CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical name: 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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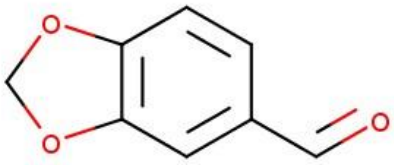
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	1,3-benzodioxole-5-carbaldehyde
Other names (usual name, trade name, abbreviation)	Piperonal 2H-1,3-benzodioxole-5-carbaldehyde 3,4-Dihydroxybenzaldehyde methylene ketal Benzo[d][1,3]dioxole-5-carbaldehyde Heliotropin
ISO common name (if available and appropriate)	Not applicable
EC number (if available and appropriate)	204-409-7
EC name (if available and appropriate)	Piperonal
CAS number (if available)	120-57-0
Other identity code (if available)	Not applicable
Molecular formula	C8H6O3
Structural formula	
SMILES notation (if available)	O=CC1=CC2=C(OCO2)C=C1
Molecular weight or molecular weight range	150.13
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	Not applicable

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3 (CLP)	Current self- classification and labelling (CLP)
piperonal; 1,3-benzodioxole-5-carbaldehyde	Mono-constituent substance	None	Skin Sens. 1B, H317 Repr. 2, H361fd

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling

No impurities relevant for classification.

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling

No additives relevant for classification.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: Proposed harmonised classification and labelling for piperonal; 1,3-benzodioxole-5-carbaldehyde according to the CLP criteria

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitter's proposal	605-RST-VW-Y	piperonal; 1,3-benzodioxole-5-carbaldehyde	204-409-7	120-57-0	Repr. 1B Skin Sens. 1	H360FD H317	GHS08 GHS07 Dgr	H360FD H317			
Resulting Annex VI entry if agreed by RAC and COM	605-RST-VW-Y	piperonal; 1,3-benzodioxole-5-carbaldehyde	204-409-7	120-57-0	Repr. 1B Skin Sens. 1	H360FD H317	GHS08 GHS07 Dgr	H360FD H317			

Table 6: Reason for not proposing harmonised classification and status under consultation

Hazard class	Reason for no classification	Within the scope of consultation
Explosives	Hazard class not assessed in this dossier.	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier.	No
Oxidising gases	Hazard class not assessed in this dossier.	No
Gases under pressure	Hazard class not assessed in this dossier.	No
Flammable liquids	Hazard class not assessed in this dossier.	No
Flammable solids	Hazard class not assessed in this dossier.	No
Self-reactive substances	Hazard class not assessed in this dossier.	No
Pyrophoric liquids	Hazard class not assessed in this dossier.	No
Pyrophoric solids	Hazard class not assessed in this dossier.	No
Self-heating substances	Hazard class not assessed in this dossier.	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier.	No
Oxidising liquids	Hazard class not assessed in this dossier.	No
Oxidising solids	Hazard class not assessed in this dossier.	No
Organic peroxides	Hazard class not assessed in this dossier.	No
Corrosive to metals	Hazard class not assessed in this dossier.	No
Acute toxicity via oral route	Hazard class not assessed in this dossier.	No
Acute toxicity via dermal route	Hazard class not assessed in this dossier.	No
Acute toxicity via inhalation route	Hazard class not assessed in this dossier.	No
Skin corrosion/irritation	Hazard class not assessed in this dossier.	No
Serious eye damage/eye irritation	Hazard class not assessed in this dossier.	No
Respiratory sensitisation	Hazard class not assessed in this dossier.	No
Skin sensitisation	Harmonised classification proposed.	Yes
Germ cell mutagenicity	Hazard class not assessed in this dossier.	No
Carcinogenicity	Hazard class not assessed in this dossier.	No
Reproductive toxicity	Harmonised classification proposed.	Yes
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier.	No
Specific target organ toxicity-repeated exposure	Hazard class not assessed in this dossier.	No
Aspiration hazard	Hazard class not assessed in this dossier.	No
Hazardous to the aquatic environment	Hazard class not assessed in this dossier.	No
Hazardous to the ozone layer	Hazard class not assessed in this dossier.	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

There is no harmonised classification and labelling for piperonal; 1,3-benzodioxole-5-carbaldehyde and it was not previously discussed by the Technical Committee for Classification and Labelling under Directive 67/548/EEC.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

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

In accordance with Article 36(1) of CLP, justification for action is not required for substances which fulfil the classification criteria for carcinogenicity, germ cell mutagenicity or reproductive toxicity. The dossier submitter proposes classification as a category 1B reproductive toxicant and therefore no justification for this hazard class is required.

Justification that action is needed at Community level is required.

In accordance with Article 36(3) of CLP, justification for action is required for hazard classes other than those referred to in Article 36(1). The REACH registrant has self-classified piperonal; 1,3-benzodioxole-5-carbaldehyde as a reproductive toxicant category 2 and skin sensitiser category 1B. The dossier submitter considers that the data presented in this dossier supports classification as category 1B reproductive toxicant. In addition, the dossier submitter considers that the available data on skin sensitisation does not allow sub-categorisation and thus considers that classification as category 1 skin sensitiser is more appropriate. Thus, harmonised classification for the hazard classes reproductive toxicant and skin sensitisation are proposed due to the disagreement by the dossier submitter with the current self-classification.

5 IDENTIFIED USES

Piperonal; 1,3-benzodioxole-5-carbaldehyde is a solid crystalline product used in the formulation of fragrances and end-products, formulation of tobacco flavours, in industrial washing and cleaning products and as a chemical intermediate. It is also used in waxes, polishes, and washing and cleaning products for professional and consumer uses. Additional consumer uses include in air care products, tobacco products, biocides and cosmetics.

6 DATA SOURCES

Data for piperonal; 1,3-benzodioxole-5-carbaldehyde are taken from:

- Publicly disseminated REACH registration dossier (ECHA dissemination site, 2023).
- Unpublished study reports provided by the registrants for the skin sensitisation and reproductive toxicity endpoints.
- Publicly available study reports for the skin sensitisation and reproductive toxicity endpoints.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid	ECHA dissemination site, 2023	-
Melting/freezing point	309.55 K	ECHA dissemination site, 2023	Measured, at 98.5 kPa.
Boiling point	546 K	ECHA dissemination site, 2023	Measured, 98.5 kPa.
Relative density	1.419 g/cm³	ECHA dissemination site, 2023	Measured, at 20°C.
Vapour pressure	1 mmHg at 87°C 760 mmHg at 263°C	ECHA dissemination site, 2023	Registrant has assigned reliability score of 4 (not assignable).
Surface tension	Not applicable		
Water solubility	1.4 g/L	ECHA dissemination site, 2023	Measured, at 20°C.
Partition coefficient n-octanol/water	log Pow 1.2	ECHA dissemination site, 2023	Measured at 35°C.
Flash point	Not applicable		
Flammability	Non flammable	ECHA dissemination site, 2023	Measured, the test substance did not burn over a length of 200 mm within 4 minutes.
Explosive properties	Not applicable	ECHA dissemination site, 2023	
Self-ignition temperature	> 400 °C	ECHA dissemination site, 2023	Measured, no self-ignition temperature could be determined up to 400 °C.
Oxidising properties	Not applicable		
Granulometry	L_{50} = 272 µm	ECHA dissemination site, 2023	Measured by laser diffraction.
Stability in organic solvents and identity of relevant degradation products	Not applicable		
Dissociation constant	Not applicable		
Viscosity	Not applicable		

