



Scientific Committee on Consumer Safety
SCCS

OPINION

**on the safety of Heliotropin
used in cosmetic products
(CAS No. 120-57-0, EC No. 204-409-7)**



The SCCS adopted this document in plenary meeting on 26 March 2026

ACKNOWLEDGMENTS

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1. ABSTRACT

The SCCS concludes the following:

1. *In light of the data provided and taking under consideration the possible classification as 'Repr.1B' under Regulation (EC) No 1272/2008 (CLP Regulation) and the conditions laid out in Article 15 (2) (d) of the Regulation (EC) No 1223/2009, does the SCCS consider Heliotropin, safe when used as an ingredient in fine fragrances for adults only up to 1.8%?*

Based on the assessment of data provided and taking into consideration the concerns related to the possible classification as 'Repr.1B' under Regulation (EC) No 1272/2008 (CLP Regulation), the SCCS considers Heliotropin safe when used up to the maximum concentration of 1.8% only in fine fragrances for adults.

2. *Alternatively, what is according to the SCCS, the maximum concentration considered safe for use of Heliotropin in cosmetic products?*

/

3. *Does the SCCS have any further scientific concerns regarding the use of Heliotropin in cosmetic products?*

/

Keywords: SCCS, scientific opinion, Heliotropin, Regulation 1223/2009, CAS No. 120-57-0, EC No. 204-409-7

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on the safety of Heliotropin (CAS No. 120-57-0, EC No. 204-409-7) used in cosmetic products, preliminary version of 26 March 2026, SCCS/1688/26

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In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide Opinions on questions concerning health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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ISSN

ISBN

Doi:

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background

Heliotropin (CAS No. 120-57-0, EC No. 204-409-7), also known as piperonal, is an aromatic compound commonly used in cosmetic products for its sweet, floral scent. Naturally found in some essential oils but mostly produced synthetically, Heliotropin is a popular fragrance ingredient in perfumes, lotions, creams, shampoos, and deodorants. In addition, Heliotropin can function as a fixative, helping to stabilize and prolong other scents in a formulation, as well as a masking agent, softening the odour of other ingredients to enhance overall product appeal.

The European Risk Assessment Committee (RAC) of ECHA issued in September 2024 an opinion recommending among others a classification for Heliotropin as 'Reprotoxic of Category 1B (H360Fd)'. Following the RAC opinion, the European Commission may propose a classification for Heliotropin as a 'Repr.1B' (CLP Regulation Annex VI entry).

According to Article 15(2) of the Cosmetics Regulation, *'The use in cosmetic products of substances classified as CMR substances, of category 1A or 1B under Part 3 of Annex VI to Regulation (EC) No 1272/2008 shall be prohibited. However, such substances may be used in cosmetic products by way of exception where, subsequent to their classification as CMR substances of category 1A or 1B under Part 3 of Annex VI to Regulation (EC) No 1272/2008, all of the following conditions are fulfilled:*

- (a) they comply with the food safety requirements as defined in Regulation (EC) No 178/2002¹ of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety;*
- (b) there are no suitable alternative substances available, as documented in an analysis of alternatives;*
- (c) the application is made for a particular use of the product category with a known exposure; and*
- (d) they have been evaluated and found safe by the SCCS for use in cosmetic products, in particular in view of exposure to these products and taking into consideration the overall exposure from other sources, taking particular account of vulnerable population groups.'*

In view of the above, regulatory measures must be adopted by the Commission services within 15 months of the classification as CMR 1A or 1B of the substance(s) concerned in Part 3 of Annex VI to Regulation (EC) No 1272/2008.

In June 2025, the Commission services received a dossier to defend the safe use of Heliotropin (CAS No. 120-57-0, EC No. 204-409-7) as an ingredient in fine fragrances for adults only according to Article 15(2) of the Cosmetics Regulation (EC) No. 1223/2009. The Commission, therefore, requests the SCCS to carry out a safety assessment on this ingredient in view of the information provided.

¹ <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L:2002:031:FULL&from=EN>

1
2 **Terms of reference**
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- 4 1. *In light of the data provided and taking under consideration the possible classification as*
5 *'Repr.1B' under Regulation (EC) No 1272/2008 (CLP Regulation) and the conditions laid*
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3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical Identity

3.1.1.1 Primary name and/or INCI name

INCI name:
HELIOTROPINE

3.1.1.2 Chemical names

1,3-Benzodioxole-5-carboxaldehyde
IUPAC name: 1,3-benzodioxole-5-carbaldehyde or 2H-1,3-Benzodioxole-5-carbaldehyde

3.1.1.3 Trade names and abbreviations

Piperonal
Heliotropine
Heliotropin
HLT (abbreviation at ECHA registration dossier)

Synonyms (RIFM assessment: Api, 2025):

- 1,3-Benzodioxole-5-carboxaldehyde
- Dioxymethylene protocatechuic aldehyde
- Heliotropin
- 3,4-Methylene dioxybenzaldehyde
- Piperonyl aldehyde
- Protocatechuic aldehyde methylene ether
- Piperonylaldehyde
- Piperonaldehyde
- 5-Formyl benzodioxole
- 3,4-Dimethylenedioxybenzaldehyde
- Helio-Tropin
- ピペロナル
- 1,3-Benzodioxole-5-carbaldehyde
- Heliotropine Crystals

3.1.1.4 CAS / EC number

CAS n°: 120-57-0
EC number: 204-409-7

3.1.1.5 Structural formula

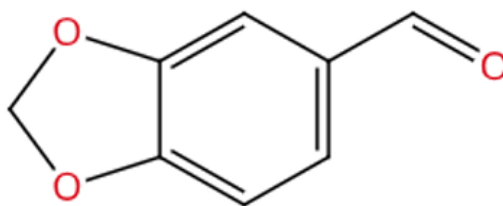


Figure 1: Structure of the substance

Ref.: Givaudan, 2025

3.1.1.6 Empirical formula

C₈H₆O₃

3.1.2 Physical form

Solid (crystal/flakes) at 20°C, colourless or white

3.1.3 Molecular weight

150.13 g/mol

3.1.4 Purity, composition and substance codes

Degree of purity: 99.9% (mono-constituent substance)

Ref: Givaudan, 2025

3.1.5 Impurities / accompanying contaminants

No impurities have been reported.

SCCS comment

To support the analytical data, a full analytical report of the chemical characterisation of the test substance in terms of purity, identity and impurities in representative batches should be provided.

3.1.6 Solubility

Water Solubility: water solubility was measured at pH > 4.8 - < 5.8 (GLP, flask method, EU A.6, OECD 105): 1.4 ± 0.008 g/L at 20°C

Table 1 provides an overview of the mole fraction solubility of Heliotropin in isopropyl alcohol, acetone, chloroform and ethanol as determined experimentally at different temperatures (Wang & Chen, 2006).

T/K	Water		Isopropyl alcohol		Acetone		Trichloromethane		Ethanol	
	$10^3 x^{expt}$	$10^3 x^{calcd}$	x^{expt}	x^{calcd}	x^{expt}	x^{calcd}	x^{expt}	x^{calcd}	x^{expt}	x^{calcd}
273.15	0.1180	0.1174	0.0174	0.0174	0.0521	0.0517	0.4275	0.4288	0.0056	0.0055
278.15	0.1530	0.1568	0.0227	0.0229	0.0655	0.0666	0.4766	0.4739	0.0143	0.0154
283.15	0.2200	0.2110	0.0332	0.0321	0.0876	0.0873	0.5308	0.5309	0.0396	0.0364
288.15	0.2770	0.2858	0.0461	0.0476	0.1176	0.1160	0.5984	0.6023	0.0697	0.0722
293.15	0.3930	0.3895	0.0753	0.0744	0.1551	0.1563	0.6934	0.6913	0.1228	0.1224

SCCS comment

The SCCS has noted that Heliotropin is soluble in different solvents, including ethanol, and is slightly soluble in water.

3.1.7 Partition coefficient (Log Pow)

1.2 at 35°C (GLP, EU Method A.8, OECD 117)

Ref: Rudio, 1997

3.1.8 Additional physical and chemical specifications

- organoleptic properties: odour balsamic, mild, powdery, aniseed-like
- melting point: 36.5 ± 0.5 °C at 98.5kPa (capillary method, EU A.1. and OECD 102)
- boiling point: 272 ± 2 °C at 98.5kPa (Siwoloboff method, EU A.2. and OECD 103)
- vapour pressure: 1 mmHg at 87.0°C and 760 mmHg at 263.0°C
- density: 1.4195 g/cm³ at 20°C (OECD 109)
- viscosity: /
- pKa: /
- pH: /
- refractive index: /
- UV/visible spectrum in methanol (0.015g/l cuvette 1 cm): λ_{max} : 230 nm, 273 nm and 312,5 nm (Kreutzer, 2019)
- particle size distribution: median particle size L50 = 272 μ m (GLP, OECD 110)

SCCS comment

The SCCS has noted that physical and chemical specifications reported above are also included in the registration dossier from ECHA: <https://echa.europa.eu/el/registration-dossier/-/registered-dossier/5265>

3.1.9 Homogeneity and Stability

The stability is confirmed under the conditions of the toxicological studies: in Polyethylene glycol 400, stability for at least 24 hours at room temperature protected from light, for at least 8 days in the refrigerator and for at least 3 weeks in the freezer (≤ -15 °C) is confirmed over the concentration range 1 to 200 mg/mL.

Ref: Kruijssen, 2019; Dassen Roland, 2021.

SCCS comment

Detailed data on the stability of the test substance under the conditions of use should be provided.

3.2 TOXICOKINETICS**3.2.1 Dermal / percutaneous absorption**

Despite physicochemical parameters (MW: 150 g/mol, LogP: 1.2 and water solubility: 1400 mg/L) that are generally considered to be compatible with dermal absorption, a high dermal absorption is not expected. This is based on the observation of toxicological studies by dermal route: no systemic toxicity was observed in rats administered an acute dermal dose of 5000 mg/kg body weight, and generally no dermal irritation has been observed in reliable studies conducted in humans, rats, and Guinea pigs.

The dermal absorption can be estimated to be 40%, for a corrected J_{max} of 8.263 $\mu\text{g}/\text{cm}^2/\text{h}$, based on MW: 150.13 g/mol, LogP: 1.2 and solubility: 1400 mg/L, and the skin absorption model developed for fragrances by (Shen *et al.*, 2014). This value is consistent with the default value of 50%. Therefore, in the absence of any experimental study, a default value of 50% is considered for safety assessment.

SCCS comment

In the absence of any proper *in vitro* dermal absorption study, the SCCS agrees with the Applicant on the use of default value of 50% for safety assessment.

3.2.2 Other studies on toxicokinetics**Oral absorption**

According to the applicant, bioavailability by oral route is expected to be high. According to REACH guidance document R7. C, oral absorption is maximal for substances with molecular weights below 500 g/mol, moderate log Kow values (between -1 and 4) and water solubility. Heliotropin has a MW: 150 g/mol, a LogP: 1.2 and a water solubility of 1400 mg/L. Experimental results, performed at dose levels below the level of saturation of the primary metabolic route, confirm a high bioavailability. In mice, nearly 90% of the dose was recovered in the urine within 12 hours of dosing, while 92.6% of the dose was recovered in the urine of rats within 24 hours of dosing. In both species, the major urinary metabolite was piperonylglycine. In rats, for which quantitative data are available, piperonylglycine accounted for 71% of the administered dose of heliotropin at 24 hours post-dosing while piperonylic acid accounted for 20% of the dose (Anonymous (REACH), 2021).

Considering this information, a bioavailability above 90% is expected and 90% of oral absorption is selected for the risk assessment in this dossier

SCCS comment

Based on the data provided, a bioavailability by oral route of 90% is selected for use in safety assessment in this dossier.

Other studies

Heliotropin can be metabolized by oxidation to piperonylic acid followed by glycine- or glucuronide conjugation. Alternatively, it can be reduced to piperonylic alcohol and conjugated to glucuronic acid or glutathione. As a third option, the methylenedioxy-ring might be cleaved leading to a catechol. These pathways were all observed in cultured rat hepatocytes (Figure 2).

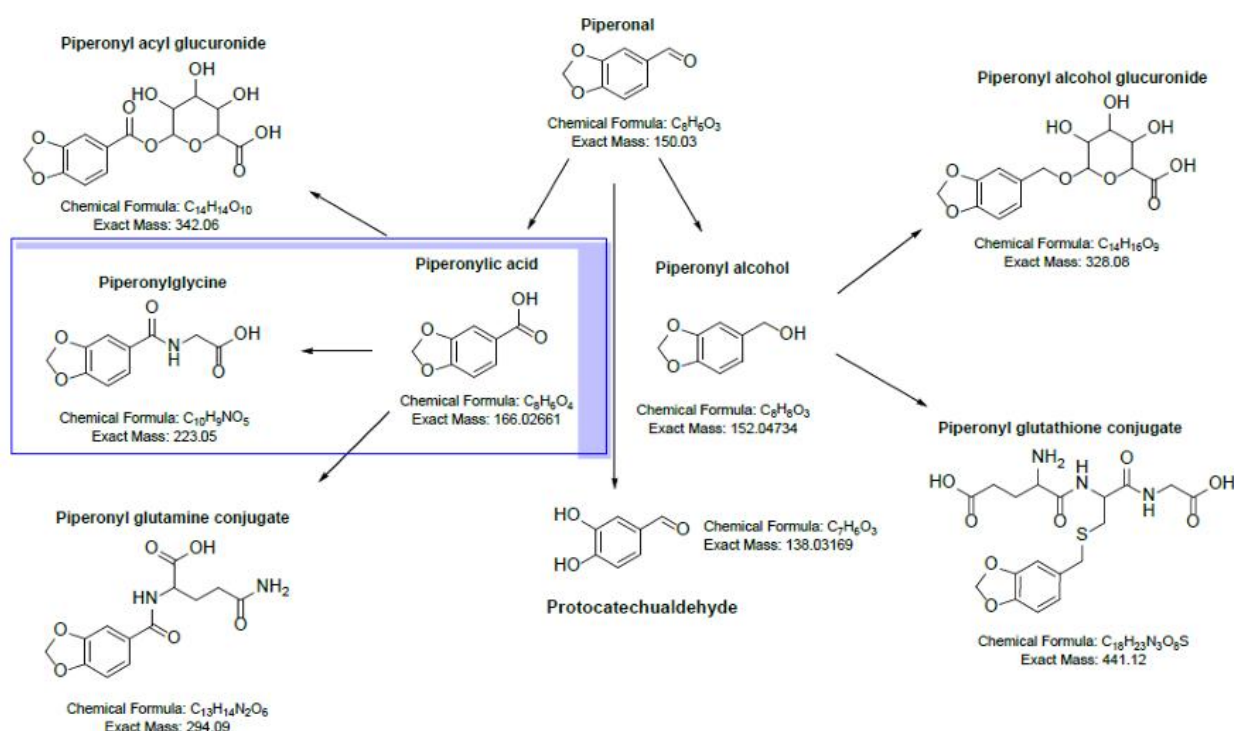


Figure 2: Metabolic pathway of Heliotropin

In an *in vivo* study in rats, heliotropin was applied by gavage at a concentration of 150 mg/kg bw (1 mmol/kg bw). Based on urinary excretion over 48 h, 71% is excreted as piperonylglycine, while 20% is excreted as piperonylic acid (Klungsoyr J & Scheline RR, 1984) at this dose. Interestingly, also after gavage administration of an equal dose of piperonyl alcohol, the elimination pathway was predominantly through heliotropin leading to a similar amount of piperonylglycine, indicating that this oxidative pathway is dominant, and that metabolism *via* the alcohol glucuronide is quantitatively not important *in vivo* in the rat. In line with the *in vitro* data, also *in vivo*, only traces of the catechol derivatives were observed (< 1%) (Klungsoyr J & Scheline RR, 1984). Only minimal contribution of the cleavage of the methylenedioxy-ring was also observed in a study with radiolabelled substrate (Kamienski & Casida, 1970).

For benzoic acid and its precursors (Beliveau & Brusilow, 1987; Gregus *et al.*, 1992; Yuan *et al.*, 1995) and salicylic acid (Patel *et al.*, 1990), it is known from multiple studies, that the glycine conjugation pathway is saturable when these chemicals are administered at a high dose, an effect which is particularly pronounced after gavage application, when a large amount of the acid is formed or delivered to the liver within a short timeframe. Saturation of the glycine-conjugation pathway leading to a peak of the free acid has been implicated in the toxicity, for e.g. benzyl acetate or salicylates (Yuan *et al.*, 1995). Glycine administration can reduce this saturation effect and hence dampen the toxic effects (Abdo *et al.*, 1998; Beliveau & Brusilow, 1987).

