:<br>Higher incidence of pups mortality (3, 3, 1 and 13 pups, resp. at 0, 20, 60 and 200 mg/kg bw/d).<br>Bw reduced on LD 4. | Anonymous, 2012 |

No human data or other studies available.

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

In an extended-one generation reproductive toxicity study (Anonymous, 2020), groups of male and female Wistar rats were given by gavage 3,5-dimethylpyrazole at a concentration of 0, 20, 50 and 100 mg/kg bw/d.

For the F0 generation, 10 weeks after the beginning of exposure, 25 males and 25 females from the same dose group were mated. Males were exposed until approximately 43/44 days post-pairing, whereas females received test substance during pairing, gestation and until the lactation day 21. Direct dosing to the F1 offspring was initiated from post-natal day 14. Pups were selected to form five cohorts of the F1-generation:

- Cohort 1A was composed of 20 males and 20 females per dose group and animals were received test substance for up to 73 days.
- Cohort 1B was composed of 20 males and 20 females per dose group and was selected to produce F2 pups. Males were dosed up to 96 days, whereas females were exposed for up to 75 days prior to pairing, through pairing and gestation and until lactation day 4.
- Cohort 2A (neurotoxicity) was composed of 10 males and 10 females per dose group and animals were exposed were dosed up to 56 days.
- Cohort 2B (neurotoxicity) was composed of 10 males and 10 females per dose group and animals were exposed for 1 day.
- Cohort 3 (immunotoxicity) was composed of 10 males and 10 females per dose group and animals were administered for up to 62 days.

F0 parental and F1 pups (before weaning):

Regarding the F0 parental generation, one male was found dead on study day 5 in control group, one male of the low dose group was found dead on study day 2 and one male of the highest dose was found dead on study day 107. While in females, one of the highest dose was sent to necropsy on lactation day 2 due to total litter loss. Clinical observation revealed that some animals exhibited increased salivation at the mid and high dose groups, sometimes also in the control and low dose groups. As indicated in the Table 10 and Table 11, no significant body weight change was observed during the study period.

**Table 10: Body weight data in males (in g)**

| Dose level (in mg/kg bw/d) | 0 | 20 | 50 | 100 |
|---|---|---|---|---|
| Pre-pairing | | | | |
| D 1 | 164.0 | 162,5 | 164,8 | 165,2 |
| D 22 | 273,4 | 273,6 | 278,1 | 275,5 |
| D 43 | 339,1 | 343,3 | 351,8 | 346,2 |
| D 71 | 389,4 | 395,1 | 405,3 | 396,9 |
| Pairing | | | | |
| D 7 | 392,6 | 398,2 | 409,0 | 402,4 |
| D 14 | 405,6 | 411,5 | 424,0 | 412,6 |
| Post-pairing | | | | |
| D 8 | 416,0 | 423,0 | 433,2 | 420,6 |
| D 29 | 436,6 | 444,8 | 454,7 | 442,4 |
| D 43 | 436,4 | 449,5 | 457,8 | 439,8 |

**Table 11: Body weight data in females (in g)**

| Dose level (in mg/kg bw/d) | 0 | 20 | 50 | 100 |
|---|---|---|---|---|
| Pre-pairing | | | | |
| D 1 | 141,6 | 141,1 | 144,5 | 139,8 |
| D 22 | 192,0 | 191,8 | 195,1 | 193,1 |
| D 43 | 220,5 | 223,3 | 226,5 | 221,8 |
| D 71 | 241,9 | 244,0 | 248,6 | 239,4 |
| Gestation | | | | |
| D 0 | 241,5 | 245,0 | 248,4 | 240,0 |
| D 10 | 271,5 | 277,8 | 282,1 | 274,7 |
| D 20 | 346,9 | 351,3 | 356,6 | 349,1 |
| Lactation | | | | |
| D 1 | 268,7 | 272,4 | 277,2 | 270,5 |

| | | | | |
|---|---|---|---|---|
| D 7 | 297,2 | 297,0 | 300,0 | 290,4 |
| D 21 | 297,3 | 299,3 | 307,1 | 296,2 |

Blood examination revealed significant haematological effects already observed in the low dose group in males and in the mid dose group in females. Thyroid hormones (IMT4 and TSHI) showed variation in males of the low and mid dose groups and in females of the low dose group (see Table 12). Regarding biological chemistry, ALT was significantly increased in males at the highest dose (also in females but not significantly), see Table 13.

**Table 12: Haematological and thyroid hormone data**

| | Males (at post pairing day 43) | | | | Females (at LD22) | | | |
|---|---|---|---|---|---|---|---|---|
| Dose level (in mg/kg bw/d) | 0 | 20 | 50 | 100 | 0 | 20 | 50 | 100 |
| Hb (g/dL) | 15.0 | 14.4* | 14.2** | 14.2** | 15.6 | 15.6 | 15.3 | 15.1 |
| RBC ($10^{12}$/L) | 8.74 | 8.23* | 8.16** | 7.64*** | 8.11 | 8.13 | 7.50* | 6.99*** |
| PCV (%) | 44.8 | 42.3** | 42.8* | 42.9* | 47.3 | 47.5 | 45.4 | 45.9 |
| MCV (fL) | 51.0 | 51.5 | 52.4* | 56.3*** | 58.3 | 58.4 | 60.7* | 65.7*** |
| MCH (pg) | 17.1 | 17.6 | 17.4 | 18.7*** | 19.3 | 19.3 | 20.4* | 21.6*** |
| MCHC (g/dL) | 33.6 | 34.1 | 33.3 | 33.2 | 33.0 | 33.0 | 33.6 | 33.0 |
| Ret (%) | 2.1 | 2.3 | 2.6** | 3.0*** | 2.4 | 2.4 | 3.4 | 3.5 |
| RDW (%) | 14.2 | 17.2 | 13.1 | 14.1 | 13.2 | 13.4 | 13.9 | 13.6 |
| HDW (%) | 2.77 | 3.19* | 2.91 | 2.49 | 1.96 | 2.04 | 1.94 | 1.69** |
| WBC ($10^9$/L) | 4.2 | 4.5 | 4.4 | 4.0 | 3.6 | 4.2 | 3.5 | 3.6 |
| Neut ($10^9$/L) | 0.84 | 0.93 | 1.03 | 0.77 | 1.96 | 2.14 | 1.62 | 1.69 |
| Leuc ($10^9$/L) | 3.31 | 3.36 | 3.20 | 3.06 | 1.44 | 1.80 | 1.73 | 1.66 |
| Mono ($10^9$/L) | 0.07 | 0.09 | 0.09 | 0.09 | 0.12 | 0.15 | 0.13 | 0.17 |
| Eos ($10^9$/L) | 0.09 | 0.09 | 0.08 | 0.07 | 0.05 | 0.04 | 0.04 | 0.04 |
| Baso ($10^9$/L) | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| Plt ($10^9$/L) | 763 | 810 | 865 | 912** | 1007 | 1025 | 1041 | 1156* |
| MPV (fL) | 7.4 | 7.4 | 7.5 | 7.6 | 7.3 | 7.3 | 7.3 | 7.4 |
| PDW (%) | 55.0 | 52.9 | 54.8 | 51.4 | 49.6 | 47.9 | 50.2 | 48.0 |
| PT (sec) | 23.1 | 22.1 | 23.6 | 28.1*** | 22.9 | 23.0 | 23.3 | 23.3 |
| APTS (sec) | 17.2 | 17.3 | 17.4 | 17.7 | 16.2 | 16.2 | 16.4 | 17.3 |
| Fib (g/L) | 1.53 | 1.53 | 1.51 | 1.45 | 1.51 | 1.56 | 1.46 | 1.43 |
| IMT4 (nmol/L) | 67 | 83 | 74 | 60 | 50 | 59 | 55 | <50 |
| TSHI (µIU/mL) | 0.23 | 0.38 | 0.44 | 0.21 | 0.24 | 0.28 | 0.22 | 0.23 |

**Table 13: Biological chemistry parameters**

| | Males (at post-pairing D 43) | | | | Females (at LD 22) | | | |
|---|---|---|---|---|---|---|---|---|
| Dose level (in mg/kg bw/d) | 0 | 20 | 50 | 100 | 0 | 20 | 50 | 100 |
| AST (IU/L) | 68 | 64 | 64 | 80 | 108 | 108 | 120 | 119 |
| ALT (IU/L) | 54 | 51 | 52 | 73* | 69 | 70 | 78 | 80 |
| ALP (IU/L) | 70 | 67 | 63 | 74 | 74 | 69 | 71 | 62 |
| Tot. chol. (mmol/L) | 1.6 | 1.8 | 2.1* | 2.0 | 1.8 | 1.4 | 1.7 | 1.9 |
| Tot. prot. (µmol/L) | 63 | 63 | 61 | 58*** | 57 | 55 | 56 | 55 |
| A/G ratio | 1.8 | 1.8 | 2.0* | 2.2** | 2.2 | 2.4 | 2.4 | 2.1 |
| Urea (mmol/L) | 8.6 | 8.9 | 9.4 | 9.2 | 11.8 | 12.0 | 12.7 | 11.3 |
| Creat (µmol/L) | 30 | 34 | 35** | 34 | 28 | 30 | 30 | 28 |

Male reproduction parameters were examined and did not reveal significant change. Sperm count (epididymis) showed a reduce but this modification was not dose-related (768.2, 707.0, 736.3 and 716.5 Mio/g, resp. at 0, 20, 50 and 100 mg/kg bw/d). Furthermore, curvilinear and straight line velocity (VCL and VSL) were slightly higher at the mid and high dose groups (VCL: 227.7, 222.1, 236.1 and 245.9 µm/s and VSL: 87.1, 84.6, 91.6 and 94.5 µm/s, resp. at 0, 20, 50 and 100 mg/kg bw/d). The percentage of abnormal sperm was very low in all tested groups (11.2, 1.3, 1.7 and 1.2 %, resp. at 0, 20, 50 and 100 mg/kg bw/d. (St. Dev. was of 31.23, 1.11, 1.48 and 1.38, resp.)). Moreover, male fertility index was not modified (96, 92, 96 and 92 %, resp. at 0, 20, 50 and 100 mg/kg bw/d).

Regarding female reproductive parameters, no significant modification was observed. Mean oestrous cycle duration was of 4.0 or 4.1 days in all dose groups. Furthermore, the mean number of implantations sites was similar in all dose groups (12.17, 11.74, 12.46 and 12.17, resp. at 0, 20, 50 and 100 mg/kg bw/d). The mean percentage of post-implantation loss was very slightly higher at the mid and high doses but this modification was not dose-dependent as it was of 0.54, 0.61, 1.29 and 1.09 %, resp. at 0, 20, 50 and 100 mg/kg bw/d. The duration of gestation was identical in all groups (23.3 days). Furthermore, the number of females with liveborn pups was similar in all dose groups (24, 23, 24 and 23 females, resp. at 0, 20, 50 and 100 mg/kg bw/d).

At necropsy, macroscopic observation did not reveal treatment-related effect. Absolute adrenal weight and absolute and relative spleen and prostate weights were significantly changed (see Table 14). Histopathology showed a higher incidence of centrilobular degeneration/regeneration of hepatocytes, which was observed in 0, 11, 24 and 24 males and in 0, 3, 12 and 11 females, resp. at 0, 20, 50 and 100 mg/kg bw/d. Furthermore, contraction was noted in prostate of 5 males exposed to 100 mg/kg bw/d, and was characterized by decreased secretions within the gland and an overall reduction in glandular size. As observed in Table 15, spleen examination showed also modifications such as increased incidence and severity of pigment in all dose groups and in both sexes.