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated as part of this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Non-guideline study and not GLP compliant. 3mM of Heliotropin (trade name of piperonal; 1,3-benzodioxole-5-carbaldehyde) (99% purity) was incubated with nasal and liver microsomes of male rats (Fisher 344, 12-16 weeks old), for 30 minutes at 37°C. The effects of oxidative metabolism of methyl N-methylanthranilate (DMA) were examined.	Percentage DMA Nasal; 23.1 ±0 % Liver; 83.6±2.6 %	The data indicates that the test substance inhibited CYP450 in nasal microsomes. Limited information on method provided. Reliability: unreliable.	Dahl, (1982), ECHA dissemination site, 2023
Non-guideline study and not GLP compliant. Male mice (Swiss Webster, unknown number and age) received 50µL of 0.75 mg/kg bw of ^{14}C radiolabelled piperonal) in DMSO and 100µL of DMSO wash via oral gavage. Treated mice were housed in a metabolism cage for 48 hours with food and water supply. The following examinations were carried out: - Total radiocarbon on expired carbon dioxide at 0.5, 1, 2, 4 and 6 hours post dosing and subsequently at 6-hour intervals. - Urine and faeces analysis at 12, 24 and 48 hours post dosing. -Intestine, liver and remaining carcass samples were analysed 48 hours post dosing. -Urine samples collected at 12 hours post dosing were used for metabolite characterisation by thin layer chromatography.	<u>12 hours post dose</u> -Volume of radiocarbon excreted was equivalent to 90% of administered dose. -4 urinary metabolites detected-Piperonylglycine (major metabolite), Piperonylic acid and 2 unknown metabolites, all in similar quantities. -Piperonal was not detected in the urine. <u>48 hours post dose</u> -95.3% of radiocarbon dose recovered -Urine – 89% -Faeces-3.2% -Carbon Dioxide- 1.1% -Intestine-0.4% -Liver-0.4% -Remaining carcass-1.2%	Limited information. Reliability: unreliable.	Kamienski, (1970), ECHA dissemination site, 2023
Non-guideline study and not GLP compliant. 3 male rats (Wistar, age unknown) were administered 150 mg/kg bw of piperonal in propylene glycol via oral gavage. Urine samples were collected at 24 hours intervals.	<u>24 hours post dose</u> 92.6 ± 5% of administered dose recovered in urine. 6 metabolites detected; -Piperonylglycine - 71 ± 5% -Piperonylic acid - 20 ± 2% -Piperonyl alcohol - 0.9 ± 0.4% -Protocatechuic acid - 0.5 ± 01% -Protocatechualdehyde - 0.1 ± 0.04% -Protocatechuyl alcohol - 0.1 ± 0.06% <u>48 hours post dose</u> 93.8 ±5 % of administered dose recovered in urine. 6 metabolites detected; -Piperonylglycine - 72 ± 0.5% -Piperonylic acid - 20.2 ± 2%	Limited information. Reliability: unreliable.	Klungsoyr, (1984), ECHA dissemination site, 2023

Method	Results	Remarks	Reference
	-Piperonyl alcohol - 0.9 ± 0.4% -Protocatechuic acid - 0.5 ± 0.1% -Protocatechualdehyde - 0.1 ± 0.04% -Protocatechuyl alcohol - 0.1 ± 0.06% There were no metabolites or parent compound detected after 48 hours.		

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

In a non-guideline, non-GLP compliant, *in vitro* study piperonal; 1,3-benzodioxole-5-carbaldehyde inhibited the oxidative metabolism of methyl N-methylanthranilate by CYP450 in rat nasal microsomes. Two non-guideline *in vivo* studies investigating metabolism and elimination of piperonal; 1,3-benzodioxole-5-carbaldehyde in mice and rats are reported in the REACH registration dossier. Following administration of ^{14}C-piperonal; 1,3-benzodioxole-5-carbaldehyde via oral gavage to male mice, 89% of radiocarbon was detected in urine at 48 hours post dosing. The urinary metabolites detected were piperonylglycine (major metabolite), piperonylic acid and 2 unknown metabolites (not reported). piperonal; 1,3-benzodioxole-5-carbaldehyde was not detected. Male rats were administered piperonal; 1,3-benzodioxole-5-carbaldehyde via oral gavage and urinary excretion was measured at 24 and 48 hours post dosing. After 24 hours, 92.6 ± 5% of the administered dose was recovered in urine and six metabolites, piperonylglycine, (major metabolite), piperonylic acid, piperonyl alcohol, protocatechuic acid, protocatechualdehyde and protocatechuyl alcohol were detected. After 48 hours, 93.8 ±5 % of the administered dose was recovered in urine and the six fore-mentioned metabolites detected.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Not evaluated as part of this dossier.

10.2 Acute toxicity - dermal route

Not evaluated as part of this dossier.

10.3 Acute toxicity - inhalation route

Not evaluated as part of this dossier.

10.4 Skin corrosion/irritation

Not evaluated as part of this dossier.

10.5 Serious eye damage/eye irritation

Not evaluated as part of this dossier.

10.6 Respiratory sensitisation

Not evaluated as part of this dossier.

10.7 Skin sensitisation

Table 9: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Test substance, species, strain, sex, no/group	Dose levels duration of exposure	Results	Reference
Similar to OECD 406: skin sensitisation (guinea pig maximisation test). GLP compliance not specified. -No concurrent or laboratory positive control. -4 control animals and different control groups used for each challenge application. -Intradermal induction protocol did not follow OECD test guideline. -Topical application challenge on day 14. -Not scored according to OECD test guideline. -Limited reporting of methods and results. Reliability: reliable.	Heliotropin (Trade name of piperonal; 1,3-benzodioxole-5-carbaldehyde) Purity: 100% Vehicle: Intradermal; 6% acetone, 20% polyethylene glycol, and 0.01% saline. Topical; Acetone. Positive control: None. Negative control: Treated and untreated animals (not further specified). Guinea pig, strain not specified. 10 (4 male and 6 female) in treatment group. 4 (sex not specified) in vehicle controls.	Range finding studies: <u>Intradermal</u> Group 1; 4 males administered 0.1ml of; 0.05%, 0.1%, 0.25%, 0.5% and 1% test substance in 0.01% saline. Group 2; 4 males administered 0.1ml of; 1%, 2%, 3% and 4% test substance in 6% acetone, 20% polyethylene glycol, and 0.01% saline. <u>Topical</u> 4 males administered 5%, 10% and 25% and 4 females administered 40%, 60% and 80% test substance in acetone. Main Study: <u>Induction</u> Day 0; Six 0.1ml intradermal injections of 1ml FCA (Freunds Complete Adjuvant) and 0.1ml of 1.5 % test substance in 6% acetone, 20% polyethylene glycol, and 0.01% saline. No further details on injection sequence were provided. Day 7; Topical application of an occluded patch of 80% test substance in acetone. The length of the treatment period was not stated. <u>Challenge</u> -Day 14; Topical application	Range finding studies: <u>Intradermal</u> - At ≥ 0.1%; signs of irritation. -At ≥ 1%; signs of oedema. -1.5% selected for main study, reported to be “suitably irritant”. -No further details were provided. <u>Topical</u> No irritation was observed. 80% selected for main study. Main Study: Result: Positive. Day 14; <u>At 24 hours</u> -2/10 (20%); positive reactions. - 1/10 “scattered, mild erythema”. -1/10 “moderate and diffuse erythema”. <u>At 48 hours</u> -4/10 (40%); positive reactions. -3/10 “moderate and diffuse erythema”. -1/10 with “intense erythema and oedema”. -No positive skin reactions observed in the vehicle control.	Anonymous, 1978

Method, guideline, deviations if any	Test substance, species, strain, sex, no/group	Dose levels duration of exposure	Results	Reference
		of 80% test substance in acetone. -Day 21 and 28; Topical application of 80% test substance in acetone. -Day 42; Topical application of 20 or 80% test substance in acetone.	Days 21 and 28 challenge -positive reactions in 4/10 (40%) at 24 and 48 hours. Day 42 challenge - At 20% in 4/10 (40%) at 48 hours. - At 80%, 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.	
Non-guideline study. Maximization Test. GLP compliance not specified. An emulsion of the test substance and FCA were administered intradermally on day 0, followed by a topical induction application on day 8. A topical challenge application was made on day 21 and assessments of the flank were made 24 and 48 hours after the challenge patch was removed. Limited reporting of methods and results. Reliability: unreliable.	Heliotropin (Trade name of piperonal;1,3-benzodioxole-5-carbaldehyde) Purity, vehicle and controls: No information available. Guinea pig, Himalayan white spotted. Male and female. 6-8 animals, sex and/or exact number per group not specified. 6-8 untreated control animals, sex and/or exact number per group not specified.	<u>Induction</u> Day 0; Animals received intradermal injections: -0.1ml FCA -0.1ml of 5 % test substance -0.1ml of 5% test substance mixed with FCA Day 8; Animals received topical applications of an occluded patch of 250 mg of test substance dissolved in 25% petrolatum to a clipped skin area of the neck. <u>Challenge</u> Day 21; Animals received topical applications of an occluded patch of the test substance at "sub-irritant" concentration in petrolatum on the flank for 24 hours. No information available for the challenge concentration.	Result; Positive. No individual animal scoring data or further details were available in the study report.	Klecak G., 1977
Non-guideline study. Open Epicutaneous test. GLP compliance not specified. <u>Undiluted test substance</u>	Heliotropin (Trade name of piperonal;1,3-benzodioxole-5-carbaldehyde) Purity and positive control: No information available.	<u>Induction</u> Day 0; Test animals received a topical application of 0.1 ml of undiluted test substance and increasingly diluted solutions (concentrations not	Result; Positive. A minimum sensitising concentration of 30% and minimum eliciting concentration of 1% was reported. No individual animal	Klecak G., 1977

