Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification: piperonal; 1,3-benzodioxole-5-carbaldehyde

EC Number: 204-409-7

CAS Number: 120-57-0

Index Number: 605-RST-VW-Y

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Version number: 2.0 **Date: Apr 2023**

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SUPPORT ON HOW TO COMPILE ANNEX I TO THE CLH REPORT

Annex I to the CLH report may be compiled from DARs, CARs and/or other sources. Non-confidential DAR/CAR can be annexed as such provided that it has sufficient level of details on the studies. The DS is encouraged to remove any irrelevant parts of the DAR/CAR. The DS must ensure that Annex I can be published during PC, i.e., it does not contain any confidential information.

For support, below is an example on how each study could be presented individually under its own subchapter including the study reference, detailed study summary and results. The format of the detailed study summary of an individual study is flexible as long as the summary is clearly reported and under a correct hazard class. Detailed support can be found below under each subchapter. If DAR/CAR is annexed to the CLH report as Annex I, it must be indicated clearly in the evaluation part of the report where in Annex I the relevant study can be found. If read-across to structurally or mechanistically similar substance is used please provide a justification for using data from this substance and, if known, present the calculations to convert dose/concentration levels from the test substance to the substance for which CLH is proposed. Please provide also a justification for providing non-testing data by any other approaches such as quantitative structure-activity relationships (QSARs) or grouping methods. Support on grouping of substances and read-across can be found in the following links:

http://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf

http://echa.europa.eu/documents/10162/13655/pg_report_qsars_en.pdf

http://echa.europa.eu/documents/10162/13655/pg_report_readacross_en.pdf

http://www.qsartoolbox.org/

http://www.oecd.org/chemicalsafety/risk-assessment/groupingofchemicalschemicalcategoriesandread-across.htm

http://echa.europa.eu/en/view-article/-/journal_content/title/assessing-read-across-how-echa-does-it

1 PHYSICAL HAZARDS

Not evaluated as part of this dossier.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Refer to CLH dossier

3 HEALTH HAZARDS

Acute toxicity

3.1 Acute toxicity - oral route

Not evaluated as part of this dossier.

3.2 Acute toxicity - dermal route

Not evaluated as part of this dossier.

3.3 Acute toxicity - inhalation route

Not evaluated as part of this dossier.

3.4 Skin corrosion/irritation

Not evaluated as part of this dossier.

3.5 Serious eye damage/eye irritation

Not evaluated as part of this dossier.

3.6 Respiratory sensitisation

Not evaluated as part of this dossier.

3.7 Skin sensitisation

3.7.1 Animal data

3.7.1.1 Study 1 Guinea Pig Skin Sensitisation test

Study reference: Anonymous, 1978, Guinea Pig Skin Sensitisation test. (Unpublished report).

Detailed study summary and results:

Test type: Similar to OECD 406; Skin Sensitisation Guinea Pig Maximisation Test and Buehler Test. The study pre-dated the adoption of the OECD test guideline and deviated from the test guideline in that;

- No concurrent or laboratory positive control used to demonstrate the reliability of the test system

- There was no data and/or information reported for animal source, housing, acclimation period or environmental conditions
- 4 control animals tested
- The induction injection protocol did not follow the OECD test guideline
- The topical application challenge was carried out on day 14 rather than day 20-22 as per OECD test guideline
- Scoring system different to that advised by OECD test guideline
- There was limited reporting of methods and results

GLP compliance not specified. Unpublished study.

Test substance

- *Name:* Heliotropin (Piperonal; 1,3-benzodioxole-5-carbaldehyde), Identical to substance identified in CLH dossier
- *Degree of purity:* 100%
- *Impurities:* No information available
- *Batch number:* No information available
- *Test substance formulation:* No information available

Test animals

- *Species/strain/sex:* Guinea pigs, strain not specified, male and female.
- *No. of animals per sex per dose:* 10 animals in treatment group (4 males and 6 females) and 4 animals in control groups (different control groups used for each challenge application).
- *Age and weight at the study initiation:*
  - *Age*: No information available
  - *Body weight*: approximately 320g

Administration/exposure

- *Vehicle:*
  - Intradermal; 6% acetone, 20% polyethylene glycol, and 0.01% saline
  - Topical; Acetone
- *Positive and Negative control:*
  - Positive control: None.
  - Negative control: The study report indicates that treated and untreated controls were included for each challenge, but no further details are provided.
- *Range finding study:*
  - <u>Intradermal</u>

    Four males were administered 0.1 ml intradermal injections of 0.05%, 0.1%, 0.25%, 0.5% and 1% test substance in 0.01% saline. A separate group of four males were administered 0.1 ml intradermal injections of 1%, 2%, 3% and 4% test substance in 6% acetone, 20% polyethylene glycol, and 0.01% saline. Animals were monitored after 24 hours for reactions. At ≥0.1% there were signs of irritation (an increase in incidence of "faint pink" reactions) and at ≥1% there were signs of "oedema". The study report indicates that 1.5% was selected for the main study as it was considered to be "suitably irritant". No further details are provided.

- Topical
  Four males were administered 5%, 10%, and 25% test substance in acetone on 8mm filter paper in 11mm aluminium patch test cups to shaved flanks for 24 hours. Four females were similarly treated with 40%, 60%, and 80% test substance in acetone. Treatment sites were examined after 24 and 48 hours. No irritation was observed. 80% concentration was selected for topical induction and challenge phase in main study.

- *Induction*
  - Day 0 – Test animals received six 0.1ml intradermal injections in a 2x4 cm area of the shoulder region:
    - 0.1 ml FCA (Freunds Complete Adjuvant)
    - 0.1 ml of 1.5% test substance in 6% acetone, 20% polyethylene glycol and 0.01% saline
    - No further details on induction injection protocol were provided
  - Day 7- Test animals received a topical application of an occluded patch of 80% test substance in acetone over the injection site. The length of the treatment period was not stated.
- *Challenge*
  - Day 14- Test animals received a topical application of an occluded patch of 80% test substance in acetone on one flank for 24 hours.
  - Day 21- Test animals received a topical application of an occluded patch of 80% test substance in acetone on one flank for 24 hours
  - Day 28- Test animals received a topical application of an occluded patch of 80% test substance in acetone on one flank for 24 hours
  - Day 42- Test animals received a topical application of an occluded patch of 20% or 80% test substance in acetone on one flank for 24 hours
- *Assessment*: Dermal assessment of all animals performed 24 and 48 hours after removal of the challenge patches
- *Grading system used:* A five-point scale (0, +/-, +, ++ and +++) was used to grade skin reactions:
  - 0= no reaction
  - +/- = barely perceptible erythema
  - + = scattered, mild erythema (faint pink)
  - ++ = moderate and diffuse erythema (pale pink)
  - +++ = intense erythema (deep pink) and oedema
- *Statistical methods:* No information available
- *Historical control Data:* No information available

Results and discussion

24 hours after the challenge on day 14, positive reactions were reported in 2/10 animals (20%) in the test group: 1/10 with “scattered, mild erythema” and 1/10 with “moderate and diffuse erythema”. 48 hours post challenge, positive reactions were reported in 4/10 animals (40%) of the test group: 3/10 with “moderate and diffuse erythema” and 1/10 with “intense erythema and oedema”. No positive reactions were reported in the treated or untreated controls at either time point.

Table 1: Summary of the skin sensitisation reactions in the guinea pig maximisation test challenge on day 14 with Heliotropin (Anonymous, 1978)

Group	No. of animals	Time (hours)	Dermal scores					
			No reaction	Barely perceptible erythema	Scattered, mild erythema (faint pink)	Moderate & diffuse erythema (pale pink)	Intense erythema (deep pink) & oedema	Total no. of positive reactions
Test substance	10	24	4	4	1	1	0	2
	10	48	4	2	3	0	1	4
Treated control (females)	4	24	0	0	0	0	0	0
	4	48	0	0	0	0	0	0
Untreated control (males)	4	24	0	0	0	0	0	0
	4	48	0	0	0	0	0	0

Three further challenges were performed at 1, 2 and 4 weeks after the original challenge (i.e., day 21, 28 and 42). At days 21 and 28, positive reactions were observed in 4/10 (40%) at 24 and 48 hours following each challenge. At day 42, positive reactions were observed at 20% test substance in 4/10 (40%) at 48 hours and at 80% test substance in 1/10 (10%) and 2/10 (20%) at 24 and 48 hours, respectively. No positive reactions were reported in the treated or untreated controls in either challenge. The study author notes that the animals with positive reactions following the day 14 challenge continued to have these reactions on days 21 and 28 to 80% test substance and on day 42 to 20% test substance. Based on the results of this study, the REACH registrants apply a self-classification as skin sensitiser category 1B.

The dossier submitter notes that the available guinea pig maximisation study has some limitations. In particular, there was no concurrent or laboratory positive control used to demonstrate the reliability of the test system, only 4 control animals were used, the induction protocol did not follow the OECD test guideline, the scoring system used was different to that advised by the OECD test guideline and there was limited reporting of the methods and results. Despite these limitations, a positivity rate of 40% was observed with a 1.5% intradermal induction dose. The dossier submitter considers that based on the results of this study, piperonal; 1,3-benzodioxole-5-carbaldehyde is a skin sensitiser.

3.7.1.2 Study 2 Maximization Test

Study reference: Klecak G., *et al,* 1977, Screening of fragrance substances for allergenicity in the guinea pig. I. Comparison of four testing methods. (Published report).

Detailed study summary and results:

Test type: Non-guideline study. A Maximization Test. Induction challenge of a 5% emulsion of test substance and Freunds Complete Adjuvant (FCA) administered intradermally in guinea pigs. On day 8, an

occlusive patch was applied to the neck for 48 hours to cause irritation and on day 21, an occlusive patch was applied to the flank for 24 hours. Assessments of the flank were made 24 and 48 hours after the patch was removed. There was limited reporting of methods and results. GLP compliance not specified. Published study.

Test substance

- *Name:* Heliotropin, identical to substance identified in CLH dossier
- *Degree of purity:* No information available
- *Impurities:* No information available
- *Batch number:* No information available
- *Test substance formulation:* No information available

Test animals

- *Species/strain/sex:* Guinea pigs, Himalayan white spotted, male and female.
- *No. of animals per dose:* 6-8. No information available for exact number of animals in each dose group.
- *Age and weight at the study initiation:*
  - *Age*: No information available
  - *Body weight*: approximately 400-500g

Administration/exposure

- *Vehicle:* No information available
- *Positive and Negative control:* No information available for positive or negative control
- *Range finding study:* No information available
- *Induction*
  - Day 0 – Test animals received intradermal injections:
    - 0.1 ml FCA
    - 0.1 ml of 5 % test substance
    - 0.1ml of 5% test substance mixed with FCA
  - Day 8- Test animals received a topical application of an occluded patch of 250mg of test substance dissolved in 25% petrolatum to a clipped skin area of the neck. The patch was kept in place for 48 hours, to cause mild to moderate skin irritation under occlusion.
- *Challenge:* Day 21-Test animals received a topical application of an occluded patch of test substance at a "sub-irritant" concentration in petrolatum on the flank for 24 hours. The challenge concentration was not reported.
- *Assessment*: Dermal assessment of all animals performed 24 and 48 hours after removal of the challenge patches
- *Grading system used:* No information available
- *Statistical methods:* No information available
- *Historical control Data:* No information available

Results and discussion

The study authors reported that the test substance was positive. No further individual animal scores were reported. No further details were available in the study report. The dossier submitter notes that this study had several limitations. There was limited information on each study's methodology, number of animals used, assessment methods, vehicles, positive and negative controls and there were no individual animal result tables reported. The dossier submitter notes that based on the lack of information and result tables in this study, it is difficult to determine if piperonal; 1,3-benzodioxole-5-carbaldehyde is a skin sensitiser. Therefore, the results of this study are provided for supporting evidence only.

3.7.1.3 Study 3 Open Epicutaneous test

Study reference: Klecak G., *et al,* 1977, Screening of fragrance substances for allergenicity in the guinea pig. I. Comparison of four testing methods. (Published report).

Detailed study summary and results:

Test type: Non-guideline study. An Open Epicutaneous test. Induction phase of daily applications of 0.1ml of undiluted test substance to a clipped flank (8cm^2 area) of guinea pigs for 21 days. Challenge phase, on days 21 and 35, 0.025ml of the "minimal irritating concentration" (not further specified) of test substance was applied to the flank (2cm^2 area), assessments were carried out at 24, 48 and 72 hours. There was limited reporting of methods and results. GLP compliance not specified. Published study.