**Table 14: Organ weight (in g or %)**

| | | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|---|
| Dose level (in mg/kg bw/d) | | 0 | 20 | 50 | 100 | 0 | 20 | 50 | 100 |
| Nb examined | | 24 | 24 | 25 | 24 | 24 | 23 | 24 | 21 |
| FBW | | 431.2 | 441.7 | 449.4 | 434.8 | 250.7 | 250.3 | 255.7 | 247.9 |
| Adrenal | Abs | 0.059 | 0.063 | 0.068* | 0.066 | 0.083 | 0.084 | 0.086 | 0.073* |
| | Rela | 0.014 | 0.014 | 0.015 | 0.015 | 0.033 | 0.034 | 0.034 | 0.030* |
| Brain | Abs | 2.059 | 2.083 | 2.120 | 2.057 | 1.891 | 1.918 | 1.939 | 1.922 |
| | Rela | 0.481 | 0.475 | 0.475 | 0.476 | 0.757 | 0.768 | 0.760 | 0.777 |
| Heart | Abs | 1.068 | 1.049 | 1.075 | 1.069 | 0.941 | 0.882 | 0.905 | 0.906 |
| | Rela | 0.248 | 0.238 | 0.240 | 0.246 | 0.375 | 0.352 | 0.355 | 0.366 |
| Kidneys | Abs | 2.492 | 2.518 | 2.558 | 2.528 | 1.934 | 1.907 | 1.982 | 1.976 |
| | Rela | 0.579 | 0.570 | 0.571 | 0.582 | 0.772 | 0.762 | 0.777 | 0.797 |
| Liver | Abs | 10.861 | 11.177 | 11.261 | 10.398 | 9.689 | 9.755 | 9.880 | 9.958 |
| | Rela | 2.511 | 2.525 | 2.506 | 2.389 | 3.869 | 3.899 | 3.872 | 4.013 |
| Pituitary | Abs | 0.010 | 0.010 | 0.010 | 0.010 | 0.012 | 0.013 | 0.013 | 0.013 |
| | Rela | 0.002 | 0.002 | 0.002 | 0.002 | 0.005 | 0.005 | 0.005 | 0.005 |
| Spleen | Abs | 0.666 | 0.661 | 0.705 | 0.701 | 0.538 | 0.541 | 0.584 | 0.610* |
| | Rela | 0.155 | 0.150 | 0.157 | 0.161 | 0.214 | 0.216 | 0.229 | 0.246** |
| Thymus | Abs | 0.318 | 0.317 | 0.324 | 0.331 | 0.153 | 0.148 | 0.163 | 0.146 |
| | Rela | 0.074 | 0.072 | 0.072 | 0.077 | 0.061 | 0.059 | 0.064 | 0.059 |
| Thyroid/parathyroid | Abs | 0.020 | 0.020 | 0.020 | 0.020 | 0.015 | 0.016 | 0.016 | 0.016 |
| | Rela | 0.005 | 0.005 | 0.005 | 0.005 | 0.006 | 0.006 | 0.006 | 0.006 |
| Epididymis | Abs | 1.574 | 1.578 | 1.633 | 1.604 | - | - | - | - |
| | Rela | 0.367 | 0.361 | 0.365 | 0.370 | - | - | - | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Prostate | Abs | 1.214 | 1.191 | 1.129 | 0.977*** | - | - | - | - |
| | Rela | 0.283 | 0.270 | 0.254 | 0.225*** | - | - | - | - |
| Seminal vesicle | Abs | 1.246 | 1.176 | 1.122 | 1.055 | - | - | - | - |
| | Rela | 0.292 | 0.270 | 0.250 | 0.242 | - | - | - | - |
| Testis | Abs | 3.579 | 3.659 | 3.734 | 3.682 | - | - | - | - |
| | Rela | 0.833 | 0.835 | 0.833 | 0.854 | - | - | - | - |
| Ovary | Abs | - | - | - | - | 0.085 | 0.084 | 0.087 | 0.086 |
| | Rela | - | - | - | - | 0.034 | 0.034 | 0.034 | 0.035 |
| Uterus | Abs | - | - | - | - | 0.517 | 0.530 | 0.635 | 0.629 |
| | Rela | - | - | - | - | 0.205 | 0.212 | 0.250 | 0.256 |

**Table 15: Incidence of splenic pigment**

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| Dose level (in mg/kg bw/d) | 0 | 20 | 50 | 100 | 0 | 20 | 50 | 100 |
| Nb animal examined | 24 | 24 | 25 | 24 | 24 | 23 | 24 | 21 |
| Grade 1 | 6 | 11 | 6 | 5 | 9 | 11 | 8 | 1 |
| Grade 2 | 3 | 10 | 16 | 14 | 2 | 12 | 15 | 14 |
| Grade 3 | - | - | 2 | 5 | - | - | 1 | 6 |

Regarding offspring examination, the mean number of pups delivered was similar in all groups (11.71, 11.26, 11.17 and 11.09, resp. at 0, 20, 50 and 100 mg/kg bw/d), and stillborn pups were only observed in the control (2 pups) and the low dose (3 pups) groups. Furthermore, sex ratio was not modified (51, 47, 52 and 51 % of males, resp. at 0, 20, 50 and 100 mg/kg bw/d). Viability index was slightly lowered at the highest dose (99, 98, 98 and 92 %, resp. at 0, 20, 50 and 100 mg/kg bw/d) as well as survival index at weaning (99, 99, 100 and 95 %, resp. at 0, 20, 50 and 100 mg/kg bw/d). Pups body weight examination did not show modification (see Table 16). Furthermore, ano-genital distance was similar in all groups (in males: 4.5, 4.6, 4.4 and 4.6 mm and in females: 2.1, 2.1, 1.8 and 1.9 mm, resp. at 0, 20, 50 and 100 mg/kg bw/d). IMT4 was significantly higher at the highest dose in males (see Table 17).

**Table 16: Mean pup weight (in g)**

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| Dose level (in mg/kg bw/d) | 0 | 20 | 50 | 100 | 0 | 20 | 50 | 100 |
| D 1 | 7.00 | 7.02 | 7.05 | 6.95 | 6.72 | 6.75 | 6.66 | 6.72 |
| D 4 | 10.69 | 10.65 | 10.39 | 10.29 | 10.43 | 10.35 | 9.86 | 10.01 |
| D 7 | 15.50 | 15.73 | 14.91 | 14.84 | 15.16 | 15.28 | 14.41 | 14.48 |
| D 14 | 28.75 | 29.43 | 27.67 | 27.24 | 28.17 | 28.63 | 26.99 | 26.79 |
| D 20 | 43.72 | 44.29 | 41.98 | 41.14 | 43.27 | 43.47 | 41.15 | 40.91 |

**Table 17: Thyroid hormone data (at PND 22)**

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| Dose level (in mg/kg bw/d) | 0 | 20 | 50 | 100 | 0 | 20 | 50 | 100 |
| IMT4 (nmol/L) | 67 | 81 | 76 | 91** | 69 | 73 | 70 | 73 |
| TSHI (µIU/mL) | <0.09 | <0.10 | <0.09 | <0.11 | <0.09 | 0.11 | <0.09 | 0.11 |

Necropsy of pups was performed and did not reveal treatment-related effects during the macroscopic observation. Final body weight, brain and spleen weight were unaffected by test substance, while absolute and relative thymus weight were significantly decreased at 100 mg/kg bw/d (see Table 18).

**Table 18: Pups organ weight data (in g)**

| | Males | Females |
|---|---|---|

| Dose level (in mg/kg bw/d) | | 0 | 20 | 50 | 100 | 0 | 20 | 50 | 100 |
|---|---|---|---|---|---|---|---|---|---|
| FBW | | 47.4 | 47.3 | 45.9 | 46.9 | 46.3 | 48.6 | 43.3 | 44.8 |
| Brain | Abs | 1.4038 | 1.2144 | 1.4270 | 1.4321 | 1.3840 | 1.3853 | 1.3845 | 1.3818 |
| | Rela | 2.9871 | 3.0123 | 3.1633 | 3.0969 | 0.0301 | 2.8967 | 3.2483 | 3.1259 |
| Spleen | Abs | 0.2131 | 0.2154 | 0.1893 | 0.1932 | 0.2161 | 0.2187 | 0.1851 | 0.1901 |
| | Rela | 0.4477 | 0.4516 | 0.4054 | 0.4109 | 0.4651 | 0.4464 | 0.4203 | 0.4226 |
| Thymus | Abs | 0.2136 | 0.2111 | 0.2052 | 0.1830* | 0.2285 | 0.2337 | 0.2104 | 0.1938* |
| | Rela | 0.4519 | 0.4460 | 0.4492 | 0.3935** | 0.4940 | 0.4819 | 0.4873 | 0.4330** |

Cohort 1A:

After weaning, animals of the cohort 1A did not exhibit body weight change (see Table 19). In females, vaginal opening was similar in all groups (35, 36, 35 and 36 days, resp. at 0, 20, 50 and 100 mg/kg bw/d). In males, balano-preputial separation was slightly higher at the mid and high dose groups (48, 48, 50 and 51 days, resp. at 0, 20, 50 and 100 mg/kg bw/d), while a slightly higher mean body weight at completion was noted (208.0, 207.1, 217.9 and 224.3 g, resp. at 0, 20, 50 and 100 mg/kg bw/d).

**Table 19: Body weight data (in g)**

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| Dose level (in mg/kg bw/d) | 0 | 20 | 50 | 100 | 0 | 20 | 50 | 100 |
| D 22 | 49.9 | 49.4 | 49.9 | 48.0 | 49.9 | 48.6 | 48.4 | 46.3 |
| D 32 | 108.8 | 108.6 | 110.1 | 108.9 | 101.3 | 99.6 | 99.5 | 99.6 |
| D 50 | 218.3 | 219.8 | 221.4 | 219.8 | 161.6 | 159.0 | 158.4 | 161.9 |
| D 64 | 291.4 | 292.6 | 291.6 | 294.1 | 189.0 | 185.5 | 186.4 | 191.8 |
| D 85 | 353.2 | 356.5 | 356.3 | 358.8 | 213.8 | 211.6 | 214.3 | 216.2 |
| D 92 | - | - | - | - | 205.5 | 201.5 | 209.4 | 204.9 |

As in the parental generation, haematological examination showed significant modifications (see Table 20). Significantly higher AST value was also observed at the highest dose in males (71, 73, 79 and 90*** IU/L, resp. at 0, 20, 50 and 100 mg/kg bw/d). Unlike the parental generation, IMT4 was not changed in males exposed to 100 mg/kg bw/d, whereas TSHI was reduced in all dose groups of males (0.46, 0.27, 0.27* and 0.28 µlU/mL, resp. at 0, 20 50 and 100 mg/kg bw/d).

**Table 20: Haematological data**

| | Males (at D 85/87) | | | | Females (at D 85) | | | |
|---|---|---|---|---|---|---|---|---|
| Dose level (in mg/kg bw/d) | 0 | 20 | 50 | 100 | 0 | 20 | 50 | 100 |
| Hb (g/dL) | 14.7 | 14.6 | 14.3 | 14.5 | 14.1 | 13.9 | 13.7 | 13.5 |
| RBC ($10^{12}$/L) | 8.18 | 8.18 | 8.08 | 7.78* | 7.82 | 7.47 | 7.24** | 6.82 |
| PCV (%) | 43.8 | 43.4 | 42.9 | 43.2 | 43.2 | 42.5 | 42.1 | 42.4 |
| MCV (fL) | 53.6 | 53.1 | 53.1 | 55.7* | 55.4 | 57.0 | 58.2** | 62.2*** |
| MCH (pg) | 18.0 | 17.8 | 17.6 | 18.6 | 18.0 | 18.6 | 18.9 | 19.8*** |
| MCHC (g/dL) | 33.6 | 33.6 | 33.2 | 33.4 | 32.6 | 32.7 | 32.5 | 31.9 |
| Ret (%) | 2.5 | 2.7 | 3.0** | 3.2*** | 3.1 | 3.7* | 3.6 | 4.0** |
| RDW (%) | 12.8 | 14.2 | 12.7 | 14.9* | 11.0 | 11.8 | 11.3 | 11.8 |
| HDW (%) | 2.76 | 3.07 | 2.93 | 2.84 | 1.97 | 2.07 | 1.95 | 1.77** |
| WBC ($10^9$/L) | 4.3 | 5.4 | 5.3 | 4.8 | 1.8 | 2.8 | 2.8 | 2.9 |
| Neut ($10^9$/L) | 0.79 | 0.92 | 0.89 | 0.77 | 0.30 | 0.45 | 0.47* | 0.37 |
| Leuc ($10^9$/L) | 3.33 | 4.25 | 4.18 | 3.79 | 1.46 | 2.25 | 2.24 | 2.41 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Plt ($10^9$/L) | 760 | 868** | 876** | 958*** | 777 | 848 | 893* | 946** |
| PDW (%) | 49.7 | 50.8 | 49.3 | 50.4 | 56.7 | 55.9 | 54.0 | 50.9*** |
| PT (sec) | 21.3 | 20.9 | 21.7 | 23.8*** | 22.8 | 23.1 | 23.0 | 23.4 |
| APTS (sec) | 15.3 | 16.2 | 15.6 | 16.5 | 15.4 | 16.0 | 16.4 | 17.5** |
| Fib (g/L) | 1.55 | 1.57 | 1.58 | 1.51 | 1.17 | 1.27 | 1.24 | 1.23 |

Regarding male fertility, as observed in Table 21, parameters were not significantly affected however changes were observed. Sperm count and % of motility were slightly reduced at the highest dose, however modification was not dose-related.

**Table 21: Sperm examination data**

| Dose level (in mg/kg bw/d) | 0 | 20 | 50 | 100 |
|---|---|---|---|---|
| Sperm count (epididymis) (Mio/g) | 835.9 | 796.3 | 831.0 | 812.6 |
| Sperm motility (%) | 76 | 81 | 79 | 70 |
| Average patch velocity (VAP) (µm/s) | 124.0 | 135.4 | 135.2 | 132.6 |
| Curvilinear velocity (VCL) (µm/s) | 220.7 | 239.7 | 239.6 | 230.9 |
| Straight line velocity (VSL) (µm/s) | 85.4 | 93.2 | 96.7 | 94.2 |
| Straightness (VSL/VAP) | 65 | 68 | 70 | 70 |
| Abnormal sperm (%) | 0.7 | 0.6 | 0.8 | 1.3 |

Concerning female fertility, average follicle count exhibited a higher number of primordial follicle at the highest dose (19 vs 17 in control group). The mean number of oestrous cycle or the mean cycle length were not significantly modified. The mean cycle length was only slightly higher at the highest dose (4.5 days compared to the others groups (4.1 days)).