In the *in vivo* toxicokinetic study only one dose of heliotropin was applied (150 mg/kg bw), which is within the non-toxic range where no effects were seen in the developmental and reprotoxicity screening studies. Therefore, from the *in vivo* toxicokinetic study, no substance-specific indication of the saturation of the detoxification pathways for heliotropin nor for non-linear kinetics can be directly drawn, although there is a clear indication from other substances as summarized above, that the glycine-conjugation pathway is saturated in rats by gavage dosing close to the limit dose, and one may assume that the capacity of a given pathway – as long as the same enzymes and co-factors are involved – is not substance specific.

According to RAC assessment

After the consultation of the CLH report, a trade association proposed a read-across approach from toxicokinetic data of benzoate compounds (i.e. benzoic acid, benzoate salts, benzaldehyde, benzyl acetate, benzyl alcohol and benzyl benzoate) to piperonal. In their submitted position paper, they argued that piperonal is mainly excreted *via* the Hippurate pathway, **that a glycine dependent saturation of the Hippurate pathway takes place, and that this is not relevant to humans.** In their view, this would support that the effects on fertility and development observed in piperonal studies at high dose levels are only due to glycine depletion and consequently high free piperonylic acid concentration.

Considering that human exposure to piperonal would clearly be below this point of saturation, **the trade association concluded that there was doubt about human relevance for effects on fertility and development observed at the limit dose.** Therefore, they considered that Category 2 would be more appropriate than Category 1B.

RAC considers that the read-across from benzoate compounds is not justified since piperonal is not a precursor of benzoic acid. Further, the hippurate pathway is not appropriate since piperonylic acid does not give rise to hippuric acid after conjugation with glycine. RAC also notes that among the reports on benzoate compounds (WHO, 2005; EFSA, 2016 and JECFA, 2022) cited by the trade association, none includes piperonal in their grouping.

Ref: Committee for Risk Assessment RAC, 2024

SCCS comment

From the very limited compound-specific toxicokinetic data, the SCCS also considers that no conclusion can be drawn on the saturation of glycine-conjugation in rats exposed to high doses of piperonal since a single dose of 150 mg/kg bw was tested in Klungsoyr (1984) and alternative metabolism pathways (e.g. glucuronidation) at higher dose levels cannot be excluded.

From the Applicant: recent study to investigate this assumption (Natsch, 2025)

To fill this gap on the saturation of the elimination pathway for heliotropin with *in vitro* data, studies in cultured rat hepatocytes were conducted applying increasingly high doses which mimic the dose-range of the key *in vivo* study, calculated as the dose per hepatocyte under the treatment regime (Table 2).

Table 2 (taken from Table S1 of (Natsch A., 2025): Calculation applied to translate the *in vivo* to *in vitro* dose.

Table S1. To translate the *in vivo*, to the *in vitro* dose, the following calculation was applied:

<i>In vivo</i> dose	1000 mg/kg	300 mg/kg	100 mg/kg
Rat liver weight	10.5 g	10.5 g	10.5 g
Rat liver hepatocellularity	4.5×10^7 cells/g	4.5×10^7 cells/g	4.5×10^7 cells/g
Rat weight	235 g	235 g	235 g
Dose: mg/g Liver	22.3 mg	6.7 mg	2.2 mg
Dose per cell (pg/cell)	494 pg/cell	148 pg/cell	49 pg/cell

<i>In vitro</i> dose	1200 μ M	600 μ M	200 μ M	50 μ M
Volume	0.15 mL	0.15 mL	0.15 mL	0.15 mL
Mg/well	0.027 mg	0.013 mg	0.0045 mg	0.0011 mg
Number of cells / well	7×10^4 cells	7×10^4 cells	7×10^4 cells	7×10^4 cells
Dose per cell (pg/cell)	386 pg /cell	193 pg /cell	64 pg /cell	16 pg /cell

In cultured rat hepatocytes, Heliotropin was eliminated completely within 7h, even after application of doses of up to 1200 μ M (data not shown). Figure 3 indicates the formation of piperonylglycine over time after application of four different doses of heliotropin to cultured rat hepatocytes. The cells were treated one day after plating, and the excreted metabolites were monitored over 24h. A second dose was then applied to the same cells, and metabolism monitored for another 48h.

After application of 50 μ M, there is quantitative formation of the glycine-conjugate after both applications over 24h. At a dose of 200 μ M, glycine-conjugation increases over 48h to reach a maximum of around 138 μ M. However, when the dose is further increased to 600 μ M or 1200 μ M, the maximal amount of the glycine conjugate is increased only to 174 μ M, indicating that there is a saturation at the 24h time point, with only partial elimination by this pathway at 600 μ M and 1200 μ M dosing.

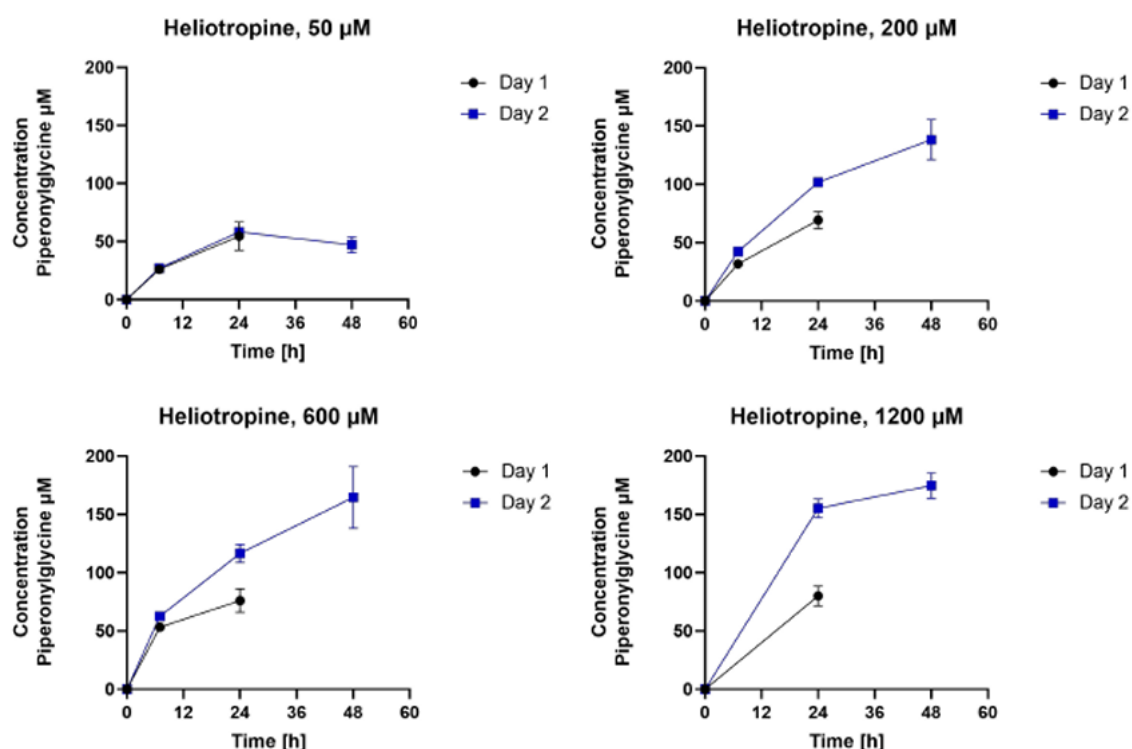


Figure 3: Formation of piperonylglycine over 24 and 48 hours after application of different doses of Heliotropin to cultured hepatocytes

Concentration of piperonylglycine on day 1 (black) and day 2 (blue) of treatment. a) 50 µM Piperonal b) 200 µM Piperonal c) 600 µM Piperonal and d) 1200 µM Piperonal treatment of cultured rat hepatocytes in sandwich culture. Data from study GLN03685-053 (Natsch A., 2025).

Figure 4 lists the analysis of the same cells for the formation of piperonylic acid. At the 50 µM dose, there is a transient peak after 7 h, and the acid is eliminated completely by 24 h, in line with the quantitative formation of the glycine conjugate. At a dose of 200 µM, piperonylic acid is formed and still present at a concentration of 80-120 µM after 24 h and only eliminated after 48h. As the dose is further increased to 600 µM, around 350 µM of piperonylic acid is formed at 24 h and it is not eliminated even after 48 h. At 1200 µM dosing an even higher concentration is observed, although the absolute amount does not quantitatively represent the applied dose. At the high-test doses, acylglucuronides were formed, however, this alternative pathway did not compensate to prevent formation of the free piperonylic acid (Natsch A., 2025).

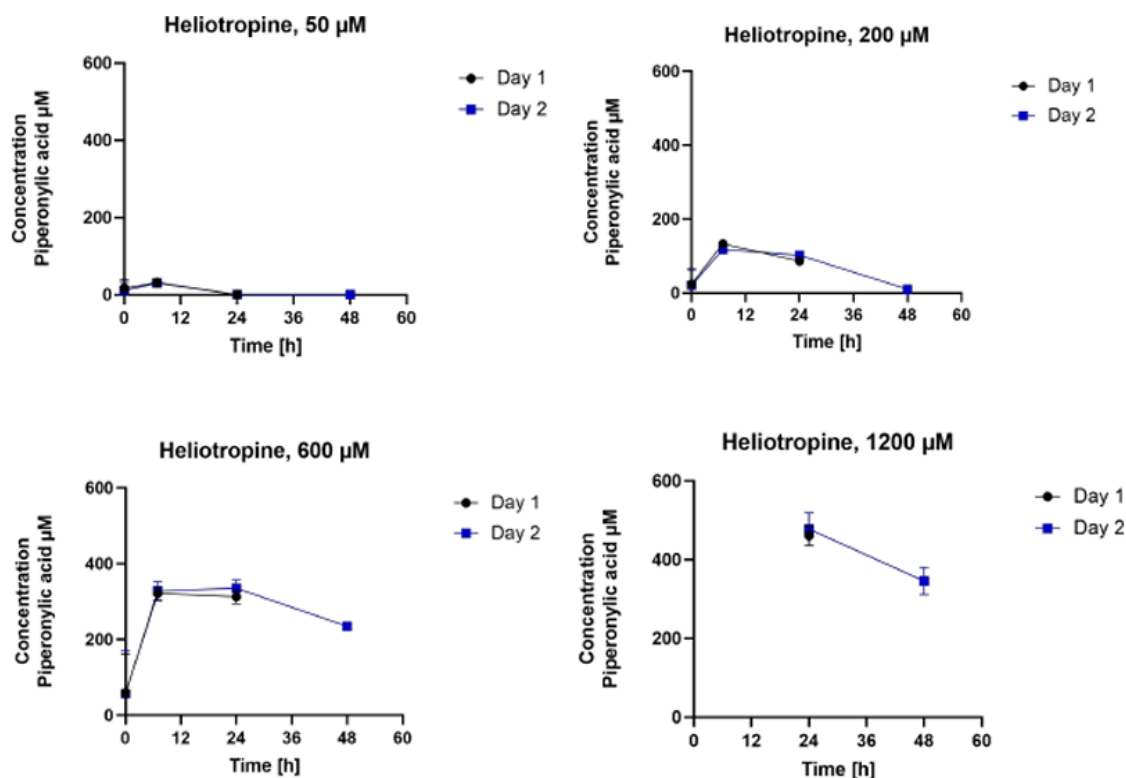


Figure 4: Formation of piperonylic acid over 24 and 48 hours after application of different doses of Heliotropin to cultured hepatocytes.

Concentration of piperonylic acid on day 1 (black) and day 2 (blue) of treatment. a) 50 µM Piperonal b) 200 µM Piperonal c) 600 µM Piperonal and d) 1200 µM Piperonal treatment of cultured rat hepatocytes in sandwich culture.

Note: The 7 h data at the top dose are missing due to too high dilution of the samples for the analysis. Data from study GLN03685-053 (Natsch A., 2025).

Conclusion from the Applicant

The *in vivo* and *in vitro* results clearly indicate a glycine-based detoxification process for heliotropin. When comparing the *in vitro* results to the *in vivo* studies, the concentration of heliotropin used *in vivo* (150 mg/kg) is similar to the dose that began to saturate detoxification *in vitro* (200 µM dose *in vitro*). As the dose is further increased, the glycine elimination pathway becomes saturated and an increasing amount of the free piperonylic acid is present in the incubations. Based on these new data and the literature evidence on benzoic acid precursors dosed by oral gavage, it is highly likely that the reprotoxic adverse effects observed in rats at a dose of 1000 mg/kg occur under conditions of metabolic saturation, where large amounts of free piperonylic acid is formed, which cannot be reached following exposure to cosmetic products.

SCCS comment

The SCCS concurs with the RAC Opinion concerning the argument for saturation of glycine-conjugation in rats to exclude the reprotoxicity concern of Heliotropin in humans is not evidence-based.

3.3 EXPOSURE ASSESSMENT

3.3.1 Function and uses

Heliotropin is 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 air care products, tobacco products, biocides and cosmetics.

Heliotropin can be used as fragrance ingredient in a range of cosmetic products, at the concentrations reported further, in several product types, from synthetic origin. It can also be used in food, as a flavouring agent. Heliotropin is also used as a fragrance ingredient in household products as well as a flavour in e-liquids and tobacco products (Anonymous (REACH), 2021). The EU Cosmetics Regulation currently doesn't list Heliotropin as a restricted substance in cosmetics and personal care products.

In the context of this mandate, the use of Heliotropin as an ingredient in fine fragrances up to 1.8% for adults is considered by the SCCS as it is the only category of cosmetic products for which the Commission received an exemption request from the Applicant.

As Heliotropin is for the moment still used in other products categories, the Applicant also provided in the dossier an assessment for the other cosmetic products, which is put in Annex to this opinion for information.

Volume of use (worldwide band): 100–1000 metric tons per year (IFRA,2019).

3.3.2 Calculation of SED/LED

As the proposed classification is Repr.1B, the overall exposure to Heliotropin has to take into account cosmetic products, but also other sources: food, household cleaning products, and e-cigarettes and tobacco products. Three routes are then considered: the dermal route, the oral route and the inhalation route.

Exposure to Cosmetic products (fine fragrances)

Exposure is calculated based on exposure scenarios by using appropriate exposure models. Thus, an iterative process that begins as simple as possible and moves to more complexity, bringing in more data as and when available to refine the assessment (Alexander-White *et al.*, 2022; Meek *et al.*, 2011). Tier 1 approach is the basis of the current SCCS Notes of Guidance to aggregate exposure assessment (SCCS - Scientific Committee on Consumer Safety, 2023). If refinement is necessary, a more realistic and refined risk assessment using a probabilistic approach (Tier 2) should be used for aggregate exposures. With good data on habits and practices of cosmetic product use and distributions of use concentration data in products, this probabilistic approach to estimating exposure can be performed with the target protection goal of the 95th percentile of the population.

Calculation of Systemic Exposure Dose (SED) of adults following dermal exposure

Category of Product	Product type	HELIOTROPIN concentration in finished product used for EXPOSURE assessment (w/w%)	Exposure product (E_{product}) (g/d)	Retention factor for dermal exposure	Eproduct normalised by body weight per product (mg/kg/d) ¹	% absorbed through dermal route	Calculated SED from DERMAL exposure per product ($\mu\text{g/kg/d}$) ³
Fine fragrances (non-spray) e.g. Eau de toilette, Parfum,...)		1.80	0.280*	1	4.67	50	42.0
Fine fragrances (spray formulation) e.g. Eau de toilette, Parfum,...)		1.80	0.618*	1	10.30	50	78.8

* Based on the P95 daily amount used reported by Ficheux & Roudot (2017) and corrected by the frequency of use.

Table 3: deterministic adult exposure to Heliotropin in Fine Fragrance**SCCS comment**

As spray products lead to higher systemic dose compared to non-spray fragrances, the systemic amount of Heliotropin following exposure to spray fragrances will be used for the safety assessment, i.e. exposure from the inhalation route will be considered together with the exposure from the dermal route.