Method, guideline, deviations if any	Test substance, species, strain, sex, no/group	Dose levels duration of exposure	Results	Reference
was applied daily to a clipped flank of guinea pigs for 21 days. On days 21 and 35, animals were challenged with the test substance and assessments were carried out at 24, 48 and 72 hours. "Allergenic" if 2/8 animals of a concentration exhibited positive reaction. Limited reporting of methods and results. Reliability: unreliable.	Vehicle: Not specified. Guinea pig, Himalayan white spotted. Male and female. 6-8 animals, sex and/or exact number per group not specified. 6-8 untreated control animals, sex and/or exact number per group not specified.	specified). Repeated application on the same skin area for 21 days and left uncovered for 24 hours. <u>Challenge</u> Day 21 and 35; Test animals received a topical application of 0.025 ml of the "minimal irritating concentration" of test substance (not further specified).	scoring data or further details were available in the study report.	
Non-guideline study. Draize test. GLP compliance not specified. On day 0 and on 9 subsequent alternate days, test substance in isotonic saline was administered intradermally to guinea pigs. On days 35 and 49, animals were challenged intradermally, with the test substance. The assessment criteria was the "mean diameter of the papular reaction." Limited reporting of methods and results. Reliability: unreliable.	Heliotropin (Trade name of piperonal; 1,3-benzodioxole-5-carbaldehyde) Purity and positive control: No information available. Vehicle: Isotonic saline. Guinea pig, Himalayan white spotted. Male and female. 6-8 animals, sex and/or exact number per group not specified. 6-8 untreated control animals, sex and/or exact number per group not specified.	<u>Induction</u> Day 0; Test animals received intradermal injections of 0.05 ml of 0.1% emulsion of test substance in isotonic saline. Animals received 0.1ml injections on 9 alternate days. The total dose administered was 0.95 mg. <u>Challenge</u> Day 35 and 49; Test and control animals received intradermal injections of 0.05 ml of 0.1% solution.	Result; Negative. No individual animal scoring data or further details were available in the study report.	Klecak G., 1977
Non-guideline study. Freunds Complete Adjuvant test. GLP compliance not specified. Animals received undiluted test substance and FCA or FCA intradermally to the neck on days 0, 2, 4, 7 and 9. On days 21 and 35, the animals were challenged with test substance applied topically to the flank. No information available	Heliotropin (Trade name of piperonal; 1,3-benzodioxole-5-carbaldehyde) Purity and positive control: No information available. Vehicle: Intradermal; FCA. Topical; No information available. Guinea pig, Himalayan white spotted.	<u>Induction</u> Day 0, 2, 4, 7 and 9; Test animals received intradermal injections of 0.05 ml of equal volumes of the test substance and FCA into the neck region. The total dose administered was 250 mg. The control animals received 5 intradermal 0.05 ml injections of FCA. <u>Challenge</u> Day 21 and 35; Test animals	Result; Negative. No individual animal scoring data or further details were available in the study report.	Klecak G., 1977

Method, guideline, deviations if any	Test substance, species, strain, sex, no/group	Dose levels duration of exposure	Results	Reference
on assessment criteria. Limited reporting of methods and results. Reliability: unreliable.	Male and female. 6-8 animals, sex and/or exact number per group not specified. Unspecified number of animals were untreated as negative controls.	received a topical application of 0.025 ml of "minimal irritating concentration" of test substance (not further specified) applied to the flank (2cm^2 area).		

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

No human data on the skin sensitising effects of piperonal; 1,3-benzodioxole-5-carbaldehyde are available.

A guinea pig maximisation test conducted with piperonal; 1,3-benzodioxole-5-carbaldehyde is reported in the REACH registration dossier. The study is similar to OECD 406 although it was conducted before the adoption of the test guideline and GLP compliance is not specified. The dose levels for the main study were selected based on the results of two intradermal and two topical range finding studies. In the intradermal studies, four males per study received intradermal injections of either 0.05%, 0.1%, 0.25%, 0.5% and 1% test substance in 0.01% saline or 1%, 2%, 3% and 4% test substance in 6% acetone, 20% polyethylene glycol and 0.01% saline. Observations were made at 24 hours post administration. In the first intradermal range finding study, reactions were observed from 0.1% test substance and as the concentration increased, there was an increase in incidences of "faint pink" reactions. In the second intradermal range finding study, 100% of animals had "faint pink" reactions from 1% test substance. At ≥ 2% test substance, incidences of "white centre" and "oedema" were observed. The study authors indicated that 1.5% was selected for the main study as it was considered "suitably irritant". No further details were provided. The dossier submitter notes that it is difficult to interpret the severity of the reactions outlined in the study report and it is not clear what is meant by "suitably irritant".

In the topical dose ranging studies, 5%, 10% and 25% test substance in acetone was applied to the flanks of 4 males and 40%, 60% and 80% test substance in acetone was applied to the flanks of 4 females for 24 hours. Observations were made at 24 and 48 hours post treatment. No irritation was reported. 80% concentration was used for topical induction and the challenge phase in the main study.

On day 0 of the main study, 10 guinea pigs (strain not specified), 4 males and 6 females, received six intradermal 0.1ml injections of Freunds Complete Adjuvant (FCA) and 1.5% test substance in 6% acetone, 20% polyethylene glycol and 0.01% saline. There were no further details provided for the injection sequence. On day 7, animals received a topical induction application of 80% test substance in acetone. On days 14, 21 and 28 animals were challenged with a topical application of 80% test substance in acetone on one flank for 24 hours and on day 42 animals were challenged with a topical application of 20% or 80% test substance in acetone on one flank for 24 hours. The study authors indicated that treated and untreated controls (4 animals/challenge) were included in the study, but no further details were provided. Dermal assessments were made 24 and 48 hours after removal of the challenge patches.

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 10: 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

At days 21 and 28, positive reactions were observed in 4/10 animals (40%) at 24 and 48 hours following each challenge. At day 42, positive reactions were observed with 20% test substance in 4/10 animals (40%) at 48 hours and with 80% test substance in 1/10 and 2/10 animals (10% and 20%, respectively) 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 registrants applied 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.

Klecak *et al* (1977) investigated the skin sensitising potential of piperonal; 1,3-benzodioxole-5-carbaldehyde in four different non-guideline test protocols. In the first study, a maximisation test, guinea pigs received intradermal injections of 5% test substance, FCA or an emulsion of 5% test substance with FCA. On day 8,

an occlusive patch of 250 mg test substance in 25% petrolatum was applied to the neck for 48 hours to cause irritation and on day 21, an occlusive patch at a "sub-irritant" concentration in petrolatum was applied to the flank for 24 hours. Assessments were made 24 and 48 hours after the patch was removed. The study author reported that the test substance was positive.

In the second study, an open epicutaneous test, guinea pigs received daily topical applications of undiluted test substance to the same area of clipped flank for 21 days. On days 21 and 35, a "minimal irritating concentration" (not further specified) of test substance was applied to the flank and assessments were conducted at 24, 48 and 72 hours. The study author reported that the test substance was positive and had a "minimum sensitising concentration" of 30% and "minimum eliciting concentration" of 1%. There was no further information provided.

In the third study, a Draize test, intradermal injections of test substance in isotonic saline were administered to guinea pigs on day 0 and subsequently on 9 alternate days. On days 35 and 49, treated and untreated animals were challenged intradermally with 0.1% test substance. The assessment criteria was the "mean diameter of a papular reaction." The study author reported that the test substance did not elicit any reaction.

In the fourth study, a Freund's Complete Adjuvant Test, guinea pigs received equal volumes of undiluted test substance and FCA intradermally to the neck on days 0, 2, 4, 7 and 9. Control animals received FCA only. On days 21 and 35 a "minimal irritating concentration of test substance" (not further specified) was applied to the flank. There was no information available on the assessment method. The study author reported that the test substance did not elicit any reaction.

The dossier submitter notes that Klecak *et al* (1977) has 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 results tables reported. The study authors reported that there were positive reactions in 2/4 non-standard studies. While the dossier submitter considers these studies to be unreliable due to the limited reporting and the lack of individual animal data, the results from the open epicutaneous test and the maximisation test provide supporting evidence of the skin sensitising potential of 1,3-benzodioxole-5-carbaldehyde.

Further details on the above studies are provided in Annex I to this report.

10.7.2 Comparison with the CLP criteria

In accordance with Annex I of the CLP Regulation, substances may be classified as skin sensitiser category 1:

(a) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons; or

(b) if there are positive results from an appropriate animal test"

No human data on the skin sensitising effects of piperonal; 1,3-benzodioxole-5-carbaldehyde are available. A positive guinea pig maximisation test with piperonal; 1,3-benzodioxole-5-carbaldehyde is available and based on the results of this study, classification as skin sensitiser category 1 is warranted.

In accordance with section 3.4.2.2.1.1 of Annex I of the CLP Regulation, '*skin sensitisers should be classified in Category 1, where data are not sufficient for sub-categorisation'*. An assessment of the need for subcategorisation is outlined below.

Annex I to the CLP Regulation states a substance must be classified as skin sensitiser category 1A where *"…a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans".* Table 3.4.3 of Annex I to the CLP Regulation outlines that, for a guinea pig maximisation test, there should be a positivity rate of ≥ 30 % at ≤ 0.1 % intradermal induction dose or ≥ 60 % at > 0.1 % to ≤ 1 % intradermal induction dose.