At necropsy, macroscopic examination did not show treatment-related findings. Kidneys, liver, pituitary and seminal vesicle exhibited significant weight change (see Table 22). Histopathology revealed an increased incidence of degeneration centrilobular of hepatocytes (minimal to slight) in all treated groups (in 1, 16, 19 and 18 males and in 0, 17, 17 and 17 females, resp. at 0, 20, 50 and 100 mg/kg bw/d). Furthermore, an increased incidence of contraction was noted in seminal vesicles, which was characterized by decreased glandular secretions and an overall reduction in glandular size (in 1, 2, 3 and 4 males, resp. at 0, 20, 50 and 100 mg/kg bw/d). As observed in the parental generation, splenic pigment was observed in all dose groups. In females, the incidence and severity were dose-dependently increased (see Table 23).

**Table 22: Organ weight (in g or %)**

| | | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|---|
| Dose level (in mg/kg bw/d) | | 0 | 20 | 50 | 100 | 0 | 20 | 50 | 100 |
| FBW | | 346.8 | 348.0 | 346.2 | 349.2 | 207.1 | 203.5 | 207.2 | 208.2 |
| Adrenal | Abs | 0.067 | 0.070 | 0.072 | 0.071 | 0.072 | 0.071 | 0.072 | 0.069 |
| | Rela | 0.0194 | 0.0201 | 0.0208 | 0.0205 | 0.0350 | 0.0346 | 0.0349 | 0.0334 |
| Brain | Abs | 1.959 | 1.959 | 1.949 | 1.950 | 1.783 | 1.790 | 1.820 | 1.815 |
| | Rela | 0.5670 | 0.5652 | 0.5648 | 0.5634 | 0.8654 | 0.8837 | 0.8827 | 0.8743 |
| Heart | Abs | 0.966 | 0.966 | 0.959 | 0.997 | 0.697 | 0.673 | 0.678 | 0.718 |
| | Rela | 0.2791 | 0.2776 | 0.2777 | 0.2872 | 0.3372 | 0.3311 | 0.3270 | 0.3451 |
| Kidneys | Abs | 2.209 | 2.274 | 2.217 | 2.309 | 1.526 | 1.501 | 1.548 | 1.634* |
| | Rela | 0.6382 | 0.6544 | 0.6412 | 0.6623 | 0.7375 | 0.7376 | 0.7463 | 0.7864* |
| Liver | Abs | 10.051 | 10.207 | 10.120 | 10.334 | 5.601 | 5.719 | 6.182** | 6.690*** |
| | Rela | 2.8913 | 2.9273 | 2.9175 | 2.9675 | 2.7104 | 2.8117 | 2.9774*** | 3.2163*** |
| Pituitary | Abs | 0.009 | 0.010 | 0.009 | 0.010 | 0.011 | 0.012 | 0.012 | 0.014** |
| | Rela | 0.0027 | 0.0029 | 0.0027 | 0.0030 | 0.0054 | 0.0058 | 0.0060 | 0.0065 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Spleen | Abs | 0.634 | 0.626 | 0.638 | 0.653 | 0.449 | 0.433 | 0.443 | 0.467 |
| | Rela | 0.1822 | 0.1800 | 0.1847 | 0.1879 | 0.2175 | 0.2130 | 0.2140 | 0.2244 |
| Thymus | Abs | 0.474 | 0.493 | 0.524 | 0.527 | 0.394 | 0.373 | 0.376 | 0.403 |
| | Rela | 0.1364 | 0.1414 | 0.1514 | 0.1519 | 0.1894 | 0.1839 | 0.1824 | 0.1933 |
| Thyroid/ parathyroid | Abs | 0.018 | 0.018 | 0.018 | 0.019 | 0.015 | 0.016 | 0.014 | 0.014 |
| | Rela | 0.0053 | 0.0051 | 0.0051 | 0.0056 | 0.0071 | 0.0077 | 0.0066 | 0.0068 |
| Epididymis | Abs | 1.281 | 1.299 | 1.347 | 1.285 | - | - | - | - |
| | Rela | 0.3704 | 0.3730 | 0.3888 | 0.3706 | - | - | - | - |
| Prostate | Abs | 0.802 | 0.827 | 0.781 | 0.775 | - | - | - | - |
| | Rela | 0.2314 | 0.2392 | 0.2253 | 0.2230 | - | - | - | - |
| Seminal vesicle | Abs | 0.964 | 0.890 | 0.810 | 0.764** | - | - | - | - |
| | Rela | 0.2805 | 0.2576 | 0.2349 | 0.2209* | - | - | - | - |
| Testis | Abs | 3.365 | 3.384 | 3.411 | 3.322 | - | - | - | - |
| | Rela | 0.9761 | 0.9761 | 0.9912 | 0.9557 | - | - | - | - |
| Ovary | Abs | - | - | - | - | 0.100 | 0.098 | 0.108 | 0.113 |
| | Rela | - | - | - | - | 0.0482 | 0.0482 | 0.0524 | 0.0543 |
| Uterus | Abs | - | - | - | - | 0.610 | 0.560 | 0.583 | 0.520 |
| | Rela | - | - | - | - | 0.2919 | 0.2768 | 0.2819 | 0.2511 |

**Table 23: Incidence of splenic pigment**

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| Dose level (in mg/kg bw/d) | 0 | 20 | 50 | 100 | 0 | 20 | 50 | 100 |
| Nb animal examined | 20 | 20 | 20 | 19 | 20 | 20 | 20 | 19 |
| Grade 1 | 2 | 8 | 6 | 7 | 8 | 8 | 6 | 1 |
| Grade 2 | - | - | - | 1 | 3 | 12 | 11 | 14 |
| Grade 3 | - | - | - | - | - | - | 2 | 4 |

In this cohort, immunophenotyping was examined and revealed a significantly lower CD161+ % of splenocytes (see Table 24).

**Table 24: Immunophenotyping**

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| Dose level (in mg/kg bw/d) | 0 | 20 | 50 | 100 | 0 | 20 | 50 | 100 |
| Spleen CD3 abs (cells/mg) | 95489 | 122316 | 125595 | 103882 | 100866 | 102712 | 116905 | 96593 |
| Spleen CD3 % of lymphocytes | 38.8 | 38.3 | 39.3 | 39.2 | 35.3 | 37.7 | 41.8 | 38.1 |
| Spleen CD4 abs (cells/mg) | 51104 | 70469 | 77509 | 60140 | 62129 | 64590 | 74453 | 59968 |
| Spleen CD4 % of lymphocytes | 19.2 | 21.7 | 24.6 | 22.3 | 21.7 | 24.0 | 26.4 | 23.6 |
| Spleen CD8 abs (cells/mg) | 40479 | 47896 | 44722 | 40888 | 36436 | 35995 | 40115 | 34901 |
| Spleen CD8 % of lymphocytes | 16.6 | 15.2 | 13.7 | 15.8 | 12.7 | 12.9 | 14.6 | 13.8 |
| Spleen CD45RA abs (cells/mg) | 114791 | 137541 | 146495 | 112989 | 128573 | 134717 | 118693 | 104316 |
| Spleen CD45RA % of splenocytes | 45.3 | 45.3 | 45.2 | 42.8 | 45.5 | 45.7 | 40.6 | 42.0 |
| Spleen CD161A abs (cells/mg) | 14143 | 17423 | 15567 | 9898 | 18399 | 15720 | 14952 | 11877 |
| Spleen CD161+ % of splenocytes | 6.8 | 5.4 | 5.1 | 4.1** | 6.8 | 5.9 | 5.3 | 4.8 |

Cohort 1B and F2 pups:

Males did not exhibit body weight change while in females exposed to the mid dose, a significantly higher body weight was observed during gestation and lactation period (see Table 25). Vaginal opening and balano-

preputial separation were similar in all groups (49, 47, 50 and 51 days in males and 34, 36, 35 and 35 days in females, resp. at 0, 20, 50 and 100 mg/kg bw/d).

**Table 25: Body weight (in g)**

| Dose level (in mg/kg bw/d) | | 0 | 20 | 50 | 100 |
|---|---|---|---|---|---|
| Pre-pairing period | D 22 | 50.0 | 49.7 | 48.3 | 47.2 |
| | D 30 | 90.0 | 90.6 | 90.7 | 88.8 |
| | D 43 | 141.5 | 144.3 | 146.3 | 142.0 |
| | D 64 | 185.6 | 192.1 | 198.8 | 193.3 |
| | D 78 | 204.8 | 213.1 | 219.5 | 212.2 |
| | D 92 | 216.1 | 226.0 | 232.7 | 222.9 |
| Gestation period | D 6 | 236.8 | 245.7 | 256.8* | 250.5 |
| | D 14 | 263.9 | 274.4 | 288.2* | 278.3 |
| | D 20 | 315.4 | 331.9 | 346.9* | 334.5 |
| Lactation period | D 1 | 250.5 | 258.6 | 274.1* | 267.1 |
| | D 4 | 258.4 | 268.4 | 278.3 | 278.3 |

Regarding female fertility, mean oestrous cycle length was slightly higher at the highest dose group (4.3, 4.2, 4.3 and 4.6 days, resp. at 0, 20, 50 and 100 mg/kg bw/d) which was correlated to a slight decrease of mean number of cycle (2.7, 3.3, 3.1 and 2.4, resp. at 0, 20, 50 and 100 mg/kg bw/d). Furthermore, mean number of implantation sites was reduced at the highest dose (12.26, 11.35, 12.28 and 9.79, resp. at 0, 20, 50 and 100 mg/kg bw/d). However, fertility index did not exhibit significant changes (100, 100, 90 and 95 %, resp. at 0, 20, 50 and 100 mg/kg bw/d). Duration of gestation was similar in all groups (between 23.2 and 23.4 days)

At necropsy, macroscopic examination did not reveal treatment-related findings. Organ weight examination showed significant modification in seminal vesicle and ovary (see Table 26).

**Table 26: Organ weight (in g or %)**

| | | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|---|
| Dose level (in mg/kg bw/d) | | 0 | 20 | 50 | 100 | 0 | 20 | 50 | 100 |
| FBW | | 410.6 | 422.3 | 422.8 | 416.2 | 258.1 | 267.7 | 280.6* | 275.7 |
| Pituitary | Abs | 0.009 | 0.010 | 0.010 | 0.009 | 0.013 | 0.014 | 0.015 | 0.015 |
| | Rela | 0.002 | 0.002 | 0.002 | 0.002 | 0.005 | 0.005 | 0.005 | 0.005 |
| Epididymis | Abs | 1.384 | 1.396 | 1.436 | 1.346 | - | - | - | - |
| | Rela | 0.338 | 0.332 | 0.341 | 0.324 | - | - | - | - |
| Prostate | Abs | 1.053 | 1.075 | 1.069 | 0.972 | - | - | - | - |
| | Rela | 0.258 | 0.255 | 0.256 | 0.233 | - | - | - | - |
| Seminal vesicle | Abs | 1.070 | 1.109 | 1.076 | 0.834* | - | - | - | - |
| | Rela | 0.261 | 0.261 | 0.255 | 0.202* | - | - | - | - |
| Testis | Abs | 3.619 | 3.541 | 1.436 | 3.519 | - | - | - | - |
| | Rela | 0.884 | 0.843 | 0.341 | 0.846 | - | - | - | - |
| Ovary | Abs | - | - | - | - | 0.097 | 0.100 | 0.111* | 0.110* |
| | Rela | - | - | - | - | 0.038 | 0.038 | 0.039 | 0.040 |
| Uterus | Abs | - | - | - | - | 0.775 | 0.755 | 0.822 | 0.794 |
| | Rela | - | - | - | - | 0.302 | 0.283 | 0.294 | 0.289 |

Total number of live pups was of 187, 220, 199 and 179, resp. at 0, 20, 50 and 100 mg/kg bw/d (mean number of pups of 10.39, 11.58, 11.06 and 9.94, resp. at 0, 20, 50 and 100 mg/kg bw/d). Live birth and viability index were not modified. Furthermore, mean pup weight was not changed in both sexes (see Table 27). At post-natal day 4, 1, 1, 3 and 6 pups, resp. at 0, 20, 50 and 100 mg/kg bw/d were missing and 2 pups

of the highest dose were found dead. Anogenital distance was also similar in all groups (5.3, 5.0, 5.1 and 5.0 mm in males and 2.6, 2.5, 2.3 and 2.5 mm in females, resp. at 0, 20, 50 and 100 mg/kg bw/d)

**Table 27: Mean pup body weight (in g)**

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| Dose level (in mg/kg bw/d) | 0 | 20 | 50 | 100 | 0 | 20 | 50 | 100 |
| D 1 | 7.16 | 7.11 | 7.21 | 7.28 | 6.73 | 6.91 | 6.82 | 6.67 |
| D 4 | 10.84 | 10.62 | 10.78 | 10.87 | 10.35 | 10.47 | 10.25 | 10.03 |

Cohort 2A:

As observed in Table 28, no significant body change was noted. Balano-preputial separation was slightly higher at the mid and high dose (48, 48, 49 and 50 days, resp. at 0, 20, 50 and 100 mg/kg bw/d; bw at completion was of 211.5, 213.5, 217.3 and 219.6 g, resp. at 0, 20, 50 and 100 mg/kg bw/d), while vaginal opening was significantly increased in the low dose group (34, 32*, 33 and 34 days, resp. at 0, 20, 50 and 100 mg/kg bw/d ; bw at completion 109.3, 96.9, 104.0 and 106.3 g, resp. at 0, 20, 60 and 100 mg/kg bw/d).