Calculation of Systemic Exposure Dose (SED) of adults following inhalation exposure

Based on the low vapor pressure of Heliotropin, inhalation exposure through excessive volatilisation is not expected. Therefore, the systemic exposure through inhalation will be performed for the sprayed fraction of finished products as below:

$$\text{SED}_{\text{inh}} = (\text{IA1} + \text{IA2}) \times G \times \text{RF} \times \text{DA}/\text{BW}$$

Where:

SED_{inh} = Systemic Exposure Dose from the inhalation route (mg/kg/day)

IA1 = the potential amount inhaled during the first 2 min (in mg)

IA1 ($\text{EA}/\text{V1} \times \text{BR} \times \text{t1}$)

IA2 = the potential amount inhaled during the subsequent 10-20 min (in mg)

IA2 ($\text{EA}/\text{V2} \times \text{BR} \times \text{t2}$)

EA = potential amount to be inhaled

$\text{EA} = (\text{A} \times \text{C} \times \text{P} \times \text{AF})/100$

A = Amount of product by application (mg/application) (user defined or default SCCS 2023)*

C = Percentage concentration of ingredient in product (%) (user defined)*

P = Proportion of non-propellant in formulation (no units) (user defined)* or default 60% (propellant,

100% pump spray) (Bremmer/RIVM 2006)

AF = airborne fraction (no units); 1 (propellant spray); 0.2 (pump spray) (Bremmer/RIVM 2006)

V1 = First step: near-field, 1m^3 (SCCS 2023)

V2 = Second step: far-field, 10m^3 (SCCS 2023)

BR = breathing rate, 13 L/min (SCCS 2021; US EPA 2011)**

t1 = 2 minutes in near field (SCCS 2021; Rothe et al 2011)
t2 = 10-20 minutes in far field (SCCS 2021; Rothe et al 2011)
G = default factor substance lung retention 0.75 (25% is exhaled) (Rothe et al 2011; SCCS 2023)
RF = respirable fraction: propellant & pump spray specific (user defined experimental value*)
DA = Daily frequency of application (user defined* or SCCS 2023)
BW = body weight = adult 60 kg (SCCS 2023)

*Product-dependent parameter value

** highest median among several adult age categories

A systemic exposure dose from the potential for inhalation (SED_{inh}) from spray products can be calculated by assuming instant release of the ingredient in a defined box (1-Box model) or by applying a 2-Box model based on principles in (Rothe *et al.*, 2011). In the 12th Notes of Guidance (2023), a deterministic approach for 2-box modelling was presented for propellant and pump spray products according to the generic equations and associated parameters in the box above.

Table 4: Deterministic systemic exposure dose after inhalation exposure (SED_{inh}) to 1.8% Heliotropin in a fine fragrance spray formulation

Parameter	Description	Parameter value	Unit
A	Amount by application ¹	618	mg/application
	bodyweight	60	kg
C	Fraction of substance in non-propellant	0.018	fraction w/w
P	Proportion of non-propellant in formulation (60% for aerosol)	1	non-propellant in formulation
AF	Airborne fraction ²	0.2	fraction
EA	Potential amount to be inhaled	2.2248	mg
V1	Near-field, 1 m ²	1000	L
BR	Breathing Rate	13	L/min
t1	First step: 2 min in near-field	2	min
IA1	Potential amount inhaled during t1	0.0578	mg
V2	Second step: 20 min in far field	10000	L
BR	Breathing Rate	13	l/min
t2	Far-field, 10 m ²	20	min
IA2	Potential amount inhaled during t2	0.0578448	mg
G	Substance availability (25% exhaled)	0.75	
RF	Respirable fraction ³	0.01	
DA	Frequency of application	1	
BW	Default body weight	60	kg
SED	SED_{inhal}	1.44612E-05	mg/kg bw/day
		0.0145	µg/kg bw /d

¹Based on the P95 daily amount used reported by Ficheux & Roudot (2017) and corrected by the frequency of use.

²Bremmer/RIVM 2006.

³Delmaar and Bremmer, 2009 (1% for hydroalcoholic fragrances).

⁴Table 4 of the 12th SCCS NoG (2023).

SCCS comment

The SCCS accepts the calculation of the SED, but points out that, in the calculation of the inhalation SED, the assumption of a respirable fraction of 0.01 should be substantiated by experimental data on the particle size distribution of the product considered. The reference provided by the Applicant does not provide data upon which a value of 0.01 may be based. The SCCS has, for pump sprays, previously accepted a value of 0.01 (SCCS 2021). There, the Applicant based this value on an internal, unpublished survey of Cosmetics Europe that was not made available to the SCCS. Although the appropriateness of the assumption of a respirable fraction of 0.01 could thus not be verified, the SCCS agrees that for pump sprays, this fraction will generally be very low. Therefore, for Heliotropin, the SED for inhalation will be negligible compared with the dermal SED.

When comparing the SED from fine fragrances in spray, based on the assumption of 50% dermal absorption (default value), a much higher exposure is foreseen *via* the dermal route compared to inhalation, which seems to be negligible. Therefore, the risk assessment of systemic exposure will be based on the systemic **exposure of 78.8 µg/kg bw/d for all fine fragrance's products.**

Exposure from food

Piperonal is reported to occur in the following foods (Api, 2025):

Capers (<i>Capparis spinosa</i>)	<i>Mangifera</i> species
Chicken	Melon
Dill (<i>Anethum</i> species)	Pepper (<i>Piper nigrum</i> L.)
Sherry (non-categorized)	Vanilla
Vaccinium species	

Exposure estimate based on the EFSA evaluation (2012)

Heliotropin (FI No. 05.016) is listed in the Commission implementing Regulation (EU) No 872/2012 adopting the list of flavouring substances as an authorised food additive that can be added to all the food categories without any restriction provided that its purity is at least 95%. Heliotropin has been evaluated by the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids in 2012 in the Scientific Opinion on Flavouring Group Evaluation 20, Revision 4 (FGE.20Rev4) (Beckman Sundh *et al.*, 2012). The EFSA Panel concluded that heliotropin does not give rise to safety concerns at its levels of dietary intake (1500 µg/person/day, i.e. 25 µg/kg bw/day), estimated on the basis of the Maximised Survey-derived Daily Intake (MSDI) approach. Average per capita intake is estimated on the assumption that the amount added to food is consumed by 10 % of the population. This is derived for heliotropin from estimates of annual volume of production provided by the food Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60%) in the industry surveys (SCF, 1999).

Exposure estimate based on the food Industry data**MSDI calculation (from the Applicant)**

To account for the most recent annual volume of production data, the CE Heliotropin consortium asked the European Flavour Association (EFFA) to provide the most up to date information regarding the food industry reported volume of production for Heliotropin.

Based on the feedback from EFFA, the updated estimate based on the MSDI approach can be calculated as follows

V: Annual volume of use for 2020 = 28 600 kg/year = 28 600.109 µg/year

P: European population size: 400 000 000 persons, corrected by a factor of 10% to consider the eaters only

SCF: Survey Correction Factor = 0.8. An SCF of 0.8 instead of 0.6 has been used to account for a more robust survey response compared to the 2011 survey.

Intake = $V / (P \cdot 0.1 \cdot 365 \cdot SCF)$ = 2449 µg/person/day, i.e. **40.8 µg/kg bw/day**.

mTAMDI calculation (from EFSA)

The EFSA Scientific Opinion on FGE.20Rev4 published in 2012 did not provide any data on the food intake estimate derived from the modified Theoretical Added Maximum Daily Intake (mTAMDI) approach.

The method for calculation of mTAMDI values is based on the approach used by Scientific Committee on Food (SCF) up to 1995 (European Commission, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table 19. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table 5: Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (European Commission, 1995)

Class of product category	Intake estimate (g/day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF mTAMDI approach (European Commission, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (Official Journal of the European Communities, 2000) and reported by the Flavour Industry in the following way:

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000)
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16 (EC, 2000)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

In a personal and confidential email communication, EFSA has provided the normal use levels reported by the food Industry for Heliotropin for the 18 food categories, as reported in the table 6 below.

Table 6: Normal use levels ($\mu\text{g/g}$) reported by EFSA for Heliotropin for the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000.

No.	Food Category	Normal use level ($\mu\text{g/g}$)
1	Dairy products and analogues	8.89
2	Fats and oils and fat and oil emulsions	/
3	Edible ices	/
4.2	Processed fruit and vegetables	/
5	Confectionery	15.98
5.3	chewing gum	4.90
6	Cereals and cereal products	5.41
7	Bakery wares	13.00
8	Meat	0.10
9	Fish and fisheries products	/
10	Eggs and egg products	/
11	Sugars, syrups, honey and table-top sweeteners	/
12	Salts, spices, soups, sauces, salads and protein products	/
13	Food intended for particular uses	/
14.1	non-alcoholic Beverages	6.61
14.2	alcoholic Beverages	4.02
15	Ready to eat savouries and snacks	/
16	Composite foods	/

These concentrations are then translated into the seven food categories from SCF according to the above-mentioned correspondence and by retaining the highest reported value when multiple food categories correspond to the same SCF category. The reported use levels in the different food categories are then multiplied by the consumption estimates and summed up to yield the total intake estimate based on the mTAMDI approach (see table 7 below).

Table 7: Total heliotropin intake estimated based on the mTAMDI approach.

Class of product category	Intake estimate (g/day)	Normal use level (µg/g)	Intake (µg/day)
Beverages (non-alcoholic)	324.0	6.61	2141.64
Foods	133.4	13.0	1734.2
Exception a: Candy, confectionery	27.0	15.98	431.46
Exception b: Condiments, seasonings	20.0	0	0
Exception c: Alcoholic beverages	20.0	4.02	80.4
Exception d: Soups, savouries	20.0	0	0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)	4.90	9.8
TOTAL			4397.5

The total heliotropin intake estimate based on the mTAMDI approach is 4397.5 µg/day, i.e. **73.3 µg/kg bw/day**.

Conclusion from the Applicant on the exposure to heliotropin from food

Based on the data provided by EFSA, the most conservative exposure to Heliotropin derived from the mTAMDI approach will be retained for the aggregated exposure to all sources, i.e. 73.3 µg/kg bw/day.

SCCS comment

The SCCS has taken note of the value reported by the Applicant from the EFSA evaluation. The proposed calculated SED of **73.3 µg/kg bw/day** will be used in the calculation of margin of safety (MoS) for Heliotropin.

Exposure from Household Products

Household products are identified as a category of daily used products which can contain Heliotropin and then constitute of source of exposure to the substance. The concentrations of Heliotropin in different categories of household products have been identified in different product types from the following categories:

- All-purpose cleaners
- Bathroom and toilet cleaning products
- Floor, carpet, and furniture products
- Laundry products
- Miscellaneous
- Air fresheners

To determine the daily exposure to these products, a deterministic approach has been selected as first step of exposure assessment and the model ConsExpo Web (ConsExpo Web version 1.2.0, 03-10-2024) and the parameters given by the RIVM ConsExpo fact sheet have been chosen (RIVM, 2016, 2021).

ConsExpo Web uses different models depending on exposure conditions (liquid, spray) and depending on the different steps of the product use (mixing and loading, application, post application exposure). In all cases, the exposure is obtained *via* the dermal route (direct

product contact) and / or the inhalation route (exposure to vapour or spray depending on product type).

For some household product, the current Fact Sheet also refers to exposure scenarios for baby and children when they can be exposed to these products.

The specific information important for the consistent and harmonized estimation and assessment of the exposure to substances from consumer products for each product type are described in its respective RIVM Fact Sheet. These parameters are integrated in the ConsExpo Web which is ready to use (RIVM, 2016, 2021). For adults, the body weight of 68.8 kg is taken into account as a default value in RIVM fact sheet and ConsExpo tool. In this assessment on Household products containing Heliotropin, the exposure is recalculated with a human body weight of 60 kg to be aligned with the 12th SCCS Notes of Guidance for the assessment of the total aggregate exposure.

The only parameters that need to be included in the tool are those concerning the substance Heliotropin: dermal absorption (50%), inhalation absorption (100%), oral absorption (90%) and vapor pressure (2 Pa).

ConsExpo Model considers specific scenarios for baby exposure to household products. The model considers a dermal exposure *via* crawling on floor and an oral exposure *via* hand to mouth behaviour. The age at which children crawl is considered by the RIVM to be 6–12-month-old child.

For other age groups, the RIVM do not consider any exposure for that kind of product (RIVM, 2016). Children can also be exposed to air fresheners products as substances released by air freshener products disperse through the indoor air of the room, reaching product users and bystanders that are also present in the room.

Exposure assessment

The Heliotropin concentrations in household products are selected from the RIFM report of Heliotropin (Piperonal) exposure from the Creme RIFM Aggregate Exposure Model for Fragrance Materials (Creme Global & RIFM, 2025). This report includes Heliotropin 95th percentile concentrations in household products, reported by the fragrance industry to RIFM in August 2024. The Table 22 lists the attribution for each RIFM product type of the suitable RIVM factsheet.

Table 8 (= 22 in the Applicant dossier): Household products containing Heliotropin and their corresponding RIVM Factsheet to use ConsExpo web model for exposure assessment**Table 22: Household products containing Heliotropin and their corresponding RIVM Factsheet to use ConsExpo web model for exposure assessment.**

RIFM Product type	Concentration of Heliotropin in Product (%)	RIVM Product type	
Washing up Liquid	0.005	Laundry products	Washing machine detergents
Hand wash detergent	0.013	Laundry products	Hand washing laundry detergents
Multipurpose cleaner cream/gel	0.0066	All-purpose cleaners	All-purpose cleaning liquid
Multipurpose cleaner dilutable	0.0066	All-purpose cleaners	All-purpose cleaning liquid
Multipurpose cleaner spray	0.0063	All-purpose cleaners	All-purpose cleaning spray
Cleaning wipes	0.0057	All-purpose cleaners	All-purpose cleaner wet tissue
Antibacterial Cleaner Cream/Gel	0.0035	All-purpose cleaners	All-purpose cleaning liquid
Antibacterial Cleaner Dilutable	0.0034	All-purpose cleaners	All-purpose cleaning liquid
Antibacterial Cleaner Spray	0.0035	All-purpose cleaners	All-purpose cleaning spray
Bleach cream gel	0.0063	All-purpose cleaners	All-purpose cleaning liquid
Bleach Dilutable	0.0066	All-purpose cleaners	All-purpose cleaning liquid
Bleach Spray	0.0066	All-purpose cleaners	All-purpose cleaning spray
Disinfectant Cream/Gel	0.006	All-purpose cleaners	All-purpose cleaning liquid
Disinfectant Dilutable	0.006	All-purpose cleaners	All-purpose cleaning liquid
Disinfectant Spray	0.0058	All-purpose cleaners	All-purpose cleaning spray
Kitchen Cleaner Cream/Gel	0.006	All-purpose cleaners	All-purpose cleaning liquid
Kitchen Cleaner Dilutable	0.006	All-purpose cleaners	All-purpose cleaning liquid
Kitchen Cleaner Spray	0.006	All-purpose cleaners	All-purpose cleaning spray
Bath Cleaner Cream/Gel	0.0053	Bathroom and toilet cleaning products	Bathroom cleaning liquid
Bath Cleaner Dilutable	0.0054	Bathroom and toilet cleaning products	Bathroom cleaning liquid
Bath Cleaner Spray	0.005	Bathroom and toilet cleaning products	Bathroom cleaner spray
Floor Cleaner Cream/Gel	0.0058	Floor, carpet, and furniture products	Floor cleaning liquid
Floor Cleaner Dilutable	0.0057	Floor, carpet, and furniture products	Floor cleaning liquid
Floor Cleaner Spray	0.006	Floor, carpet, and furniture products	Floor polish spray
Furniture Polish Spray	0.0065	Floor, carpet, and furniture products	Furniture polish spray
Furniture Polish Liquid	0.0063	Floor, carpet, and furniture products	Furniture polishing liquid
Furniture Polish Cream/Gel	0.0063	Floor, carpet, and furniture products	Furniture polishing liquid
Window cleaner spray	0.0024	Miscellaneous	Glass cleaners
Air fresheners aerosol	0.068	Air fresheners	Instant air refreshment sprays
Air freshener plug-in	2.3	Air fresheners	Electric plug-in evaporators
Scented candles	0.31	Air fresheners	Scented candles

A total of 31 product types are considered. Their respective contributions to the exposure to heliotropin is estimated and fully detailed in an annex named "ConsExpo report on household products exposure adults and babies" that is provided separately to the SCCS (industry consortium, 2025). This annex also integrates the compilation of the ConsExpo Web reports with all the parameters used by ConsExpo Web to calculate the exposure assessment. Household products can be presented through different product types, for example the all-purpose cleaning product can be a liquid or a spray. It is assumed that same product category is not used in the same day by consumer. Therefore, to calculate the deterministic aggregate

exposure of Heliotropin in Household products, only the worst-case of each product category has been considered. This data is summarized in Table 23 for adults.