Annex I to the CLP Regulation states a substance must be classified as skin sensitiser category 1B where *"…a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans."* Table 3.4.3 of Annex I to the CLP Regulation outlines that, for a guinea pig maximisation test, there should be a positivity rate of ≥ 30 % - < 60 % at > 0.1 % to ≤ 1 % intradermal induction dose or ≥ 30 % at > 1 % intradermal induction dose.

In the available guinea pig maximisation test with piperonal; 1,3-benzodioxole-5-carbaldehyde, a positivity rate of 40% (at 48-hour assessment) was observed with an intradermal dose of 1.5 %. These results are within the range for classification in category 1B. As discussed in section 10.7.1, the dossier submitter notes that in the dose range finding studies for the selection of the intradermal induction dose for the guinea pig maximisation test, positive reactions described as 'faint pink' were described from 0.1 %, but there was no further information on the severity of these reactions. The study report indicates that 1.5 % was selected for the main study as it was considered "suitably irritant", but no further details were provided on the rationale for this dose selection. The dossier submitter considers that as it is difficult to interpret the severity of the reactions, and thus if a lower intradermal induction dose should have been selected for the study, it cannot be excluded that a lower (i.e., ≤ 1 %) intradermal induction dose would have led to positive skin reactions supporting classification as a skin sensitiser category 1A.

ECHA's Guidance on the application of the CLP criteria (version 5.0, July 2017) (CLP Guidance), states, *"when category 1A cannot be excluded, category 1 should be applied instead of category 1B."* Based on the available data, the dossier submitter considers that sub-categorisation is not appropriate and classification as skin sensitiser category 1 is warranted.

10.7.3 Conclusion on classification and labelling for skin sensitisation

Based on the available data, classification of piperonal; 1,3-benzodioxole-5-carbaldehyde as a skin sensitiser category 1 (without sub-categorisation) is warranted. Based on the available data, the assignment of a specific concentration limit is not warranted.

10.8 Germ cell mutagenicity

Not evaluated as part of this dossier.

10.9 Carcinogenicity

Not evaluated as part of this dossier.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 11: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD 422; Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test. GLP compliant. Rat, Wistar CrLl. WI (Han), male and female, 10/sex/dose. Deviated from OECD 422; -10 females instead of 12-13. -Only parental males were fasted prior to blood sampling.]-Pup T4 blood sampled on PND 14-16 instead of PND 4.	Piperonal or Heliotropine (tradename of piperonal;1,3-benzodioxole-5-carbaldehyde) Purity: 99.9% Oral gavage, once daily. 0, 100, 300 and 1000 mg/kg bw/day. Vehicle; PEG 400. Males - 7 days/week, for 10 weeks pre-mating period and 2-week mating period. Females- 7 days/week, for 10 weeks pre-mating period, 2-week mating period, throughout gestation period and until at least PND 13. Reliability: reliable.	- No test substance related deaths reported. - Males at 1000 mg/kg bw/day: ↓ in mean body weight and mean body weight gain during premating and mating. Females at 1000 mg/kg bw/day: ↓ in mean body weight and mean body weight gain during gestation. - Females: ↑ in food consumption at ≥ 300 mg/kg bw/day during gestation and at 300 mg/kg bw/day during lactation (no information for 1000 mg/kg bw/day, no pups survived at this dose). Males: no effects observed. - Males at ≥ 300 mg/kg bw/day: statistically significant ↓ in total T4 levels. -At 1000 mg/kg bw/day: ↓ in absolute and relative prostate weight and absolute and relative epididymides weight. A statistically significant ↑ in absolute and relative ovary weight and absolute and relative uterus weight. - At 1000 mg/kg bw/day: ↓ in fertility index, gestation index and mean number of implantation sites. No effect on pairing or mating indices, pre-coital interval and duration of gestation. - No significant effect on oestrous cycle and spermatogenesis. - No significant effect on parturition or maternal care. For information on development related parameters, please refer to Table 15: Summary table of animal studies on adverse effects on development. For information on non-reproductive system related repeat dose parameters, please refer to Table 22; Summary table of animal studies on STOT RE.	Anonymous, 2020a
Non-Guideline study. GLP status unknown. 10 female Sprague-Dawley rats per dose received a vehicle or test substance via oral gavage for 39 days. Male rats were untreated. Animals were mated 1:1. Parental females were examined for mating, fertility	Piperonal; 1,3-benzodioxole-5-carbadehyde (purity unknown) Oral gavage, once daily. 0, 250, 500 and 1000 mg/kg bw/day. Vehicle; methylcellulose or corn oil. Females treated for 39 days total: 7 days of	- At 1000 mg/kg bw/day, ↑ in mortality. No further details reported. - At ≥ 500mg/kg bw/day, ↑ incidence of clinical signs (not further specified). -↓ in body weight in females at 1000 mg/kg bw/day and ↓ in body weight gain in females at 500 mg/kg bw/day. - At 1000 mg/kg bw/day, ↓ in food consumption. - At 1000 mg/kg bw/day, ↓ in fertility. No further details reported.	Vollmuth T.A., (1990), ECHA dissemination site, 2023

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
and gestation indices, delivery of litter, number of offspring per litter, oestrous cycle, 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.	premating, 7 days of mating, 21-day gestation period and 4-day lactation period. Reliability: unreliable.	-No information on oestrous cycle, duration of gestation or parturition and maternal care. -Sperm parameters, precoital interval, number of implantations, pre and post implantation loss, corpora lutea not assessed. For information on development related parameters, please refer to Table 15: Summary table of animal studies on adverse effects on development	

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

In an OECD 422 Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test, 10 Wistar CrLl. WI (Han) rats per sex per dose were administered 0, 100, 300 and 1000 mg/kg bw/day of piperonal; 1,3-benzodioxole-5-carbaldehyde. Males were treated for 7 days/week for the 10 week pre-mating and 2 week mating periods. Females were treated 7 days/week for the 10 week pre-mating and 2 week mating periods and throughout the gestation period, until at least post-natal day (PND) 13. There were no test substance related deaths and no significant clinical observations reported for either sex.

In males at 1000 mg/kg bw/day, there was a statistically significant decrease in mean body weight from week 7 of pre-mating and in mean body weight gain from week 4 of pre-mating until the end of the mating period (week 14). At week 14 the mean body weight was 352g compared with 401g in the control and the mean body weight gain was 58g compared with 82g in the control group. There was also a statistically significant decrease in mean terminal body weight in males at 1000 mg/kg bw/day, the mean terminal body weights were 375, 385, 364 and 322g at 0, 100, 300 and 1000 mg/kg bw/day, respectively.

Females did not show any changes in mean body weight or mean body weight gain during the pre-mating or mating periods. At 1000 mg/kg bw/day, there was a decrease in mean body weight and mean body weight gain observed from gestation day (GD) 14. Mean body weight changes reached statistical significance on GD 17 and 20. At this dose, the mean body weight on GD 17 was 93% of the control value (284g at 1000 mg/kg bw/day compared to 306g in the control group) and the mean body weight on GD 20 was 84% of the control value (291g at 1000 mg/kg bw/day compared to 348g in the control group). The changes in mean body weight gain reached statistical significance on GD 20. The mean body weight gain at GD 20 in this group was 22g compared to 44g in the control group.

Table 12: 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

There was also a statistically significant decrease in mean terminal body weight in females at 1000 mg/kg. The mean terminal body weight at the highest dose was 16% lower than the control, the mean values were 292, 279, 282 and 244g at 0, 100, 300 and 1000 mg/kg bw/day, respectively.

To further assess if the effect on body weight observed at 1000 mg/kg bw/day was due to maternal toxicity or an intrauterine effect, the dossier submitter attempted to calculate the corrected mean maternal body weight changes in accordance with Annex I, 3.7.2.4.4 of CLP. However, it was not possible to calculate the corrected maternal body weights at 1000 mg/kg bw/day, as there was no gravid uterine weight or pup weight data available at this dose. It should also be noted that only the female who gave birth at 1000 mg/kg bw/day was weighed on LD 1, therefore there is no mean body weight for this treatment group. However, this individual dam had a higher body weight on LD 1 than the mean control value (284g compared to 267g in the control). Although the data is limited, it provides some indication that the effect on maternal body weight may be an intrauterine effect, rather than maternal toxicity.

There were no effects observed on food consumption for males or females during pre-mating. There was a statistically significant increase in food consumption in females during the GD 11-14 at 300 mg/kg bw/day (26g compared to 23g in the control) and GD 4-7 at 1000 mg/kg bw/day (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. This further supports that effects seen at 1000 mg/kg bw/day were likely to be intrauterine effects, rather than secondary to maternal toxicity.

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 result 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 on total T4 levels in treated females or total T3 and TSH levels in either sex. There was a statistically significant increase in absolute thyroid weight (0.018g compared to 0.015g in control) and relative thyroid weight (0.007g compared to 0.005g) in females at 1000 mg/kg bw/day. No effect on thyroid weight was observed in males.