**Table 28: Body weight (in g)**

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| Dose level (in mg/kg bw/d) | 0 | 20 | 50 | 100 | 0 | 20 | 50 | 100 |
| D 22 | 52.5 | 50.8 | 51.4 | 47.9 | 51.0 | 49.0 | 49.5 | 45.0 |
| D 32 | 111.7 | 110.9 | 113.1 | 108.4 | 101.7 | 98.9 | 99.5 | 96.7 |
| D 43 | 180.5 | 182.4 | 183.3 | 177.8 | 143.8 | 142.0 | 142.0 | 141.8 |
| D 57 | 264.3 | 266.1 | 274.0 | 266.6 | 178.5 | 173.5 | 178.0 | 176.9 |
| D 64 | 289.4 | 300.5 | 309.3 | 301.9 | 190.7 | 187.2 | 188.7 | 189.9 |
| D 71 | 310.8 | 324.8 | 335.9 | 326.6 | 199.4 | 197.4 | 201.9 | 199.6 |

Neurological parameters were examined. Acoustic startle examination did not reveal any treatment-related effects for mean startle amplitude and for pre-pulse inhibition. Furthermore, motor activity parameters (total activity and the number of rears) and functional observational battery were not significantly affected by the treatment.

At necropsy, no macroscopic treatment-related effect were observed. As noted in Table 29, final body weight was unaffected, while brain weight exhibited a trend of increase. No treatment-related changes in brain measurements, macroscopic or neuropathological effects were observed.

**Table 29: FBW and brain weight (in g or %)**

| | | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|---|
| Dose level (in mg/kg bw/d) | | 0 | 20 | 50 | 100 | 0 | 20 | 50 | 100 |
| FBW | | 325.1 | 342.6 | 353.3 | 341.5 | 208.1 | 203.2 | 208.0 | 206.3 |
| Brain | Abs | 1.925 | 1.945 | 1.976 | 1.916 | 1.738 | 1.837* | 1.818* | 1.776 |
| | Rela | 0.5969 | 0.5702 | 0.5610 | 0.5641 | 0.8425 | 0.9107 | 0.8772 | 0.8692 |

Cohort 2B:

Clinical examination did not reveal treatment-related effects. Furthermore, body weight was unaffected by treatment (at PND 22: 49.0, 47.8, 48.6 and 46.7 g in males and 49.1, 49.9, 47.7 and 45.5 g in females, resp. at 0, 20, 50 and 100 mg/kg bw/d).

At necropsy, macroscopic and microscopic examination did not reveal treatment-related effects, as well as brain measurements.

Cohort 3:

A slight increased incidence of excessive salivation was only observed. Furthermore, as observed in Table 30, body weight examination did not reveal significant change. Balano-preputial separation was not significantly modified (50, 48, 49 and 49 days, resp. at 0, 20, 50 and 100 mg/kg bw/d; bw at completion: 215.6, 208.2, 220.4 and 218.7 g, resp. at 0, 20, 50 and 100 mg/kg bw/d). While vaginal opening was increased dose-dependently and was significantly higher at the highest dose (32, 33, 34 and 36 days, resp. at 0, 20, 50 and 100 mg/kg bw/d; bw at completion: 99.0, 101.1, 107.2 and 115.1* g, resp. at 0, 20, 50 and 100 mg/kg bw/d).

**Table 30: Body weight (in g)**

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| Dose level (in mg/kg bw/d) | 0 | 20 | 50 | 100 | 0 | 20 | 50 | 100 |
| D 22 | 49.4 | 48.6 | 50.4 | 45.7 | 49.9 | 49.2 | 48.6 | 44.7 |
| D 32 | 108.8 | 108.1 | 113.4 | 107.0 | 99.3 | 98.6 | 99.9 | 97.8 |
| D 43 | 176.8 | 178.1 | 188.0 | 176.7 | 140.5 | 141.7 | 143.8 | 141.0 |
| D 57 | 259.7 | 260.5 | 273.1 | 265.7 | 177.5 | 177.4 | 182.7 | 181.5 |
| D 78 | 338.4 | 341.3 | 354.5 | 350.2 | 205.6 | 209.4 | 214.9 | 212.4 |

In this cohort, evaluation of the anti-KLH IgM and IgG response was performed. The results showed slight modification in the secondary IgG response to KLH, with an increase in males (more marked at the mid dose) and a decrease in females at the mid and high doses groups. Same trend was noted in the secondary IgM response to KLH.

At necropsy, no macroscopic treatment-related findings were noted and the final body weight and the spleen weight were unaffected by treatment.

In a combined repeated dose toxicity with the reproduction/developmental toxicity screening test (Anonymous, 2012), groups of 10 male and 10 female Wistar rats were exposed by gavage to 3,5-dimethylpyrazole at a concentration of 0, 20, 60 and 200 mg/kg bw/d. Males were treated during 29 to 31 days (2 weeks prior mating, during mating and up to the day prior to scheduled necropsy). While females were exposed 45 to 56 days (2 weeks prior mating, during pairing, gestation and up to lactation day 4).

During the study period, one female exposed to 200 mg/kg bw/d was euthanized due to total litter loss. Clinical observation did not reveal treatment-related effects. In males, body weight and body weight gain were significantly lowered at the highest dose on PMD 8 and through the entire mating period. In females, body weight gain was significantly reduced at the highest dose on PMD 8 and on PCD 11, and was also slightly decreased on PCD 7, 14 to 17 and 20 (no more information available).

Female reproductive parameters exhibited modifications. At the highest dose, the fertility index was of 60 %, compared to 80 % in the control group (see Table 31). Other parameters, such as precoital time, numbers of corpora lutea, and number of implantation sites were unaffected by the treatment (no more information available).

**Table 31: Female reproductive parameters**

| Dose level (in mg/kg bw/d) | 0 | 20 | 60 | 200 |
|---|---|---|---|---|
| Nb of female paired | 10 | 10 | 10 | 10 |
| Nb of females mated | 10 | 10 | 10 | 9 |
| Nb of females non-mated | 0 | 0 | 0 | 1 |
| Nb of pregnant females | 8 | 9 | 9 | 6 |
| Nb of non-pregnant females | 2 | 2 | 1 | 3 |

At necropsy, macroscopic examination revealed findings at the highest dose. In females, an enlarged spleen was observed in 6 animals out of 10 and a reduced size of thymus was noted in 5 animals out of 10. While in males, effects were observed in reproductive organs. One male exhibited reduced size of the epididymides, testes and seminal vesicles. Histopathology confirmed that reproductive organs were affected by the treatment as, at 200 mg/kg bw/d, oligospermia (in 1 male out of 6, marked) and an increased incidence and/or severity of seminiferous cell debris (in 3 males out of 6, up to slight) were observed. Furthermore, degeneration/depletion of spermatocytes (in 6 males out of 6, up to massive) and an increased incidence and/or severity of spermatic giant cells (in 5 males out of 6, up to moderate) were also noted at the highest dose group. In thymus, an increased severity of lymphoid atrophy was observed at 200 mg/kg bw/d in 3 males out of 5 and in 5 females out of 5. Liver examination showed also modifications, as hepatocellular basophilia (up to slight) and/or apoptosis/single cell necrosis (up to marked) in the area directly around the central veins were noted in males exposed to 60 mg/kg bw/d and in both sexes exposed to 200 mg/kg bw/d. Moreover, males, exposed to 200 mg/kg bw/d, exhibited hepatocellular karyomegaly (in 5 males out of 5) and midzonal hepatocellular vacuolation (in 3 males out of 5). Males exposed to the highest dose had also higher severity of hematopoietic foci (up to marked).

During the first days of lactation, a higher incidence of pup mortality was observed at the highest dose (3, 3, 1 and 13 pups, resp. at 0, 20, 60 and 200 mg/kg bw/d). 7 pups out of 13 dead pups of the highest dose were attributable to one female who had a total litter loss by day 3. At the highest dose, treatment-related body weight effects were observed as pup body weight was significantly lower than control group on lactation day 4 (9.1, 8.7, 8.8 and 7.3** g, resp. at 0, 20, 60 and 200 mg/kg bw/d).

Pups necropsy exhibited only incidental findings as small lower jaw was only observed in a single pup of the mid dose group. Furthermore, necropsy of the pups which were found dead showed autolysis, absence of milk in the stomach and partial cannibalism (abdominal organs missing).

The registration dossier mentions in conclusion of this study “On the basis of the effects seen, the material should be classified as Repr. 2: H361: Suspected of damaging fertility or the unborn child in accordance with Regulation (EC) No. 1272/2008.”

### 10.10.3 Comparison with the CLP criteria

**Table 32: Comparison with the CLP criteria regarding fertility**

| Criteria for Category 1 | Criteria for category 2 |
|---|---|
| “Known or presumed human reproductive toxicant<br><br>Substances are classified in category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human (category 1A) or from animal data (category 1B).<br><br>Category 1A: known human reproductive toxicant. The classification is largely based on evidence from humans<br><br>Category 1B: presumed human reproductive | “Suspected human reproductive toxicant<br><br>Substances are classified in category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in category 1. If deficiencies in the study make the quality of evidence less convincing, category 2 could be the more appropriate classification.<br><br>Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects." |

| | |
|---|---|
| toxicant. The classification is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in category 2 may be more appropriate." | |

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

Two available rodent studies (of reliability 1) examined male and female fertility parameters.

- **In males:**

In the EOGRTS (Anonymous, 2020) as well as the combined repeated dose toxicity with the reproduction/developmental toxicity screening test (Anonymous, 2012), male reproductive organs were affected by exposure to the test-substance. Significant organ weight changes and microscopic findings were observed at the highest tested doses (100 mg/kg bw/d and 200 mg/kg bw/d, resp. in the EOGRTS and in the combined study).

**Table 33: Summary of male reproductive parameters**

| Dose level (in mg/kg bw/d) | | 0 | 20 | 50 | 60 | 100 | 200 |
|---|---|---|---|---|---|---|---|
| **Sperm count (M/g)** | | | | | | | |
| EOGRTS | F0 | 768.2 | 707.0 | 736.3 | / | 716.5 | / |
| | C1A | 835.9 | 796.3 | 831.0 | / | 812.6 | / |
| Combined | | NE | NE | / | NE | / | NE |
| **Sperm motility (%)** | | | | | | | |
| EOGRTS | F0 | 85 | 83 | 90 | / | 87 | / |
| | C1A | 76 | 81 | 79 | / | 70 | / |
| **VCL (µm/s)** | | | | | | | |
| EOGRTS | F0 | 227.7 | 222.1 | 236.1 | / | 245.9 | / |
| | C1A | 220.7 | 239.7 | 239.6 | / | 230.9 | / |
| **VSL (µm/s)** | | | | | | | |
| EOGRTS | F0 | 87.1 | 84.6 | 91.6 | / | 94.5 | / |
| | C1A | 85.4 | 93.2 | 96.7 | / | 94.2 | / |
| **% of abnormal sperm** | | | | | | | |
| EOGRTS | F0 | 11.2 | 1.3 | 1.7 | / | 1.2 | / |
| | C1A | 0.7 | 0.6 | 0.8 | / | 1.3 | / |
| **Male fertility index (%)** | | | | | | | |
| EOGRTS | F0 | 96 | 92 | 96 | / | 92 | / |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | C1B | | 100 | 100 | 90 | / | 94 | / |
| **Epididymis weight (g or %)** | | | | | | | | |
| EOGRTS | F0 | Abs | 1.574 | 1.578 | 1.633 | / | 1.604 | / |
| | | Rela | 0.367 | 0.361 | 0.365 | / | 0.370 | / |
| | C1A | Abs | 1.281 | 1.299 | 1.347 | / | 1.285 | / |
| | | Rela | 0.3704 | 0.3730 | 0.3888 | / | 0.3706 | / |
| | C1B | Abs | 1.384 | 1.396 | 1.436 | / | 1.346 | / |
| | | Rela | 0.338 | 0.332 | 0.341 | / | 0.324 | / |
| Combined | | | No info | No info | / | No info | / | Lower epididymides weight (abs, but FBW also lower) |
| **Prostate weight (g or %)** | | | | | | | | |
| EOGRTS | F0 | Abs | 1.214 | 1.191 | 1.129 | / | 0.977*** | / |
| | | Rela | 0.283 | 0.270 | 0.254 | / | 0.225*** | / |
| | C1A | Abs | 0.802 | 0.827 | 0.781 | / | 0.775 | / |
| | | Rela | 0.2314 | 0.2392 | 0.2253 | / | 0.2230 | / |
| | C1B | Abs | 1.053 | 1.075 | 1.069 | / | 0.972 | / |
| | | Rela | 0.258 | 0.255 | 0.256 | / | 0.233 | / |
| Combined | | | No info | No info | / | No info | / | Lower prostate weight (abs, but FBW also lower) |
| **Seminal vesicle weight (g or %)** | | | | | | | | |
| EOGRTS | F0 | Abs | 1.246 | 1.176 | 1.122 | / | 1.055 | / |
| | | Rela | 0.292 | 0.270 | 0.250 | / | 0.242 | / |
| | C1A | Abs | 0.964 | 0.890 | 0.810 | / | 0.764** | / |
| | | Rela | 0.2805 | 0.2576 | 0.2349 | / | 0.2209* | / |
| | C1B | Abs | 1.070 | 1.109 | 1.076 | / | 0.834* | / |
| | | Rela | 0.261 | 0.261 | 0.255 | / | 0.202* | / |
| Combined | | | No info | No info | / | No info | / | Lower seminal vesicle weight (abs + rela) |
| **Testis weight (g or %)** | | | | | | | | |
| EOGRTS | F0 | Abs | 3.579 | 3.659 | 3.734 | / | 3.682 | / |
| | | Rela | 0.833 | 0.835 | 0.833 | / | 0.854 | / |
| | C1A | Abs | 3.365 | 3.384 | 3.411 | / | 3.322 | / |
| | | Rela | 0.9761 | 0.9761 | 0.9912 | / | 0.9557 | / |
| | C1B | Abs | 3.619 | 3.541 | 1.436 | / | 3.519 | / |
| | | Rela | 0.884 | 0.843 | 0.341 | / | 0.846 | / |
| Combined | | | No info | No info | / | No info | / | Lower testes weight (abs + rela) |
| **Microscopic findings** | | | | | | | | |
| EOGRTS | F0 | | | | | / | Prostate: contraction in 5 M<br>Characterized by lower | / |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | secretions within the gland and an overall reduction in glandular size. | |
| | C1A | | | | / | Seminal vesicles: increased inc of contraction (1, 2, 3 and 4 M, resp. at 0, 20, 50 and 100 mg/kg bw/d) and was characterized by decreased glandular secretions and an overall reduction in glandular size. | / |
| Combined | | No info | No info | / | No info | / | Epididymides: Oligospermia (1 M/6, marked), seminiferous cell debris (3 M/6, up to slight)<br>Testes: degeneration/depletion of spermatocytes (6 M/6, up to massive) + increase inc and/or severity of spermatic giant cells (5 M/6, up to moderate) |