Table 9 (= 23 in the Applicant dossier): Aggregate exposure of adults to household products for each product category estimated by ConsExpo Web

Table 23: Aggregate exposure of Adults to household products for each product category estimated by ConsExpo Web

RIVM Product types* (RIVM FACT SHEET)		Concentration of HELIOTROPIN in product (%)	Exposure type	Application type	Exposure (µg/day)
Laundry products	Washing machine detergent	0.005	Direct exposure	mixing and loading	0.29
				application: hanging machine washed laundry	0.20
	Hand washing laundry detergent	0.013	Post exposure	migration from fabric	1.74
				mixing and loading	0.11
			Direct exposure	application: hand washing laundry	2.06
				application: hanging hand washed laundry	0.85
All-purpose cleaners	All-purpose cleaning spray	0.0066	Direct exposure	migration from fabric	52.3
				application: spraying	61.9
Bathroom and toilet cleaning products	Bathroom cleaner spray	0.005	Direct exposure	application: rinsing	11.8
				application: spraying	79.1
Floor, carpet, and furniture products	Floor polish spray	0.006	Direct exposure	application: spraying	14.5
				application: spot polishing	2.70
	Furniture polish spray	0.0065	Direct exposure	Application: spraying	4.66
				Application polishing	0.057
Miscellaneous	Glass cleaner	0.0024	Direct exposure	Application: spraying	6.52
				Application: cleaning	20.6
Air fresheners	Instant air refreshment spray	0.068	Direct exposure	Application: spraying	274
	Electric plug-in evaporator	2.3	Direct exposure	Application: vapour	777
			Direct exposure	Refilling	115
	Scented candle	0.31	Direct exposure	Application: vapour	2208
DAILY ADULTS AGGREGATE EXPOSURE to HOUSEHOLD PRODUCTS (µg/day)					3640
ADULTS AGGREGATE EXPOSURE to HOUSEHOLD PRODUCTS for an adult of 60 kg bw (µg/kg bw/day)					60.7

*For each product category, the combination product/exposure/application type inducing the highest exposure has been reported in the table and used for aggregate exposure.

SCCS comment

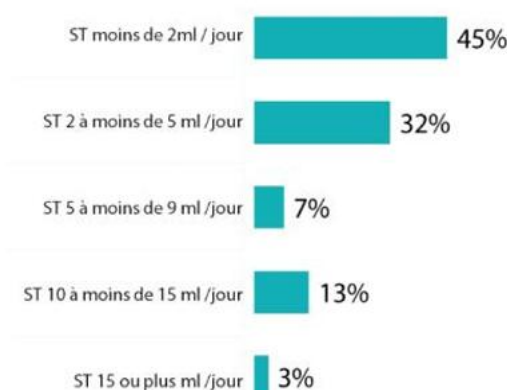
The SCCS has taken note of the value reported by the Applicant from ConsExpo. The proposed calculated SED of **60.7 µg/kg bw/day** will be used in the calculation of margin of safety (MoS) for Heliotropin.

Exposure to e-cigarettes and Tobacco products

Heliotropin in e-cigarettes

Habits and practices data

The French Agency for Food, Environmental, and Occupational Health (Anses) has published in 2022 a study on the consumption practices of electronic cigarette users in France (Anses, 2022). The daily e-liquid consumption for users of 10 mL refill bottles has been estimated on the basis of a survey (see figure 5 below). For this subset of respondents (n = 456), an average consumption of 4.1 mL of e-liquid per day was found, with a median of 3 mL. These users therefore consume, on average, just over two bottles per week.



* ST moins de 2 ml / jour = user of < 2 mL per day; ST 2 à moins de 5ml/jour = user of 2 to < 5mLper day ; ST 5 à moins de 9 ml/jour = user of 5 to < 9mL per day ; ST 10 à moins de 15 ml/jour = user of 10 to <15 mL per day; ST 15 ou plus ml/jour = user of 15 mL or more per day.

Figure 5: Quantities of e-liquid consumed per day (users of 10 mL refill bottles, n = 456)
(OpinionWay survey for Anses). Excerpt from figure 14 of the Anses report.

From this figure, it can be concluded that 84% of the surveyed population consumes 9 mL or less of e-liquid per day (84th percentile) and 97% consumes 15 mL of e-liquid or less per day (97th percentile). The appropriate value to consider for the assessment would be the 95th percentile. However, it is not possible to derive the 95th percentile quantity from the reported data. Therefore, the conservative estimate of 15 mL of e-liquid consumed per day will be chosen for the exposure assessment to Heliotropin contained in e-liquid, covering 97% of the e-cigarette's consumers habits and practices.

Concentration data

The SCHEER Opinion on electronic cigarettes (SCHEER Scientific Committee on Health, 2021) provides some information on the concentration of the most common ingredients found in e-liquids in the Netherlands. **Heliotropin has been reported to be present in 9.6% of the e-liquids, and the third quartile amount is estimated to be 6 mg (see table 10 below).**

Table 10 (= 28 in the Applicant dossier): Heliotropin content in e-liquids. Excerpt from the Annex 2 of the SCHEER Opinion on electronic cigarettes.

Table 28: Heliotropin content in e-liquids. Excerpt from the Annex 2 of the SCHEER Opinion on electronic cigarettes.

Ingredient name	% present	1st Qu. amount (mg)	Median amount (mg)	3rd Qu. amount (mg)
Piperonal (Heliotropin)	9.6	0.15	0.47	6

It is however not clear from the SCHEER Opinion if the reported amounts were found in a volume of 10 mL or less. In disposable electronic cigarettes or in single use cartridges, the cartridge or tank does not exceed a volume of 2 mL. In refill containers, the volume can be much higher (> 10 mL). However, nicotine-containing liquid is only placed on the market in dedicated refill containers not exceeding a volume of 10 mL. Considering a lower volume would have as a consequence an overestimation of Heliotropin's concentration, and conversely, a high volume would result in an underestimated concentration. However, according to the Anses study on the consumption practices of electronic cigarette users in France (see figure 6 below), the majority of the non disposable devices users use 10 mL refill containers.

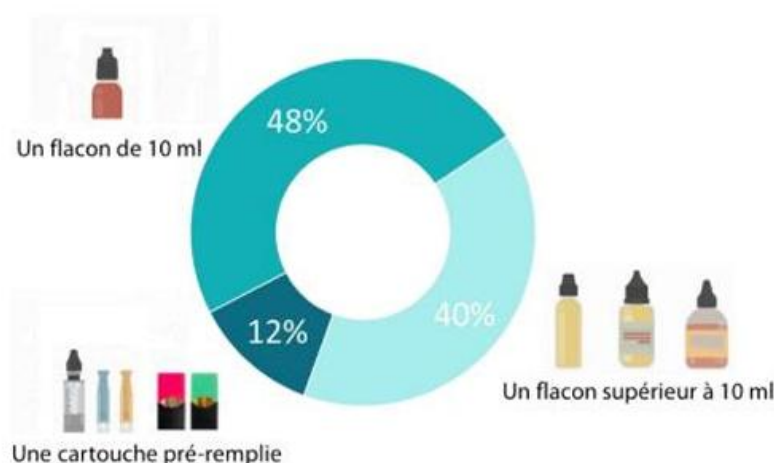


Figure 6: Container most often used to refill electronic cigarettes (users of non-disposable devices, n = 949) (OpinionWay survey for Anses). Excerpt from figure 24 of the Anses report. 48% of the e-cigarettes users use a 10 mL container, 40% use a container larger than 10 mL, and 12% use a pre-filled cartridge of less than 2 mL.

Therefore, for the purpose of the calculation of Heliotropin's concentration in e-liquids, the assumption will be made that the third quartile amount of 6 mg is found in a volume of 10 mL.

In another report from Anses (Anses, 2020), the P95 concentration of Heliotropin in e-liquids marketed in France has been estimated to be 3672 ppm (see figure 7 below), with a presence rate of 8.3% (consistent with the value of 9.6% reported for e-liquids in the Netherlands in the SCHEER opinion). The vast majority (95.2%) of products from which this concentration has been derived are 10 mL refill containers (Anses, 2020).

CAS No	Ingredient	Rate of presence (% of products incl. ingredient)	Concentration (ppm)		
			median	Min (P05)	Max (P95)
120-57-0	HELIOTROPIN	8.3	173	0	3672

Figure 7: Presence of Heliotropin in e-liquids (from Annex 1 of the Anses report on the List of ingredients in e-liquids with a maximum concentration greater than 1000 ppm)

Exposure assessment

1) **Based on the SCHEER data** and on conservative estimates, the systemic exposure to Heliotropin in e-liquids can be calculated as follows:

Q_{liq} : Quantity of e-liquid consumed/day = 15 mL
 $C_{heliotropin}$: P75 amount of Heliotropin found in 10 mL = 6 mg, corresponding to a concentration of 600 µg/mL
 P : Percentage of presence in e-liquids = 9.6%
 F_{inh} : Inhalable fraction = 100%
 Abs : Bioavailability *via* the inhalation route: 100%
 BW : Adult bodyweight = 60 kg

$$SED_{heliotropin} = (Q_{liq} \cdot C(heliotropine) \cdot P \cdot F_{inh} \cdot Abs) / BW = 14.4 \text{ µg/kg bw/day}$$

SCCS comment

The SCCS has taken note of the value reported by the Applicant for the exposure to Heliotropin in e-cigarettes. However, the SCCS noticed that a correction for the market presence rate (9.6% as cited above) was applied. As some consumers might always buy the same product, and because the future trend of the market cannot be predicted, the SCCS prefers not to apply this correction which might underestimate the exposure for some consumers. Therefore, keeping a note of the margin of safety (MoS) calculated by the Applicant, the SCCS will also calculate the MoS using an SED of 150 µg/kg bw/day for the exposure from e-cigarettes.

2) **Based on the Anses data and on conservative estimates, the systemic exposure to Heliotropin in e-liquids can be calculated as follows:**

Q_{liq} : Quantity of e-liquid consumed/day = 15 mL
 $C_{heliotropin}$: P95 concentration of Heliotropin found in 10 mL is 3672 ppm, corresponding to a concentration of 3672 µg/mL
 P : Percentage of presence in e-liquids = 8.3%
 F_{inh} : Inhalable fraction = 100%
 Abs : Bioavailability *via* the inhalation route: 100%
 BW : Adult bodyweight = 60 kg

$$SED_{heliotropin} = (Q_{liq} \cdot C(heliotropine) \cdot P \cdot F_{inh} \cdot Abs) / BW = 76.2 \text{ µg/kg bw/day}$$

SCCS comment

The SCCS has taken note of the value reported by the Applicant for the exposure to Heliotropin in e-cigarettes. However, the SCCS noticed that a correction for the market presence rate (8.3% as cited above) was applied. As some consumers might always buy the same product, and because the future trend of the market cannot be predicted, the SCCS prefers not to apply this correction as this might underestimate the exposure for some consumers (see previous SCCS comment). Therefore, keeping a note of the margin of safety (MoS) calculated by the Applicant, the SCCS will recalculate MoS using an **SED of 918 µg/kg bw/day** for the exposure from e-cigarettes.

For this assessment, the SCCS only considered Heliotropin as such, not side-products that may have been produced upon heating and for which no information was made available.

Heliotropin in tobacco products***Habits and practices data***

No data have been found on the consumers' practices and habits for tobacco products. The conservative assumption of 20 cigarettes smoked per day will therefore be used for the calculation of exposure to Heliotropin from tobacco products.

Concentration data

The SCENIHR Final Opinion on Additives used in tobacco products (SCENIHR Scientific Committee on Emerging and Newly Identified Health Risks, 2016) provides information on the reported tobacco industry uses. Heliotropin is added as a flavour to tobacco. However, no exposure calculation is possible, as the reported figures are imprecise and difficult to interpret. Another opinion from the SCENIHR on the addictiveness and attractiveness of Tobacco Additives has been published in 2010 (SCENIHR Scientific Committee on Emerging and Newly Identified Health Risks, 2010). In this opinion, the reported maximal use level of heliotropin is 35 µg/cigarette (see figure 8 below).

Ingredient	maximal use level (w/w%)	maximal use level (mg/cigarette (700 mg))
piperonal	0.005	0.035

Figure 8: Ingredients added to the tobacco based on a table presented by Philip Morris International (PMI) on cigarettes manufactured for sale in Germany. Excerpt from table 2 of the SCENIHR opinion.

Exposure assessment

Based on the SCENIHR data and on conservative estimates, the systemic exposure to Heliotropin in tobacco products can be calculated as follows:

Q_{cig} : Quantity of cigarettes consumed/day = 20
 $C_{\text{heliotropin}}$: maximum use level of Heliotropin found in a cigarette = 35 µg, corresponding to a concentration of 35 µg/cigarette
 F_{inh} : Inhalable fraction = 100%
 A_{bs} : Bioavailability *via* the inhalation route: 100%
 BW : Adult bodyweight = 60 kg

$$SED_{\text{heliotropin}} = (Q_{\text{cig}} \cdot C_{\text{heliotropin}} \cdot F_{\text{inh}} \cdot A_{\text{bs}}) / BW = 11.67 \mu\text{g/kg bw/day}$$

SCCS comment

The SCCS has taken note of the value reported by the Applicant for the exposure to Heliotropin from tobacco products. The proposed calculated SED of **11.67 µg/kg bw/day** will be used in the calculation of the margin of safety (MoS) for Heliotropin in tobacco products.

Conclusion on the exposure to Heliotropin from e-cigarettes and tobacco products

For the purpose of exposure calculation to Heliotropin from e-cigarettes and tobacco products, it has been decided to consider only the highest exposure value either from e-cigarettes or from tobacco products, as it is not realistic to be a high consumer of both these product types at the same time. Therefore, the conservative systemic exposure dosage to Heliotropin retained for the total aggregate exposure to all sources is 76.2 µg/kg bw/day, corresponding to the exposure from e-cigarettes.

SCCS comment

The SCCS agrees with the Applicant's suggestion that it is highly unlikely that someone would use both of these product types at the same time and unrealistic that they would be simultaneously a high consumer of both. Therefore, the exposure values cover both. As noted in the SCCS comment above, the SCCS will recalculate the MoS using an **SED of 918 µg/kg bw/day** for the exposure coming from e-cigarettes, without applying any correction for the market presence rate.

Overall Exposure

The overall exposure to Heliotropin takes into account Fine Fragrances, but also other sources: food, household products, and e-cigarettes and tobacco products. Thus, 3 routes of exposure are considered: the dermal route, the oral route and the inhalation route. In this approach, the exposures for individual product types are calculated. The results are shown in Table 11 and 12. **The overall exposure of Adults to Heliotropin is estimated to 289 µg/kg bw/d if a market presence rate for e-cigarettes is applied and 1130 µg/kg bw/d if not.**

Table 11: Overall exposure to Heliotropin, taking into account a market presence rate for e-cigarettes (as used by the applicant)

Product category	Systemic Exposure Dose (SED) for Adults (µg/kg bw/day)
Fine fragrances	78.8
Food	73.3
Household products	60.7
e-cigarettes and tobacco products (only exposure to e-cigarettes is considered as explained above; a correction for market presence rate is applied as suggested by the applicant)	76.2
Total SED (µgkg bw/d)	289

Table 12: Overall exposure to Heliotropin, not taking into account a market presence rate for e-cigarettes (as calculated by the SCCS)

Product category	Systemic Exposure Dose (SED) for Adults (µg/kg bw/day)
Fine fragrances	78.8
Food	73.3
Household products	60.7
e-cigarettes and tobacco products (only exposure to e-cigarettes is considered as explained above; no correction for market presence rate is applied as suggested by the applicant)	918
Total SED (µgkg bw/d)	1130

3.4 TOXICITY

3.4.1. Irritation and corrosivity

3.4.1.1 Skin irritation

The skin irritation potential of heliotropin was investigated in a study performed similar to OECD Guideline 402 in Sprague-Dawley rats. A single dose of 5000 mg/kg bw (volume of 1.0 mL/100 g body weight) of the test substance was applied evenly using a B-D disposable syringe over the back of sixteen (8 male and 8 female) young adult albino rats for 24 hours.

Results: No signs of skin irritation were observed.

Conclusion: In an OECD 402 study in rats, undiluted, Heliotropin is not irritating to rat skin.

Ref: Anonymous (REACH), 2021

3.4.1.2 Mucous membrane irritation / eye irritation

The eye irritation potential of Heliotropin was investigated in a study performed similar to OECD Guideline 405 in albino New Zealand rabbits. 0.1g of the test substance was applied once to the right eye of each of six rabbits. The eyes were not washed after application of the test substance. The observations were made at 24, 48 and 72 hours and at 4, 7 days after dosing & the effects were scored according to the Draize method.

Results: No signs of eye irritation were noted during 7-day observation period. The primary ocular irritation; scores were 0 at all readings.

Conclusion: In an OECD 405 study in rabbits, undiluted, Heliotropin is not irritating to the eyes.

Ref: Anonymous (REACH), 2021

Table 13: Skin and eye irritation results

Method	Dose	Species	Results
Acute Dermal Toxicity (similar to OECD 402) GLP compliant	5000 mg/kg bw, applied in a volume of 1.0 mL/100 g body weight	Rats (n = 8/sex)	No irritation was observed

Method	Dose	Species	Results
Acute Eye Irritation/Corrosion (similar to OECD 405) GLP compliant	100%; 0.1g test substance	Rabbits (n = 6)	No irritation was observed; primary ocular irritation scores = 0 at all readings

SCCS comment

Based on data made available to the SCCS, Heliotropin is considered as non-irritant for the skin and for mucous membrane.