Test substance

- *Name:* Heliotropin, identical to substance identified in CLH dossier
- *Degree of purity:* No information available
- *Impurities:* No information available
- *Batch number:* No information available
- *Test substance formulation:* No information available

Test animals

- *Species/strain/sex:* Guinea pigs, Himalayan white spotted, male and female.
- *No. of animals per dose:* 6-8. No information available for exact number of animals in each dose group.
- *Age and weight at the study initiation:*
  - *Age*: No information available
  - *Body weight*: approximately 400-500g

Administration/exposure

- *Vehicle:* The study reports acetone, ethanol, diethyl phthalate, Vaseline, polyethylene glycol and water were all used as vehicles, but the study does not specify which one was used for the test substance.

- *Positive and Negative control:* No positive control specified. 6-8 animals were untreated as negative controls, no information on the exact number of animals used as negative controls for the test substance.
- *Dose response curve concentrations:* 0.03%, 0.1%, 1%, 3%, 10% and 30%
- *Range finding study:* None reported for method, dose response curve used to determine the tolerance threshold. No specific details on skin reactions for test substance reported.
- *Induction:* Day 0 – Test animals received a topical application of 0.1ml of undiluted test substance and increasingly diluted solutions to an $8cm^2$ area of clipped flank skin. This application was repeated on the same skin area for 21 days and left uncovered for 24 hours
- *Challenge:* Day 21 and 35 – Test animals received a topical application of 0.025ml of the "minimal irritating concentration" of test substance to a $2cm^2$ area. This concentration was not specified
- *Assessment*: Dermal assessment of all animals performed at 24, 48 and 72 hours after application of challenge dose
- *Grading system used:* Number of reactions/ number of animals in group, using a 6-point scale (0, 0.5, 1, 2, 3 and 4)
- *Conclude whether the test substance is positive, negative or equivocal:* Allergenic if 2/8 animals of a concentration exhibited positive reaction
- *Statistical methods:* No information available
- *Historical control Data:* No information available

Results and discussion

The study authors reported that the test substance was positive. A minimum sensitising concentration of 30% and minimum eliciting concentration of 1% was reported. No further individual animal scores were reported. No further details were available in the study report. The dossier submitter notes that this study had several limitations. There was limited information on each study's methodology, number of animals used, assessment methods, vehicles, positive and negative controls and there were no individual animal result tables reported. The dossier submitter notes that based on the lack of information and result tables in this study, it is difficult to determine if piperonal; 1,3-benzodioxole-5-carbaldehyde is a skin sensitiser. Therefore, the results of this study are provided for supporting evidence only.

3.7.1.4 Study 4 Draize test

Study reference: Klecak G., *et al,* 1977, Screening of fragrance substances for allergenicity in the guinea pig. I. Comparison of four testing methods. (Published report).

Detailed study summary and results:

Test type: Non-guideline study. Draize test. Induction phase, 0.05ml of 0.1% test substance in isotonic saline was injected intradermally in guinea pigs, with further intradermal 0.1ml treatments on 9 alternate days. On days 35 and 49, treated and untreated guinea pigs were challenged intradermally, with 0.05ml of 0.1% test substance. The assessment criteria was the "mean diameter of the papular reaction". There was limited reporting of methods and results. GLP compliance not specified. Published study.

Test substance

- *Name:* Heliotropin, identical to substance identified in CLH dossier
- *Degree of purity:* No information available
- *Impurities:* No information available
- *Batch number:* No information available
- *Test substance formulation:* No information available

Test animals

- *Species/strain/sex:* Guinea pigs, Himalayan white spotted, male and female.
- *No. of animals per dose:* 6-8. No information available for exact number of animals in each dose group.
- *Age and weight at the study initiation:*
  - *Age*: No information available
  - *Body weight*: approximately 400-500g

Administration/exposure

- *Vehicle:* Isotonic saline
- *Positive and Negative control:* No positive control specified. An unspecified number of animals were untreated as negative controls
- *Range finding study:* No information available
- *Induction:* Day 0 – Test animals received intradermal injections of 0.05 ml of 0.1% test substance and isotonic saline. Animals received 0.1 ml injections on nine alternate days; the total dose administered was 0.95mg.
- *Challenge:* Day 35 and 49 – Test and control animals received intradermal injections of 0.05 ml of 0.1% solution.
- *Assessment*: "Mean diameter of a papular reaction"
- *Grading system used:* No information available
- *Conclude whether the test substance is positive, negative or equivocal:* No information available
- *Statistical methods:* No information available
- *Historical control Data:* No information available

Results and discussion

The study authors reported that the test substance did not elicit any reaction. No further individual animal scores were reported. No further details were available in the study report. The dossier submitter notes that this study had several limitations. There was limited information on each study's methodology, number of animals used, assessment methods, vehicles, positive and negative controls and there were no individual animal result tables reported. The dossier submitter notes that based on the lack of information and result tables in this study, it is difficult to determine if piperonal; 1,3-benzodioxole-5-carbaldehyde is a skin sensitiser. Therefore, the results of this study are provided for supporting evidence only.

3.7.1.5 Study 5 Freund's Complete Adjuvant Test

Study reference: Klecak G., *et al,* 1977, Screening of fragrance substances for allergenicity in the guinea pig. I. Comparison of four testing methods. (Published report).

Detailed study summary and results:

Test type: Non-guideline study. Freund's Complete Adjuvant Test. Guinea pigs received equal volumes of 0.05ml of undiluted test substance and FCA intradermally to the neck on days 0, 2, 4, 7 and 9. Control animals received 5x0.05ml of FCA. On days 21 and 35, 0.025ml of the "minimal irritating concentration" (not further specified) of test substance was applied to the flank (2cm^2 area). GLP compliance not specified. Published study.

Test substance

- *Name:* Heliotropin, identical to substance identified in CLH dossier
- *Degree of purity:* No information available
- *Impurities:* No information available
- *Batch number:* No information available
- *Test substance formulation:* No information available

Test animals

- *Species/strain/sex:* Guinea pigs, Himalayan white spotted, male and female.
- *No. of animals per dose:* 6-8. No information available for exact number of animals in each dose group.
- *Age and weight at the study initiation:*
  - *Age*: No information available
  - *Body weight*: approximately 400-500g

Administration/exposure

- *Vehicle:* FCA for intradermal injection. No information available for topical application.
- *Positive and Negative control:* No positive control specified. An unspecified number of animals were untreated as negative controls
- *Range finding study:* No information available
- *Induction:* Day 0, 2, 4, 7 and 9 – Test animals received intradermal injections of 0.05ml of equal volumes of the test substance and FCA into the neck region. The total dose administered was 250mg. The control animals received 5 intradermal 0.05ml injections of FCA
- *Challenge:* Day 21 and 35 – Test animals received a topical application of 0.025ml of "minimal irritating concentration" of test substance to a 2cm^2 area. This concentration was not specified
- *Assessment*: No information available
- *Grading system used:* No information available
- *Conclude whether the test substance is positive, negative or equivocal:* No information available
- *Statistical methods:* No information available
- *Historical control Data:* No information available

Results and discussion

The study authors reported that test substance did not elicit any reaction. No further individual animal scores were reported. No further details were available in the study report. The dossier submitter notes that this study had several limitations. There was limited information on each study's methodology, number of animals used, assessment methods, vehicles, positive and negative controls and there were no individual animal result tables reported. The dossier submitter notes that based on the lack of information and result tables in this study, it is difficult to determine if piperonal; 1,3-benzodioxole-5-carbaldehyde is a skin sensitiser. Therefore, the results of this study are provided for supporting evidence only.

3.7.2 Human data

No information available

3.7.3 Other data

No information available

3.8 Germ cell mutagenicity

Not evaluated as part of this dossier.

3.9 Carcinogenicity

Not evaluated as part of this dossier.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Study 1 OECD 422: Combined Screening Test

Study reference: Anonymous, 2020a. Combined Repeated Dose Toxicity Study with Reproduction/Development Toxicity Screening Test of Piperonal or Heliotropine by Oral Gavage in Rats. (Unpublished report).

Detailed study summary and results:

Test type

OECD Guideline 422: Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test. The study deviated from the test guideline in that it used 10 females instead of 12-13, only parental males were fasted prior to blood sampling and pups' thyroxine (T4) blood sampling was carried out on post-natal day (PND) 14-16 not PND 4. GLP compliant. Unpublished study.

Test substance

- *Name:* Piperonal or Heliotropine. Identical to substance identified in CLH dossier.
- *Degree of purity:* 99.9%
- *Impurities:* Not reported
- *Batch number:* 1810017
- *Test substance formulation:* The dosing formulations were prepared weekly.

Test animals

- *Species/strain/sex:* Rat Wistar CrLl. WI (Han), male and female
- *No. of animals per sex per dose:* 10/sex/dose
- *Age and weight at the study initiation*: 7-9 weeks; 194-243g (male) and 140-172g (female)

Administration/exposure

- *Route of administration:* Oral; gavage
- *Duration and frequency of test/exposure period:* Daily administration until at least post-natal day (PND) 13.
- *Doses and rationale for dose level selection:* 0, 100, 300 and 1000 mg/kg bw/day. Dose selection was based on the results of a 10-day dose range finding study in which the test material was administered daily to three female rats via oral gavage at 500 or 1000 mg/kg bw/day. No deaths were observed. 1/3 females at 500 mg/kg bw/day had fluid in their uterus and 1/3 females at 1000 mg/kg bw/day had a diaphragmatic hernia in their liver during macroscopic examinations. In females at 1000 mg/kg bw/day, there was an increase in absolute and relative liver weights and absolute kidney weights.
- *Control group and treatment:* 10 animals/sex administered polyethylene glycol (PEG) 400 via oral (gavage)
- *Vehicle and rationale:* PEG 400.
- *Actual doses (mg/kg bw/day):* 0, 100, 300 and 1000 mg/kg bw/day.

Description of test design:

- *Details on mating procedure:* Male:female 1:1. The length of cohabitation was up to 14 days. Proof of pregnancy was determined by vaginal plug or sperm in vaginal smear. If pregnancy was not achieved in 14 days, a second male with proven fertility replaced the male.
- *Premating exposure period for males and females (P and F1)*: 10 weeks
- *Dosing schedule:*
  - Males were treated once daily, 7 days/week, for a minimum of 10 weeks pre-mating period and a 2-week mating period
  - Females were treated once daily, 7 days/week for a minimum of 10 weeks pre-mating period, a 2-week mating period, throughout the gestation period and until at least PND 13
- *Parameters assessed for parental animals:*
  - Clinical and mortality observations: 1/day and 2/day, respectively
  - Body weight: Monitored weekly up to mating in male and females. In females monitored on days 0, 4, 7, 11, 14, 17 and 20 after mating and on PND 1, 4, 7 and 17. A terminal weight for all animals was measured on day of necropsy

  - Food consumption: Monitored weekly up to mating in male and females. In females monitored on days 0, 4, 7, 11, 14, 17 and 20 after mating and during lactation on PND 1, 4, 7 and 17
  - Ophthalmoscpic examinations: Assessed pre-dosing and during week 13 in males and the lactation period in females
  - Functional tests: Hearing ability, pupillary reflex, static righting reflex, fore and hind-limb grip strength and locomotor activity assessed in males at week 13 and females during PND 6-13
  - Haematology and clinical chemistry: Blood samples collected on day of necropsy
  - Thyroid hormones: Blood samples collected on day of necropsy and analysed for Triiodothyronine (T3), Thyroxine (T4), and Thyroid Stimulating Hormone (TSH).

- *Oestrous cycle:* Daily examinations of vaginal cytology of all females from 14 days before mating until evidence of mating.
- *Post mortem examinations:*
  - Males were sacrificed at a minimum of 13 weeks
  - Females who successfully delivered a litter were sacrificed on PND 14-16
  - A full post mortem was carried out on all animals and the reproductive organs were the focus of the examination. Organs and tissue were weighed and microscopically examined.
- *Assessment of male reproductive organs:* At necropsy, testis and epididymis were weighed. Spermatogenesis was assessed at 0 and 1000 mg/kg bw/day.
- *Parameters assessed for F1:* Pups were monitored daily for clinical observations, general health and mortality. The number of live and dead pups were recorded on PND 1 and daily until end of study. Live pups were weighed on PND 1, 4, 7 and 13. All pups were sexed on PND 1 and 4. Anogenital distance (AGD) was determined for live pups on PND 1. Male pups' nipples/areolae were determined on PND 13.
- *Standardisation of litters:* Litters standardised on PND 4 to 8 pups/litter (4/sex/litter).
- *Post exposure observation period*: None.
- *Historical control data*: The study report provides historical control data (HCD) for the time range from 2015-2019.
- *Statistical methods:* Datasets were compared using Dunnett test (t-test) and group means were compared using parametric (ANOVA) tests coupled with the Bonferroni correction for several testing. Non-parametric datasets were analysed using the Steel test and the Fisher exact test.