At 1000 mg/kg bw/day, absolute prostate and epididymides weights were statistically significantly decreased. There was also a biologically significant decrease in the relative weights of these organs. At the same dose, there were statistically significant increases in absolute and relative ovary weights and absolute and relative uterus weights. There were no histopathological findings in the reproductive organs of either sex.

Table 13: Reproductive 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	-	-	-	-
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**

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

There was a decrease in the fertility index noted at 1000 mg/kg bw/day, the fertility indices were 100%, 90%, 90% and 40% at 0, 100, 300 and 1000 mg/kg bw/day, respectively. There were no effects on the oestrous cycle or spermatogenesis or on the pairing or mating indices.

A decrease in the mean number of implantation sites was observed at 1000 mg/kg bw/day, the mean values were 12.4, 12.2, 12.3 and 2.3 at 0, 100, 300 and 1000 mg/kg bw/day, respectively.

At 1000 mg/kg bw/day, the gestation index was decreased, with the indices reported as 100%, 89%, 100% and 0% at 0, 100, 300 and 1000 mg/kg bw/day, respectively.

For effects relevant for classification for development seen in this study, please see section: 10.10.4 Adverse effects on development.

Table 14: 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

In a 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: 7 days of pre-mating, 7 days of mating, 21-day gestation period and 4-day lactation period. 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 robust study summary indicated that there was a statistically significant increase in mortality in dams at 1000 mg/kg bw/day and an increased incidence of clinical signs in females ≥ 500mg/kg bw/day, but no details were provided. It was reported that in females at 500 mg/kg bw/day, there was a decrease in body weight gain and at 1000 mg/kg bw/day there was a statistically significant decrease in body weight. A non-statistically significant decrease in food consumption in females at 1000 mg/kg bw/day was reported. No further details were reported for body weight or food consumption data.
A non-statistically significant decrease in the fertility index was reported at 1000 mg/kg bw/day but no data was provided. No information was reported on oestrous cycle, parturition and/or maternal care.

The robust study summary indicated that this study was not reliable, and that the documentation was insufficient for assessment. The dossier submitter agrees that this study has limited reporting of methods and results and thus it is presented only as supporting evidence.

For effects relevant for classification for development seen in this study, please see section: 10.10.4 Adverse effects on development.

Further details on the above studies are provided in Annex I to this report.

10.10.3 Comparison with the CLP criteria

In accordance with Annex I to the CLP Regulation, substances may be classified as category 1A reproductive toxicants if they are known "*human reproductive toxicants*". There is no epidemiological data available to demonstrate reproductive toxicity in humans of piperonal; 1,3-benzodioxole-5-carbaldehyde; therefore, classification at category 1A is not warranted.

In accordance with Annex I to the CLP Regulation, substances may be classified as category 1B reproductive toxicants if presumed to be a human reproductive toxicant. The classification of a substance as category 1B reproductive toxicant '*…is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate'*.

In an OECD 422 Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test with piperonal; 1,3-benzodioxole-5-carbaldehyde, a statistically significant decrease in absolute prostate and epididymides weights was observed at 1000 mg/kg bw/day. There was also a biologically significant decrease in the relative weights of these organs. At the same dose, absolute and relative ovary and uterus weights were statistically significantly increased. Although there were no histopathological findings in these organs, the effect on reproductive organ weights in males and females is indicative of a treatment related effect at 1000 mg/kg bw/day.

A significant decrease in the fertility index, mean implantation sites and gestation index was observed at 1000 mg/kg bw/day. The dossier submitter considers these findings to be treatment related.

The dossier submitter notes that no significant maternal toxicity was observed and thus considers that the effects observed were not secondary non-specific consequences of other toxic effects. Therefore, the effects observed are indicative of an effect on sexual function and fertility. Based on the available information, the dossier submitter considers that classification in category 1B is warranted for effects on sexual function and fertility.

In accordance with Annex I to CLP Regulation, a substance may be classified as category 2 if it is a suspected human reproductive toxicant. The classification of a substance as category 2 reproductive toxicant is warranted *"…where there is some evidence from humans or experimental animals…of an adverse effect on sexual function and fertility, or on development…if deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification"*.

The OECD 422 study with piperonal; 1,3-benzodioxole-5-carbaldehyde provides clear evidence of an effect on sexual function and fertility, which is not considered a secondary non-specific consequence of other toxic effects. Therefore, a classification of piperonal; 1,3-benzodioxole-5-carbaldehyde in category 2 is not considered appropriate.

10.10.4 Adverse effects on development

Table 15: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD Guideline 414: Prenatal developmental toxicity. Rat Wistar CrLl. WI (Han), female, 22/dose. GLP compliant.	Piperonal or Heliotropin (tradename of piperonal; 1,3-benzodioxole-5-carbaldehyde) Purity: 99.9% Oral gavage, once daily. 0, 100, 300 and 1000 mg/kg bw/day. Vehicle; PEG 400. Females treated from GD 6-20. Reliability: reliable.	<u>Maternal</u> - No mortality observed. - At ≥300 mg/kg bw/day: piloerection, hunched posture and salivation. - At 1000 mg/kg bw/day: statistically significant ↓ in mean body weight gain, absolute and relative food consumption. - At 1000 mg/kg bw/day: biologically significant ↓ in mean gravid uterus weight. - At 1000 mg/kg bw/day: biologically significant ↑ in early and late resorptions and post-implantation loss. - No effect observed on number of pregnancies, number of implantations, pre-implantation loss or corpora lutea. <u>Foetuses</u> - At 1000 mg/kg bw/day: biologically significant ↓ in litter size and viable foetuses. - At 1000 mg/kg bw/day: statistically significant ↓ in mean foetal weight. - At ≥300 mg/kg bw/day: a biologically significant ↑ incidence of dilated ureter. At 1000 mg/kg bw/day: a biologically significant ↑ incidence of absent or small renal papilla. - At 1000 mg/kg bw/day: statistically significant ↑ in the incidence of rib anomaly and a biologically significant ↑ in vertebral anomaly (with or without rib anomaly), vertebral centra anomaly, sternoschisis and costal cartilage. - At ≥ 100 mg/kg bw/day: statistically significant ↑ in the incidence of unossified metacarpal and/or metatarsal. - At ≥ 300mg/kg bw/day: statistically significant ↑ in the incidence of reduced ossification of the skull. At 300mg/kg bw/day: statistically significant ↑ incidence of bent ribs. - At 1000 mg/kg bw/day: statistically significant ↑ in the incidence of reduced ossification of vertebral centra and ventral arches, unossified sternebrae at positions 1-4 and 5-6 and seventh cervical full rib and ossification sites. Biologically significant ↑ the incidence of fourteenth rib and malaligned sternebrae.	Anonymous, 2020b

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD 422; Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test. GLP compliant. Rat Wistar CrLl. WI (Han), male and female, 10/sex/dose. Deviated from OECD 422; -10 females instead of 12-13. -Only parental males were fasted prior to blood sampling. -Pup T4 blood sampled on PND 14-16 instead of PND 4.	Piperonal or Heliotropine (tradename of piperonal; 1,3-benzodioxole-5-carbaldehyde) Purity: 99.9% Oral gavage, once daily. 0, 100, 300 and 1000 mg/kg bw/day. Vehicle; PEG 400. Males - 7 days/week, for 10 weeks pre-mating period and 2-week mating period. Females- 7 days/week, for 10 weeks pre-mating period, 2-week mating period, throughout gestation period and until at least PND 13. Reliability: reliable.	<u>Parental</u> - No test substance related deaths. - Males at 1000 mg/kg bw/day: ↓ in mean body weight and mean body weight gain during premating and mating. Females at 1000 mg/kg bw/day: ↓ in mean body weight and mean body weight gain during gestation. - Females: ↑ in food consumption at ≥ 300 mg/kg bw/day during gestation and at 300 mg/kg bw/day during lactation (no information for 1000 mg/kg bw/day, no pups survived at this dose). Males: no effects observed. - Males at ≥ 300 mg/kg bw/day: statistically significant ↓ in total T4 levels. - ≥ 300 mg/kg bw/day: ↓ in post implantation survival index. At 1000 mg/kg bw/day: ↓ live birth and pup viability indices. - At 1000 mg/kg bw/day: ↓ in litter numbers and mean litter size. The lactation index could not be calculated for this group as there were no surviving pups at PND 1. There was no effect on lactation index and maternal care in females at ≤ 300 mg/kg bw/day. For information on sexual function and fertility related parameters, please refer to Table 11: Summary table of animal studies on adverse effects on sexual function and fertility. <u>Pups</u> - At 1000 mg/kg bw/day: ↓ litter numbers and mean live litter size. - At 1000 mg/kg bw/day: only 1 pup born in 1 litter, this pup was dead at first litter check on PND 1. - At ≥ 300 mg/kg bw/day: ↓ in post implantation survival index. - At 1000 mg/kg bw/day: no live pups on PND 4. - Females pups at ≥ 100 mg/kg bw/day: statistically significant, ↓ in mean body weight at PND 1. No effects observed in females pups during PND 4-13 or in male pups during PND 1-13. -No effects on AGD, nipple retention, T4 levels or macroscopic findings in pups. For information on non-reproductive system related repeat dose parameters, please refer to Table 22; Summary table of animal studies on STOT RE.	Anonymous, 2020a
Non-Guideline study. GLP status unknown. 10 female Sprague-Dawley	Piperonal, 1,3-benzodioxole-5-carbadehyde (purity unknown)	<u>Maternal</u> - ↓ in body weight in females at 1000 mg/kg bw/day and ↓ in body weight gain in females at	Vollmuth T.A., (1990), ECHA dissemination