3.4.2 Skin sensitisation

Heliotropin has been recently proposed for harmonised classification and labelling at EU level as Skin Sens. 1; H317 (May cause an allergic skin reaction) without sub-categorisation by Committee for Risk Assessment RAC (Committee for Risk Assessment RAC, 2024).

3.4.2.1 IN SILICO AND IN VITRO

Structural analysis

Heliotropin was predicted *in silico* to be reactive to skin proteins directly (Toxtree v3.1.0; OECD Toolbox v4.5).

Ref: Api *et al.*, 2025

In Chemico and in vitro tests for skin sensitisation

Direct Peptide Reactivity Assay (DPRA)

The DPRA was conducted with Heliotropin in triplicate, following protocol similar to OECD 442C. A 19.5% mean depletion of cysteine and lysine peptide was observed, demonstrating low reactivity of Heliotropin to skin proteins. Therefore, the results of the DPRA were concluded to be positive.

Ref: Miller Adele & Lamm Megan, 2016

KeratinoSens assay

The KeratinoSens assay was conducted with Heliotropin in 3 separate runs, following the OECD 442D.

Results: In 2/3 runs, Heliotropin induced ARE-dependent luciferase gene activity in KeratinoSens cells. The fold induction for 1/3 runs was between 1.35-fold and 1.67-fold compared to the solvent control. The fold induction for 1/3 runs was >1.67-fold compared to the solvent control. Imax values (maximal induction factor of luciferase activity compared to solvent control) of 1.76, 1.06, and 1.57 were obtained. Viability at the lowest concentration with >1.35-fold induction was >70%.

Conclusion: Under the conditions of the study, the test results for Heliotropin were concluded to be borderline positive based on OECD 497.

Ref: Natsch, 2020

Human Cell Activation Test (h-CLAT)

The h-CLAT assay was conducted with Heliotropin in 2 separate runs, following the protocol similar to OECD 442E.

Results: In each run, Heliotropin significantly induced the expression of CD86 and CD54. Viability at the concentration with >255% induction of CD54 and >184% induction of CD86 was at least 50%. The EC200 values were 273.21 µg/mL and 281.48 µg/mL, and the EC150 values were 76.94 µg/mL and 130.01 µg/mL for runs 1 and 2, respectively.

Conclusion: Under the conditions of the study, the test results for Heliotropin were considered to be positive. The minimum induction threshold was determined to be 76.94 µg/mL.

Ref: Oberfrank C., 2018

Table 14: Skin sensitisation studies for Heliotropin (*in silico* and *in vitro*)

Method	Methods & Observations	Outcome
<i>In silico</i>		
Toxtree v3.1.0; OECD Toolbox v4.5. <i>In silico</i> predictions	Heliotropin was predicted to be reactive to skin proteins directly.	Positive
<i>In chemico and in vitro</i>		
<i>In chemico</i> Direct Peptide Reactivity Assay (DPRA) (similar to OECD 442C)- Miller, 2016	Heliotropin analysed in triplicate; 19.5% mean depletion of cysteine and lysine peptide was observed, demonstrating low reactivity of heliotropin to skin proteins.	Positive – key event 1
<i>In vitro</i> KeratinoSens assay (OECD 442D) - key event 2	Heliotropin analysed in 3 separate runs. In 2/3 runs, heliotropin induced ARE-dependent luciferase gene activity in KeratinoSens cells. The fold induction for 1/3 runs was between 1.35-fold and 1.67-fold compared to the solvent control. The fold induction for 1/3 runs was >1.67-fold compared to the solvent control. Imax values (maximal induction factor of luciferase activity compared to solvent control) of 1.76, 1.06, and 1.57 were observed. Viability at the lowest concentration with >1.35-fold induction was >70%.	Borderline
<i>In vitro</i> Human Cell Activation Test (h-CLAT) (similar to OECD 442E) – key event 3 Oberfrank, 2018	Heliotropin was analysed in 2 separate runs. In each run, heliotropin significantly induced the expression of CD86 and CD54. Viability at the concentration with >255% induction of CD54 and >184% induction of CD86 was at least 50%. The EC200 values were 273.21 µg/mL and 281.48 µg/mL, and the EC150 values were 76.94 µg/mL and 130.01 µg/mL for runs 1 and 2, respectively. The minimum induction threshold was determined to be 76.94 µg/mL.	Positive

Conclusion from the applicant on *in silico* analysis and *in vitro* studies

In silico, Heliotropin is predicted to directly interact with skin proteins. It was studied in the *in vitro* DPRA, KeratinoSens, and h-CLAT tests, which represent key events 1, 2, and 3, respectively, of the skin sensitization adverse outcome pathway developed by OECD. It is positive in the studies representative of the activation of KE1 and 3 (DPRA and h-CLAT), and it has borderline results in the Keratinosens assay representative of KE2. According to the OECD Guideline No. 497 (Defined Approaches on Skin Sensitisation) and based on the 2 out of 3 Defined Approach, Heliotropin is a skin sensitizer.

According to RAC on *in chemico/in vitro* data

In a publication of the Research Institute for Fragrance Materials (RIFM) (Lee *et al.*, 2022), dedicated to the assessment of the skin sensitisation potential of 67 fragrance ingredients using the U-SENS™ assay, piperonal is reported to be:

- * Positive in a direct peptide reactivity assay (DPRA) addressing key event 1 “Covalent interaction with skin proteins”,
- * Positive in the KeratinoSens™ assay addressing key event 2 “Keratinocyte responses”,
- * Positive in the human cell line activation test (h-CLAT) while negative in the U-SENS™ assay, both addressing key event 3 “Dendritic cell responses” of the adverse outcome pathway (AOP) for skin sensitisation.

While only the results are reported, hampering a full assessment of these in chemico/*in vitro* assays (considered of non-assignable reliability), RAC notes that applying the "2 out of 3" Defined Approach (OECD TG 497) criterium, piperonal is predicted to be a skin sensitizer.

Ref: Committee for Risk Assessment-RAC, 2024

SCCS comments on NAM data

The SCCS agrees with the Applicant that considering the results from the *in silico*, *in chemico* and *in vitro* data, Heliotropin is a skin sensitiser.

3.4.2.2 Animal Data

Guinea-pig Maximisation

Test: The skin sensitisation potential of Heliotropin was evaluated in the guinea-pig maximisation test (GPMT) performed similar to OECD Guideline 406 in 10 guinea pigs (GLP conditions: unknown). Induction consisted of intradermal injection followed by an occluded patch one week later. On day 0 of the main study, test animals received six 0.1 mL intradermal injections of FCA (Freund's Complete Adjuvant) and 0.1 mL of 1.5% test substance in a mix solvent of 6% acetone, 20% polyethylene glycol, and 0.01% saline in an area of the shoulder region. There were no further details provided for the injection sequence. Seven days later, test animals received topical application of an occluded patch of 80% test substance in acetone over the injection site. 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. Dermal assessments were made 24 and 48 hours after removal of the challenge patches.

Results: 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". At days 21 and 28, positive reactions were observed in 4/10 (40%) at 24 and 48 hours following each challenge. At day 42, positive reactions were observed at 20% test substance in 4/10 (40%) at 48 hours and at 80% test substance in 1/10 (10%) and 2/10 (20%) at 24 and 48 hours, respectively. No positive reactions were reported in the treated or untreated controls in either challenge.

Conclusion: Under the conditions of the study, Heliotropin was considered to have skin sensitising potential.

Ref: Study 1978 (Anonymous (REACH), 2021)

Guinea-pig Maximisation Test

The skin sensitisation potential of Heliotropin was evaluated in a guinea-pig maximisation test (GPMT) conducted according to the method described by Magnusson and Kligman using 5 test guinea pigs (GLP conditions: unknown). The main study consisted of an induction phase and a challenge phase. The concentration suitable for intradermal injection was found to be 30% Heliotropin in Freund's complete adjuvant, 25% Heliotropin in petrolatum for topical induction, and 10% Heliotropin in petrolatum for topical challenge. For the induction phase, 3 pairs of 0.1 mL intradermal injections were made simultaneously into a shoulder area. Injections for the test group animals were as follows: 1) Freund's complete adjuvant; 2) 30% Heliotropin in an unknown vehicle; and 3) 30% Heliotropin in Freund's complete adjuvant.

One week after the injections, the 25% Heliotropin in petrolatum was applied over the clipped area. This patch was covered by an occlusive bandage and left in place for 48 hours. Two weeks later, the animals were challenged topically with 10% Heliotropin in petrolatum. The patch was sealed and left for 24 hours. The challenge site was evaluated 24, 48, and 72 hours after patch application.

Results: Reactions indicative of sensitisation was observed in 4/5 (80%) animals tested. No information about the type of reactions was provided.

Conclusion: Under the conditions of the study, Heliotropin showed sensitisation reactions in 4/5 (80%) tested animals.

Ref: Anonymous, 1977

Open Epicutaneous Test

The skin sensitisation potential of Heliotropin has been evaluated in the Open Epicutaneous Test in guinea pigs. Concentrations inducing irritation are tested with 0.025 ml at 100%, 30%, 10% and 3% onto flank skin, and read after 24 hours. 10% is the lowest test irritant concentration and 3% is the highest non-irritant concentration. During the induction phase, from D0 to D20, the test item is daily applied for 3 weeks or 5 times during 4 weeks, on the clipped flanked skin. The challenge is provoked at D21 and D35, onto the contralateral flank at the minimal irritating concentration. At 60% in ethanol: 5 animals /5 have positive reactions at D21, 5/5 at D35; at 30%: 2 animals /5 have positive reactions at D21, 4/5 at D35; at 10% and 3%: 0 animals /8 have positive reactions at D21, 0/8 at D35.

Results: Reactions indicative of sensitisation are observed at 60% and 30%. No reaction is observed at 10% and 3%.

Ref: Klecak, 1977

Local Lymph Node Assay

The skin sensitisation potential of Heliotropin has been evaluated in the murine LLNA study according to OECD 429 and in compliance with GLP. For the main study, 5 treated groups of 5 animals received Heliotropin at 1, 2.5, 5, 10, and 25% w/v in diethylphtalate: ethanol (DEP:EtOH; 3:1). A negative control group of 5 animals received the vehicle. A positive control group of 3 treated groups of 5 animals received 5, 15, and 35% α -hexylcinnamaldehyde (HCA), a moderate sensitizer, in DEP:EtOH (3:1). During the induction phase, 25 μ L of Heliotropin or vehicle or HCA were applied to each ear for 3 consecutive days. Following 2 days of rest, each animal received a single intravenous injection of 250 μ L of saline containing 20 μ Ci of ^{3} H-TdR. Approximately 5 hours later, auricular lymph nodes were excised, and lymphocyte proliferation was quantified by β scintillation counting. The Stimulation Index (SI) was obtained by calculating the ratio of disintegrations per minute (dpm) of the treated group divided by the dpm of the vehicle control group.

Results: SI of 1.1, 1.9, 2.2, 2.1, and 3.0 were observed for 1, 2.5, 5, 10, and 25% w/v Heliotropin, respectively. The EC₃ for Heliotropin was reported as 25% (6250 μ g/cm²). The positive control HCA was found to be sensitizing with SI values of 5.5, 6.1, and 19.5 for 5, 15, and 35% v/v in DEP:EtOH (3:1).

Conclusion: Under the conditions of the study, Heliotropin was considered to have skin sensitising potential.

Ref: Vasquez R.E., 2012

According to RAC on Animal Data

In a GPMT conducted with piperonal (Anonymous, 1978, considered reliable with restrictions), the tested concentrations were 1.5 % for the intradermal induction, 80 % (in acetone maximum possible concentration, non-irritant) for topical induction and challenge (day 14), and 80 % (day 21, day 28 and day 42) or 20 % (day 42) for re-challenges. Piperonal induced positive reactions in the same 4 out of 10 animals (40 %) on days 14, 21 and 28 with 80 % test substance and on day 42 with 20 % test substance. Among the limitations of this pre-guideline GPMT that could lead to an underestimation of the skin sensitising potential of piperonal, is that the selected concentration of 1.5 % for intradermal induction was not clearly justified. In the preliminary study for the selection of the intradermal induction dose, positive skin reactions described as 'faint pink' were already observed at lower concentrations. Furthermore, for topical induction, sodium lauryl sulphate should have been applied in order to create a local irritation since piperonal 80 % in acetone was not irritant in the preliminary test (OECD TG 406).

In a published screening of 32 fragrance ingredients for skin sensitisation using four different methods in Guinea pigs (Klecak *et al.*, 1977), piperonal was found to be positive in two tests (Open Epicutaneous test and Maximisation test) and negative in the two other tests (Draize test and Freuds Complete Adjuvant test). Despite the limited reporting of these non-guideline studies, RAC considers that the positive Open Epicutaneous test and the Maximisation tests provide some supporting evidence for the skin sensitising potential of piperonal.

In the OECD Toolbox, an EC3 of 25 % in a Local Lymph Node Assay from the RIFM database is reported which would support a moderate potency. However, this assay is not published nor submitted in the REACH dossier and therefore its reliability cannot be independently verified.

Ref: Committee for Risk Assessment-RAC, 2024

SCCS comment on animal data

The available tests in guinea pigs show inconsistent results. Some of these are old non-guideline studies. The LLNA is positive, which is in line with the *in vitro* and *in silico* results. The EC3 value of 25% shows that Heliotropin is a moderate skin sensitiser.

3.4.2.3 Clinical Data

Human skin sensitisation data Human Maximisation Test (HMT)

A human maximisation test was carried out with 6% Heliotropin in 25 adult volunteers (sex not reported). Occluded applications with 6% Heliotropin (or 4140 µg/cm²) in an unspecified vehicle were made to the same site on the subject's forearms for 5 alternate-days, 48-hour periods over 15 days. In each case, the sites were pretreated for 24 hours with 5% aqueous sodium lauryl sulfate (SLS) under occlusion. After a 10-day rest period, occluded challenge patches with 6% Heliotropin were applied to fresh sites for 48 hours. The sites were pretreated with 10% aqueous SLS for 1 hour under occlusion. The reactions were evaluated at the time of patch removal and also at 24 and 48 hours after the patch removal.

Results: No evidence of skin sensitisation was observed in any of the subjects.

Conclusion: Under the conditions of the study, no sensitisation reactions were observed when 6% (4120 µg/cm²) Heliotropin in an unknown vehicle was used for induction and challenge.

Ref: Greif, 1967

Human Repeat Insult Patch Test (HRIPT)

A repeat insult patch test was conducted in 112 subjects to evaluate the sensitising potential of Heliotropin at 2.5% in EtOH: DEP (1:3). A total of 120 subjects (47 males and 73 females) were enrolled. Out of the enrolled subjects, 112 subjects completed the study (the other 8 subjects withdrew due to personal reasons; none withdrew due to test material reactions). During the induction phase, a volume of 0.3 mL of 2.5% Heliotropin in 1:3 ethanol: diethyl phthalate was applied to designated 25 mm patches that were adhered occlusively to one side of the back of each subject. This represented a dose of 2953 µg/cm². Patches were applied as such on Mondays, Wednesdays, and Fridays for 3 consecutive weeks (9 total applications), and were removed approximately 24 hours after each application (on Tuesdays, Thursdays, and Saturdays). Patches were repeatedly applied to the same sites, which were evaluated on Mondays, Wednesdays, and Fridays for possible reactions. A 2-week rest period followed the induction phase, after which subjects were challenged with a similar application of patches on the opposite side of the back. Patches were removed 24 hours later at which time scoring for reactions was conducted; reaction scoring also was conducted 48, 72 and 96 hours post-patching.

Results: No evidence of skin sensitisation was observed in any of the subjects.

Conclusion: Under the conditions of the study, no sensitisation reactions were observed when 2.5% (2953 µg/cm²) Heliotropin in EtOH:DEP (1:3) was used for induction and challenge.

Ref: Study, 2011 (Anonymous (REACH), 2021)

Human Repeat Insult Patch Test (HRIPT)

A repeat insult patch test was conducted in 41 subjects (14 males and 27 females) to evaluate the sensitising potential of Heliotropin at 5% in ethanol. During the induction phase, 0.5 mL aliquot of 5% Heliotropin in ethanol applied to a Webril pad which was then affixed to an adhesive elastic bandage. The patches were applied to the upper arm of each subject for 24 hours under semi occlusion. The applications were conducted on a Monday-Wednesday-Friday schedule. Nine induction applications were made over a period of 3 weeks. After a rest period of approximately 2 weeks, a 24-hour semi-occluded challenge application was made to a virgin site. Reactions were evaluated at 24 and 72 hours after patch removal.