Results and discussion

Observations of effects in Parental Generation

- *Mortality:* 1/10 male at 100 mg/kg bw/day died on day 21 of premating. At necropsy, isolated dark red foci in glandular mucosa of stomach, enlarged liver, watery clear fluid in the thoracic cavity and autolysis of several organs were observed but the cause of death was not established. 1/10 females at 1000 mg/kg bw/day died on lactation day (LD) 1. Total litter loss was observed but there were no abnormalities found at necropsy. All other animals survived to scheduled sacrifice.
- *Clinical signs:* Slight salivation was observed from week 2 in animals treated with ≥ 300mg/kg bw/day and from week 8 in all treated animals.
- *Body weight:*
  Males: At 1000 mg/kg bw/day, a statistically significant decrease in mean body weight was observed from week 7 of premating through to the end of the mating period (week 14). At week 14, the mean body weight in this group was 352g compared with 401g in the control. At 1000 mg/kg bw/day, there was also a statistically significant decrease in mean body weight gain from week 4 of pre-mating until the end of the mating period. The mean body weight gain at week 14 in this group was 58g

compared with 82g in the control group. Males at 1000 mg/kg bw/day also had a statistically significant decrease in mean body weight at end of treatment check, the mean body weights were 375, 385, 364 and 322g at 0, 100, 300 and 1000 mg/kg bw/day, respectively.
Females: No effect on mean body weight or body weight gain was noted during the pre-mating or mating periods. At 1000 mg/kg bw/day, there was a decrease in mean body weight observed from gestation day (GD) 14, which reached statistical significance on GD 17 and 20. The mean body weight on GD 17 was 284g at 1000 mg/kg bw/day compared to 306g in the control group and the mean body weight on GD 20 was 291g at 1000 mg/kg bw/day compared to 348g in the control group. At 1000 mg/kg bw/day, there was also a decrease in mean body weight gain from GD 14 with significance on GD 20. The mean body weight gain at GD 20 in this group was 22g compared to 44g in the control group. The study authors notes that the change in body weight at 1000 mg/kg bw/day could be related to abnormal pregnancies observed in four females. Three females only had implantation sites and one female had complete litter loss on lactation day (LD) 1. During the lactation period, there was no effect on mean body weight gain at ≤300 mg/kg bw/day and no data for females at 1000mg/kg bw/day. The mean terminal body weights were measured on PND 14-16 for females who delivered a litter, on gestation day 25-38 for females who failed to deliver and 24 hours after last pup was found dead for females with complete litter loss. Overall, there was a statistically significant decrease in mean terminal body weight in females at 1000 mg/kg (16% lower than controls). The mean terminal body weights were 292, 279, 282 and 244g at 0, 100, 300 and 1000 mg/kg bw/day, respectively.

Table 2: Female mean body weight (g) data measured during Combined Repeated Dose Toxicity Study with Reproduction/Development Toxicity Screening Test of Piperonal or Heliotropine by Oral Gavage in Rats. (Anonymous 2020a).

Dose (mg/kg bw/day)	0	100	300	1000
Gestation Period	Mean body weight (g)			
GD 0	242±13.1	237±16.7	233±15.2	244±5.1
GD 4	254±11.5	249±14.1	250±11.1	261±11.5
GD 7	261±10.8	256±10.4	252±17.7	267±13.6
GD 11	273±12.0	267±11.2	270±14.2	275±9.7
GD 14	283±12.8	278±11.8	282±15.7	276±3.7
GD 17	306±13.9	298±10.8	305±19.1	284±9.8*
GD 20	348±18.4	334±18.0	344±23.2	291±21.0**
GD 27	290±13.2	282±14.9	280±7.4	265±--
GD 34	-	261±--	-	261±--

*p<0.05; **p < 0.01, --; N= 1 no St. Dev, –; no data, N=0

- *Food consumption:*
  Males: No effect observed
  Females: No effect during the pre-mating period. Food consumption was statistically significantly increased on GD 11-14 at 300 mg/kg bw/day (26g compared to 23g in the control) and 1000 mg/kg bw/day at GD 4-7 (26g compared to 20g in the control). Food consumption relative to body weight was also statistically significantly increased on GD 11-14 and LD 4-13 at 300 mg/kg bw/day and on GD 4-7 and GD 14-20 at 1000 mg/kg bw/day. There was no data reported for females at 1000mg/kg bw/day during the lactation period.

- *Water consumption:* No information reported.
- *Functional observation battery:*
  Males: A significant decrease in mean total movement was observed at 1000 mg/kg bw/day, although it is noted that while all treated males exhibited total movement less than the concurrent control, the values were within the historical control range of the laboratory (Wistar Han rats, 90-day study, N; 424, mean; 3609, range; 1990 – 5497). Therefore, the biological significance of the effect was unclear. No effect on mean ambulation was observed in males.
  Females: A significant increase in mean total movement and mean ambulation was observed at 100mg/kg bw/day. In the absence of a dose response, this effect was not considered treatment related. No effects on hearing ability, pupillary reflex, static righting reflex, fore and hind limb grip strength were reported.
- *Ophthalmological examination:* No effect observed.
- *Haematology:* At 1000 mg/kg bw/day, a statistically significant decrease in eosinophils was observed in males and females and a statistically decrease in neutrophils was observed in females.
  In males at 1000 mg/kg bw/day, the mean number of reticulocytes was statistically significantly increased. In females at 1000 mg/kg bw/day, a statistically significant decrease in haematocrit and haemoglobin was observed. There was a non-statistically significant decrease in the mean number of red blood cells, with the study authors noted that the mean value ($8.06 \pm 0.45 \times 10^{12}$/L) was below the lower limit of the historical control data range from a 90-day repeated dose toxicity studies (Rat Crl:Wl (Han) males, (2018), N; 159, mean; 9.06×10^{12}/L, range; $8.16- 9.86 \times 10^{12}$/L).

  At 1000 mg/kg bw/day, the mean corpuscular volume (MCV) was statistically significantly increased in males and decreased in females, while the mean corpuscular haemoglobin concentration (MCHC) was statistically significantly increased in females. The mean platelet count was statistically significantly decreased in females at 1000 mg/kg bw/day, with a non-statistically significant decrease observed in males at this dose. The study authors reported that the mean platelet count in males at 1000 mg/kg bw/day ($564 \pm 57 \times 10^{9}$/L) was was below the lower limit of the testing laboratory historical control range from a 90-day repeated dose toxicity studies (Rat Crl:Wl (Han) males, (2018), N; 158, mean; 730×10^{9}/L, range; $563-883 \times 10^{9}$/L). The prothrombin time (PT) was also statistically significantly increased in males at this dose.

Table 3: Haematology data measured during the Combined Repeated Dose Toxicity Study with Reproduction/Development Toxicity Screening Test of Piperonal or Heliotropine by Oral Gavage in Rats. (Anonymous 2020a)

Haematology data	Dose (mg/kg bw/day)							
	Males				Females			
	0	100	300	1000	0	100	300	1000
Eosinophils (10^9/L)	0.1±0.0	0.1±0.0	0.1±0.0	0.0±0.0**	0.1±0.0	0.1±0.0	0.1±0.0	0.0±0.0**
Neutrophils (10^9/L)	1.1± 0.2	1.2± 0.5	1.4± 0.6	1.0± 0.3	1.7±0.8	1.3± 0.8	1.6±0.5	0.6± 0.3**
Reticulocytes (10^9/L)	171.1± 24.2	168.2± 9.7	177.2± 22.5	223.1± 49.0*	191.1± 47.1	217.0± 35.4	210.2± 22.2	222.5± 80.9
Haematocrit (L/L)	0.453± 0.031	0.449± 0.039	0.459± 0.037	0.442 ±0.024	0.435± 0.012	0.422± 0.016	0.418± 0.012	0.395± 0.022**
Haemoglobin (mmol/L)	9.7± 0.6	9.6± 0.7	9.8± 0.5	9.3± 0.4	8.9±0.3	8.9±0.3	8.7±0.2	8.5±0.3*
Red blood cells (10^9/L)	8.65± 0.83	8.42± 0.77	8.44± 0.63	8.06± 0.45	7.26± 0.30	7.30± 0.43	6.97± 0.23	7.08± 0.54
MCV (fL)	52.5± 1.9	53.3± 1.5	54.3± 1.0	54.8± 2.2*	59.9± 1.7	57.9± 2.4	60.1± 2.3	55.9± 1.5**
MCHC (mmol/L)	21.37± 0.55	21.40± 0.55	21.39 ± 0.74	21.15 ± 0.43	20.48± 0.28	21.02± 0.48*	20.68± 0.39	21.57± 0.48**
Platelets (10^9/L)	702± 151	661± 67	660± 155	564± 57	733± 138	672±153	682±90	511±118**
PT (S)	17.6± 0.7	16.9± 0.7	16.9± 0.7	18.8± 1.1*	17.6±1.0	17.5±0.6	17.3±1.3	18.1±0.7

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

- *Clinical chemistry:* At 1000 mg/kg bw/day, a statistically significant decrease in mean aspartate aminotransferase activity was observed in females and a statistically significant increase in mean alkaline phosphatase activity in males. At the same dose, mean alanine aminotransferase activity was statistically significantly increased in males and decreased in females.
  Mean cholesterol, HDL cholesterol and LDL cholesterol were all statistically significantly decreased in males at 1000 mg/kg bw/day, with LDL cholesterol also statistically significant decreased in females in this treatment group. At 100 and 1000 mg/kg bw/day, mean albumin levels were statistically significantly increased in females. At 1000 mg/kg bw/day, mean total protein was statistically significantly decreased in males and increased in females.

  At 1000 mg/kg bw/day, mean potassium was statistically significantly increased in males and decreased in females. In males, there was a statistically significant increase in mean chloride at ≥300mg/kg bw/day and in mean inorganic phosphate levels at 1000 mg/kg bw/day. In females, at 1000 mg/kg bw/day there were statistically significant increases in calcium and glucose levels.

In males at 1000 mg/kg bw/day, bile acids were statistically significantly increased. In females, mean urea levels were statistically significantly decreased at ≥ 300 mg/kg bw/day and total bilirubin was statistically significantly increased at 1000 mg/kg bw/day.

Table 4: Measured Clinical Biochemistry data from the Combined Repeated Dose Toxicity Study with Reproduction/Development Toxicity Screening Test of Piperonal or Heliotropine by Oral Gavage in Rats (Anonymous 2020a)

Clinical Biochemistry	Dose (mg/kg bw/day)							
	Males				Females			
	0	100	300	1000	0	100	300	1000
ASAT (U/L)	102.3± 29.4	87.5± 12.5	97.1± 17.8	115.7± 13.0	113.9±21.4	102.8± 11.0	106.5± 24.4	93.2± 10.0*
ALP (U/L)	96± 23	110±34	124±19	192± 75**	232±155	215±174	269±111	153±54
ALAT (U/L)	51.2± 13.7	42.7±5.6	56.4± 12.8	75.3± 20.9**	146.8± 41.2	120.6± 51.1	116.6± 38.4	54.0± 14.8**
Cholesterol (mmol/L)	1.80± 0.34	2.05± 0.32	1.97± 0.25	1.18± 0.37**	1.89± 0.41	2.16± 0.35	2.16± 0.34	1.47± 0.40
HDL Cholesterol (mmol/L)	0.94± 0.13	1.05± 0.16	1.04± 0.10	0.71± 0.17**	0.78± 0.24	0.88± 0.21	0.94± 0.26	0.66± 0.20
LDL Cholesterol (mmol/L)	0.30± 0.03	0.31± 0.05	0.33± 0.09	0.22± 0.03**	0.47± 0.08	0.56± 0.22	0.50± 0.11	0.18± 0.07**
Albumin (g/L)	33.3±1.1	33.2±1.3	33.8±0.8	33.2±1.1	29.4± 2.0	31.7± 2.4*	31.1± 1.8	34.1± 1.1**
Total protein (g/L)	64.7±2.7	64.0±3.6	64.4±2.1	59.4±1.6	54.9± 3.8	58.7± 5.1	57.9± 3.2	63.0 ±2.9**
Potassium (mmol/L)	3.95± 0.17	3.88± 0.19	4.13± 0.28	4.28± 0.24**	4.96± 0.57	4.62± 0.68	4.80± 0.48	3.90± 0.25**
Chloride (mmol/L)	104± 1	104±1	105±1*	106±1**	103±2	103±2	102±1	103±2
Inorganic Phosphate (mmol/L)	1.70± 0.19	1.75± 0.28	1.81± 0.19	2.27± 0.46**	0.91± 0.54	0.64± 0.42	0.72± 0.49	1.36± 0.21
Calcium (mmol/L)	2.57± 0.04	2.62± 0.06	2.59± 0.07	2.53± 0.06	2.41± 0.08	2.50± 0.14	2.48± 0.12	2.60± 0.11**
Glucose (mmol/L)	8.86± 1.03	8.72± 1.68	8.83± 1.08	8.79± 0.99	7.82± 0.92	9.27± 1.97	8.41± 1.74	11.80±1.74 **
Bile acids (µmol/L)	24.8± 14.0	33.5± 14.9	38.9± 17.2	70.9± 54.3**	26.9± 11.7	20.2± 14.7	35.3± 21.3	36.3± 22.5
Urea (mmol/L)	6.5±0.5	6.6±1.1	7.3±1.3	7.7±1.6	10.1±1.2	8.8±1.6	8.5±1.2*	7.1±0.5**
Total bilirubin (µmol/L)	2.3±0.4	2.1±0.3	2.3±0.3	2.5±0.3	2.0±0.3	2.1±0.3	2.1±0.3	2.3±0.2*

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

- *Thyroid hormones:*

  A decrease in total T4 levels was observed in males. The total T4 levels were 4.25, 3.63, 3.05 and 1.57 µg/dl at 0, 100, 300 and 1000 mg/kg bw/day, respectively. While statistical significance was achieved at ≥ 300 mg/kg bw/day, the level at 1000 mg/kg bw/day was the only one outside the test laboratory historical control range ((Rat Crl: Wl (Han) males, (2017-2019), N; 557, mean; 4.51 µg/dl, range; 2.85-6.37 µg/dl). There was no effect noted on total T4 levels in treated females or total T3 and TSH levels in either sex. The dossier submitter notes there was no total T3 value for control females, so it was not possible to do a direct comparison, but the treated females were all within the historical control range of the test laboratory (see table 5).