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
rats per dose received a vehicle or test substance via oral gavage for 39 days. Male rats were untreated. Animals were mated 1:1. Parental females were examined for mating, fertility and gestation indices, delivery of litter, and number of offspring per litter, oestrous cycle, 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.	Oral gavage, once daily. 0, 250, 500 and 1000 mg/kg bw/day. Vehicle; methylcellulose or corn oil. Females treated for 39 days total: 7 days of pre-mating, 7 days of mating, 21-day gestation period and 4-day lactation period. Reliability: unreliable.	500 mg/kg bw/day. - At 1000 mg/kg bw/day, ↓ in food consumption. For information on sexual function and fertility related parameters, please refer to Table 11: Summary table of animal studies on adverse effects on sexual function and fertility. <u>Pups</u> - At 1000 mg/kg bw/day: ↑ in pup mortality and a ↓ in viability of offspring. No further details reported. -Survival index at weaning, AGD, organ weight, nipple retention, T4 levels, macroscopic and microscopic examinations were not assessed. -No information available on number of litters, dead pups, live birth and live litter size on PND 1 or sex ratios.	site, 2023

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

In an OECD 414 prenatal developmental toxicity study, 22 Wistar CrLl. WI (Han) female rats per dose were administered 0, 100, 300 and 1000 mg/kg bw/day of piperonal; 1,3-benzodioxole-5-carbaldehyde via oral gavage from GD 6-20. There were no deaths observed. Clinical observations at ≥300 mg/kg bw/day included salivation from GD1, piloerection from GD7 and hunched posture from GD 13-17.

There were no effects on mean maternal body weight. From GD 9-21 there was a statistically significant decrease in mean body weight gain at 1000 mg/kg bw/day (40% compared to 48% in controls at GD 21).

Table 16: 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**

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

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 GD 15-18 (11% above control values).

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. Therefore, the dossier submitter considers the toxicological significance of the decrease in total T3 and T4 to be unclear. There was no effect observed on absolute or relative thyroid weight.

At 1000 mg/kg bw/day, there was a biologically significant decrease in mean gravid uterus weight. 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.

At 1000 mg/kg bw/day, there was a biologically significant increase in early and late resorptions. The mean percentage 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, respectively. The mean percentage of late resorptions were 0%, 0%, 0% and 1.1% per litter at 0, 100, 300 and 1000 mg/kg bw/day, respectively. Both the mean early and 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; early resorptions; mean; 5.0%, percentile range; 1.9%-9.9% and late resorptions; mean; 0.1%, percentile range; 0%-0.4%)). The increase in resorptions, in particular the early resorptions, contributed to 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, with the incidence at 1000 mg/kg bw/day 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%).

There was no effect observed on the number of pregnancies, mean number of implantation sites, mean pre-implantation loss or mean number of corpora lutea.

Table 17: 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

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. At the same dose, 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%). There were no dead foetuses observed. At the highest dose, there was a statistically significant decrease in mean foetal weight; the mean weights were 5.3, 5.2, 5.0 and 3.9 g at 0, 100, 300 and 1000 mg/kg bw/day, respectively, and the mean foetal weight at 1000 mg/kg bw/day was below 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).

Table 18: 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

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 if they were treatment related or not.

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 concludes that these variations are treatment related.

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 also 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, which were also outside the test laboratory's historical control range (Rat Crl: WI (Han) on GD 21, (2014 – 2018). The incidences and laboratory's historical control data of these malformations are reported in Table 19 below. The dossier submitter noted that these malformations are all located in the same thoracic region and thus were 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 19: 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

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 20: 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

Variations		Dose (mg/kg bw/day)				
		0	**100**	**300**	**1000**	**HCD** **Litter incidence (%)**
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=-#
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

In an OECD 422 Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test, 10 Wistar CrLl. WI (Han) rats per sex per dose were administered 0, 100, 300 and 1000 mg/kg bw/day of piperonal; 1,3-benzodioxole-5-carbaldehyde. Males and females were treated for 7 days/week for the 10-week pre-mating and 2-week mating periods. Females were subsequently treated throughout the gestation period until at least PND 13.

In males at 1000 mg/kg bw/day, there was a statistically significant decrease in mean body weight from week 7 of pre-mating, mean body weight gain from week 4 of pre-mating until the end of the mating period (week 14) and mean terminal body weights.

In females at 1000 mg/kg bw/day, there was a decrease in mean body weight and mean body weight gain observed from GD 14. At this dose, mean body weight changes reached statistical significance on GD 17 and 20 and changes in mean body weight gain reached statistical significance on GD 20. For further details see Table 12 in section 10.10.2.

To further assess if the effect on maternal body weight observed at the highest dose was due to maternal toxicity or an intrauterine effect, the dossier submitter attempted to calculate the corrected mean maternal body weight changes in accordance with Annex I, 3.7.2.4.4 of CLP. However, there were no gravid uterine weights or pup weight data, and it was not possible to calculate the corrected maternal body weights at 1000 mg/kg bw/day. It should also be noted that the only female that gave birth at 1000 mg/kg bw/day was weighed on LD1 and therefore it was not possible to calculate a mean body weight for this treatment group. However, this individual dam had a higher body weight on LD1 than the mean control value (284g compared

to 267g). The dossier submitter notes that although there is limited data, it provides some indication that the effect on maternal body weight may be an intrauterine effect rather than maternal toxicity.

For other general toxicity effects and observations relevant for classification for sexual function and fertility seen in this study, please see section: 10.10.1 Adverse effects on sexual function and fertility.

A decrease in post implantation survival index was observed at ≥ 300 mg/kg bw/day. The indices 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 for Wistar Han rats (2015-2019), N; 118, mean; 92%, range; 83%-98%).

A decrease in the live birth index and pup viability was observed 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. The viability indices were 100%, 100%, 99% and 0% at 0, 100, 300 and 1000 mg/kg bw/day, respectively. There was only one pup born at 1000 mg/kg bw/day, and this pup did not survive past the first litter check on PND 1. At the same dose, there was a decrease in the total number of pups born (117, 93, 90 and 1 at 0, 100, 300 and 1000 mg/kg bw/day, respectively) and the number of live pups on PND 1 (117, 93, 87 and 0 at 0, 100, 300 and 1000 mg/kg bw/day, respectively). At the highest dose, there was also a decrease in the number of litters (10/10, 8/10, 9/10 and 1/10 at 0, 100, 300 and 1000 mg/kg bw/day, respectively) and the mean live litter size (11.7, 11.6, 9.7 and 0 living pups/litter at 0, 100, 300 and 1000 mg/kg bw/day, respectively). The number of live pups on PND 4 was 117, 93, 86 and 0 at 0, 100, 300 and 1000 mg/kg bw/day and at ≤ 300mg/kg bw/day all pups survived from PND 4 (after culling) until live pup check on PND 13. As there were no surviving pups at 1000 mg/kg bw/day, there was no data available for the lactation index or the sex ratio at this dose, therefore these parameters could not be assessed for this dose. At ≤ 300 mg/kg bw/day, there was no effect on lactation index on PND 13 and there were no differences noted in the male to female ratio before culling on PND 4 or on PND 13.

Table 21: 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

In female pups at 100 and 300 mg/kg bw/day, there was a statistically significant decrease in mean body weights (6.5, 5.8 and 5.9 g 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 is unclear if this effect was treatment related. There were no significant effects on male pup weight on PND 1 or the pup weight of either sex on PND 4, 7 or 13 at 0, 100 and/or 300 mg/kg bw/day.

There were no significant differences in T4 levels, anogenital distance (AGD), areola/nipple retention observed in pups in either sex at 0, 100 and/or 300 mg/kg bw/day.

In a 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: 7 days of pre-mating, 7 days of mating, 21-day gestation period and 4-day lactation period. 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.

The robust study summary indicated that there was a statistically significant increase in mortality of dams at 1000 mg/kg bw/day and an increased incidence of clinical signs ≥ 500mg/kg bw/day, but no details were

provided. It was reported that at 500 mg/kg bw/day there was a decrease in body weight gain and at 1000 mg/kg bw/day there was a statistically significance decrease in body weight. A non-statistically significant decrease in food consumption at 1000 mg/kg bw/day was reported. No further details were reported for body weight or food consumption data.