Results: No evidence of skin sensitisation was observed in any of the subjects.

Conclusion: Under the conditions of the study, no sensitisation reactions were observed with 5% Heliotropin in ethanol.

Ref: Study 1964 (Anonymous (REACH), 2021)

Confirmation of No Induction in Humans (CNIH) test

A CNIH was conducted on 99 male and female volunteer subjects to evaluate the skin sensitisation potential of Heliotropin. Prior to the main test, a preliminary irritation test was conducted. Six volunteer subjects were patched on the back with 0.3 mL of 3.48% (4109 µg/cm²) Heliotropin in EtOH:DEP (1:3) under occlusion using a 25-mm Hill Top Chamber System. Each patch was allowed to volatilize for at least 15 minutes but no longer than 40 minutes before being applied to the subject. Each subject was instructed to keep the patches in place and dry for approximately 48 hours. The test sites were scored approximately 48, 72, 96 and 168 hours post-patch application.

No skin reactions were observed in any of the volunteer subjects.

The main test consisted of an induction phase and a challenge phase. For the induction, 0.3 mL of 3.48% Heliotropin in EtOH:DEP (1:3) was applied to each designated patch on the left side of the back for 24 hours on a Monday, Wednesday, and Friday schedule. The identical patch site was re-patched until 9 such induction patchings were completed over the course of 3 weeks. A 2-week rest period, during which no material was applied, followed the final induction patching. At the start of the challenge phase, the subjects were queried as to whether any reaction was experienced, and the original induction test site was observed. For the challenge application, 0.3 mL of 3.48% Heliotropin was applied to each designated patch on the right side of the back to a previously unexposed site. The subjects were instructed to keep the patch on and dry. The challenge patches were removed 48 hours after the patch application. The volunteer subjects returned to the lab for challenge site scoring at 48, 72, 96 and 168 hours post-patch application. Results No reactions indicative of skin sensitisation was observed.

Conclusion: Under the conditions of the study, no sensitisation reactions were observed when 3.48% or 4109 µg/cm² Heliotropin in EtOH: DEP (1:3) was used for induction and challenge. The CNIH NOEL was therefore 4109 µg/cm².

Ref: Saeid Amini-Nik, 2023

Diagnostic patch tests

Heliotropin was tested among other fragrances in 1606 consecutive patients of contact dermatitis clinics at 6 European dermatology departments. Heliotropin induced 2 (0.1%) and 6 (0.4%) positive reactions at concentrations of 1% and 5% in petrolatum, respectively. The 6 patients had no history of adverse reactions to scented products.

Ref: Committee for Risk Assessment-RAC, 2024

In a review on fragrance allergy, the reported frequency of occurrence of skin sensitisation in routine testing for Heliotropin ranged from 0.4% to 1.0 %, and a frequency of 5 % (1/20) in selected patients (contact allergy related to cosmetics) is mentioned in a single study.

Ref: Committee for Risk Assessment-RAC, 2024

A 62-year-old woman developed perfume dermatitis on face. When tested with 94 liquid fragrance components, she reacted positively to 11 of them including Heliotropin.

Ref: Committee for Risk Assessment-RAC, 2024)

Table 15: Skin sensitisation studies for Heliotropin (*in silico*, *in vitro*, *in vivo* and clinical studies)

Assay type	Methods & Observations	Outcome
<i>In vivo</i> animal studies		
Guinea-pig Maximisation Test (GPMT) (similar to OECD Guideline 406) GLP compliance: not specified	10 test and 4 control guinea pigs Dose range finding study: <u>Intradermal</u> Group 1: 4 males administered 0.1 mL of 0.05, 0.1, 0.25, 0.5 and 1% test substance in 0.01% saline Group 2: 4 males administered 0.1 mL of 1, 2, 3 & 4% test substance in a mix solvent of 6% acetone, 20% polyethylene glycol, and 0.01% saline <u>Topical</u> 4 males applied 5, 10 and 25% and 4 females applied 40, 60 and 80% test substance in acetone Main study: <u>Induction</u> Day 0: Six 0.1mL intradermal injections of 1mL FCA (Freund's Complete Adjuvant) and 0.1 mL of 1.5% test substance in a mix solvent of 6% acetone, 20% polyethylene glycol, and 0.01% saline Day 7: Topical application of an occluded patch of 80% test substance in acetone <u>Challenge</u> Day 14, 21 and 28: Topical application of 80% test substance in acetone Day 42: Topical application of 20 or 80% test substance in acetone. At day 14, positive reactions were reported in 2/10 (20%) and in 4/10 (40%) animals after challenge at 24 and 48 hours, respectively. At days 21 and 28, positive reactions were observed in 4/10 (40%) animals after challenge at 24 and 48 hours. At day 42, positive reactions were observed with 20% test substance in 4/10 (40%) animals at 48 hours and with 80% test substance in 1/10 (10%) and 2/10 (20%) animals at 24 and 48 hours, respectively	Positive

Opinion on the safety of Heliotropin (CAS No. 120-57-0, EC No. 204-409-7)

Assay type	Methods & Observations	Outcome
Guinea-pig Maximisation Test	5 test guinea pigs <u>Induction</u> For the induction phase, 3 pairs of 0.1 mL intradermal injections were made simultaneously into a shoulder area. Injections for the test group animals were as follows: 1) Freund's complete adjuvant; 2) 30% heliotropin in an unknown vehicle; and 3) 30% heliotropin in Freund's complete adjuvant. One week after the injections, the 25% heliotropin in petrolatum was applied over the clipped area. <u>Challenge</u> Two weeks later, the animals were challenged topically with 10% heliotropin in petrolatum. The patch was sealed and left for 24 hours. The challenge site was evaluated 24, 48, and 72 h after patch application. Reactions indicative of sensitisation was observed in 4/5 (80%) animals tested. No information about the type of reactions was provided.	Positive
Open Epicutaneous Test	6 to 8 guinea pigs per group Skin irritation: 0.025 ml of test concentrations 100%, 30%, 10% and 3% tested onto flank skin. Reactions read after 24h. 10% is the lowest test irritant concentration, 3% is the highest non-irritant concentration. <u>Induction</u> For the induction phase (D0-20), daily application of undiluted or diluted solutions, daily for 3 weeks or 5 times during 4 weeks, on the clipped flanked skin. <u>Challenge</u> At D21 & 35: challenge onto the contralateral flank at the minimal irritating concentration. At 60% in ethanol: 5 animals /5 have positive reactions at D21, 5/5 at D35; at 30%: 2 animals /5 have positive reactions at D21, 4/5 at D35; at 10% and 3%: 0 animals /8 have positive reactions at D21, 0/8 at D35. Reactions indicative of sensitisation are observed at 60% and 30%. No reaction is observed at 10% and 3%.	Positive
Murine local lymph node assay (OECD 429) GLP compliant	Mice; 5 treated groups of 5 animals received heliotropin at 1, 2.5, 5, 10 and 25% w/v in 3:1 DEP:EtOH. Negative control group of 5 animals received the vehicle only. Positive control group of 3 treated groups of 5 animals received 5, 15 and 35% α-hexylcinnamaldehyde (HCA) in 3:1 DEP:EtOH. Stimulation Index (SI) of 1.1, 1.9, 2.2, 2.1, and 3.0 were observed for 1, 2.5, 5, 10, and 25% w/v heliotropin, respectively. The EC3 value was calculated to be 25% (6250 μg/cm²)	Positive
Clinical studies		
Human Maximisation Test (HMT)-	n = 25 subjects; 6% heliotropin applied in an unspecified vehicle under occlusion on the subject's forearms for 5 alternate-days, 48-hour periods over 15 days. After a 10-day rest period, occluded challenge patches with 6% heliotropin were applied to fresh sites for 48 hours. No evidence of skin sensitisation was observed in any of the subjects.	Negative at 4140 μg/cm²

Opinion on the safety of Heliotropin (CAS No. 120-57-0, EC No. 204-409-7)

Assay type	Methods & Observations	Outcome
Human Repeat Insult Patch Test (HRIPT)	n = 112 subjects; 25 mm Hill Top Chamber System was used occlusively. 0.3 mL of 2.5% (or 2953 µg/cm ²) heliotropin in 1:3 ethanol: diethyl phthalate was applied to one side of the back of each subject for 24 hours; Mon, Wed, Fri schedule. 9 induction patches over 3 weeks in total. 2-week rest then challenge with 0.3 mL of 2.5% heliotropin. No evidence of skin sensitisation was observed in any of the subjects.	Negative at 2.5% (or 2953 µg/cm ²)
Human Repeat Insult Patch Test (HRIPT)	n = 41 subjects; 0.5 mL aliquot of 5% heliotropin in ethanol applied to a Webril pad which was then affixed to an adhesive elastic bandage for 24 hours under semi-occlusion. Mon, Wed, Fri schedule. 9 induction patches over 3 weeks in total. After approximately 2-week rest period, a challenge application was made to a virgin site. No evidence of skin sensitisation was observed in any of the subjects.	Negative
Confirmation of No Induction in Humans (CNIH) test	n = 99 subjects; 0.3 mL of 3.48% (or 4109 µg/cm ²) heliotropin in 1:3 EtOH:DEP was applied on the left side of the back for 24 hours on a Monday, Wednesday, and Friday schedule under occlusion. 9 induction patches over 3 weeks in total. 2-week rest then challenge with 0.3 mL of 3.48% heliotropin on the right side of the back to a previously unexposed site. No reactions indicative of sensitisation was observed.	Negative A WoE No Expected Sensitisation Induction Level (WoE NESIL) was derived as 4100 µg/cm².
Human diagnostic patch tests	Heliotropin was tested among other fragrances in 1606 consecutive patients of contact dermatitis clinics at 6 European dermatology departments. Heliotropin induced 2 (0.1%) and 6 (0.4%) positive reactions at concentrations of 1% and 5% in petrolatum, respectively. The 6 patients had no history of adverse reactions to scented products.	Positive
Human diagnostic patch tests	In a review on fragrance allergy, the reported frequency of occurrence of skin sensitisation in routine testing for heliotropin ranged from 0.4% to 1.0 %, and a frequency of 5 % (1/20) in selected patients (contact allergy related to cosmetics).	Positive
Human diagnostic patch tests	A 62-year-old woman developed perfume dermatitis on face. When tested with 94 liquid fragrance components, she reacted positively to 11 of them including heliotropin.	Positive

Animal and clinical data

From well conducted studies in guinea pigs or mice, including following OECD TG 406 and 429, GLP compliant, Heliotropin is sensitizing. The LLNA assay show a dose-dependent increase of the Stimulating Index, at least at the first 3 concentrations, leading to an EC3 value of 25% or 6250 µg/cm². This EC3 value indicates a potential of Heliotropin to be a weak sensitizer. Although testing for skin sensitization in humans is not considered as appropriate to characterize skin sensitization hazard, the results of well conducted studies in humans can help to identify a no effect level. Four studies performed in 25 to 112 subjects confirm the absence of skin reactions at 2.5%, 3.48%, 5% and 6%.

According to RAC on Human DataHuman Repeated Insult Patch Test (HRIPT) & Human Maximisation Test (HMT)

An HMT with piperonal 6 % in petrolatum (3 724 µg/cm²) induced no reaction in 25 subjects (Greif *et al.*, 1967 reported in the database of human predictive patch test data for skin sensitisation from NICEATM and BfR; Strickland *et al.*, 2023).

In a HRIPT (unpublished) performed by the RIFM to confirm a no-effect level obtained from other non-human tests, piperonal in ethanol and diethyl phthalate (1:3) was negative in 112 human volunteers (2953 µg/cm²) as reported by Na *et al.* (2021) and Lee *et al.* (2022).

Diagnostic patch tests

Piperonal was tested among other fragrances in 1606 consecutive patients of contact dermatitis clinics at 6 European dermatology departments. Piperonal induced 2 (0.1 %) and 6 (0.4 %) positive reactions at concentrations of 1 % and 5 % in petrolatum, respectively. The 6 patients had no history of adverse reactions to scented products (Frosch, 2002; SCCS, 2012).

In a review on fragrance allergy (de Groot, 2020), the reported frequency of occurrence of skin sensitisation in routine testing for piperonal ranged from 0.4 % to 1.0 % (no reference available), and a frequency of 5 % (1/20) in selected patients (contact allergy related to cosmetics) is mentioned in a single study (Larsen, 1977).

Larsen (1975) described a 62-year-old woman with a perfume dermatitis on the face. When tested with 94 liquid fragrance components, she reacted positively to 11 of them including piperonal.

RAC notes that relatively low/moderate to high frequency of occurrences of skin sensitisation are observed in the available human data. However, in view of their limited number and in the absence of data to estimate exposure, the classification and sub-categorisation are primarily based on the available animal studies.

Ref: Committee for Risk Assessment-RAC, 2024

Overall conclusion from the Applicant on skin sensitisation

Heliotropin is identified as a skin sensitizer with a weak potential. In deriving a No Expected Sensitization Induction Level (NESIL), an overall Weight of Evidence (WoE) approach is utilized, which considers all available data, i.e., from structural analysis, *in vitro* studies, animal studies, and human studies. A WoE NESIL is an exposure to a skin sensitizer which should not result in the induction of sensitization in humans. For Heliotropin, the WoE NESIL is 4100 µg/cm² from the CNIH performed on 99 subjects and which is lower than the EC3 value (6250 µg/cm²). This value is used to provide the maximum acceptable concentrations in finished products, which take into account skin sensitisation and application of the Quantitative Risk Assessment (QRA2) methodology.

CLP criteria applied by RAC

Human, animal and *in vitro* data provide consistent evidence that piperonal is a skin sensitizer. According to section 3.4.2.2.1.1 of Annex I of the CLP Regulation, 'skin sensitizers should be classified in Category 1, where data are not sufficient for sub-categorisation'.

In the GPMT (Anonymous, 1978), 40 % animals responded at 1.5 % intradermal induction concentration which fulfils the CLP criteria for Category 1B. However, due to the uncertainties linked to the selected induction concentration, RAC considers that Category 1A cannot be excluded and Category 1 should be applied instead of Category 1B in accordance with the CLP guidance (2024).

Other available animal, human and *in vitro* data do not allow to propose any sub-categorisation.

Therefore, based on a weight of evidence approach and in line with the DS proposal, RAC concluded that classification as Skin Sens. 1; H317 (May cause an allergic skin reaction) without sub-categorisation, is warranted.

The generic concentration limit (1%) for Category 1 with no sub-categorisation should apply.

SCCS overall comment on skin sensitisation

Taking all the provided data together, Heliotropin is considered to be a skin sensitiser. The LLNA results in an EC3 value of 25%, which points to a weak to moderate skin sensitising potency. The validity of this study could not be evaluated by the SCCS, since it had no access to the full study report.

QRA2 for Skin sensitisation as applied by the Applicant

Heliotropin is recognized to present a potential for skin sensitization and has been proposed for classification by RAC as skin sensitizer H317 cat.1. The classification of a substance according to the criteria of the CLP Regulation, or even its potency is not sufficient to identify the acceptable exposure levels of sensitizing substances.

The most relevant toxicity data to assess skin sensitization is a concentration without any effect in humans. Considering all available results, showing a reactivity *in silico*, a low potency *in vitro* and *in vivo*, the no-effect concentration in humans was confirmed in several studies, confirming a NESIL of 4100 µg/cm².

The QRA is a model for dermal sensitization quantitative risk assessment (QRA) developed and implemented by the International Fragrance Association (IFRA) in the current standards providing recommendations of limitation of use for some fragrance substances. Since its first development (Api & Vey, 2008), the method has been refined (QRA2) reviewed (Api *et al.*, 2020) and commented by the SCCS in 2018 and finally, in 2024 with the specific assessment of Citral (SCCS, 2018, 2024b).

The QRA2 approach uses:

- Categories of products, grouped according to quantity of their exposures (in mg/cm²/day)
- For each product category, the product type showing the highest consumer exposure level (CEL) is selected as default value for QRA2 calculation
- Safety assessment factors (SAF), from 30 to 300 (considering inter-individual SAF, product composition SAF, frequency and skin condition)
- QRA2 Aggregate Adjustment Factor, to consider the use of the substance in different categories of products and which is based on the practices of use of the population as described in Api *et al.* 2020 and in the SCCS Opinion on Citral (Api *et al.*, 2020; SCCS, 2024b).

The following steps are followed to obtain the maximal concentration in each product type:

Step 1: Determine the WoE NESIL

- Here the NESIL is 4100 µg/cm² (see above)

Step 2: Calculate the Acceptable Exposure Level (AEL)

- $AEL = NESIL / SAF$
- Each AEL is calculated for each product category. The SAFs are 30, 100 or 300.