Table 5: Thyroid hormone data measured during the Combined Repeated Dose Toxicity Study with Reproduction/Development Toxicity Screening Test of Piperonal or Heliotropine by Oral Gavage in Rats. (Anonymous 2020a)

Thyroid hormone	Dose (mg/kg bw/day)									
	Males					Females				
	0	100	300	1000	HCD	0	100	300	1000	HCD
TSH (ulU/mL)	0.145± 0.145	0.130± 0.114	0.104± 0.127	0.185± 0.213	No data	0.240± 0.177	0.278± 0.207	0.271± 0.176	0.204± 0.109	No data
Total T3 (ng/dl)	52.4± 12.1	57.3± 8.5	48.6± 5.8	48.3± 6.5	No data	-	54.6±1.6	46.9±6.2	49.9± 11.0	Mean= 59.7 Range= 42.29-78.91 N=53
Total T4 (µg/dl)	4.25± 0.97	3.63± 0.50	3.05± 0.56**	1.57± 0.50**	Mean= 4.51 Range= 2.85-6.37 N=557	2.38± 0.60	2.49± 056	2.50± 0.64	2.71± 1.04	No data

**p < 0.01, HCD; T3; Rat Crl: Wl (Han) females, 90-day study (2018-2019); T4; Rat Crl: Wl (Han) males, (2017-2019)

- *Organ weights:*

  In males at 1000 mg/kg bw/day, absolute prostate weight was statistically significantly decreased (0.743g compared to 0.956g in control group) and there was a biologically significant decrease in relative prostate weight (0.229g compared to 0.257g in control group). There was also a statistically significant decrease in absolute epididymides weight (0.950g compared to 1.205g in control group) and a biologically significant decrease in relative epididymides weight (0.296g compared to 0.323g in control group).

  In females at 1000 mg/kg bw/day, there was a statistically significant increase in absolute ovary weight (0.171g compared to 0.110g in control group) and relative ovary weight (0.071g compared to 0.038g in control group). At the same dose there was also statistically significant increase in absolute uterus weight (0.960g compared to 0.363g in control group) and relative uterus weight ((0.385g compared to 0.125g in control group).

In both sexes, a statistically significant decrease in absolute pituitary gland weight was observed. In females at ≥ 300mg/kg bw/day (0.0013, 0.012, 0.011 and 0.011 g at 0, 100, 300 and 1000 mg/kg bw/day, respectively and in males at 1000 mg/kg bw/day (0.008 g compared to 0.009 g in control).
A statistically significant decrease in absolute adrenal weight was observed in both sexes at 1000 mg/kg bw/day: in males the mean absolute weight was 0.044g compared to 0.054g in the control and in females, the mean absolute weight was 0.057g compared to 0.073g in the control.
A statistically significant increase in relative kidney weight was observed in males at ≥300 mg/kg bw/day and in females at 1000 mg/kg bw/day. In males, the relative kidney weights were 0.65, 0.66, 0.71 and 0.74g at 0, 100, 300 and 1000 mg/kg bw/day, respectively. In females the relative kidney weights was 0.78 g at 1000 mg/kg bw/day compared to 0.70g in the control.
In females, at 1000 mg/kg bw/day a statistically significant increase was observed in absolute thyroid weight (0.018g compared to 0.015g in the control) and relative thyroid weight (0.007g compared to 0.005g in the control). The same effect was not observed in males.
In males at 1000 mg/kg bw/day, there was a statistically significantly decrease in absolute brain weights (1.96g compared to 2.05g in the control). In males and females at 1000 mg/kg bw/day, relative brain weights were statistically significantly increased, 0.61g compared to 0.55g in control in males and 0.77g compared to 0.66g in the control in females.
At 1000 mg/kg bw/day, absolute liver weights were statistically significantly increased in males (10.21g compared to 8.47 g in control) and decreased in females, (10.56 g compared to 12.55 g in control). Relative liver weights were statistically significantly increased in males at ≥300 mg/kg bw/day (2.25, 2.27, 2.46 and 3.17g at 0, 100, 300 and 1000 mg/kg bw/day, respectively).
In females, at 1000 mg/kg bw/day there was a statistically significant decrease in absolute heart weight (0.803g compared to 0.892g in control). In males, at ≥300mg/kg bw/day, there was a statistically significant increase in relative heart weight (0.260, 0.259, 0.278 and 0.286g at 0, 100, 300 and 1000 mg/kg bw/day, respectively) and in females at 1000 mg/kg bw/day (0.331g compared to 0.306g in control).
In males, at 1000 mg/kg bw/day, there was a statistically significant decrease in absolute thymus weight (0.183g compared to 0.269g in control) and in relative thymus weight (0.057g compared to 0.071g in control). In females, there was an increase from 100mg/kg bw/day in absolute and relative thymus weight with statistical significance achieved at ≥300 mg/kg bw/day. The absolute thymus weights were 0.161, 0.190, 0.227 and 0.233g at 0, 100, 300 and 1000 mg/kg bw/day, respectively. The relative thymus weights were 0.055, 0.068, 0.081 and 0.096g at 0, 100, 300 and 1000 mg/kg bw/day, respectively. The study authors noted that the changes observed in the thymus for both males and females were 'test-item related'. The dossier submitter notes these changes and agrees that these changes may be related to the test substance.

Table 6: Organ weight data measured during the Combined Repeated Dose Toxicity Study with Reproduction/Development Toxicity Screening Test of Piperonal or Heliotropine by Oral Gavage in Rats (Anonymous 2020a)

Organ weights	Dose (mg/kg bw/day)							
	Males				Females			
	0	100	300	1000	0	100	300	1000
Absolute prostate (g)	0.956± 0.123	0.958± 0.180	0.948± 0.108	0.743± 0.171**	-	-	-	-
Relative prostate (g/100 g)	0.257± 0.048	0.248± 0.043	0.261± 0.031	0.229± 0.036	-	-	-	-

Organ weights	Dose (mg/kg bw/day)							
	Males				Females			
	0	100	300	1000	0	100	300	1000
Absolute Epididymides (g)	1.205± 0.068	1.179± 0.099	1.121± 0.088	0.950± 0.073**	-	-	-	-
Relative Epididymides (g/100 g)	0.323± 0.030	0.306± 0.021	0.309± 0.030	0.296± 0.019	-	-	-	-
Absolute ovary (g)	-	-	-	-	0.110± 0.024	0.117± 0.016	0.115± 0.018	0.171± 0.088**
Relative ovary (g/100 g)	-	-	-	-	0.038± 0.009	0.042± 0.008	0.041± 0.007	0.071± 0.039**
Absolute uterus (g)	-	-	-	-	0.363± 0.048	0.435± 0.198	0.518± 0.294	0.960± 0.650**
Relative uterus (g/100 g)	-	-	-	-	0.125± 0.017	0.161± 0.088	0.189± 0.119	0.385± 0.235**
Absolute Brain (g)	2.05± 0.09	2.03± 0.07	2.03± 0.07	1.96± 0.05*	1.91± 0.09	1.89± 0.05	1.93± 0.04	1.87± 0.08
Relative brain (g/100 g)	0.55± 0.04	0.53± 0.03	0.56± 0.03	0.61± 0.05**	0.66± 0.06	0.68± 0.05	0.69± 0.04	0.77± 0.05**
Absolute Pituitary gland (g)	0.009± 0.001	0.009± 0.001	0.009± 0.001	0.008± 0.001**	0.013± 0.002	0.012± 0.002	0.011± 0.002**	0.011± 0.001*
Absolute liver (g)	8.47± 1.14	8.74± 0.89	8.95± 0.76	10.21± 1.06	12.55± 1.14	11.81± 2.06	12.44± 1.78	10.56± 1.37*
Relative liver (g/100 g)	2.25± 0.16	2.27± 0.17	2.46± 0.13*	3.17± 0.21**	4.30± 0.21	4.21± 0.54	4.41± 0.44	4.33± 0.33
Absolute heart (g)	0.971± 0.062	1.000± 0.060	1.015± 0.102	0.919± 0.050	0.892± 0.072	0.839± 0.067	0.886± 0.071	0.803± 0.068*
Relative heart (g/100 g)	0.260± 0.018	0.259± 0.010	0.278± 0.018*	0.286± 0.015**	0.306± 0.010	0.301± 0.014	0.315± 0.011	0.331± 0.025**
Absolute Thyroid(g)	0.019± 0.004	0.017± 0.003	0.018± 0.004	0.016 ±0.003	0.015±0.003	0.015±0.002	0.016±0.003	0.018±0.002*
Relative thyroid (g/100 g)	0.005± 0.001	0.004± 0.001	0.005± 0.001	0.005± 0.001	0.005± 0.001	0.005± 0.001	0.006± 0.001	0.007± 0.001**
Absolute Thymus(g)	0.269±0.051	0.290±0.036	0.236±0.032	0.183±0.057**	0.161±0.038	0.190±0.037	0.227±0.035**	0.233±0.042**
Relative thymus (g/100 g)	0.071± 0.010	0.076± 0.011	0.065± 0.008	0.057± 0.017*	0.055± 0.012	0.068± 0.014	0.081± 0.017**	0.096± 0.019**
Absolute Adrenal (g)	0.054±0.006	0.051±0.007	0.051±0.008	0.044±0.007*	0.073±0.012	0.071±0.011	0.064±0.010	0.057±0.006**
Relative kidneys (g/100 g)	0.65± 0.07	0.66± 0.05	0.71± 0.05*	0.74± 0.05**	0.70± 0.05	0.74± 0.04	0.73± 0.03	0.78± 0.07**

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

- *Macroscopic examination:*
  2/10 females at 1000mg/kg bw/day had discolouration in the adrenal glands. 1/10 males in the same treatment group had nodules in their epididymides.
  The study authors concluded that the gross observations were within the range of background findings for Wistar Hans rats of this age and were not related to the test substance.
- *Histopathology examinations*
  Lymphoid atrophy of the thymus, graded as minimal, was observed in 0/10, 0/10, 0/10 and 4/10 males at 0, 100, 300 and 1000 mg/kg bw/day, respectively and in 2/10, 2/10, 0/10 and 1/10 females at 0, 100, 300 and 1000 mg/kg bw/day, respectively. In females, incidence of cystic epithelial hyperplasia of the thymus were observed. The incidence was 3/10, 3/10, 3/10 and 9/10 at 0, 100, 300 and 1000 mg/kg bw/day, respectively and at the top dose the effects were graded from minimal (4/10) to moderate (1/10).
  At 1000 mg/kg bw/day, incidence of hepatocellular hypertrophy, graded as minimal, were observed in 7/10 males and 1/10 females. The single incidence in females was observed in a female who exhibited total litter loss.
  Increased trabecular bone in both the sternum and femur was observed in males at 1000 mg/kg bw/day and females at ≥300 mg/kg bw/day. The incidence in the sternum were 0/10, 0/10, 1/10 and 7/10 in males and 0/10, 0/10, 2/10 and 10/10 in females at 0, 100, 300 and 1000 mg/kg bw/day, respectively. The incidence in the femur were 0/10, 0/10, 0/10 and 9/10 in males and 0/10, 0/10, 2/10 and 10/10 in females at 0, 100, 300 and 1000 mg/kg bw/day, respectively. The study authors reported the increase in the femur bone was most evident just below the growth plate in the metaphysis region and in the femur head. The increase resulted in a slight decrease in the bone marrow space. The bone adjacent to the intervertebral discs were those most affected in the sternum, and this has previously been related to oestrogen toxicity. This finding was considered adverse in 1000 mg/kg bw/day by the study authors.