As observed in Table 33, in the EOGRTS (Anonymous, 2020), a dose-related lower prostate weight (abs and rela) was observed in the F0 generation. This modification was statistically significant at the highest dose and was correlated with microscopic findings as contraction was noted in prostate of 5 males (out of the 24 exposed to 100 mg/kg bw/d). This contraction was characterized by decreased secretions within the gland and an overall reduction in glandular size. In the cohort 1A, seminal vesicle weight change and microscopic findings (contraction) were also observed.

However, sperm parameters examination did not exhibit treatment-related modifications. Furthermore, at the highest dose, the male fertility index was slightly reduced but the change was not dose-related.

In the combined study (Anonymous, 2012), which had a high dose higher than the EOGRTS, effects were more pronounced. Toxicity to reproduction was observed at the highest tested dose (200 mg/kg bw/d). Microscopic modifications were observed in the epididymides and in the testes, characterized by a marked oligospermia in 1 male (out of 6 examined), the presence of seminiferous cell debris in 3 males, and a degeneration/depletion of spermatocytes in all males of this highest dose and described as up to massive. All these modifications were correlated with the lower fertility (60 % vs 80 % in control group) and conception (66.7 % vs 80 % in control group) indices (no information about the statistic). These results come from the registration dossier, as the full study report was not made available to the DS.

The two available studies demonstrated effects on the male reproductive system.

Furthermore, balano-preputial separation was dose-dependently delayed in the cohort 1A (48, 48, 50 and 51 days, resp. at 0, 20, 50 and 100 mg/kg bw/d). While, mean body weight at completion was higher at the 2 highest dose. Furthermore, delay was also observed in the cohort 1B (49, 47, 50 and 51 days, resp. at 0, 20, 50 and 100 mg/kg bw/d), while mean body weight at completion was also slightly increased. In the cohort 2A, similar slight increased delay of balano-preputial separation was noted as well as a slight increased mean body weight at completion.

As mentioned in the guidance on the application of the CLP criteria (version 5.0 – July 2017), *"Adverse effects on fertility and reproductive performance seen only at dose levels causing marked systemic toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma) are not relevant for classification purposes. There is no established relationship between fertility effects and less marked systemic toxicity. Therefore it should be assumed that effects on fertility seen at dose levels causing less marked systemic toxicity are not a secondary consequence of this toxicity. However, mating behaviour can be influenced by parental effects not directly related to reproduction (e.g. sedation, paralysis), and such effects on mating behaviour may not warrant classification."*

In the 2 available studies, no marked systemic toxicity (such as lethality, dramatic reduction in absolute body weight, coma) can explain the disturbance of the male reproductive system. Furthermore, the effects were observed at relatively low doses.

The registration dossier of the combined study (Anonymous, 2012) indicate that *"At 200 mg/kg, treatment related effects on body weights, food and water consumption, functional observations, clinical pathology, macroscopic findings and microscopic findings in the thymus, liver spleen, testes and epididymides were seen. Females at 60 mg/kg had a trend towards increased water consumption and males at this dose level had toxicologically relevant liver findings at the microscopic examination. There was no parental mortality in the study."*. These information are not enough to disregarded the male reproductive adverse effects. Furthermore, the registration dossier mention also that *"On the basis of the effects seen, the material should be classified as Repr. 2: H361: Suspected of damaging fertility or the unborn child in accordance with Regulation (EC) No. 1272/2008."*.

- **In females:**

As observed in Table 34, in the cohort 1A and 1B of the EOGRTS (Anonymous, 2020), estrous cycle length was slightly higher at the highest dose. This modification was not observed in the parental generation. At the highest dose of this study, a lower mean number of implantation sites was observed in the cohort 1B as well as a lower mean number of pups. However, fertility index was not affected in any generation.

The combined repeated dose toxicity study (Anonymous, 2012) does not allow to confirm or infirm the results of the EOGRTS as estrous cycle was not examined. The only information available about the implantation sites was "unaffected" without more data available. However, in this study, the fertility index was of 60 % at the highest dose (1 non-mated female and 3 non-pregnant females after mating) (no information about the statistical analysis).

Regarding the female reproductive organ weight, no dose-related modification was observed in the EOGRTS (Anonymous, 2020). In the cohort 1B, absolute ovaries weight was significantly higher at the mid and highest doses compared to control group and the relative weight showed also a slight increase at these 2 doses. The absolute ovaries weight in the cohort 1A was also modified but the change was not significant.

**Table 34: Summary of female reproductive effects**

| Dose level (in mg/kg bw/d) | | 0 | 20 | 50 | 60 | 100 | 200 | |
|---|---|---|---|---|---|---|---|---|
| **Estrous cycle length (in d)** | | | | | | | | |
| EOGRTS | F0 | 4.1 | 4.1 | 4.0 | / | 4.1 | / | |
| | C1A | 4.1 | 4.1 | 4.1 | / | 4.5 | / | |
| | C1B | 4.3 | 4.2 | 4.3 | / | 4.6 | / | |
| Combined | | NE | NE | / | NE | / | NE | |
| **Mean nb of implantation sites** | | | | | | | | |
| EOGRTS | F0 | 12.17 | 11.74 | 12.46 | / | 12.17 | / | |
| | C1B | 12.26 | 11.35 | 12.28 | / | 9.79 | / | |
| Combined | | | | | | | | Unaffected (no more info) |
| **Fertility index (in %)** | | | | | | | | |
| EOGRTS | F0 | 96 | 92 | 96 | / | 92 | / | |
| | C1B | 100 | 100 | 90 | / | 95 | / | |
| Combined | | 80 | No info | / | No info | / | 60 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Duration of gestation** | | | | | | | | | |
| EOGRTS | F0 | | 23.3 | 23.3 | 23.3 | / | 23.3 | / | |
| | C1B | | 23.3 | 23.4 | 23.2 | / | 23.4 | / | |
| Combined | | | No info | No info | / | No info | / | No info | |
| **Mean nb of pups per dam** | | | | | | | | | |
| EOGRTS | F0/F1pups | | 11.71 | 11.26 | 11.17 | / | 11.09 | / | |
| | C1B/F2 pups | | 10.39 | 11.58 | 11.06 | / | 9.94 | / | |
| **Ovary weight (in g or %)** | | | | | | | | | |
| EOGRTS | F0 | Abs | 0.085 | 0.084 | 0.087 | / | 0.086 | / | |
| | | Rela | 0.034 | 0.034 | 0.034 | / | 0.035 | / | |
| | C1A | Abs | 0.100 | 0.098 | 0.108 | / | 0.113 | / | |
| | | Rela | 0.0482 | 0.0482 | 0.0524 | / | 0.0543 | / | |
| | C1B | Abs | 0.097 | 0.100 | 0.111* | / | 0.110* | / | |
| | | Rela | 0.038 | 0.038 | 0.039 | / | 0.040 | / | |
| Combined | | | No info | No info | / | No info | / | No info | Weight but not info |
| **Uterus weight (in g or %)** | | | | | | | | | |
| EOGRTS | F0 | Abs | 0.517 | 0.530 | 0.635 | / | 0.629 | / | |
| | | Rela | 0.205 | 0.212 | 0.250 | / | 0.256 | / | |
| | C1A | Abs | 0.610 | 0.560 | 0.583 | / | 0.520 | / | |
| | | Rela | 0.2919 | 0.2768 | 0.2819 | / | 0.2511 | / | |
| | C1B | Abs | 0.775 | 0.755 | 0.822 | / | 0.794 | / | |
| | | Rela | 0.302 | 0.283 | 0.294 | / | 0.289 | / | |
| Combined | | | No info | No info | / | No info | / | No info | Weight but not info |

- **Conclusion:**

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

In the EOGRTS (Anonymous, 2020), although no adverse effects on mating performance and fertility were observed, male reproductive organ were affected by the treatment. Furthermore, the combined study (Anonymous, 2012), which has a reliability of 1 in the registration dossier, revealed histopathological effects such as oligospermia, presence of seminiferous cell debris and degeneration/depletion of spermatocytes (up to massive).

Based on the available results and the disturbance of the male reproductive system, a classification as **Repr. 1B for fertility** is suggested.

### 10.10.4 Adverse effects on development

**Table 35: Summary table of animal studies on adverse effects on development**

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| Prenatal developmental toxicity study<br>Oral (gavage) | 3,5-DMP<br>Purity: 99.98 %<br>Conc.: 0, 20, 60 and 200 mg/kg | Dams:<br>No mortality nor treatment-related clinical signs. | Anonymous, 2018 |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| Wistar rats<br>22 females/group<br>OECD TG 414<br>GLP<br>Rel. 1 (mentioned in the registration dossier) | bw/d<br>Duration of exposure: GD 6 to 20 | Bw not sign. modified but lower at GD 15 and 20 at the highest dose. BWG sign. lower at the highest dose (see Table 36).<br>1 F in control group was not pregnant and 1 of the highest dose had no viable fetuses.<br>% of post-implantation loss: 3.6, 8.9, 6.0 and 10.7 %, resp. at 0, 20, 60 and 200 mg/kg bw/d).<br>Necropsy: no treatment-related macroscopic findings.<br>Gravid uterus weight + tot weight change (D 3-21): lower but not sign. At 200 mg/kg bw/d (approx. -9.5 % for gravid weight and -16.5 % for tot weight change compared to control group). Corrected bw change (D 3-21) of 39.2, 36.3, 35.3 and 27.7 g, resp. at 0, 20, 60 and 200 mg/kg b/w<br>Pups:<br>Mean nb of live pups: 11, 9, 10 and 10 pups, resp. at 0, 20, 60 and 200 mg/kg bw/d.<br>Mean fetal weight: reduced of approx. 10 % at the highest dose (see Table 38).<br>Higher incidence of malformations and variations observed at 200 mg/kg bw/d | |
| EOGRTS with DNT and DIT cohorts<br>Gavage<br>Rat (Wistar)<br>25 rats/sex/group in the F0 generation<br>20 rats/sex/group in the cohort 1A and 1B<br>10 rats/sex/group in the cohort 2A, 2B and 3<br>OECD TG 443<br>GLP<br>Rel. 1 (mentioned in the registration dossier) | 3,5-dimethylpyrazole<br>Purity: 99.98 %<br>Conc.: 0, 20, 50 and 100 mg/kg bw/d<br>Duration of exposure:<br>- F0 generation: up to 127 days in M (10 w prior mating, during pairing and until 42/43 days post-pairing) and up to 122 days in F (10 w prior pairing, during pairing and gestation and until LD 21)<br>- F1 pups: PND 14 to 21<br>- C1A: up to 73 days<br>- C1B: up 96 days for M and up to 75 days prior pairing, during | Results presented in Table 9 | Anonymous, 2020 |

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| | pairing and gestation and until LD 4 for F<br>- C2A: up to 56 days<br>- C2B: 1 day<br>- C3: up to 62 days | | |
| Combined repeated dose toxicity with the reproduction/developmental toxicity screening test<br>Oral (gavage)<br>Wistar rats<br>10/sex/group<br>OECD TG 422<br>GLP<br>Rel. 1 in the registration dossier. However, full study report not made available to DS | 3,5-DMP<br>Purity: unspecified<br>Conc.: 0, 20, 60 and 200 mg/kg bw/d<br>Duration of exposure: 29 to 31 days in M (2 w prior mating, during mating and up to the day prior scheduled necropsy) and 45 to 56 days in F (2 w prior mating, during pairing, gestation and until lactation day 4) | Results described in Table 9 | Anonymous, 2012 |

No human data or other studies available.

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

In a prenatal developmental toxicity study (Anonymous, 2018), groups of 22 female Wistar rats were exposed to 3,5-dimethylpyrazole at a concentration of 0, 20, 60 and 200 mg/kg bw/d. Animals received the test substance from gestation day 6 to gestation day 20, and were sacrificed to gestation day 21.