Step 3: QRA2 Aggregate Adjustment factors: they depend on the relative contribution of exposure of each product category based on the uses and practices of consumers.

Step 4: the Upper Concentration Level (UCLagg) considering aggregate exposure can be derived. The formula below shows the results of calculation of the UCLagg for sensitization risk assessment by using the QRA2 approach for Fine Fragrances containing Heliotropin:

$UCLagg = AEL/CEL \times \text{Aggregate Adjustment factor}$

In the Category 4 of IFRA (such as Fine fragrance products), the concentration of Heliotropin associated with an acceptable exposure regarding skin sensitization is calculated this way:

- The NESIL is 4100 µg/cm²
- The Acceptable Exposure Level is: $AEL = NESIL / SAF$
- For Fine fragrance, the SAF is 100 (10 for inter-individual SAF, 1 for Product composition SAF, 3 for Frequency/ duration, and 3 for Skin condition)
- $AEL: 4100 \mu\text{g}/\text{cm}^2 / 1000 / 100 = 0.041 \text{ mg}/\text{cm}^2$
- The category Consumer Exposure Level is 2.21 mg/cm² for Fine fragrance (Api *et al.*, 2020)
- The Upper Concentration Level considering aggregate exposure (UCLagg) is:
 $UCLagg = AEL / CEL \times \text{Aggregate Adjustment factor}$
- the Aggregate Adjustment factor for this category is 0.95
- $UCL \text{ Based on QRA2} = 0.041 / 2.21 \times 0.95 = 1.8\%$

Therefore, based on the expected NESIL of Heliotropin of 4100 µg/cm² and the QRA2 methodology described above, Heliotropin is safe in relation to the induction of sensitization until 1.8% in Fine Fragrance products. All the UCLagg calculated with the QRA2 methodology for each product category are above the concentrations reported in the Heliotropin CE consortium survey.

Table 16: Heliotropin Upper Concentration Level for each product category defined with the QRA2 approach

IFRA Category	Category description	CEL for the product category (mg/cm ² /day)	SAF	AEL (mg/cm ²) for the substance	QRA2 Aggregate Adjustment factor	UCLagg
Category 1	Lip products	11.8	100	0.0410	0.91	0.32%
Category 2	Deodorants/ Antiperspirants	9.1	300	0.0137	0.63	0.094%
Category 3	Products applied to the face using fingertips	2.17	100	0.0410	1	1.9%
Category 4	Fine fragrance products	2.21	100	0.0410	0.95	1.8%
Category 5A, 5B, 5C	Products applied to the face and body using the hands (palms), primarily leave-on	3.02	100	0.0410	0.33	0.45%
Category 6	Products with oral and lip exposure	1.27	100	0.0410	0.32	1.0%
Category 7	Products applied to the hair with some hand contact	2.2	30	0.1367	0.58	3.6%
Category 9	Products with body and hand exposure, primarily rinse off	0.2	300	0.0137	0.5	3.4%

SCCS comment

The SCCS could not evaluate the assessment through QRA2, since the Applicant did not provide details on several aspects of the approach as applied to Heliotropin. The SCCS could therefore not evaluate if Heliotropin could induce skin-sensitisation when used up to 1.8% in

fine fragrance products. For future submissions, the SCCS encourages the Applicant to provide a more detailed description on how QRA2 was applied, to enable a conclusion on this aspect.

Taking into consideration all the available evidence, which indicates that Heliotropin is a weak to moderate skin sensitiser, the SCCS considers the risk of induction of skin sensitisation in consumers to be low.

3.4.3 Acute toxicity

3.4.3.1 Acute oral toxicity

The acute oral toxicity of Heliotropin was evaluated in a study performed similar to OECD Guideline 401 in Osborne-Mendel rats. Groups of 10 young adult Osborne-Mendel rats evenly divided by sex (five males and five females per dose) were fasted for approximately 18 hours prior to treatment. Then the rats were administered 25% Heliotropin in corn oil. All animals were maintained under close observation for recording toxic signs and time of death. Such observation was continued until animals appeared normal and showed weight gain. The observation period was one week. Results Deaths occurred from 2 hours to 5 days post-dosing. Tremors for several hours followed by depression and ataxia were observed. Conclusion Under the conditions of this study, the oral LD50 of Heliotropin was 2700 mg/kg bw (95% CL: ≥ 2350 – ≤ 3100 mg/kg bw) for rats, confirming a very low acute toxicity by oral route.

Ref: Study 1964 and 1965 cited in Anonymous (REACH), 2021

3.4.3.2 Acute dermal toxicity

The acute dermal toxicity of Heliotropin was evaluated in a study performed similar to OECD Guideline 402 in Sprague-Dawley rats. Single dose of 5.0g of the test substance was applied evenly using a B-D disposable syringe over the back of sixteen (8 male and 8 female) young adult albino rats for an exposure period of 24 hours. The animals were observed for mortality, clinical signs, body weight and macroscopic pathology for 14 days.

Results: No mortalities were observed during the study and all animals remained healthy throughout the study and gained weight in a normal manner, except for one female which lost 10g over the course of the study. Also, there were no clinical signs and gross pathological changes indicative of toxicity.

Conclusion: Under the conditions of this study, the dermal LD50 of Heliotropin was greater than 5000 mg/kg bw for rats, confirming a very low acute toxicity by dermal route.

Ref: Study 1980 cited in Anonymous (REACH), 2021

3.4.3.3 Acute inhalation toxicity

There are no acute inhalation toxicity data available in animals for Heliotropin. However, according to the Guidance on information requirements and chemical safety assessment – Endpoint specific guidance, Chapter R.7.a (ECHA, 2017), no acute inhalation tests need to be performed for substances with a vapour pressure (below 1×10^{-5} kPa to 1×10^{-4} kPa). Heliotropin has a low vapour pressure (2 Pa at 25°C) and a boiling of 272°C, so the potential for the generation of inhalable forms is low. Heliotropin induces no concern regarding inhalation. Moreover, use of Heliotropin in consumer products for decades has revealed no adverse effects in the lung. Heliotropin is not irritating to the skin and eye at the

concentrations used in cosmetic products and therefore, is not expected to be irritating or toxic to respiratory tract and lung at the concentrations used in cosmetic products.

Table 17 Acute toxicity studies

Method	Species	Dosing	LD50 (mg/kg bw)	Observed effects
Acute Oral Toxicity (similar to OECD Guideline 401) Pre-GLP	Rats	Oral dose of 25% solutions of heliotropin in corn oil; n = 5/sex	2700	Deaths occurred from 2 hours to 5 days post-dosing. Tremors for several hours followed by depression and ataxia were observed
Acute Dermal Toxicity (similar to OECD Guideline 402) GLP compliant	Rats	Single dermal dose of 5.0g of heliotropin; n = 8/sex	>5000	No deaths & clinical signs and gross pathological changes indicative of toxicity

Taken from EFSA Journal (2012)

Table 18 (= IV.1 in EFSA): Acute Toxicity

TABLE IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD ₅₀ (mg/kg bw)	Reference
(Piperonal [05.016])	Rat	M, F	Gavage	2700	(Jenner et al., 1964)
	Rat	M, F	Gavage	2700	(Taylor et al., 1964)
	Rat	M, F	Gavage	2700	(Hagan et al., 1965)

SCCS comment

SCCS considers Heliotropin as a low acute toxicant via any route of exposure.

3.4.4 Repeated dose toxicity

3.4.4.1 Repeated dose (28 days) oral / dermal / inhalation toxicity

No specific 28-day toxicity data are available, but a combined OECD 422 & 90-d study is available which is described below.

3.4.4.2 Sub-chronic (90 days) oral / dermal / inhalation toxicity

Based on Article 41 of Regulation (EC) No 1907/2006 (REACH-Regulation), the European Chemicals Agency (ECHA) has requested an oral sub-chronic toxicity study in rats according to EU BH.26/OECD TG 408, and an oral screening for reproductive/developmental toxicity in rats according to OECD TG 421 or 422 (ECHA, 2018). An oral combined repeated dose toxicity

study (90-day) with the reproduction/developmental toxicity screening test (OECD TG 422) was performed and its results were discussed in detail in the CLH report for the Proposal for Harmonised Classification and Labelling of Heliotropin (piperonal; 1,3-benzodioxole-5-carbaldehyde) (ECHA, 2023). The repeated dose toxicity part of this study is described briefly below.

Guideline:	OECD Test Guideline 408/422
Species/ strain:	Rat Wistar CrLI. WI (Han), 7-9 weeks; 194-243g (male) and 140-172g (female)
Group size:	10/sex/dose
Test substance:	Piperonal or Heliotropin
Batch:	1810017
Purity:	99.9%
Vehicle:	PEG 400
Dose levels:	0, 100, 300 and 1000 mg/kg bw/day
Administration:	Oral gavage
GLP:	In compliance
Study period:	03 Feb 2019 - 18 Oct 2019

An OECD Guideline 422 Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test was performed in compliance with GLP in Wistar CrLI. WI (Han) rats (10 per sex per dose). The test item was administered daily *via* oral gavage in doses of 0, 100, 300 and 1000 mg/kg bw/day in PEG 400. Males were treated for 7 days/week for the 10 weeks pre-mating and 2 weeks mating periods. Females were treated 7 days/week for the 10 weeks pre-mating and 2 weeks mating periods and throughout the gestation period, until at least post-natal day (PND) 13. Parental animals were observed for clinical and mortality observations for once/day and twice/day, respectively. Body weights and food consumption were monitored weekly up to mating in male and females. In females, body weights and food consumption were monitored on days 0, 4, 7, 11, 14, 17 and 20 after mating and on PND 1, 4, 7 and 17. A terminal weight for all animals was measured on day of necropsy. Ophthalmoscopic examinations were assessed pre-dosing and during week 13 in males and the lactation period in females. Functional tests including hearing ability, pupillary reflex, static righting reflex, fore and hind-limb grip strength and locomotor activity were assessed in males at week 13 and females during PND 6-13. Oestrous cycle was monitored by daily examinations of vaginal cytology of all females from 14 days before mating until evidence of mating. Blood samples were collected on day of necropsy for haematology and clinical chemistry, and analysis for Triiodothyronine (T3), Thyroxine (T4), and Thyroid Stimulating Hormone (TSH). Males were sacrificed at a minimum of 13 weeks, and females who successfully delivered a litter were sacrificed on PND 14-16. A full postmortem was carried out on all animals and the reproductive organs were the focus of the examination. Organs and tissue were weighed and microscopically examined. At necropsy, testis and epididymis were weighed. Spermatogenesis was assessed at 0 and 1000 mg/kg bw/day.

Litters were standardised on PND 4 to 8 pups/litter (4/sex/litter). Pups were monitored daily for clinical observations, general health and mortality. The number of live and dead pups were recorded on PND 1 and daily until end of study. Live pups were weighed on PND 1, 4, 7 and 13. All pups were sexed on PND 1 and 4. Anogenital distance (AGD) was determined for live pups on PND 1. Male pups' nipples/areolae were determined on PND 13.

The study report provides historical control data (HCD) for the time range from 2015-2019.

Results:

Mortality and clinical signs

No test substance related deaths and no significant clinical observations were reported for either sex. Food Consumption and Body weight

In males, there was no significant changes in food consumption at any dose level. At 1000 mg/kg bw/day, absolute body weights in males were decreased from Day 22 onwards (up to 12% compared with concurrent controls at end of treatment), reaching statistical significance from Day 43 onwards. Moreover, a statistically significant reduced body weight gain was recorded for males treated at 1000 mg/kg bw/day from Day 22 onwards (up to 29% lower than concurrent controls at end of treatment).

Female rats treated at 1000 mg/kg bw/day showed no effects on body weights and body weight gain during the pre mating and mating period. From post-coitum Day 14 onwards, a lower mean body weight and body weight gain was observed, reaching statistical significance on Day 17 and/or Day 20, respectively. This could be explained by the reduced weight gain and/or weight loss in 4/4 pregnant females (Nos. 74, 76, 78, and 79), which had abnormal pregnancies (three females with implantation sites only and one female with total litter loss on PND 1). At Day 1 of lactation, mean body weight of the one female (No. 79) that delivered one pup (with total litter loss on PND 1) was similar to that of controls. In female rats treated up to 300 mg/kg bw/day, during lactation from Day 4-7 onwards, relative food consumption was decreased in females treated at 300 mg/kg bw/day (8% lower than concurrent controls, statistically significant on Days 4-7 and 7-13), which was considered not toxicologically relevant, due to the minimal magnitude of the change, and/or the absence of an effect on body weight. During the post coitum period, a higher absolute and relative food consumption was noted in females treated at 300 mg/kg bw/day on Days 11-14 (up to 13% higher than concurrent controls). This change was considered to be unrelated to treatment with the test item, due to the minimal magnitude of the change, and/or no clear trend was apparent regarding dose and duration of treatment. Female rats treated at 1000 mg/kg bw/day showed food consumption, before or after correction for body weight, was increased during pre mating Day 8-71, although no statistical significance was achieved. In addition, absolute food consumption (of 4 pregnant females only) was increased on post coitum Days 4-7 and relative food consumption was increased from post coitum Days 4-7 onwards, reaching statistical significance on Days 4-7, 14-17 and 17-20. These changes were considered not toxicologically relevant, due to the minimal magnitude and direction of change.

Functional observation battery (FoB)

No test substance related biologically significant adverse effects were noted on FoB parameters.

Ophthalmological examination

No effects were observed.

Haematology

At 1000 mg/kg bw/day, a statistically significant decrease in eosinophils and increase in mean corpuscular volume (MCV) was observed in males and females. In females at 1000 mg/kg bw/day, a statistically significant decrease in neutrophils, haematocrit and haemoglobin, and increase in mean corpuscular haemoglobin concentration (MCHC) was observed. In males at 1000 mg/kg bw/day, the mean number of reticulocytes and prothrombin time (PT) was statistically significantly increased.

Clinical chemistry

In females at 1000 mg/kg bw/day, a statistically significant increase in mean total protein, total bilirubin, calcium and glucose levels, and a decrease in mean aspartate aminotransferase activity, mean alanine aminotransferase activity, LDL cholesterol and mean potassium levels were noted.

In males at 1000 mg/kg bw/day, a statistically significant increase in mean alkaline phosphatase activity, mean alanine aminotransferase activity, bile acids, mean inorganic phosphate levels and mean potassium levels, and a decrease in mean cholesterol, HDL cholesterol, LDL cholesterol and mean total protein were noted.

At ≥ 300 mg/kg bw/day, a statistically significant increase in mean chloride in males, and a decrease in mean urea levels in females were noted.

At 100 and 1000 mg/kg bw/day, mean albumin levels were statistically significantly increased in females.

Thyroid hormones

A decrease in total T4 levels was observed in males. The total T4 levels were 4.25, 3.63, 3.05 and 1.57 $\mu\text{g/dl}$ 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: WI (Han) males, (2017-2019), N; 557, mean; 4.51 $\mu\text{g/dl}$, range; 2.85-6.37 $\mu\text{g/dl}$). 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.

Organ weights and Histopathology examinations

At 1000 mg/kg bw/day, absolute prostate weights (0.743g compared to 0.956g in control group) and epididymides weights (0.950g compared to 1.205g in control group) were statistically significantly decreased. There was also a decrease (not statistically significant) in the relative weights of these organs (relative prostate weight: 0.229g compared to 0.257g in control group; relative epididymides weight: 0.296g compared to 0.323g in control group). At the same dose, there were statistically significant increases in absolute and relative ovary weights (absolute: 0.171g compared to 0.110g in control group; relative: 0.071g compared to 0.038g in control group) and absolute and relative uterus weights (absolute: 0.960g compared to 0.363g in control group; relative: 0.385g compared to 0.125g in control group). However, the non-pregnant status of the majority of the females was considered to be the cause of these differences. There were no histopathological findings in the reproductive organs of either sex.

In males, there were dose-related reductions in relative thymus weights that were significant at 1000 mg/kg bw/day ($p < 0.01$), together with minimal lymphoid atrophy. However, in females the observations were different, in this case there was a dose related increase in thymus organ weight that was significant ($p < 0.01$) at both 300 and 1000 mg/kg bw/day, together with (cystic) epithelial hyperplasia. In this case the relationship of these observations at 1000 mg/kg bw/day with the failure of pregnancy is unclear because similar dose-related effects were observed in all three dose groups. However, in the pathology report it states "Treatment-related epithelial hyperplasia has been seen with diethylstilbesterol administration in mice and epithelial cyst formation has been recorded in rats treated with exogenous estrogen (Pearse, 2006)." Pearce also states "In pregnant females, an early increase in thymic weight is followed by a marked reduction in cellularity of the cortex. The level of cellularity returns to normal once pregnancy is over (Schuurman *et al.*, 1994). Increased levels of progesterone during pregnancy have a negative effect on thymic weight, whereas increased prolactin occurring during lactation has a stimulatory effect on the thymus. Thyroxine and growth hormone also have a stimulatory effect, and decreased levels of growth hormone have been associated with a reduction in thymic weight." It is difficult to make concrete conclusions about the adversity of the thymus organ weight changes because of the complexity of the organ's responses to multiple factors.