For effects relevant for classification for sexual function and fertility seen in this study, please see section: 10.10.1 Adverse effects on sexual function and fertility.

No information was reported on parturition and/or maternal care, number of litters, dead pups and live birth, litter size on PND 1 or sex ratio.

The robust study summary reported an increase in pup mortality and a statistically significant decrease in viability of offspring at 1000 mg/kg bw/day. A non-statistically significant decrease in body weight gain during PND 1-4 was reported at ≥ 500mg/kg bw/day. No clinical signs of toxicity were reported. There were no further details reported for pup mortality, viability or body weight.

The robust study summary indicated that this study was not reliable, and that the documentation was insufficient for assessment. The dossier submitter agrees that this study has limited reporting of methods and results and thus the study is used only as supporting evidence.

Further details on the above studies are provided in Annex I to this report.

10.10.6 Comparison with the CLP criteria

In accordance with Annex I to the CLP Regulation, substances may be classified as category 1A reproductive toxicants if they are known "*human reproductive toxicants*". There is no epidemiological data available to demonstrate reproductive toxicity in humans of piperonal; 1,3-benzodioxole-5-carbaldehyde; therefore, classification at category 1A is not warranted.

In accordance with Annex I to the CLP Regulation, substances may be classified as category 1B reproductive toxicants if they are a presumed human reproductive toxicant. The classification of a substance as category 1B reproductive toxicant '*…is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate'*.

In the available OECD 414 Prenatal developmental toxicity study with piperonal; 1,3-benzodioxole-5-carbaldehyde, a significant increase in early and late resorptions and post implantation loss, and a significant decrease in foetal weight, mean litter size and viable foetuses per litter was observed at 1000 mg/kg bw/day.

At ≥ 300 mg/kg bw/day, there was an increased incidence of visceral variations, dilated ureter and absent or small renal papilla. It was noted that these variations were outside the range of the historical control data of the test laboratory, and it is concluded that they are treatment related.

A significant increase in the incidence of skeletal malformations: rib anomaly, vertebral anomaly (with or without rib anomaly), vertebral centra anomaly, sternoschisis and costal cartilage was observed at 1000 mg/kg bw/day. The incidences of these malformations were outside the historical control range of the test laboratory and were associated with the same thoracic region, which supports the conclusion they were treatment related. An increased incidence of skeletal variations including unossified metacarpal and/or metatarsal at ≥ 100 mg/kg bw/day, reduction of ossification of the skull and bent ribs at ≥ 300 mg/kg bw/day and reduced ossification of vertebral centra and ventral arches, unossified sternebrae at positions 1-4 and 5-6, seventh cervical full rib and ossification sites, caudal shift in the pelvic girdle, increases in the fourteenth rib and malaligned sternebrae at 1000 mg/kg bw/day was observed. The incidence of these skeletal variations were also outside the historical control range of the test laboratory and therefore support the conclusion that these variations are treatment related.

The dossier submitter notes that the observed effects occurred in the absence of significant maternal toxicity and are therefore not considered to be a secondary non-specific consequence to other toxic effects.

In the available OECD 422, Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test with piperonal; 1,3-benzodioxole-5-carbaldehyde, there was a decrease in the number of litters, mean litter size and number of viable pups on PND 1 at 1000 mg/kg bw/day and on the post-implantation survival index at ≥ 300 mg/kg bw/day. At 1000 mg/kg bw/day, only one pup was born, and it did not survive past the first litter check at PND 1, and thus the pup viability was 0%.

Based on the available information, the dossier submitter considers that classification in category 1B is warranted for effects on development.

In accordance with Annex I to CLP Regulation, a substance may be classified as category 2 if it is a suspected human reproductive toxicant. The classification of a substance as category 2 reproductive toxicant is warranted *"…where there is some evidence from humans or experimental animals…of an adverse effect on sexual function and fertility, or on development…if deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification".*

Both guideline studies with piperonal; 1,3-benzodioxole-5-carbaldehyde provide clear evidence of an effect on development, which is not considered a secondary non-specific consequence of other toxic effects. Therefore, a classification of piperonal; 1,3-benzodioxole-5-carbaldehyde in category 2 is not considered suitable.

10.10.7 Adverse effects on or via lactation

In an OECD 422 Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test, at 1000 mg/kg bw/day, there were no surviving pups and therefore there was no data available for the lactation index to be assessed at this dose. The study author reported 100% for lactation index at 0, 100 and 300 mg/kg bw/day. There was no other data available in relation to effects on or via lactation. For further details see section: 10.10.4 Adverse effects on development.

10.10.8 Conclusion on classification and labelling for reproductive toxicity

Based on the available data, classification of piperonal; 1,3-benzodioxole-5-carbaldehyde as a reproductive toxicant category 1B for effects on sexual function and fertility and effects on development is warranted.

10.11 Specific target organ toxicity-single exposure

Not evaluated as part of this dossier.

10.12 Specific target organ toxicity-repeated exposure

No classification proposed.

The repeated dose toxicity studies reported below are provided only as supporting information for reproductive toxicity assessment.

Table 22: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
OECD 422; Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test. GLP compliant. Rat, Wistar CrLl. WI (Han), male and female, 10/sex/dose. Deviated from OECD 422; -10 females instead of 12-13. -Only parental males were fasted prior to blood sampling. -Pup T4 blood sampled on PND 14-16 instead of PND 4.	Piperonal or Heliotropine (tradename of piperonal; 1,3-benzodioxole-5-carbaldehyde) Purity 99.9% Oral gavage, once daily. 0, 100, 300 and 1000 mg/kg bw/day Vehicle; PEG 400. Males - 7 days/week, for 10 week pre-mating period and 2-week mating period. Females- 7 days/week, for 10 week pre-mating period, 2-week mating period, throughout gestation period and until at least PND 13. Reliability: reliable.	<u>Parental animals</u> - Males at 100 mg/kg bw/day: 1/10 died on day 21 of premating. Females at 1000 mg/kg bw/day: 1/10 died on lactation day 4. No abnormalities found or cause of death established at necropsy for either animal. - Slight salivation was observed from week 2 at ≥ 300mg/kg/bw/day and from week 8 in all treated animals. - Males at 1000 mg/kg bw/day: ↓ in mean body weight and mean body weight gain during premating and mating. Females at 1000 mg/kg bw/day: ↓ in mean body weight and mean body weight gain during gestation. - Females: ↑ in food consumption at ≥ 300 mg/kg bw/day during gestation and at 300 mg/kg bw/day during lactation (no information for 1000 mg/kg bw/day, no pups survived at this dose). Males: no effects observed. - Males at 1000 mg/kg bw/day: ↓ in mean total movement. - Both sexes at 1000 mg/kg bw/day: ↓ in red blood cells and platelets. Males at 1000 mg/kg bw/day: ↓ in eosinophils and an ↑ in reticulocytes and mean corpuscular volume. Females at 1000 mg/kg bw/day: ↓ in neutrophils, haematocrit, haemoglobin, mean corpuscular volume and ↑ in mean corpuscular haemoglobin. - Both sexes at 1000 mg/kg bw/day: ↓ LDL cholesterol and ↑ in mean total protein. Males at 1000 mg/kg bw/day: ↑ mean alkaline phosphatase, mean alanine aminotransferase activity, mean potassium levels, mean	Anonymous, 2020a

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
		inorganic phosphate, bile acids and ↓ mean HDL. Males at ≥ 300 mg/kg bw/day: ↑ mean chloride. Females at 1000 mg/kg bw/day: ↑ in calcium and glucose levels and a ↓ mean aspartate aminotransferase, mean alanine aminotransferase activity, mean potassium levels and mean urea levels. Females at 100 and 1000 mg/kg bw/day: ↑ mean albumin levels. Females ≥300 mg/kg bw/day: ↓ in total bilirubin. -Males at ≥ 300 mg/kg bw/day: statistically significant ↓ in total T4 levels. - Both sexes at 1000 mg/kg bw/day: ↓ absolute pituitary weight, absolute adrenal weight and ↑ in relative kidney weight, relative heart weight and relative brain weight. - Males at 1000 mg/kg bw/day: ↓ in absolute and relative prostate weight, absolute and relative epididymides weight, absolute brain weight, absolute and relative thymus weight and ↑ absolute and relative liver weight. - Females: ↑ in absolute and relative ovary weight, absolute and relative uterus weight absolute thyroid weight, absolute and relative thymus weight and ↓ absolute liver and heart weights. - Discolouration of the adrenal glands in 2/10 females at 1000 mg/kg bw/day. Nodules in the epididymides in 1/10 males at 1000 mg/kg bw/day. - Both sexes at 1000 mg/kg bw/day: hepatocellular hypertrophy. Males at 1000 mg/kg bw/day and females at 0, 100 and 1000 mg/kg bw/day: lymphoid atrophy of the thymus. Males at 1000 mg/kg bw/day and females at ≥ 300mg/kg bw/day: increased trabecular bone in both the sternum and femur. Females at 1000 mg/kg bw/day: cystic epithelial hyperplasia of the thymus. See Table 11: Summary table of animal studies on adverse effects on sexual function and fertility and section 10.10.2 for short summary of reproductive parameters.	
A non-guideline study. Not GLP compliant. 10 Male and female rats (strain not specified, 27-29 days old) were administered the test substance in a mixture, (heliotropin (22ppm) in a combined total mixture of 145 ppm), orally via feed daily for 12 weeks. No information on control available. Animals were observed for effects on clinical observations, body weight, food consumption, food efficiency and	Heliotropin (Trade name of piperonal; 1,3-benzodioxole-5-carbaldehyde) No information available on form or purity. Oral; feed. 22 ppm approximately equivalent to 17 mg/kg bw. Reliability: unreliable.	- No effect observed on clinical signs, mortality, body weight, body weight changes, food consumption, food efficiency, urinalysis, organ weight or gross pathology. - NOAEL = 17 mg/kg bw/day	Anonymous, (1958), ECHA dissemination site, 2023