During the study period, no mortality occurred and no treatment-related clinical signs were observed as thinning fur, minimal lesions, fur staining and pale teeth were noted in control and treated groups. As observed in Table 36, body weight gain (GD 6 to 21) was significantly lower at the highest dose compared to control group.

**Table 36: Body weight (in g)**

| Dose level (in mg/kg bw/d) | 0 | 20 | 50 | 200 |
|---|---|---|---|---|
| GD 3 | 207.5 | 211.1 | 208.4 | 208.2 |
| GD 6 | 220.2 | 222.9 | 218.7 | 221.5 |
| GD 15 | 258.4 | 256.0 | 253.4 | 243.5 |
| GD 21 | 322.5 | 314.2 | 314.4 | 302.4 |
| BWG GD 6-21 | 102.3 (n= 22) | 91.3 (n= 21) | 95.6 (n= 22) | 80.9*** (n=21) |

N: nb of animals examined

The number of pregnant females was of 22, 21, 22 and 21, resp. at 0, 20, 60 and 200 mg/kg bw/d, as 1 female of the low dose group was not pregnant and 1 female of the highest had no viable fetuses. The percentage of post-implantation was higher in all treated groups (3.6, 8.9, 6.0 and 10.7 %, resp. at 0, 20, 60 and 200 mg/kg bw/d), however the modification was not dose-related.

At necropsy, no treatment-related macroscopic findings were observed. Gravid uterus weight was reduced in treated groups as well as the total weight change (D 3-21) (see Table 37).

**Table 37: Body weight change and uterus weight (in g)**

| Dose level (in mg/kg bw/d) | 0 | 20 | 60 | 200 |
|---|---|---|---|---|
| Nb animals examined | 22 | 21 | 22 | 21 |
| Gravid uterine weight | 72.0 | 63.1 | 67.3 | 65.2 |
| Corrected bw (carcass weight) | 246.7 | 247.5 | 243.7 | 235.9 |
| Corrected weight change D 3-21 | 39.2 | 36.3 | 35.3 | 27.7 |
| Tot. weight change D 3-21 | 111.2 | 99.4 | 102.6 | 92.9 |

Litters were examined and did not demonstrate significant changes as the mean number of live pups was of 11, 9, 10 and 10 pups, resp. at 0, 20, 60 and 200 mg/kg bw/d. As observed in Table 38, mean fetal weight was reduced at the highest dose group.

**Table 38: Mean fetal weight (in g)**

| Dose level (in mg/kg bw/d) | 0 | 20 | 60 | 200 |
|---|---|---|---|---|
| Mean fetal weight | 5.17 | 5.12 | 5.18 | 4.62 |
| Mean male fetuses weight | 5.27 | 5.31 | 5.35 | 4.74 |
| Mean female fetuses weight | 4.99 | 4.89 | 5.02 | 4.53 |

A higher incidence of fetuses with malformations was observed at the highest dose compared to control groups (16 fetuses from 9 litters vs 4 fetuses from 4 litters). Supernumerary digit/polydactyly was noted in 3 fetuses from 2 litters of the highest dose. Bent scapula blade was noted in 1 fetus of the low dose group and in 6 fetuses from 3 litters of the highest dose. Furthermore, diaphragmatic cyst and short humerus were observed in litters of the highest dose (no more information about incidence). Other malformations were observed and considered to be unrelated to treatment as the incidence was more important in control group or in low dose group (malformations in the sternebra, kidney hydronephrosis, ventricular septum defect of the heart associated major blood vessels).

Variations were also noted during fetal observation. As observed in Table 39, a significant increased incidence of umbilical artery variation, sutural bones, abnormal pelvic gridle alignment, additional ossification site on the sternebra, incomplete ossification on vertebra and on metatarsal.

**Table 39: Incidence of variation**

| Dose level (in mg/kg bw/d) | | | 0 | 20 | 60 | 200 | HCD[A] |
|---|---|---|---|---|---|---|---|
| Blood vessel | Umbilical artery – left sided | % litter | 41 | 43 | 45 | 86** | 59.34 |
| | | % fetal | 8.54 | 9.41 | 10.74 | 29.27 | 17.09 |
| Skull | Presphenoid – incomplete ossification | % litter | 5 | 15 | 0 | 24 | NP |
| | | % fetal | 0.79 | 3.00 | 0.00 | 6.63 | |
| | Suture – sutural bone | % litter | 5 | 5 | 0 | 33* | NP |
| | | % fetal | 0.68 | 1.67 | 0.00 | 7.30 | |
| Pelvic gridle | Iliac alignment – abnormal | % litter | 19 | 20 | 18 | 95*** | 15 |
| | | % fetal | 3.62 | 3.83 | 5.91 | 89.59 | 5 |
| Sternebra | Additional ossification site | % litter | 0 | 0 | 0 | 24* | 0.82 |
| | | % fetal | 0.00 | 0.00 | 0.00 | 7.30 | 0.15 |
| | Incomplete ossification | % litter | 0 | 10 | 5 | 38** | 14.50 |
| | | % fetal | 0.00 | 1.83 | 1.82 | 18.87 | 3.43 |
| Vertebra | Cervical centrum – incomplete | % litter | 43 | 55 | 36 | 81* | 37.50 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | ossification | % fetal | 10.60 | 18.08 | 12.66 | 30.71 | 10.67 |
| | Cervical centrum - unossified | % litter | 67 | 80 | 45 | 86 | 30 |
| | | % fetal | 26.29 | 37.17 | 19.70 | 59.16 | 9.59 |
| | Thoracic centrum – incomplete ossification | % litter | 19 | 5 | 14 | 48 | 27.50 |
| | | % fetal | 4.85 | 0.83 | 2.47 | 12.32 | 6.11 |
| Hindlimb | Metatarsal – incomplete ossification | % litter | 5 | 10 | 14 | 33* | 17.50 |
| | | % fetal | 0.95 | 3.00 | 2.69 | 8.15 | 4.02 |
| | Metatarsal - unossified | % litter | 19 | 30 | 32 | 43 | 2.50 |
| | | % fetal | 3.89 | 8.33 | 11.82 | 16.43 | 0.63 |

[A]: no information available e.g. time range, laboratories, species

An extended-one generation reproductive toxicity study (Anonymous, 2020) was performed, method and results are presented in section 10.10.2.

A combined repeated dose toxicity with the reproduction/developmental toxicity screening test (Anonymous, 2012) was performed, method and results are described in section 10.10.2.

### 10.10.6 Comparison with the CLP criteria

**Table 40: Comparison with the CLP criteria regarding development**

| Criteria for Category 1 | Criteria for category 2 |
|---|---|
| "Known or presumed human reproductive toxicant<br><br>Substances are classified in category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human (category 1A) or from animal data (category 1B).<br><br>Category 1A: known human reproductive toxicant. The classification is largely based on evidence from humans<br><br>Category 1B: presumed human reproductive toxicant. The classification is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in category 2 may be more | "Suspected human reproductive toxicant<br><br>Substances are classified in category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in category 1. If deficiencies in the study make the quality of evidence less convincing, category 2 could be the more appropriate classification.<br><br>Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects." |

| | |
|---|---|
| appropriate.” | |

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

In the combined repeated dose toxicity study (Anonymous, 2012), higher pups mortality was observed at the highest tested dose (200 mg/kg bw/d). Indeed, 3, 3, 1 and 13 pups were found dead or went missing during the first day of lactation at respectively 0, 20, 60 and 200 mg/kg bw/d. At the highest dose, 7 pups out of 13 were attributable to one female who had a total litter loss by day 3. DS did not have more information about this dam. Furthermore, a treatment-related effect on pups body weight was mentioned in the registration dossier as a significant lower body weight was observed on lactation day 4 (7.3** g at the highest dose vs 9.1 g in control group). The conclusion available in the registration dossier indicate *“Under the conditions of the test developmental toxicity was observed at 200 mg/kg, based on treatment related effects observed on pup mortality (postnatal loss) and lower pup body weights at 200 mg/kg. No treatment-related changes were noted in any of the remaining developmental parameters investigated in this study, i.e. gestation index and duration, parturition, maternal care and clinical signs and macroscopy of pup. Based on the observed results the developmental NOAEL was determined to be 60 mg/kg b.w./day. On the basis of the effects observed, the material should be classified as Repr. Cat. 2: H361: Suspected of damaging fertility or the unborn child, in accordance with Regulation (EC) No. 1272/2008.”.*

In the F1 pups generation of the EOGRTS (Anonymous, 2020), a slightly higher incidence of pup deaths and the higher number of missing pups (presumed cannibalized) of the highest dose (11 vs 3 in control group) were observed. At the highest dose, all pups of one litter died. Viability index as well as survival index tend to decrease at the highest dose (viability: 92 % vs 99 % in control ; survival: 95 % vs 99 % in control) compared with controls (99 %). In the F2 pups generation, viability index tend to decrease at the highest dose (100, 100, 99 and 95 %, resp. at 0, 20, 50 and 100 mg/kg bw/d). At this dose, 2 pups died and 6 pups were missing at PND 4 (compared to only 1 in control group).

In 2 different studies, higher pups mortality was observed at the highest doses (which were 200 mg/kg bw/d for both studies).

Moreover, in the prenatal developmental toxicity study (Anonymous, 2018), the number of malformations and variations was increased at the highest dose (200 mg/kg bw/d). Regarding observed malformations (supernumerary digit/polydactyly, bent scapula blade, diaphragmatic cyst, short humerus), 16 fetuses from 9 litters exhibited them compared to only 4 fetuses from 4 litters in the control group. Furthermore, few types of variations were significantly observed at the highest dose and the incidence was outside the historical control data (left sided umbilical artery, abnormal iliac alignment, additional ossification site in sternebra, incomplete ossification in sternebra, incomplete ossification in vertebra).

The conclusion of the study mention in the registration dossier indicate that *“Test article-related effects on the maternal animal and subsequent litters following 200 mg/kg/day administration were considered adverse. Maternal effects following 20 or 60 mg/kg/day were considered not to represent an adverse health effect, and no effect on the fetuses was evident. As such, the maternal and fetal No Observed Adverse Effect Level (NOAEL) is considered to be 60 mg/kg/day.”.*

As mentioned in the CLP Regulation (version 5.0 – July 2017),

*“Annex I: 3.7.2.4.2. Based on pragmatic observation, maternal toxicity may, depending on severity, influence development via non-specific secondary mechanisms, producing effects such as depressed foetal weight, retarded ossification, and possibly resorptions and certain malformations in some strains of certain species. However, the limited number of studies which have investigated the relationship between developmental effects and general maternal toxicity have failed to demonstrate a consistent, reproducible relationship across species.* ***Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity.*** *Moreover,*

*classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant postnatal functional deficiencies.*

*Annex I: 3.7.2.4.3. Classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally-mediated mechanism has been demonstrated. In such a case, classification in Category 2 may be considered more appropriate than Category 1. However, when a substance is so toxic that maternal death or severe inanition results, or the dams are prostrate and incapable of nursing the pups, it is reasonable to assume that developmental toxicity is produced solely as a secondary consequence of maternal toxicity and discount the developmental effects. Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity."*

In the 2 available studies, no marked systemic toxicity (such as lethality, dramatic reduction in absolute body weight, coma) can explain the higher pups mortality or the increased incidence of malformations/variations. Furthermore, the effects were observed at relatively low doses.

Based on the available results, DS is of the opinion that maternal toxicity is not severe enough to explain the observed effects on pups mortality or the malformations/variations. In conclusion, a classification as **Repr. 1B for development** is suggested.

### 10.10.7 Adverse effects on or via lactation

**Table 41: Summary table of animal studies on effects on or via lactation**

| Method, guideline, deviations if any, species, strain, sex, no/group | Test substance, dose levels duration of exposure | Results | Reference |
|---|---|---|---|
| EOGRTS with DNT and DIT cohorts<br>Gavage<br>Rat (Wistar)<br>25 rats/sex/group in the F0 generation<br>20 rats/sex/group in the cohort 1A and 1B<br>10 rats/sex/group in the cohort 2A, 2B and 3<br>OECD TG 443<br>GLP<br>Rel. 1 (mentioned in the registration dossier) | 3,5-dimethylpyrazole<br>Purity: 99.98 %<br>Conc.: 0, 20, 50 and 100 mg/kg bw/d<br>Duration of exposure:<br>- F0 generation: up to 127 days in M (10w prior mating, during pairing and until 42/43 days post-pairing) and up to 122 days in F (10w prior pairing, during pairing and gestation and until LD 21)<br>- F1 pups: PND 14 to 21<br>- C1A: up to 73 days<br>- C1B: up 96 days for M and up to 75 days prior pairing, during pairing and gestation and until LD 4 for F<br>- C2A: up to 56 days<br>- C2B: 1 day<br>- C3: up to 62 days | Results presented in Table 9 | Anonymous, 2020 |

No human data or other data available.

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

An extended-one generation reproductive toxicity study (Anonymous, 2020) was performed, method and results are presented in section 10.10.2.

### 10.10.9 Comparison with the CLP criteria

Based on the CLP Regulation and the Table 3.7.1 (b):

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

*(a) human evidence indicating a hazard to babies during the lactation period; and/or*

*(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or*

*(c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.*"

No human evidence is available regarding 3,5-dimethylpyrazole.