Table 7: Summary of microscopic finding from Combined Repeated Dose Toxicity Study with Reproduction/Development Toxicity Screening Test of Piperonal or Heliotropine by Oral Gavage in Rats. (Anonymous 2020a)

Organ Microscopic finding Degree of finding	Dose (mg/kg bw/day)							
	Males				Females			
	0	100	300	1000	0	100	300	1000
Thymus								
Lymphoid atrophy								
Minimal	0	0	0	4	2	2	0	1
Cystic Epithelial hyperplasia								
Minimal	3	1	0	0	3	2	2	4
Slight	0	0	0	0	0	1	1	4
Moderate	0	0	0	0	0	0	0	1
Liver								
Hepatocellular hypertrophy								
Minimal	0	0	0	7	0	0	0	1
Bone-Sternum								
Increased bone								
Minimal	0	0	0	7	0	0	2	1
Slight	0	0	0	0	0	0	0	9
Bone-Femur								
Increased bone, metaphysis								
Minimal	0	0	0	1	0	0	1	0
Slight	0	0	0	6	0	0	1	2
Moderate	0	0	0	2	0	0	0	8

N of litters=10 for all treatment groups

Reproductive parameters

- *Oestrous cycle:* There was no significant effect on the oestrous cycle. 2/10, 1/10, 0/10 and 2/10 at 0, 100, 300 and 1000 mg/kg bw/day, respectively had irregular cycles. 1/10, 1/10, 0/10 and 1/10 at 0, 100, 300 and 1000 mg/kg bw/day, respectively were acyclic.
- *Effects on sperm:* No abnormalities reported for spermatogenesis.
- *Toxic response data including indices of mating, fertility, gestation, live birth, viability, post-implantation survival and lactation, abortions, resorptions; indicate the numbers used in calculating the indices*:
  - There was no effect on pairing and/or mating as all groups exhibited pairing and mating indices of 100%
  - There was a decrease in the fertility index at 1000 mg/kg bw/day. The fertility index was 100%, 90%, 90% and 40% at 0, 100, 300 and 1000 mg/kg bw/day, respectively

- There was a decrease in the gestation index at 1000 mg/kg bw/day. The gestation index was 100%, 89%,100% and 0% at 0, 100, 300 and 1000 mg/kg bw/day, respectively
- A decrease in post implantation survival index[1] was observed at ≥ 300 mg/kg bw/day. The indices observed were 94%, 85%, 81% and 11% at 0, 100, 300 and 1000 mg/kg bw/day, respectively. Post implantation survival indices at ≥ 300 mg/kg bw/day were also outside the test laboratory's historical control range (Rats, Crl:Wl (Hans) females, (2015- 2019), N; 118, mean; 92%, range; 83-98%).
- There was a decrease in the live birth index at 1000 mg/kg bw/day. The live birth index was 100%, 100%, 97% and 0% at 0, 100, 300 and 1000 mg/kg bw/day, respectively
- There was an effect on pup viability at 1000 mg/kg bw/day on PND 1 and 4. The viability indices were 100%, 100%, 99% and 0% at 0, 100, 300 and 1000 mg/kg bw/day, respectively.
- There was no data for lactation index at 1000 mg/kg bw/day as no pup survived past PND 1. There was no effect on lactation index at ≤ 300 mg/kg bw/day on PND 13. The lactation index was 100%, at 0, 100 and 300 mg/kg bw/day treatment groups.
- There was no information available for abortions or resorptions.

- *Precoital interval*: There was no effect on mean pre-coital time. The mean coital time was, 2.3, 2.6, 2.0 and 1.8 days at 0, 100, 300 and 1000 mg/kg bw/day, respectively in the first pairing period. 2/10 females at 1000 mg/kg bw/day did not achieve pregnancy in the first pairing period. These females were re-paired after 14 days, and their mean pre-coital time was 2.0 days.
- *Number of implantations:* There was a decrease in the number of implantation sites at 1000 mg/kg bw/day. The mean number of implantation sites were 12.4, 12.2, 12.3 and 2.3 at 0, 100, 300 and 1000 mg/kg bw/day, respectively.
- *Duration of gestation:* There was no significant effect on the duration of gestation. A statistically significant decrease was observed at 100 mg/kg bw/day, but in the absence of a dose response, this effect was not considered to be treatment related.
- *Parturition and maternal care:* Pregnant females did not exhibit any signs of a difficult or prolonged parturition and there was no indications of abortions or premature births on analysis of pregnant females' cage debris or reduced maternal care.

[1] Post-implantation survival index (%) was calculated as follows; (Total number of offspring born/Total number of uterine implantation sites) * 100

Table 8: Summary of reproductive parameters relevant for sexual function and fertility from the Combined Repeated Dose Toxicity Study with Reproduction/Development Toxicity Screening Test of Piperonal or Heliotropine by Oral Gavage in Rats. (Anonymous 2020a)

Reproductive parameters				
Dose (mg/kg bw/day)	**0**	**100**	**300**	**1000**
Number of litters	10	8	9	1
Mating index (%)	100	100	100	100
Fertility index (%)	100	90	90	40
Gestation index (%)	100	89*	100	0[#]
Mean duration of gestation (days)	21.8±0.4	21.3±0.5*	21.7±0.7	22.0±--
Total implantation sites	124	110	111	9
Mean implantation sites	12.4	12.2	12.3	2.3

*= $p<0.05$; #= No data for litters at 1000mg/kg bw/day; --; N= 1 no St. Dev

Observations in Offspring

- *Number of litters on PND 1:* There was a decrease of litter numbers observed at 1000 mg/kg bw/day. The incidences were 10/10, 8/10, 9/10 and 1/10 at 0, 100, 300 and 1000 mg/kg bw/day, respectively.
- *Mean live litter size on PND 1:* At 1000 mg/kg bw/day, there were no surviving pups at PND 1. The mean live litter size was 11.7, 11.6, 9.7 and 0 living pups/litter at 0, 100, 300 and 1000 mg/kg bw/day, respectively.
- *Number of dead pups on PND 1:* There was only one pup born at 1000 mg/ kg bw/day and this individual pup was dead at the first litter check on PND 1. There is no data for pups at the high dose, past this check, for any other parameter. Three pups in 1/9 litters at 300 mg/kg bw/day did not survive past the first litter check on PND 1, there was no further details on these dead pups. There were no other deaths or complete litter losses seen in the control and/or other treatment groups.
- *Number of live births on PND 1:* There were no surviving pups at 1000 mg/kg bw/day at PND 1. The number of live pups on PND 1 were 117, 93, 87 and 0 at 0, 100, 300 and 1000 mg/kg bw/day.
- *Number of pre- and post-implantation loss:* There was no information available on number of pre and post implantation losses, but the study authors reported post implantation survival index. There was a decrease observed at ≥ 300 mg/kg bw/day. The indices observed were 94%, 85%, 81% and 11% at 0, 100, 300 and 1000 mg/kg bw/day, respectively and the decrease at ≥ 300 mg/kg bw/day were outside the test laboratory's historical control range for Wistar Han rats (2015-2019), N; 118, mean; 92%, range; 83%-98%).
- *Number of live births on PND 4 (before culling):* There were no surviving pups at 1000 mg/kg bw/day at PND 4. One pup at 300 mg/kg bw/day was missing on PND 2. The number of live pups on PND 4 were 117, 93, 86 and 0 at 0, 100, 300 and 1000 mg/kg bw/day.
- *Sex ratio:* As there were no surviving pups at 1000 mg/kg bw/day, the sex ratio could not be assessed for this concentration. There were no differences noted in the male to female ratio at ≤ 300 mg/kg bw/day before culling on PND 4 or on PND 13. *Survival index at weaning (PND 13):* There were no surviving pups at 1000 mg/kg bw/day at PND 13, as no pup at this dose survived past PND 1. At ≤ 300mg/kg bw/day all pups survived from PND 4 (after culling) until PND 13.
- *Clinical observations:* No effects observed

Table 9: Summary of developmental parameters in the offspring from PND 1-13 in the Combined Repeated Dose Toxicity Study with Reproduction/Development Toxicity Screening Test of Piperonal or Heliotropine by Oral Gavage in Rats. (Anonymous 2020a)

Developmental parameters				
Dose (mg/kg bw/day)	**0**	**100**	**300**	**1000**
Total number of pups born	117	93	90	1
Post-implantation survival index (%)	94	85	81	11
Live birth index (%)	100	100	97	0#
Viability index (%)	100	100	99	0#
Lactation index	100	100	100	0#
Number of litters	10	8	9	1
Mean live litter size	11.7±2.3	11.6±1.1	9.7±2.7	0.0#
No. of dead pups on PND 1	0	0	3	1
No. of live pups on PND 1(after littering)	117	93	87	0#
No. of live pups on PND 4 (Before culling)	117	93	86	0#
No. of live pups on PND 4(After culling)	80	64	66	0#
Mean live pups on PND 4 (after culling)	8.0±0.0	8.0±0.0	7.3±1.4	0.0#
No. of live pups on PND 13 (after littering)	80	64	66	0#
Mean live pups on PND 13 (after littering)	8.0±0.0	8.0±0.0	7.3±1.4	0.0#
Sex Ratio M:F	56/44	51/49	57/43	0/0#

#= No data for litters at 1000mg/kg bw/day

- *Mean litter or pup weight by sex:*
  At 1000 mg/kg bw/day, no pup survived past PND 1, so there is no body weight data available for this dose group. In female pups at 100 and 300 mg/kg bw/day there was a statistically significant decreases in mean body weights, (6.5g, 5.8g and 5.9g at 0, 100 and 300 mg/kg bw/day, respectively) on PND 1. However, the mean values were within the test laboratory historical control range for female rats, (Wistar Han (2017-2019) female PND 1, N; 2623, mean; 6.0 g, range; 5.0–7.3 g), therefore, it unclear if these effects were treatment related. There were no effects on male pup body weight, and they were within the test laboratory historical control range for male rats (Wistar Han (2017-2019) male PND 1, N; 2590, mean; 6.0 g, range; 5.3–7.6 g). There were no significant differences noted for pup weights for either sex on PND 4, 7 or 13 at 0, 100 and/or 300 mg/kg bw/day.

Table 10: Pup mean body weights in both sexes on PND 1, 4, 7 and 13 observed in Combined Repeated Dose Toxicity Study with Reproduction/Development Toxicity Screening Test of Piperonal or Heliotropine by Oral Gavage in Rats. (Anonymous 2020a)

Dose (mg/kg bw/day)	**0**	**100**	**300**	**100**
Post-natal development period Sex	**Mean body weight (g)**			
PND 1				
M	6.6±0.4	6.1±0.5	6.1±0.6	-
F	6.5±0.5	5.8±0.4*	5.9±0.6*	-
PND 4		9.4±1.1	9.8±1.3	-
M	10.2±0.9			
		9.2±1.0	9.6±1.3	-
F	10.1±1.1			
PND 7				
M	16.8±1.4	15.9±1.4	16.0±1.7	-
F	16.2±1.7	15.2±1.3	15.5±1.8	-
PND 13				
M	31.2±2.2	30.6±2.0	30.3±2.5	-
F	30.4±2.5	29.7±2.1	29.6±2.7	-

-= No data for 1000mg/kg bw/day, *p < 0.05, N= 10, 8, 9 and 0 litters for 0, 100, 300 and 1000 mg/kg bw/day

- *Anogenital Distance:* No effect observed
- *Areola/Nipple Retention:* No effect observed
- *Clinical Biochemistry (T4 levels):* Pups at 1000 mg/kg bw/day did not survive past PND 1, so there is no T4 data available for this dose group. In male pups, the mean T4 levels were 6.59, 6.50 and 6.93 µg/dL at 0, 100 and 300 mg/kg bw/day, respectively. In female pups, the mean T4 levels were 5.65, 6.16 and 6.26 µg/dL at 0, 100 and 300 mg/kg bw/day, respectively. There was no significance difference observed in either sex.