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
urinalysis. Necropsy examinations were performed. Limited information available.			
A non-guideline study. Not GLP compliant. The test substance was administered orally via feed daily to rats (Osborne-Mendel) male and female, (5/sex/dose for dose groups and 10/sex for control). No information provided on vehicle. Animals were observed for effects body weight, food consumption, food efficiency and haematology. Necropsy examinations were performed	Piperonal No information available on form or purity. Oral; feed. 1000 ppm (approximately equivalent to 50 mg/kg bw/day) daily for 27-28 weeks. 10,000 ppm (approximately equivalent to 500 mg/kg bw/day) daily for 15 weeks. Reliability: unreliable.	- No effect observed on mortality, body weight, body weight changes, food consumption, haematology, organ weight or gross pathology. - NOAEL= 50 mg/kg bw/day for 28 weeks dietary exposure and 500 mg/kg bw/day for 15 weeks dietary exposure.	Hagan E.C, (1967), ECHA dissemination site, 2023
A non-guideline study. Not GLP compliant. The test substance was administered orally via feed daily to an unspecified number of male and female rats (Osborne-Mendel). No information was provided on number of animals/ doses, vehicle or any details of the study methodologies e.g., observations and examination, necropsy or gross pathology. No details provided for results of examinations.	Piperonal No information available on form or purity. Oral; feed. 1000 ppm (approximately equivalent to 50 mg/kg bw/day) daily for 28 weeks. 10,000 ppm (approximately equivalent to 500 mg/kg bw/day) daily for 16 weeks. Reliability: unreliable.	- Study summary reports “Quantitative data not provided. The study authors concluded, "No adverse effects occurred in the rats fed piperonal”.	Hagan E.C, (1965), ECHA dissemination site, 2023
A non-guideline study. Not GLP compliant. The test substance was administered orally via feed daily to 5 rats/sex/dose (strain not specified). Gross pathology and histopathology were carried on liver, kidneys, testis, spleen, adrenal and thyroid. No information provided on vehicle or details of the study methodologies e.g., observations and examination.	Piperonal No information available on form or purity. Oral; feed. 0.1% (approximately equivalent to 50 mg/kg bw/day), daily for 28 weeks. 1% (approximately equivalent to 500 mg/kg bw /day), daily for 16 weeks. Reliability: unreliable.	- Study summary reports “based on the findings from the 28-week exposure period, the NOAEL for this study can be considered as 50 mg/kg body weight/day (a higher NOAEL of 500 mg/kg body weight/day can be considered for the shorter exposure period of 16 weeks)”.	Anonymous, (1954), ECHA dissemination site, 2023
A non-guideline study. Not GLP compliant. The test substance was administered orally via feed daily to 30 rats/sex at 0.5% and 10 rats/sex at 0.1% (strain not specified) for 2 years. Animals were examined for changes in appearance, behaviour, weight. Liver, kidneys, adrenal glands, heart, spleen, pancreas, cerebellum, and any identified tumours were examined histopathologically. No information available on food consumption, ophthalmology,	Piperonal No information available on form or purity. Oral; feed. 0.1% (approximately equivalent to 50 mg/kg bw/day), daily for 2 years. 0.5% (approximately equivalent to 250 mg/kg bw /day), daily for 2 years. Reliability: unreliable.	- No effect on clinical signs, mortality, body weight, and body weight changes. No gross pathological or histopathological changes observed. - The study summary reports no effects on growth or carcinogenicity and the NOAEL was 250mg/kg bw/day.	Bar F., (1967), ECHA dissemination site, 2023

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
haematology, clinical biochemistry, behaviour, organ weights and urinalysis.			

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

In an OECD 422; Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test, 10 Wistar CrLl. WI (Han) rats per sex per dose were administered 0, 100, 300 and 1000 mg/kg bw/day piperonal; 1,3-benzodioxole-5-carbaldehyde. Males and females were treated for 7 days/week for the 10 week pre-mating and 2 week mating periods. Females were then treated throughout the gestation period, until at least PND 13.

There were 2 deaths observed, 1 male at 100 mg/kg bw/day and 1 female at 1000 mg/kg bw/day, the cause of death for both was not established. The only clinical sign noted was slight salivation.

At 1000 mg/kg bw/day, there was a decrease in mean body weight and mean body weight gain in males during pre-mating and mating and in females during gestation. Females had an increase in food consumption at ≥ 300 mg/kg bw/day during gestation and at 300 mg/kg bw/day during lactation. There was no information for females at 1000 mg/kg bw/day during lactation, as no pups survived at this dose. There were no effects observed in males. Males at ≥ 300 mg/kg bw/day had a statistically significant decrease in total T4 levels. There was no effect on total T4 levels in treated females or total T3 and TSH levels in either sex. Effects on body weight, food consumption and thyroid hormones are further discussed in section 10.10.2.

In the functional observation battery, there were statistically significant decreases in mean total movement reported for males 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 effects in males is unclear. There were some statistically and non-statistically significant changes reported in haematology, including a decrease in red blood cells and platelets in both sexes at 1000 mg/kg bw/day. There were also statistically significant changes reported in other clinical chemistry parameters including a decrease in LDL cholesterol in both sexes at 1000 mg/kg bw/day.

At 1000 mg/kg bw/day, absolute prostate and epididymides weights were statistically significantly decreased. There was also a biologically significant decrease in the relative weights of these organs. At the same dose, there were statistically significant increases observed in absolute and relative ovary weights and absolute and relative uterus weights. There were no histopathological findings in the reproductive organs in either sex.

There was a statistically significant increase in absolute pituitary weights in females at ≥ 300 mg/kg bw/day and in males at 1000 mg/kg bw/day. In both sexes at 1000 mg/kg bw/day, a statistically significant decrease

in absolute adrenal weights was observed. At 1000 mg/kg bw/day, there was a statistically significant decrease in absolute brain weight in males and a statistically significant increase in relative brain weight in both sexes. 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 at 1000 mg/kg bw/day, there was a statistically significant decrease in absolute and relative thymus weight and a statistically significant increase in absolute and relative liver weight. In females at 1000 mg/kg bw/day, there was a statistically significant increase in absolute thyroid weight, absolute and relative thymus weight and statistically significant decrease in absolute liver and heart weights.

Incidences of lymphoid atrophy of the thymus, graded as minimal, were observed in females at 0, 100 and 1000 mg/kg bw/day and in males at 1000 mg/kg bw/day and cystic epithelial hyperplasia of the thymus, graded from minimal to moderate, was observed in females at 1000 mg/kg bw/day. Incidences of hepatocellular hypertrophy, graded as minimal, were observed in both sexes at 1000 mg/kg bw/day. 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, which was considered adverse at 1000 mg/kg bw/day by the study author.

Further details on the above study are provided in Annex I to this report.

A number of non-guideline oral repeated dose toxicity studies with piperonal; 1,3-benzodioxole-5-carbaldehyde are available with exposure periods from 5 weeks to 2 years. All of these studies reported limited information on methods and results and are therefore considered unreliable by the dossier submitter and not considered further.

10.12.2 Comparison with the CLP criteria

Not evaluated as part of this dossier. The information is provided as supportive information for the reproductive toxicity assessment (see section 10.10).

10.12.3 Conclusion on classification and labelling for STOT RE

Not evaluated as part of this dossier. The information is provided as supportive information for the reproductive toxicity assessment (see section 10.10).

10.13 Aspiration hazard

Not evaluated as part of this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated as part of this dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated as part of this dossier.

13 ADDITIONAL LABELLING

Not applicable.

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15 ANNEXES

Detailed study summaries for studies referenced in this report.