Only one available study, the EOGRTS (Anonymous, 2020), examined pups until weaning. During the lactation period, pups body weight decreased at the highest dose (100 mg/kg bw/d). As observed in Table 42, the reduction was higher at the end of the lactation period.

**Table 42: Pups body weight during the lactation period in F1 pups (in g)**

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| Dose level (in mg/kg bw/d) | 0 | 20 | 50 | 100 | 0 | 20 | 50 | 100 |
| D 1 | 7.00 | 7.02 | 7.05 | 6.95 (- 0.7 %) | 6.72 | 6.75 | 6.66 | 6.72 |
| D 4 | 10.69 | 10.65 | 10.39 | 10.29 (- 3.7 %) | 10.43 | 10.35 | 9.86 | 10.01 (- 4.02 %) |
| D 7 | 15.50 | 15.73 | 14.91 | 14.84 (- 4.25 %) | 15.16 | 15.28 | 14.41 | 14.48 (- 4.48 %) |
| D 14 | 28.75 | 29.43 | 27.67 | 27.24 (- 5.25 %) | 28.17 | 28.63 | 26.99 | 26.79 (- 4.89 %) |
| D 20 | 43.72 | 44.29 | 41.98 | 41.14 (- 5.9 %) | 43.27 | 43.47 | 41.15 | 40.91 (- 5.45 %) |

In the registration dossier, a toxicokinetic study is mentioned and conclude that *"The fate of the test material when administered orally was shown to have no potential for bioaccumulation. The test material was determined to be completely absorbed in the gastrointestinal tract, converted into four metabolites and almost entirely excreted via the urine within 24 hours."*.

The available information is **not enough to warrant a classification for lactation**.

### 10.10.10 Conclusion on classification and labelling for reproductive toxicity

Based on the available results, a classification as **Repr. 1B H360FD** is warranted.

## 10.11 Specific target organ toxicity-single exposure

Hazard class not assessed in this dossier.

## 10.12 Specific target organ toxicity-repeated exposure

**Table 43: Summary table of animal studies on STOT RE**

| **Method, guideline, deviations if any, species, strain, sex, no/group** | **Test substance, route of exposure, dose levels, duration of exposure** | **Results** | **Reference** |
|---|---|---|---|
| Combined repeated dose toxicity with the reproduction/developmental toxicity screening test<br>Gavage<br>Rat (Wistar)<br>10 animals/sex/group<br>OECD TG 422<br>GLP<br>Rel. 1 in the registration dossier. However, full study report not made available to DS. | 3,5-dimethylpyrazole<br>Purity: unspecified<br>Conc.: 0, 20, 60 and 200 mg/kg bw/d<br>Duration of exposure: 29 to 31 days in M (2 w prior mating, during mating and up to the day prior scheduled necropsy) and 45 to 56 days in F (2 w prior mating, during pairing, gestation and up to LD 4) | Parental generation:<br>Mortality: 1 female euthanized (due to total litter loss)<br>Bw: in M: slightly lower at the highest dose on PMD 8 and during the entire mating period.<br>In F: bwg sign. reduced at the highest dose on PMD 8, PCD 11 and slightly lower on PCD 7, 14 to 17 and 20.<br>Necropsy: in M, lower FBW, testes, seminal vesicles, prostate and epididymides weight + findings at the histopathology in thymus, liver, spleen, epididymides and testes. | Anonymous, 2012 |

| | | | |
|---|---|---|---|
| EOGRTS with DNT and DIT cohorts<br>Gavage<br>Rat (Wistar)<br>25 rats/sex/group in the F0 generation<br>20 rats/sex/group in the cohort 1A and 1B<br>10 rats/sex/group in the cohort 2A, 2B and 3<br>OECD TG 443<br>GLP<br>Rel. 1 | 3,5-dimethylpyrazole<br>Purity: 99.98 %<br>Conc.: 0, 20, 50 and 100 mg/kg bw/d<br>Duration of exposure:<br>- F0 generation: up to 127 days in M (10 w prior mating, during pairing and until 42/43 days post-pairing) and up to 122 days in F (10 w prior pairing, during pairing and gestation and until LD 21)<br>- F1 pups: PND 14 to 21<br>- C1A: up to 73 days<br>- C1B: up 96 days for M and up to 75 days prior pairing, during pairing and gestation and until LD 4 for F<br>- C2A: up to 56 days<br>- C2B: 1 day<br>- C3: up to 62 days | F0:<br>Bw: not significantly changed (see Table 10 and Table 11).<br>Haematological effects already observed at the lowest dose in males and at the mid dose in females.<br>Necropsy: adrenal, spleen and prostate weight were sign. modified (see Table 14).<br>Histology: higher incidence of degeneration/regeneration centrilobular of hepatocytes in all male treated groups and at the mid and high doses in females.<br>+ prostate: contraction in 5 males at 100 mg/kg bw/d and lower secretion<br>+ spleen: increased incidence of splenic pigment<br>Cohort 1A:<br>Bw: not modified (see Table 19).<br>Haematological effects observed (see Table 20).<br>Necropsy: Kidneys, liver, pituitary and seminal vesical weight sign. modified (see Table 22).<br>Histology: increased incidence of degeneration centrilobular of hepatocytes in all treated groups.<br>+ seminal vesicles: increased incidence of contraction and decreased glandular secretions<br>+ spleen: increased incidence of splenic pigment<br>Immunophenotyping (see Table 24): % of spleen CD161+ sign. lower.<br>Cohort 1B:<br>Bw: sign. higher in F at the mid dose group during gestation and lactation periods (see Table 25).<br>Necropsy: seminal vesicle and ovary weight sign. affected (see Table 26).<br>Cohort 2A:<br>Bw: not sign. affected (see Table 28).<br>Cohort 2B:<br>Clinical examination: no treatment-related effects.<br>Bw: unaffected.<br>Necropsy: no effects observed.<br>Cohort 3:<br>Bw: unaffected.<br>Changes in the secondary IgM/IgG response to KLH.<br>FBW and spleen weight: unaffected. | Anonymous, 2020 |

No human data or other studies available.

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

An extended-one generation reproductive toxicity study (Anonymous, 2020) was performed, method and results are presented in section 10.10.2.

A combined repeated dose toxicity with the reproduction/developmental toxicity screening test (Anonymous, 2012) was performed, method and results are presented in section 10.10.2.

**Table 44: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days**

| Study reference | Effective dose (mg/kg/d) | Length of exposure | Extrapolated effective dose to 90-day exposure | Classification supported by the study |
|---|---|---|---|---|
| **Liver Toxicity** | | | | |
| EOGRTS (Anonymous, 2020)<br>Parental generation | Centrilob degen./regen. of hepatocytes in all treated groups (in M: inc and severity dose-related)<br>In males: 50 mg/kg bw/d (almost every M affected) | M: approx. 127 days | Approx. 38 mg/kg bw/d | STOT RE 2 |
| EOGRTS (Anonymous, 2020)<br>Cohort 1A | Centrilob degen in M + F already at 20 mg/kg bw/d<br>( + Hepatic enzymes sign increased at the highest dose in M<br>Liver weight sign higher at the 2 highest dose in F) | 73 days | Approx. 24 mg/kg bw/d | STOT RE 2 |
| Combined repeated dose toxicity with the reproduction/developmental toxicity screening test (Anonymous, 2012) | Liver effects (hepatocellular basophilia + apoptosis/single cell necrosis)<br>60 mg/kg bw/d in M | Approx. 30 days in M | 20 mg/kg bw/d | STOT RE 2 |
| **Blood – Haematological system** | | | | |
| EOGRTS (Anonymous, 2020)<br>Parental generation | Haematological parameters modified and microscopic modification in spleen already observed at the | M: approx. 127 days<br>F: approx. 122 days | Approx 15 mg/kg bw/d | STOT RE 2 |

| Study reference | Effective dose (mg/kg/d) | Length of exposure | Extrapolated effective dose to 90-day exposure | Classification supported by the study |
|---|---|---|---|---|
| | lowest dose<br>20 mg/kg bw/d | | | |
| EOGRTS (Anonymous, 2020)<br>Cohort 1A | Haematological parameters modified and microscopic modification in spleen already observed at the lowest dose<br>20 mg/kg bw/d | 73 days in both sexes | Approx. 24 mg/kg bw/d | STOT RE 2 |
| Combined repeated dose toxicity with the reproduction/developmental toxicity screening test (Anonymous, 2012) | Hematopoietic foci in 6 M out of 6 exposed to 200 mg/kg bw/d (No more information about the other tested dose).<br>Hematology not examined | Approx. 30 days in M<br>45 to 56 days in F | In M: 66.6 mg/kg bw/d<br>In F: between 100 to 125 mg/kg bw/d | STOT RE 2 for M<br>In F: borderline |

### 10.12.2 Comparison with the CLP criteria

**Table 45: Comparison with the CLP criteria regarding STOT RE**

<table>
<tr><th>Criteria for STOT RE 1</th><th>Criteria for STOT RE 2</th></tr>
<tr><td>“Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure.<br><br>Substance are classified in category 1 for target organ toxicity (repeat exposure) on the basis of:<br>▪ Reliable and good quality evidence from human cases or epidemiological studies; or<br>▪ Observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations.”<br><br>“Classification in category 1 is applicable, when significant toxic effects observed in a 90-day repeated dose study conducted in experimental animals are seen to occur at or below the guidance value (C) as indicated in table 3.9.2”<br><table><tr><td>Route of exposure</td><td>Units</td><td>Guidance value</td></tr></table></td><td>Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure.<br><br>Substances are classified in category 2 for target toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.”<br><br>“Classification in category 2 is applicable, when significant toxic effects observed in a 90-day repeated dose study conducted in experimental animals are seen to occur within the guidance value range as indicated in table 3.9.3”<br><table><tr><td>Route of exposure</td><td>Units</td><td>Guidance value range</td></tr><tr><td>Oral (rat)</td><td>mg/kg bw/d</td><td>10 < C ≤ 100</td></tr></table></td></tr>
</table>

| | | | | | |
|---|---|---|---|---|---|
| | Oral (rat) | mg/kg bw/d | C≤10 | | |

- **Liver:**

In the EOGRTS (Anonymous, 2020), the parental generation was exposed during approximately 127 days for females and 122 days males. A classification in category 2 is warranted when an organ is disrupted after an exposure period of 90 days and the concentration range is between 10 and 100 mg/kg bw/d. Based on the exposure period in the parental generation, the extrapolated dose range was comprised between, 7 and 70 mg/kg bw/d in males and 7.3 and 73 mg/kg bw/d in females.

As observed in Table 46, centrilobular degeneration/regeneration of hepatocytes was observed already at the lowest dose (20 mg/kg bw/d). Furthermore, incidence and severity increased in a dose-related manner in males. At the mid dose (50 mg/kg bw/d), almost all males (24 males out of 25) were affected by this change.

Regarding the cohort 1A, animals of both sexes were exposed to the test substance during 73 days. Based on this duration of exposure, extrapolated dose range to classify in category 2 was comprised between 12.3 and 123 mg/kg bw/d. This range cover all the tested dose.

As observed in Table 46, AST and ALT were significantly increased in males exposed to the highest dose (100 mg/kg bw/d). These 2 enzymes are markers of hepatic disruption. In males, AST was dose-dependently increased. In females, enzymes did not exhibit any modifications, however absolute and relative liver weights were significantly higher in the 2 highest dose groups. As in the parental generation, degeneration centrilobular was observed in nearly all animals in all treated groups.

In the combined reproductive toxicity study (Anonymous, 2012), males were exposed during approximately 30 days. Based on this duration of exposure, extrapolated dose range, corresponding to a classification in category 2, is comprised between 30 and 300 mg/kg bw/d. While, females were exposed during a study period of 55 days. The extrapolated dose range in females was then of 16.4 to 164 mg/kg bw/d.

The registration dossier mention that enzymes were not examined and no information was available about liver weight. However, histopathology was described and revealed hepatocellular basophilia as well as apoptosis/single cell necrosis in males exposed to 60 and 200 mg/kg bw/d and in females exposed to 200 mg/kg bw/d. Apoptosis and single cell necrosis severity was noted as “up to marked”. Furthermore, hepatocellular karyomegaly was noted in all males exposed to 200 mg/kg bw/d. Registration dossier conclude that *“males at this dose level* (60 mg/kg bw/d) *had toxicologically relevant liver findings at the microscopic examination.”*.

These 2 available studies described liver toxicity at doses corresponding to a classification in Category 2.