Table 11: Total T4 levels detected in pups (PND 14-16) in the Combined Repeated Dose Toxicity Study with Reproduction/Development Toxicity Screening Test of Piperonal or Heliotropine by Oral Gavage in Rats. (Anonymous 2020a)

Dose (mg/kg bw/day)	0	100	300	1000	HCD
Sex	Total T4 level (µg/dL)				
M	6.59	6.50	6.93	-	Mean =4.51 Range = 2.85-6.37 (N=557)
F	5.65	6.16	6.26	-	No data for females

-= No data for 1000mg/kg bw/day; HCD; Rats, Crl:Wl (Hans) males F0 animals (2017-2019), N= 10, 8, 9 and 0 litters for 0, 100, 300 and 1000 mg/kg bw/day, respectively.

- *Macroscopic Findings:* No effect observed

3.10.1.2 Study 2 Non-Guideline Reproductive and developmental screening test

Study reference: Vollmuth T.A., Bennett M.B., Hoberman A.M., and Christian M.S., (1990) '*An evaluation of food flavoring ingredients using an in vivo reproductive and developmental toxicity screening test',* Teratology 41(5):597 [Abstract no. P114 & extended abstract], ECHA dissemination site, 2023.

Detailed study summary and results:

Test type

Non-guideline study. 10 female Sprague-Dawley rats per dose received piperonal; 1,3-benzodioxole-5-carbaldehyde at 0, 250, 500 and 1000 mg/kg bw/day via oral gavage for 39 days. Male rats were not treated. Animals were mated 1:1. The parental females were examined for mating, fertility and gestation indices, delivery of litter, number of offspring per litter, oestrous cyclicity, gross lesions and histopathology. The litters were examined for viability, sex, clinical signs, body weight, gross external malformations, and behaviour. There was limited reporting of methods and results. GLP status unknown. Published study abstract to poster presentation.

Test substance

- *Name:* Piperonal; 1,3-benzodioxole-5-carbadehyde. Identical to substance identified in CLH dossier.
- *Degree of purity:* No information available
- *Impurities:* No information available

- *Batch number:* No information available

Test animals

- *Species/strain/sex:* Rats, Sprague-Dawley, male and female
- *No. of animals per sex per dose:* 10 /sex/dose
- *Age and weight at the study initiation:* 60 days. No information available for body weights.

Administration/exposure

- *Route of administration* – oral (gavage)
- *Duration and frequency of test/exposure period:* Females only, daily for 39 days
- *Doses and rationale for dose level selection*: 0, 250, 500 and 1000 mg/kg bw/day. The study summary reports that dose selection was based on historical toxicology data. No further information available.
- *Control group and treatment:* Yes, 10/sex. The study summary reports that the control animals received the vehicle via oral gavage. Two vehicles are specified, methylcellulose or corn oil, without further explanation.

Description of test design:

- *Details on mating procedure:* Animals were mated male: female 1:1 for a maximum of 7 days. Proof of pregnancy determined by vaginal plug or sperm in vaginal smear, and this was referred to as Day 0 of pregnancy.
- *Premating exposure period for males and females (P and F1):* 7 days (females only)
- *Dosing schedule:* Females were treated for 39 days total: 7 days of premating, 7 days of mating, 21 day gestation period and 4 day lactation period. Males were untreated.
- *Parameters assessed for parental animals:* The following parameters were assessed in females;
  - Mortality
  - Clinical observations: Daily, before and within one hour of dosing
  - Body weight: Daily during the dosing period and on days 0, 6, 10, 14, 20, 21 and 25 of assumed gestation and on PND 1 and 4.
  - Food: Daily per animal and mean daily diet consumption was determined.
  - Delivery of a litter
  - Maternal behaviour: observations on PND 1 and 4

  No assessments reported for males.
- *Oestrous cycle length and pattern:* No information available
- *Sperm examination:* Not assessed
- *Assessment of male reproductive organs:* Not assessed
- *Reproductive indices:* The mating index, fertility index, gestation index and number of pups per litter were examined.

- *Parameters assessed for F1:* Pups were examined for viability (daily examinations), sex, clinical signs, body weight and gross external malformations. The interactions between mother and pups were also examined
- *Standardization of litters (yes/no and if yes, how and when):* No information available
- *Necropsy examinations:* Performed in maternal animals and offspring.
  - Dams who failed to deliver a litter on Day 25 of assumed gestation
  - Dams who delivered a litter on PND 4
  - Offspring on PND 4
- *Historical control data if available:* No information available
- *Statistical analysis:* Maternal and pup body weight, maternal body weight changes and food consumption, sex ratio, percentage of mortality per litter were assessed using Bartlett's Test of Homogeneity of Variances and an Analysis of Variance (ANOVA) followed by a Dunnett's Test. Where the data was not homogenous, it was assessed using Fisher's Exact Test or the Kruskal-Wallis Test followed by Dunn's Method of Multiple Comparisons. The Kruskal-Wallis Test was used to assess natural delivery data and the Variance Test of Homogeneity of the Binomial Distribution was used to analyse all proportion data.

Results and discussion

Observations of effects in Parental Generation

- *Mortality:* The robust study summary indicated there was a statistically significant increase in mortality in dams at 1000 mg/kg bw/day. No further details are reported.
- *Clinical signs:* An increased incidence of clinical signs (not further specified) was reported in females at ≥ 500mg/kg bw/day.
- *Body weight:* It was reported that in females at 500 mg/kg bw/day there was a decrease in body weight gain and in females at 1000 mg/kg bw/day there was a statistically significance decrease in body weight. No further details were reported.
- *Food consumption:* A non-statistically significant decrease in food consumption was reported in females at 1000 mg/kg bw/day. No further details reported.
- *Water consumption:* Not assessed
- *Organ weights:* Not assessed
- *Haematology:* Not assessed
- *Clinical biochemistry:* Not assessed
- *Thyroid hormones:* Not assessed
- *Macroscopic examination:* Not assessed
- *Microscopic examination:* Not assessed

Reproductive parameters

- *Oestrous cycle:* No information available.
- *Sperm parameters:* Not assessed
- *Toxic response data including indices of mating, fertility, gestation, birth, viability and lactation; indicate the numbers used in calculating the indices*: A non-statistically significant decrease in the fertility index was reported at 1000 mg/kg bw/day. No further details are reported.
- *Precoital interval:* Not assessed
- *Number of implantations:* Not assessed
- *Pre and post implantation loss:* Not assessed
- *Corpora lutea:* Not assessed

- *Duration of gestation:* No information available
- *Parturition and maternal care:* No information available

Observations in Offspring

- *Number of litters, dead pups, live birth and live litter size on PND 1:* No information available
- *Mortality/Viability index:* It was reported at 1000 mg/kg bw/day, there was an increase in pup mortality and a statistically significant decrease in viability of offspring. No further details are reported.
- *Body weight:* A non-statistically significant decrease in body weight gain during PND 1-4 was reported at ≥ 500mg/kg bw/day. No further details are reported.
- *Sex ratio:* No information available
- *Survival index at weaning:* Not assessed
- *Clinical observations:* No effects observed
- *Anogenital Distance:* Not assessed
- *Areola/Nipple Retention:* Not assessed
- *Organ weights:* Not assessed
- *Clinical Biochemistry (T4 levels):* Not assessed
- *Macroscopic Findings:* Not assessed
- *Microscopic examination:* Not assessed
- *Gross External malformations:* No information available
- *Litter/maternal interactions:* No information available

The dossier submitter notes that this study had limited reporting of methods and results, so this study is only used for supporting evidence.

3.10.1.3 Study 3 OECD 414: Prenatal developmental toxicity

Study reference: Anonymous, 2020b. Prenatal Developmental Toxicity Test of Piperonal or Heliotropine by Oral Gavage in Rats. (Unpublished report).

Detailed study summary and results:

Test type

OECD Guideline 414: Prenatal developmental toxicity. GLP compliant. Unpublished study.

Test substance

- *Name:* Piperonal or Heliotropine. Identical to substance identified in CLH dossier.
- *Degree of purity:* 99.9%
- *Impurities:* Not reported
- *Batch number:* 1810017
- *Test substance formulation:* The dosing formulations were prepared weekly.

Test animals

- *Species/strain/sex:* Rat Wistar CrLl. WI (Han), female
- *No. of animals per sex per dose:* 22/ females/dose
- *Age and weight at the study initiation*: 10-14 weeks, 177-225g

Administration/exposure

- *Route of administration:* oral gavage
- *Duration and frequency of test/exposure period:* Daily administration from gestation day (GD) 6 to 20
- *Doses and rationale for dose level selection:* 0, 100, 300 and 1000 mg/kg bw/day. Dose selection was based on the results of a 10-day dose range finding study for the OECD 422 study and the main OECD 422 study outlined in section 3.10.1.1.
- *Control group and treatment:* Yes, 22 females administered PEG 400 via oral gavage
- *Vehicle and rationale:* PEG 400. Chosen from previous studies as suitable vehicle.
- *Actual doses (mg/kg bw/day):* 0, 100, 300 and 1000 mg/kg bw/day.

Description of test design:

- *Details on mating procedure:* The mating procedure, length of cohabitation or how pregnancy was determined in females were not reported. Pregnant females were received in the lab on pregnancy day 0 or 1.
- *Assessment:*
  - Females were sacrificed on GD 21. The ovaries and uterine were examined for number of corpora lutea and implantation sites, the weight of the uterus was measured, the number and distribution of live and dead foetuses, embryo-foetal deaths and the sex of each foetus were determined. The thyroid gland was weighed, and tissue samples were collected and examined.
  - Foetuses: live foetuses were euthanised and examined for visceral anomalies, thoracic and abdominal changes and the heart, all major vessels and kidneys were examined for abnormalities. Tissues were collected and examined for malformations. Some pups were examined for skeletal abnormalities.
- *Historical control data*: The study report provides historical control data (HCD) for Rat Crl:Wl (Han) (outbred SPF-Quality) for a study data range from 2014-2019.
- *Statistical methods:* Parametric datasets were compared using Dunnett test (t-test) and non-parametric datasets were analysed using the Steel test, Mann Whitney test or Kruskal-Wallis non-parametric ANOVA test. If the ANOVA test showed statistical significance, a Dunn's test was employed to compare the control to the treatment groups.

Results and discussion

Observations of effects in Parental Generation

Mortality: None observed.

Clinical signs: In females, piloerection was observed from GD 14 at 300 mg/kg bw/day (2/22) and from GD 7 at 1000 mg/kg bw/day (15/22). Hunched posture was observed in 2/22 females at 300 and 1000 mg/kg bw/day from GD 13-17. Salivation was observed from GD 1 in 13/22 and 22/22 at 300 and 1000 mg/kg bw/day treated females, respectively.

Body weight and body weight gain: From GD 9-21 there was a statistically significant decrease in mean body weight gain in females at 1000 mg/kg bw/day. The mean body weight gain was 48% in controls compared to 40% at 1000 mg/kg bw/day at GD 21. There were no significant effects observed in mean body weight.

Table 12: Mean body weight gain measured during the Prenatal Developmental Toxicity Study of Piperonal or Heliotropine by Oral Gavage in Rats (Anonymous 2020b)

Dose (mg/kg bw/day)	0	100	300	1000
Gestation Period	**Mean body weight gain (%)**			
GD 6	0±0	0±0	0±0	0±0
GD 9	5±1.4	4±1.5	5±1.6	1±3.0**
GD 12	12±2.3	11±2.7	13±2.2	9±3.0**
GD 15	18±2.8	17±2.3	19±3.0	16±4.2*
GD 18	32±2.8	31±4.9	33±3.9	28±6.4**
GD 21	48±4.8	45±9.5	48±5.8	40±8.8**

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

Food consumption: At 1000 mg/kg bw/day there was a statistically significant decrease in absolute and relative food consumption during GD 6-9 (22% and 19% below control values respectively), and a statistically significant increase in relative food consumption during GD 12-15 (10% above control values) and 15-18 (11% above control values). There were no effects noted in other treatment groups.

Water consumption: Not reported.

Thyroid hormones: Dose-related decreases in maternal T3 and T4 levels were observed with statistical significance achieved at 1000 mg/kg bw/day. Mean total T3 levels were 43.3, 38.2, 36.3 and 28.3 ng/dl at 0, 100, 300 and 1000 mg/kg bw/day, respectively. Mean total T4 levels were 2.35, 2.02, 1.93 and 1.71 µg/dl at 0, 100, 300 and 1000 mg/kg bw/day, respectively. The study authors reported that several of the total T3 values across all dose groups and the total T4 values at 1000 mg/kg/day were below LLOQ and reported as LLOQ/2 and advised that the mean values reported for T3 and T4 at these concentrations should be interpreted with caution. The dossier submitter considers that toxicological significance of the decrease in total T3 and T4 to be unclear. There was an increase observed in mean TSH levels (0.350m 0.384, 0.418 and 0.431 ulU/mL at 0, 100, 300 and 1000 mg/kg bw/day, respectively). The dossier submitter notes that the mean TSH levels in all dose levels are within the historical control range of the test laboratory (Female Rats, Wistar Han (2018-2019), N; 249, mean; 0.381, percentile range; 0.129-0.724 ulU/mL) and in the absence of statistical significance the increase in TSH is not test item related.

Table 13: Thyroid hormone data measured during the Prenatal Developmental Toxicity Study of Piperonal or Heliotropine by Oral Gavage in Rats. (Anonymous 2020b).

Thyroid hormone	Dose (mg/kg bw/day)				
	0	100	300	1000	HCD
TSH (ulU/mL)	0.350±0.212	0.384±0.241	0.418±0.331	0.431±0.199	Mean = 0.381 Percentile Range = 0.1290-0.7240 N= 249
Total T3 (ng/dl)	43.3±15.0	38.2±20.2	36.3±15.3	28.3±17.4*	Mean = 60.3 Percentile Range = 43.20-81.90 N= 216
Total T4 (µg/dl)	2.35±0.50	2.02±0.56	1.93±0.36	1.71±1.33*	Mean = 2.24 Percentile Range = 1.470-3.300 N= 245

* $p < 0.05$, Percentile range = P5-P95; HCD; Female Rats, Wistar Han (2018-2019), N= number of animals tested.

Macroscopic finding: No effects observed
Organ weights: At 1000mg/kg bw/day, there is a biologically significant decrease in mean gravid uterus weight compared to the control. Mean gravid uterus weights were 75.3, 72.6, 74.0 and 61.6g at 0, 100, 300 and 1000 mg/kg bw/day, respectively. There was no further information on uterus weight. There was no effect observed on absolute or relative thyroid weight.
Histopathology examinations: 1/22 and 3/22 females in the control and high dose groups, respectively exhibited grade 1 ultimobranchial cyst in the thyroid gland. Due to the low incidence in both groups and no information on the historical incidence, the relevance to treatment is unclear.

Reproductive parameters
Number of pregnancies: There was no effect observed on the number of pregnancies in the treated females. The number of pregnant females 21/22, 20/22, 20/22 and 22/22 at 0, 100, 300, and 1000 mg/kg bw/day, respectively.
Resorptions: At 1000mg/kg bw/day, there was a biologically significant increase observed in early and late resorptions. The mean number of early resorptions were 4.3%, 3.6%, 3.9% and 13.8 % per litter at 0, 100, 300 and 1000 mg/kg bw/day treated females respectively. The mean early resorptions per litter at 1000 mg/kg bw/day were outside the historical control range of the test laboratory (Rat Crl:WI (Han), (2014–2018), N; 1097, mean; 5.0%, percentile range; 1.9%-9.9%). The mean number of late resorptions were 0% 0%, 0% and 1.1 % per litter at 0, 100, 300 and 1000 mg/kg bw/day, respectively. The mean late resorptions per litter at 1000 mg/kg bw/day were outside the historical control range of the test laboratory (Rat Crl:WI (Han), (2014–2018), N; 1097, mean; 0.1%, percentile range; 0%-0.4%).
Number of implantations: There was no effect observed on the number of implantation sites in treated females. The mean number of implantation sites were in 11.1, 10.9, 11.4 and 11.3 at 0, 100, 300 and 1000 mg/kg bw/day, respectively.
Pre and post implantation loss: There was no effect observed in pre-implantation loss, the mean percentage pre-implantation loss per litter was 6.3%, 7.9%, 5.5% and 6.7% at 0, 100, 300 and 1000 mg/kg bw/day, respectively. There was a biologically significant increase in post-implantation loss at 1000 mg/kg bw/day.

The mean percentage post-implantation loss per litter was 4.3%, 3.6%, 3.9% and 14.9 % at 0, 100, 300 and 1000 mg/kg bw/day, respectively. At 1000 mg/kg bw/day, 2/22 females had only implantation sites, with 100% implantation loss compared with 0/22 females in the control group. It was also noted that the mean post implantation loss per litter at 1000 mg/kg bw/day was outside the historical control range of the test laboratory (Rat Crl:WI (Han), (2014–2018), N; 1097; mean; 5.1%, percentile range; 1.9%-10.1%).
Corpora lutea: There was no effect on corpora lutea. The mean number of corpora lutea were 11.9, 11.5, 12.1 and 11.9 at 0, 100, 300 and 1000 mg/kg bw/day treated females respectively.

Table 14: Maternal reproduction parameters examined during the Prenatal Developmental Toxicity Study of Piperonal or Heliotropine by Oral Gavage in Rats. (Anonymous 2020b).

Reproductive Parameter					
Dose (mg/kg bw/day)	**0**	**100**	**300**	**1000**	**HCD**
Number of pregnant females (%)	21 (95.5)	20 (90.9)	20 (95.2)	22 (100)	Mean % pregnant females- 98.4% Percentile Range= 90.9-100
Mean % of early resorptions per litter	4.3±4.62	3.6±4.56	3.9±5.25	13.8±28.66^{+}	Mean =5.0 Percentile Range=1.9-9.9
Mean % of late resorptions per litter	0±0	0±0	0±0	1.1±2.94^{+}	Mean =0.1 Percentile Range=0.0-0.4
Mean number of implantation sites	11.1±1.37	10.9±2.74	11.4±1.69	11.3±3.21	Mean=11.2 Percentile Range=10.3-12.1
Mean % pre-implantation loss	6.3±8.13	7.9±14.64	5.5±8.76	6.7±11.13	Mean=6.4 Percentile Range=2.1-13.4
Mean % post-implantation loss	4.3±4.62	3.6±4.56	3.9±5.25	14.9±28.66^{+}	Mean=5.1 Percentile Range=1.9-10.1
Mean number of corpora lutea	11.9±1.31	11.5±2.50	12.1±1.67	11.9±3.12	Mean =12.0 Percentile Range= 11.2-13.2

Percentile range= P5-P95; + biologically significant; HCD; Rat Crl:WI (Han), Study Date Range: 2014 – 2018, Number of animals in control group=1097, Number of studies=49

Foetal observations

Litter size: There was a biologically significant decrease in mean litter size, observed at 1000 mg/kg bw/day, the mean litter size was 10.6, 10.5, 10.9 and 9.4 at 0, 100, 300 and 1000 mg/kg bw/day, respectively. The study authors noted that the litter size at 1000 mg/kg bw/day was slightly below the lower limit of the historical control range of the test laboratory (Rat Crl:WI (Han), (2014 – 2018), N; 1097, mean; 10.7, percentile range; 9.6-11.7), and this was due to 2/22 females having smaller litters (1 and 4 foetuses per litter respectively) and 2/22 females having no foetuses. The other treatment groups were within the historical control range of the laboratory and not considered to be affected by treatment.
Number of viable:
Viable foetuses: At 1000 mg/kg bw/day, there was a biologically significant decrease in the percentage of viable foetuses per litter (95.7%, 96.4%, 96.1% and 85.1% at 0, 100, 300 and 1000 mg/kg bw/day,

respectively). The mean percentage of viable foetuses per litter at 1000 mg/kg bw/day was outside the historical control range of the test laboratory (Rat Crl: WI (Han), (2014 – 2018), N;1097, mean; 94.9%, percentile range; 90%-98.2%). The study authors reported that at 1000 mg/kg bw/day mean post implantation loss was high and this was due to 2/22 females having no foetuses and complete litter loss at this dose.

Dead foetuses: None observed

Sex ratio: There was no statistically significant difference noted in the male to female ratio for the control and treatment groups.

Mean foetal weight: There was a statistically significant decrease in mean foetal weight at 1000 mg/kg bw/day. The mean foetal weights were 5.3, 5.2, 5.0 and 3.9g at 0, 100, 300 and 1000 mg/kg bw/day. The study authors considered the dose related trend towards lower foetal weights in the dose groups to be treatment related since the mean foetal weight of 1/21, 4/20, 10/20 and 20/20 of the viable litters at 0, 100, 300 and 1000 mg/kg bw/day were less than the lower limit of the historical control range of the test laboratory (Rat Crl:WI (Han), (2014 – 2018), N; 1097, mean; 5.2g, percentile range; 5.0-5.4 g).

Anogenital Distance (AGD): No effect was observed for male or female foetuses for AGD.

Table 15: Foetal parameters examined during the Prenatal Developmental Toxicity Study of Piperonal or Heliotropine by Oral Gavage in Rats. (Anonymous 2020b).

Foetal Parameter					
Dose (mg/kg bw/day)	**0**	**100**	**300**	**1000**	**HCD**
Mean Litter size	10.6±1.40	10.5±2.70	10.9±1.74	9.4±4.23^{+}	Mean=10.7 Percentile Range=9.6-11.7
% Viable foetuses per litter	95.7±4.62	96.4±4.56	96.1±5.25	85.1±28.66^{+}	Mean = 94.9 Percentile Range = 90.0-98.2
% Dead foetuses per litter	0±0 N=21	0±0 N=20	0±0 N=20	0±0 N=22	Mean=0 Percentile Range=0.0-0.4
Mean foetal weight (g)	5.3±0.39 N=21	5.2±0.31 N=20	5.0±0.51 N=20	3.9±0.38** N=20	Mean=5.2 Percentile Range=5.0-5.4
AGD male (mm)	2.92±0.266 N=21	2.87±0.215 N=20	2.95±0.276 N=20	2.86±0.283 N=19	Mean=2.9 Percentile Range=-$^{\#}$
AGD female (mm)	1.34±0.278 N=21	1.33±0.189 N=19	1.33±0.252 N=20	1.27±0.224 N=19	Mean=1.4 Percentile Range=-$^{\#}$

** $p < 0.01$; Percentile range = P5-P95; + biologically significant; # insufficient data for calculation; HCD; Rat Crl:WI (Han), Study Date Range: 2014 – 2018, Number of animals in control group=1097, Number of studies=49

External examinations: There were no external malformations or variations observed.

Visceral examination: One foetus at 1000 mg/kg bw/day had a small kidney and malpositioned testes. The study authors reported that although neither malformation was previously observed in historical control foetuses, as these malformations were only observed in one foetus, they were not considered to be treatment related. The dossier submitter considers that, as these malformations were not observed in the historical control data for the test laboratory, it is unclear they were treatment related.

There was an increase in the litter incidence of dilated ureter at ≥ 300 mg/kg bw/day; the litter incidences were 0%, 0%, 1.3% and 3.8% at 0, 100, 300 and 1000 mg/kg bw/day, respectively. An increase in the litter incidence of absent or small renal papilla was observed at 1000 mg/kg bw/day, the incidence was 2% compared to 0% in the control and other treatment groups. The dossier submitter notes that the incidence of dilated ureter and absent or small renal papilla were outside the range of the historical control data of the test laboratory (Rat Crl:WI(Han) on GD 21, (2014 – 2018), Number of foetuses/litters examined; 6234; dilated ureter; mean; 0.5%, percentile range; 0.0-2.3% and renal papilla; mean; 0.1, percentile range; 0.0-0.9%) and it is concluded that these variations are treatment related.

Table 16: Foetal visceral examinations during the Prenatal Developmental Toxicity Study of Piperonal or Heliotropine by Oral Gavage in Rats. (Anonymous 2020b)

Variation	Dose (mg/kg bw/day)				
	0	**100**	**300**	**1000**	**HCD** **Litter incidence (%)**
Ureter-dilated					Mean=0.5
Foetal incidence	0	0	1	4	Percentile Range=0.0-2.3
Litter incidence (%)	0±0	0±0	1.3±5.59	3.8±10.22	
Ureter-convoluted					Mean=0.9
Foetal incidence	0	0	1	1	Percentile Range=0.0-4.2
Litter incidence (%)	0±0	0±0	1.3±5.59	1.0±4.47	
Renal papilla- Absent and/or small					Mean=0.1
Foetal incidence	0	0	0	2	Percentile Range=0.0-0.9
Litter incidence (%)	0±0	0±0	0±0	2.0±8.94	

Percentile range = P5-P95; HCD; Rat Crl:WI(Han) on GD 21, Study Date Range: 2014 – 2018, Number of foetuses/litters examined=6234, Number of studies=49

Skeletal examination:

Malformations

There was a statistically significant increase in the incidence of rib anomaly at 1000 mg/kg bw/day, the litter incidence was 4.7% per litter compared to 0% at 0, 100 and 300 mg/kg bw/day, and was outside the historical control range of the test laboratory (Rat Crl: WI (Han) on GD 21, (2014 – 2018), Number of foetuses/litters examined; 6219, mean; 0.1%, percentile range; 0.0%-1.0%). At the same dose there were biologically significant increases in the litter incidence of vertebral anomaly (with or without rib anomaly), vertebral centra anomaly, sternoschisis and costal cartilage. The incidences and laboratory's historical control data (Rat Crl: WI (Han) on GD 21, (2014 – 2018)) of these malformations are reported in Table 17 below. The study author reported that incidence of these findings was outside the test laboratory's historical

control range and these malformations are all located in the same thoracic region and thus considered treatment related.