**In conclusion,** based on the available results, a **classification as STOT RE cat. 2 for liver** is warranted.

**Table 46: Summary of liver parameters and effects observed**

| | | | In males | | | | | | In females | | | | | | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dose level (in mg/kg bw/d) | | | 0 | 20 | 50 | 60 | 100 | 200 | 0 | 20 | 50 | 60 | 100 | 200 | |
| **Biological examination** | | | | | | | | | | | | | | | |
| EOGRTS | P0 | AST | 68 | 64 | 64 | / | 80 | / | 108 | 108 | 120 | / | 119 | / | |
| | | ALT | 54 | 51 | 52 | / | 73* | / | 69 | 70 | 78 | / | 80 | / | |
| | | ALP | 70 | 67 | 63 | / | 74 | / | 74 | 69 | 71 | / | 62 | / | |
| | C1A | AST | 71 | 73 | 79 | / | 90*** | / | 65 | 71 | 76 | / | 66 | / | |
| | | ALT | 44 | 49 | 48 | / | 60* | / | 38 | 48 | 41 | / | 39 | / | |
| | | ALP | 107 | 97 | 91 | / | 86 | / | 57 | 51 | 51 | / | 47 | / | |
| | C1B | | NE | NE | NE | / | NE | / | NE | NE | NE | / | NE | / | |
| Combined | | | NE | NE | / | NE | / | NE | NE | NE | / | NE | / | NE | |
| **Organ weight (g or %)** | | | | | | | | | | | | | | | |
| EOGRTS | P0 | Abs | 10.861 | 11.177 | 11.261 | / | 10.398 | / | 9.689 | 9.755 | 9.880 | / | 9.958 | / | |
| | | Rela | 2.511 | 2.525 | 2.506 | / | 2.389 | / | 3.869 | 3.899 | 3.872 | / | 4.013 | / | |
| | C1A | Abs | 10.051 | 10.207 | 10.120 | / | 10.334 | / | 5.601 | 5.719 | 6.182** | / | 6.690*** | / | |
| | | Rela | 2.8913 | 2.9273 | 2.9175 | / | 2.9675 | / | 2.7104 | 2.8117 | 2.9774*** | / | 3.2163*** | / | |
| | C1B | Abs | NE | NE | NE | / | NE | / | NE | NE | NE | / | NE | / | |
| | | Rela | NE | NE | NE | / | NE | / | NE | NE | NE | / | NE | / | |
| Combined | | | NI | NI | / | NI | / | NI | NI | NI | / | NI | / | NI | |
| **Histopathological findings** | | | | | | | | | | | | | | | |
| EOGRTS | P0 | Centrilob degen./regen. of hepatocytes | 0/24 | 11/24 | 24/25 (18 of grade 1 + 6 of | / | 24/24 (10 of grade 1 + 11 of | / | 0/24 | 3/23 | 12/24 (all grade 1) | / | 11/21 (all of grade 1) | / | ↘ inc and severity<br>Dose- |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | grade 2) | | grade 2 + 3 of grade 3) | | | | | | | | related<br>More sever in M |
| | | Hepatocellular karyomegaly/multinucleation | 0/24 | 0/24 | 0/25 | / | 0/24 | / | 1/25 | 1/25 | 2/25 | / | 6/23 | / | ↘ inc in F |
| | C1 A | Degen centrilob of hepatocytes (minimal to slight) | 1/20 | 16/20 | 19/20 | / | 18/20 | / | 0/20 | 17/20 | 17/20 | / | 17/20 | / | ↘ inc |
| | C1 B | | | | | | | | | | | | | | No effects |
| Combined | | All liver findings | NI | NI | / | Hepato-cellular basophilia (up to slight) and/or apoptosis/single cell necrosis (up to marked) in the area directly around the central veins | / | Hepato-cellular basophilia (up to slight) and/or apoptosis/single cell necrosis (up to marked) in the area directly around the central veins<br>+ hepatocellular karyomegaly (in 5M/5)<br>+ midzonal hepatocellular vacuolation (in 3M/5) | NI | NI | / | NI | / | Hepato-cellular basophilia (up to slight) and/or apoptosis/single cell necrosis (up to marked) in the area directly around the central veins | |

- **Haematology system:**

Hematological parameters were only examined in the EOGRTS (Anonymous, 2020). In this study, examination was performed in the parental generation and in the cohort 1A. Significant modification was observed in both generations and in both sexes.

**As observed in**

Table 47, platelet count was dose-dependently and significantly increased in both sexes and in both generations. Change was already significantly modified at the lowest dose in males of the cohort 1A.

Furthermore, signs of anemia were noted as:

- Hemoglobin was reduced in both sexes and both generations. The reduction was significant in males of the parental generation and already observed at 20 mg/kg bw/d. In females of this generation as well as in females of the C1A, a decrease was observed but it was not significant.
- RBC was also significantly reduced in the parental generation. The significant change was already observed in males exposed to 20 mg/kg bw/d and in females exposed to 50 mg/kg bw/d. In the C1A, the decrease was more pronounced and dose-related in females, while in male, it was significant only at 100 mg/kg bw/d.
- PCV was also affected and the modification was significant in males of the parental generation already at 20 mg/kg bw/d.
- MCV and MCH were also significantly modified in both generation.
- Plt significantly increased in both sexes. At the end of the study period (D 85), mean Plt level of the highest dose group was significantly higher of approx. more than 21 - 26 %, resp.in females and males, compared to the control group. Same effect was already observed at the post-pairing D 43 for males and lactation day 22 for females
- PT and APTS exhibited also significant changes.

**Table 47: Summary of haematological parameters**

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| Dose level (in mg/kg bw/d) | 0 | 20 | 50 | 100 | 0 | 20 | 50 | 100 |
| | At post-pairing day 43 | | | | At LD 22 | | | |
| Hb (g/dL) | 15.0 | 14.4* (- 4.0 %) | 14.2** (- 5.33 %) | 14.2** (- 5.33 %) | 15.6 | 15.6 | 15.3 | 15.1 (- 3.2 %) |
| RBC ($10^{12}$/L) | 8.74 | 8.23* (- 5.8 %) | 8.16** (- 6.64 %) | 7.64*** **(- 12.59 %)** | 8.11 | 8.13 | 7.50* (- 7.52 %) | 6.99*** **(- 13.81 %)** |
| PCV (%) | 44.8 | 42.3** | 42.8* (- 4.46 %) | 42.9* (- 4.24 %) | 47.3 | 47.5 | 45.4 | 45.9 (- 2.96 %) |
| MCV (fL) | 51.0 | 51.5 | 52.4* (+ 2.75 %) | 56.3*** **(+ 10.39 %)** | 58.3 | 58.4 | 60.7* (+ 4.12 %) | 65.7*** **(+ 12.69 %)** |
| MCH (pg) | 17.1 | 17.6 | 17.4 | 18.7*** (+ 9.36 %) | 19.3 | 19.3 | 20.4* (+ 5.7 %) | 21.6*** **(+ 11.92 %)** |
| MCHC (g/dL) | 33.6 | 34.1 | 33.3 | 33.2 | 33.0 | 33.0 | 33.6 | 33.0 |
| Ret (%) | 2.1 | 2.3 | 2.6** | 3.0*** | 2.4 | 2.4 | 3.4 | 3.5 |
| Plt ($10^9$/L) | 763 | 810 | 865 **(+ 13.36 %)** | 912** **(+ 19.53 %)** | 1007 | 1025 | 1041 | 1156* **(+ 14.8 %)** |
| PT (sec) | 23.1 | 22.1 | 23.6 | 28.1*** **(+ 21.65 %)** | 22.9 | 23.0 | 23.3 | 23.3 |
| APTS (sec) | 17.2 | 17.3 | 17.4 | 17.7 | 16.2 | 16.2 | 16.4 | 17.3 (+ 6.79 %) |
| | At D 85/87 | | | | At D 85 | | | |
| Hb (g/dL) | 14.7 | 14.6 | 14.3 | 14.5 | 14.1 | 13.9 | 13.7 | 13.5 (- 4.26 %) |
| RBC ($10^{12}$/L) | 8.18 | 8.18 | 8.08 | 7.78* (- 4.89 %) | 7.82 | 7.47 | 7.24** (- 7.42 %) | 6.82 **(- 12.79 %)** |
| PCV (%) | 43.8 | 43.4 | 42.9 | 43.2 | 43.2 | 42.5 | 42.1 | 42.4 |
| MCV (fL) | 53.6 | 53.1 | 53.1 | 55.7* (+ 3.92 %) | 55.4 | 57.0 | 58.2** (+ 5.05 %) | 62.2*** **(+ 12.27 %)** |
| MCH (pg) | 18.0 | 17.8 | 17.6 | 18.6 | 18.0 | 18.6 | 18.9 (+ 5.0 %) | 19.8*** **(+ 10.0 %)** |
| MCHC (g/dL) | 33.6 | 33.6 | 33.2 | 33.4 | 32.6 | 32.7 | 32.5 | 31.9 |
| Ret (%) | 2.5 | 2.7 | 3.0** | 3.2*** | 3.1 | 3.7* | 3.6 | 4.0** |
| Plt ($10^9$/L) | 760 | 868** **(+ 14.2 %)** | 876** **(+ 15.26 %)** | 958*** **(+ 26.05 %)** | 777 | 848 (+ 9.14 %) | 893* **(+ 14.93 %)** | 946** **(+ 21.75 %)** |
| PT (sec) | 21.3 | 20.9 | 21.7 | 23.8*** **(+ 11.74 %)** | 22.8 | 23.1 | 23.0 | 23.4 |

[04.01-MF-003.01]

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| APTS (sec) | 15.3 | 16.2 | 15.6 | 16.5 (+ 7.84 %) | 15.4 | 16.0 | 16.4 (+ 6.49 %) | 17.5** **(+ 13.64 %)** |

Hematological examination in the EOGRTS study revealed that many parameters (Plt, PT, APTS, MCV, MCH, RBC) were disrupted by the test substance. These parameters were not examined in the combined repeated dose toxicity study (Anonymous, 2012).

Furthermore, at necropsy, spleen showed also modification which supported that the test substance affected the hematological system. In the EOGRTS (Anonymous, 2020), in the parental generation as well as in the cohort 1A, an increased incidence and severity of presence of splenic pigment. was observed already at the lowest dose (20 mg/kg bw/d). Splenic pigment was characterized by yellow-brown, granular pigment within the splenic red pulp. In the combined repeated dose toxicity study (Anonymous, 2012), the registration dossier only mentioned a higher severity of hematopoietic foci in males exposed to 200 mg/kg bw/d (observed in 6 out of 6 males and a modification which was described as up to marked), no information was available regarding the other tested doses.

Based on the effects observed in the EOGRTS (Anonymous, 2020), a classification is warranted. Furthermore, spleen was also affected in the combined repeated dose toxicity study (Anonymous, 2012) at a tested dose borderline to a classification in category 2. However, the available information in the registration dossier doesn't allow to conclude on the lower tested dose.

**In conclusion,** based on the available results, a **classification as STOT RE cat. 2 for blood** is suggested.

### 10.12.3 Conclusion on classification and labelling for STOT RE

Based on the liver and haematological toxicity observed in 2 different studies, a classification as **STOT RE Cat. 2 H373 (liver, blood)** is suggested.

### 10.13 Aspiration hazard

Hazard class not assessed in this dossier.

## 11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in this CLH dossier.

## 12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this CLH dossier.

## 13 ADDITIONAL LABELLING

Not applicable.

## 14 REFERENCES

Dewitt J.B. *et al.*, 1953, Relationship between chemical structure and toxic action on rats, Chemical biological coordination centre, review no. 5 national research council, Washington D.C.

Full study reports

Registration dossier: https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/5791/1/1

## 15 ABBREVIATIONS

*: $p < 0.05$

**: $p < 0.01$

***: $p < 0.001$

A/G: albumin/globulin ratio

Abs: absolute

AGD: ano-genital distance

APTS: activated partial thromboplastin time

ALP: alkaline phosphatase

ALT: alanine aminotransferase

Approx.: approximately

AST: aspartate aminotransferase

ATE: acute toxicity estimated

Baso: basophils

Bw: body weight

Bwg: body weight gain

Cat.: category

Centrilob: centrilobular

Conc.: concentration

Create: creatinine

Degen: degeneration

DIT: developmental immunotoxicity

DNT: developmental neurotoxicity

DS: dossier submitter

EOGRTS: extended one generation toxicity study

Eos: eosinophils

F: female

FBW: final body weight

Fib: fibrinogen

GD: gestation day

GLP: good laboratory practice

Hb: haemoglobin

HCD: historical control data

HDW: haemoglobin distribution width

Ig: immunoglobulin

IMT4: imulite total T4

Inc: incidence

Irrit: irritation

KLH: keyhole limpet hemocyanin

LD: lactation day

LD50: lethal dose 50%

Leuc: leucocyte

Lymph: lymphocytes

M: males

MMAD: mass median aerodynamic diameter

MCH: mean cell haemoglobin

MCHC: mean cell haemoglobin concentration

MCV: mean cell volume

Mono: monocytes

MPV: mean platelet volume

Nb: number

NC: not classified

NE: not examined

Neut: neutrophils

NI: no information

No: number

NP: not present

PCD: post-coital day

PCV: packed cell volume

PDW: platelet distribution width

Plt: platelets

PMD: post-mating day

PND: post-natal day

PT: prothrombine time

RBC: red blood cells

RDW: red cell distribution width

Regen: regeneration

Rel.: reliability

Rela: relative

Repr.: reprotoxic

Resp.: respectively

Ret: reticulocytes

SD: Sprague Dawley

Sign.: significant

St. Dev.: standard deviation

STOT RE: specific target organ toxicity – repeated exposure

STOT SE: specific target organ toxicity – single exposure

TBD: to be defined

TG: test guideline

Tot: total

Tot. chol.: total cholesterol

Tot. prot.: total protein

Tox.: toxicity

TSHI: rapid thyroid stimulating hormone

VAP: average patch velocity

VCL: curvilinear velocity

VSL: straight line velocity

WBC: white blood cell

## 16 ANNEXES

Annex I to the CLH report