At the highest dose, there was a statistically significant increase in the percentage of total skeletal malformations per litter observed, the litter incidence was 1.6%, 1.8%, 1.8% and 15% at 0, 100, 300 and 1000 mg/kg bw/day, respectively.

Table 17: Foetal skeletal malformations observed during the Prenatal Developmental Toxicity Study of Piperonal or Heliotropine by Oral Gavage in Rats (Anonymous 2020b).

Malformation		Dose (mg/kg bw/day)				
		0	100	300	1000	HCD Litter incidence (%)
Vertebral anomaly (with or without rib anomaly)	Foetal incidence Litter incidence (%)	0 0±0	0 0±0	0 0±0	5 5.1±14.50	Mean=0.3 Percentile Range=0.0-1.6
Vertebral centra anomaly	Foetal incidence Litter incidence (%)	0 0±0	0 0±0	0 0±0	4 3.7±12.12	Mean=0.0 Percentile Range=0.0-0.4
Rib anomaly	Foetal incidence Litter incidence (%)	0 0±0	0 0±0	0 0±0	5 4.7±8.19*	Mean=0.1 Percentile Range=0.0-1.0
Sternoschisis	Foetal incidence Litter incidence (%)	0 0±0	0 0±0	0 0±0	3 3.0±7.11	Mean=0.1 Percentile Range=0.0-0.8
Costal cartilage anomaly	Foetal incidence Litter incidence (%)	0 0±0	0 0±0	0 0±0	1 1.1±4.59	Mean=0.1 Percentile Range=0.0-0.5

*p<0.05, **p<0.01; Percentile range= P5-P95; HCD; Rat Crl: WI (Han) on GD 21, Study Date Range: 2014 – 2018, Number of foetuses/litters examined=6219 Number of studies=49

Variations

There was a statistically significant increase in the incidence of unossified metacarpal and/or metatarsal at ≥ 100 mg/kg bw/day. The litter incidence was 0%, 5.5%, 13.0% and 87.3% at 0, 100, 300 and 1000 mg/kg bw/day, respectively. The litter incidence at ≥ 300mg/kg bw/day was outside the historical control range of the test laboratory (Rat Crl: WI(Han) on GD 21, (2014 – 2018), Number of foetuses/litters examined; 6219, mean; 3.3%, percentile range; 0.0%-12.4%).

There was a statistically significant increase in the incidence of reduced ossification of the skull observed at ≥ 300mg/kg bw/day. The litter incidence was 23.5%, 21.8%, 46.8%, and 57.3% at 0, 100, 300 and 1000 mg/kg bw/day. The dossier submitter notes that the incidence in all treatment groups was outside the range of the historical control data of the test laboratory (Rat Crl: WI(Han) on GD 21, (2014 – 2018), Number of foetuses/litters examined; 6219, mean; 8.5%, percentile range; 0.4%-18.8%) but considers that the clear increase in the incidence at ≥ 300mg/kg bw/day is indicative of a treatment related effect.

There was a statistically significant increase in the incidence of reduced ossification of vertebral centra and vertebral arches at 1000 mg/kg bw/day, which was outside the historical control range of the test laboratory. The litter incidence of reduced ossification of the vertebral centra was 0.8%, 0%, 1.4% and 31.4 % at 0, 100, 300 and 1000 mg/kg bw/day, respectively, and the test laboratory historical control (Rat Crl: WI(Han) on GD 21, (2014 – 2018), Number of foetuses/litters examined; 6219) mean was 0.8% and percentile range was 0.0%-3.2%. The litter incidence of reduced ossification of vertebral arches was 0%, 1.0%, 0% and 12.6 % at 0, 100, 300 and 1000 mg/kg bw/day, respectively, and the test laboratory historical control (Rat Crl: WI(Han) on GD 21, (2014 – 2018), Number of foetuses/litters examined; 6219) mean was 0.1% and the percentile range was 0.0%-1.1%.

There was an increase in the incidence of rib variations. These variations were observed in the seventh cervical full rib, seventh cervical ossification sites, bent ribs and fourteenth rib. At 1000 mg/kg bw/day, there was a statistically significant increase in the incidence of seventh cervical full rib (1.9%, 0%, 1% and 16.2 % at 0, 100, 300 and 1000 mg/kg bw/day, respectively) and in the seventh cervical ossification sites (5.4%, 4.7%, 11.4% and 32.8% at 0, 100, 300 and 1000 mg/kg bw/day, respectively) and a biologically significant increase in the incidence of fourteenth rib (8.3%, 12.7%, 5.5% and 21.0% at 0, 100, 300 and 1000 mg/kg bw/day, respectively). Incidences of seventh cervical full rib at 1000 mg/kg bw/day, of ossification sites at ≥ 300mg/kg bw/day and of fourteenth rib at 100 and 1000 mg/kg bw/day were outside the historical control range of the test laboratory (Rat Crl: WI(Han) on GD 21, (2014 – 2018), Number of foetuses/litters examined; 6219, mean; 0.4% and percentile range; 0.0%-1.7% for seventh cervical full rib, mean; 3.8% and percentile range; 0.0%-8.7% for seventh cervical ossification sites and mean; 6.3% and percentile range; 0.7%-12.1% for fourteenth full rib). An increased incidence of bent ribs was observed at ≥ 300 mg/kg bw/day, which was statistically significant only at 300 mg/kg bw/day. The litter incidence was 27.6%, 28.5%, 61.3% and 48.4% at 0, 100, 300 and 1000 mg/kw/bw/day, respectively and incidences in the control and treatment groups were outside the historical control range of the test laboratory (Rat Crl: WI(Han) on GD 21, (2014 – 2018), Number of foetuses/litters examined; 6219, mean; 13.7%, percentile range; 2.1%-25.8%).

At 1000 mg/kg bw/day, there was a statistically significant increase in the incidence of caudal shift of the pelvic girdle (7.3%, 8.9%, 5.9% and 28.1% at 0, 100, 300 and 1000 mg/kg bw/day, respectively). The incidence at the highest dose was outside the historical control range of the test laboratory (Rat Crl: WI(Han) on GD 21, (2014 – 2018), Number of foetuses/litters examined; 6219, mean; 5.9%, percentile range; 1.9%-12.3%).

There was an increased incidence of sternebrae variation observed at 1000 mg/kg bw/day. These variations were malaligned sternebrae and unossified sternebrae at positions 1-4 and 5-6. The increases observed in the highest dose for unossified sternebrae at positions 1-4 (0%, 0%, 0% and 6.9% at 0, 100, 300 and 1000 mg/kg bw/day, respectively) and 5-6 (0%, 0%, 0% and 44.8% at 0, 100, 300 and 1000 mg/kg bw/day, respectively) reached statistical significance and were outside the test laboratory's historical control range (Rat Crl: WI(Han) on GD 21, (2014 – 2018), Number of foetuses/litters examined; 6219, mean; 0.1%, percentile range; 0.0%-0.8% for positions 1-4 and mean; 0.4% and percentile range; 0.0%-2.5% for position 5-6). The litter incidence of malaligned sternebrae were 22.9%, 24.5%, 25.35% and 39.8% at 0, 100, 300 and 1000 mg/kg bw/day, respectively. There was no historical control data for this parameter reported and therefore, it is unclear if the increased incidence at 1000 mg/kg bw/day is biologically significant.

At 1000 mg/kg bw/day, there was reduced ossification of the pubis observed in 1 pup, resulting in a litter incidence of 0%, 0%, 0% and 0.9% at 0, 100, 300 and 1000 mg/kg bw/day, respectively. The incidence was outside the historical control range of the test laboratory (Rat Crl: WI(Han) on GD 21, (2014 – 2018),

Number of foetuses/litters examined; 6219, mean; 0.0%, percentile range; 0.0%-0.0%) and therefore it is unclear if this effect is treatment related.

Table 18: Foetal skeletal variations observed during the Prenatal Developmental Toxicity Study of Piperonal or Heliotropine by Oral Gavage in Rats (Anonymous 2020b).

Variations		Dose (mg/kg bw/day)				
		0	100	300	1000	HCD Litter incidence (%)
Unossified metacarpal and/or metatarsal	Foetal incidence Litter incidence (%)	1 1.0±4.36	6 5.5±8.67*	16 13.0±22.86**	97 87.3±28.10**	Mean=3.3 Percentile Range=0.0-12.4
Reduced ossification of the skull	Foetal incidence Litter incidence (%)	28 23.5±24.78	25 21.8±29.16	50 46.8±28.39*	64 57.3±28.89**	Mean=8.5 Percentile Range=0.4-18.8
Reduced ossification of vertebral centra	Foetal incidence Litter incidence (%)	1 0.8±3.64	0 0±0.0	2 1.4±6.39	32 31.4 ±28.73**	Mean=0.8 Percentile Range=0.0-3.2
Reduced ossification of vertebral arches	Foetal incidence Litter incidence (%)	0 0±0.0	1 1.0±4.47	0 0±0.0	14 12.6 ±22.21*	Mean=0.1 Percentile Range=0.0-1.1
Seventh cervical full rib	Foetal incidence Litter incidence (%)	2 1.9±6.02	0 0±0	1 1.0±4.47	21 16.2±27.67*	Mean=0.4 Percentile Range=0.0-1.7
Seventh cervical ossification site	Foetal incidence Litter incidence (%)	6 5.4±14.04	5 4.7±8.34	13 11.4±17.20	35 32.8±25.63**	Mean=3.8 Percentile Range=0.0-8.7
Bent ribs	Foetal incidence Litter incidence (%)	32 27.6±28.13	31 28.5±26.74	66 61.3±36.05**	54 48.4±36.83	Mean=13.7 Percentile Range=2.1-25.8
Fourteenth full rib	Foetal incidence Litter incidence (%)	8 8.3±20.07	13 12.7±20.45	6 5.5±10.83	20 21.0 ±27.95	Mean=6.3 Percentile Range=0.7-12.1
Pelvic girdle (Caudal shift)	Foetal incidence Litter incidence (%)	8 7.3±18.03	9 8.9±17.39	7 5.9±15.62	29 28.1 ±23.58**	Mean=5.9 Percentile Range=1.9-12.3
Malaligned sternebrae	Foetal incidence Litter incidence (%)	26 22.9±16.02	22 24.5±22.66	27 25.3±17.34	41 39.8 ±26.07	Mean=7.9 Percentile Range=-#

Variations		Dose (mg/kg bw/day)				
		0	**100**	**300**	**1000**	**HCD** **Litter incidence (%)**
Unossified sternebrae #1,2, 3, 4	Foetal incidence Litter incidence (%)	0 0±0.0	0 0±0.0	0 0±0.0	8 6.9 ±11.00**	Mean=0.1 Percentile Range=0.0-0.8
Unossified sternebrae #5,6	Foetal incidence Litter incidence (%)	0 0±0.0	0 0±0.0	0 0±0.0	51 44.8 ±37.64**	Mean=0.4 Percentile Range=0.0-2.5
Unossified /reduced ossification pubis-	Foetal incidence Litter incidence (%)	0 0±0.0	0 0±0.0	0 0±0.0	1 0.9 ±3.82	Mean=0.0 Percentile Range=0.0-0.0
Reduced ossification of the rib(s)	Foetal incidence Litter incidence (%)	0 0±0.0	0 0±0.0	3 2.5±11.18	0 0±0.0	Mean=0.0 Percentile Range=0.0-0.0

*p<0.05, **p<0.01; Percentile range= P5-P95; # insufficient data for calculation; HCD; Rat Crl: WI(Han) on GD 21, Study Date Range: 2014 – 2018, Number of foetuses/litters examined=6219, Number of studies=49

3.10.2 Human data

No information available

3.10.3 Other data

No information available

3.11 Specific target organ toxicity – single exposure

Not evaluated as part of this dossier.

3.12 Specific target organ toxicity – repeated exposure

Refer to CLH dossier

3.13 Aspiration hazard

Not evaluated as part of this dossier.

4 ENVIRONMENTAL HAZARDS

Not evaluated as part of this dossier