Effects on trabecular bone were seen in both sexes, although of greater severity in females, judged by the pathologist to be adverse at 1000 mg/kg bw/day but non-adverse at 300 mg/kg bw/day. Note that also in the pathology report it states "The increased trabecular bone was most obvious in the femur, just below the growth plate in the metaphysis area and to a lesser extent in the femur head. Due to the increase in bone, the spaces for the bone marrow appeared to be slightly diminished. However, this was not (yet) expressed in hematology parameters or hematopoiesis/ myelopoiesis in other organs.

For the sternum, the bone adjacent to the intervertebral discs was the most affected. Treatment-related increased trabecular bone has been seen in rats with estrogen (Dodd & Port, 1987; Hoenerhoff Mark J. & Brix Amy, n.d.). Given the nature and severity, this finding was considered adverse at 1000 mg/kg/day and non-adverse at 300 mg/kg/day." The link with estrogen toxicity is a possibility and may explain the reduced fertility and pregnancy failures. However, the references used by the pathologist to support this link are difficult to follow and do not support the involvement of estrogen. Hoenerhoff mentions estrogen toxicity as a cause of decreased bone resorption without defining or providing a specific reference, whilst Dodd's publication is on synthetic prostaglandin E1 effects in mice, and they postulated that "the hyperostosis was the result not only of the effects of misoprostol but also of endogenous estrogen. Since misoprostol did not cause hyperostosis in either rats or dogs, it is probable that this effect in mice is unique." So, the relevance of this reference is unclear and the statement of the pathologist about the involvement of estrogen appears to be unsupported by the literature. Alterations in weights of pituitary gland, kidney, brain, liver and heart were noted but considered as biologically not significant due to absence of associated histopathological changes.

Reproductive parameters

See section 3.4.5

Conclusion from the applicant

Under the test conditions of this OECD Guideline 422 Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test, based on an adverse increase in trabecular bone in both sexes at 1000 mg/kg bw/day, the study authors derived the parental NOAEL as 300 mg/kg bw/day. However, trabecular bone (essentially = bone density) may be considered as an adaptive response. In humans the bone density can increase and decrease in response to various factors, exercise, diet, pregnancy, etc. Whatever the mechanism of action it is reasonable to assume that the femur and sternum would return to normal after cessation of exposure to Heliotropin, i.e., the effect is likely to be reversible and not adverse. Reference (Kruijssen, 2019)

RAC Assessment

on Trabecular Bone

Increased trabecular bone (i.e. hyperostosis in sternum and femur) was observed in 300 mg/kg bw/d females (2/10 up to slight) and in 1 000 mg/kg bw/d rats (9/10 males and all females, up to moderate) slightly diminishing the spaces for the bone marrow. However, there was no impact in haematology parameters or haematopoiesis in other organs and therefore RAC considers the increased trabecular bone of doubtful adversity. Regarding the causes of such lesions, increased bone results from either decreased resorption of bone by osteoclasts or increased bone formation by osteoblasts. According to the NTP Nonneoplastic Lesion Atlas, increased bone is rarely observed as a treatment-related effect, an increased amount of bone is an occasional background lesion seen in aged F344 rats, with females more commonly affected than males. In rats, oestrogen toxicity results in decreased bone resorption from metaphyseal trabeculae, leading to densely thickened medullary bone. Treatment-related increased trabecular bone has been observed in adult female Wistar rats treated with 17 β -

oestradiol (Thobias *et al.*, 1991). RAC notes that fertility impairment was observed in high-dose animals also exhibiting increased trabecular bone. However, the correlation between the incidences of increased trabecular bones with the observed effects on fertility is not a causal proof that these changes are secondary to systemic toxicity. On the opposite, the bone effects may be secondary to reproductive system alteration (oestrogenic toxicity).

On Thymus

Minimal lymphoid atrophy in the thymus of 4 males was recorded in relation with a lower thymus weight. In view of the low severity, RAC considers those effects as non-adverse. Furthermore, based on the individual data there is no evidence of a relationship between these findings and fertility impairment.

In high-dose females, increased incidence and severity of epithelial hyperplasia in the thymus (9/10 up to moderate compared to 3/10 minimal in controls) were recorded and were associated with increased thymus weights. Epithelial hyperplasia is often associated with involution and/or atrophy of the thymus and may occur at relatively high incidences in some rat strains such as the Wistar rats (NTP Nonneoplastic Lesion Atlas). A variety of factors modulate the proliferative and secretory activity of medullary thymus epithelial cells (TEC). For example, TECs are stimulated by oestrogen and inhibited by testosterone (goReni, version 3). Treatment-related epithelial hyperplasia has been observed with diethylstilbesterol administration in mice and epithelial cyst formation has been recorded in rats treated with exogenous oestrogen (Pearse, 2006). RAC notes that fertility impairment was observed in high-dose females also exhibiting increased epithelial hyperplasia in the thymus. Despite the correlation, a causal relationship between thymic effects and the failure of pregnancy is not established. On the contrary, increased incidence and severity of epithelial hyperplasia may be secondary to reproductive system alteration (oestrogenic toxicity) or may reflect the different physiological status of high-dose females (cycling) versus controls (lactating).

Liver effects

In high-dose males, absolute and relative liver weights were increased (+21 % and +41 % compared to controls, respectively), which were associated with minimal hepatocellular hypertrophy (7/10) and slight alterations of clinical pathology parameters related to hepatic function. With respect to the low severity of the liver effects, RAC considers them an adaptive and reversible response.

Thyroids

There was a dose-related reduction in T4 levels observed in males, which is not considered adverse in the absence of significant changes in T3 and TSH levels, thyroid histopathology or thyroid weight.

Overall conclusion from RAC

Overall, RAC considers that the severe fertility impairments observed in the OECD TG 422 study (Anonymous, 2020a) are not secondary non-specific consequences of other toxic effects. There is no mechanistic data to support that the effects on sexual function and fertility are not relevant to humans.

Ref: Committee for Risk Assessment-RAC, 2024

SCCS comment

The SCCS agrees with the NOAEL of 300 mg/kg derived from the OECD Guideline 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test.

3.4.4.3 Chronic (> 12 months) toxicity

No data available

Taken from EFSA Journal (2012)

Table 19 (= IV.2 in EFSA): Subacute/subchronic/carcinogenicity studies

TABLE IV.2: SUBACUTE / SUBCHRONIC / CHRONIC / CARCINOGENICITY STUDIES

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration	NOAEL (mg/kg bw/day)	Reference	Comments
(Piprenal [05.016])	Rat; M, F 20	Oral	0, 16 mg/kg bw	12 weeks	16 ^{1,2}	(Trubek Laboratories Inc., 1958f)	Unpublished study of poor quality with insufficient study protocol and report of data; Eugenol: JECFA evaluation; NOAEL 250 mg/kg bw/day in rat (diet), ADI 2.5 mg/kg bw (See footnote 4).
	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day) ^{2,1}	16 weeks	500 ²	(FDA, 1954)	Unpublished study of limited quality. Insufficient study protocol and report of data; part of screening of 50 flavouring substances.
	Rat; M, F 10	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day) ^{2,1}	28 weeks	50 ²	(FDA, 1954)	Unpublished study of limited quality. Insufficient study protocol and report of data; part of screening of 50 flavouring substances.
	Rat; M, F 10	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day) ^{2,1}	15 weeks	500 ²	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F 10	Oral	0, 0.1 % (0, 50 mg/kg bw/day) ^{2,1}	27 - 28 weeks	50 ²	(Hagan et al., 1967)	Published summary of subacute and/or chronic toxicity studies on 48 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. Results not reported in detail but summarised in a table only.
	Rat; M, F NR	Oral	0, 0.1 % in the diet (0, 50 mg/kg bw/day) ^{2,1}	28 weeks	50 ²	(Hagan et al., 1965)	Published report of subchronic and/or chronic toxicity studies on 7 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. No detailed results reported.
	Rat; M, F NR	Oral	0, 1 % in the diet (0, 500 mg/kg bw/day) ^{2,1}	16 weeks	500 ²	(Hagan et al., 1965)	Published report of subchronic and/or chronic toxicity studies on 7 food flavourings carried out by the FDA. Validity of the results cannot be evaluated. No detailed results reported.
	Rat; M, F 20 - 60	Oral	0, 0.1, 0.5 % in the diet (0, 50, 250 mg/kg bw/day) ^{2,1}	1.5 - 2 years	250 ²	(Bär and Griepentrog, 1967)	Published study with incomplete report of experimental details and results.

3.4.5 Reproductive toxicity

3.4.5.1 Fertility and reproduction toxicity

In the OECD Guideline 422 Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test, 10 Wistar CrLI. WI (Han) rats per sex per dose were administered 0, 100, 300 and 1000 mg/kg bw/day of Heliotropin. 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 (see section 3.4.4.2 above).

Results

Effects on parental animals (See section 3.4.4.2 above)

Reproductive parameters

No significant effects were noted on oestrous cycle, spermatogenesis, pairing or mating indices, mean pre-coital time, duration of gestation, parturition or maternal care.

A decrease in the fertility index was 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. The study report was ambiguous about whether one female at 300 mg/kg bw/day was not pregnant or was pregnant but that the number of implantation sites were not recorded in error. If the

animal was pregnant then the fertility index values would be 100%, 90%, 100% and 40%, indicating no evidence of a dose response relationship.

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 related to developmental toxicity, see section 3.4.5.2.

Table 20 (= 34 in the Applicant dossier): Overview of reproductive toxicity results with Heliotropin (piperonal): Key study (Kruijssen, 2019)

Table 34: Overview of reproductive toxicity results with heliotropin (piperonal): Key study
(Kruijssen, 2019)

Study Type, Species	Doses and exposure period	Key findings	Reported results/point of departure
OECD 422; Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test. GLP compliant. Rat, Wistar CrLI. WI (Han), male and female, 10/sex/dose. Deviations from OECD 422; -10 females instead of 12-13. -Only parental males	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	- No test substance related deaths reported. - Males at 1000 mg/kg bw/day: Statistically significant ↓ in mean body weight and mean body weight gain during pre-mating 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:	Parental NOAEL: 300 mg/kg/day (based on an adverse increase in trabecular bone in both sexes at 1000 mg/kg/day). Reproduction NOAEL: 300 mg/kg/day (based on the absence of healthy offspring at 1000 mg/kg/day).

Study Type, Species	Doses and exposure period	Key findings	Reported results/point of departure
were fasted prior to blood sampling. -Pup T4 blood sampled on PND 14-16 instead of PND 4.	until at least PND 13. Reliability: reliable.	statistically significant ↓ in total T4 levels but within HCD. -At 1000 mg/kg bw/day: ↓ in absolute and relative prostate weight and absolute and relative epididymides weight, but not statistically significant. 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.	

Conclusion

Under the test conditions of this OECD Guideline 408/422 Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test, based on the absence of healthy offspring at 1000 mg/kg bw/day, **the study authors derived the NOAEL for reproduction as 300 mg/kg bw/day.**

Reproductive and developmental screening test (No Guideline)

Guideline: No Guideline
Species/strain: Rat Sprague-Dawley
Group size: 60-days old male and female 10/sex/dose
Test substance: Piperonal or Heliotropin
Batch: No data
Purity: No data
Vehicle: Methylcellulose or corn oil (no further information)
Dose levels: 0, 250, 500 and 1000 mg/kg bw/day
Administration: Oral gavage
GLP: No data
Study period: 1990

Reference (Kruijssen, 2019)

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 mortality and clinical observations daily, before and within one hour of dosing. Body weight was noted daily during the dosing period and on days 0, 6, 10, 14, 20, 21 and 25 of assumed gestation and on PND 1 and 4. Food consumption was monitored daily per animal and mean daily diet consumption was determined. Maternal behaviour was observed

on PND 1 and 4. The mating index, fertility index, gestation index and number of pups per litter were examined. F1 pups were examined for viability (daily examinations), sex, clinical signs, body weight and gross external malformations. The interactions between mother and pups were also examined. Necropsy examinations were performed on dams who delivered a litter on PND 4 or who failed to deliver a litter on Day 25 of assumed gestation. Necropsy examinations were performed on offspring on PND 4.

Results

Effects on parental animals

A statistically significant increase in mortality in dams at 1000 mg/kg bw/day and an increased incidence of clinical signs in females ≥ 500 mg/kg bw/day were reported but no details were specified. In females, a decrease in body weight gain at 500 mg/kg bw/day, a statistically significance decrease in body weight at 1000 mg/kg bw/day, and a non-statistically significant decrease in food consumption at 1000 mg/kg bw/day were noted but no details were specified.

Reproductive parameters

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.

No significant effects were noted on oestrous cycle, spermatogenesis, pairing or mating indices, mean pre-coital time, duration of gestation, parturition or maternal care. A decrease in the fertility index was 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. 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 related to developmental toxicity, see section 3.4.5.2.

Conclusion

Considering the limited reporting of methods and results, the non-guideline study was considered not reliable and only used for supporting evidence.

Reference (Vollmuth *et al.*, 1990)

Taken from RAC (2024)

In conclusion, RAC notes the clear, severe adverse effects on sexual function and fertility observed in rats after exposure to piperonal, which were not secondary to paternal toxicity. There are no compound-specific mechanistic data to support that the effects on sexual function and fertility are not relevant to humans. Regarding toxicokinetic considerations, RAC considered that the putative involvement of glycine depletion as the unique cause of the observed effects has not been substantiated (see for further details in the section RAC general comment at the start of this opinion). Therefore, in line with the DS proposal, RAC concludes that classification in Category 1B is warranted for adverse effects on sexual function and fertility.

Ref: Committee for Risk Assessment-RAC, 2024

3.4.5.2 Developmental Toxicity

Oral Prenatal developmental toxicity study (OECD TG 414)

Guideline: OECD Test Guideline 414
Species/strain: Rat Wistar CrLI. WI (Han), female: 10-14 weeks, 177-225g
Group size: 22 females/dose
Test substance: Piperonal or Heliotropin
Batch: 1810017
Purity: 99.9%
Vehicle: PEG 400
Dose levels: 0, 100, 300 and 1000 mg/kg bw/day
Administration: Oral gavage
GLP: In compliance
Study period: 03 Sept 2019 – 2020 (End date: not specified)

An OECD Guideline 414 Prenatal Developmental Toxicity Study was performed in compliance with GLP in Wistar CrLI. WI (Han) female rats (22 per dose). The test item was administered daily via oral gavage in doses of 0, 100, 300 and 1000 mg/kg bw/day in PEG 400 from gestation day (GD) 6-20.

Females were sacrificed on GD 21. The ovaries and uterine were examined for number of corpora lutea and implantation sites, the weight of the uterus was measured, the number and distribution of live and dead fetuses, embryo-foetal deaths and the sex of each fetus were determined. The thyroid gland was weighed, and tissue samples were collected and examined. Live fetuses were euthanised and examined for visceral anomalies, thoracic and abdominal changes and the heart, all major vessels and kidneys were examined for abnormalities. Tissues were collected and examined for malformations. Some pups were examined for skeletal abnormalities. The study report provides historical control data (HCD) for Rat CrLI:WI (Han) (outbred SPF-Quality) for a study data range from 2014-2019 (Dassen Roland, 2021).

Results

Mortality and clinical signs

No deaths were observed.
Clinical observations at ≥ 300 mg/kg bw/day included salivation from GD1, piloerection from GD7 and hunched posture from GD 13-17.

Food Consumption and Body weights

Food consumption was reduced by $\sim 22\%$ between post-coitum Days 6 to 9 (0.78x of control mean) at 1000 mg/kg bw/day. Although mean food intake recovered to control levels as treatment progressed, several animals at 1000 mg/kg bw/day also showed a notable reduction in food intake over post-coitum Days 15-21.
Body weight gain was reduced by $\sim 17\%$ at 1000 mg/kg bw/day (0.83x of the control mean at the end of the treatment period).
No effects on food consumption or body weight gain were observed at 100 or 300 mg/kg bw/day.
The effects at 1000 mg/kg bw/day were considered to be adverse by the Study Director, which is considered to be a valid conclusion because effects on food consumption and body weight are considered to be evidence of maternal toxicity (OECD, 2008). It is logical to consider that the maternal reductions in bodyweight gain were a direct consequence of the reduced food intake.

Table 21: Mean body weight gain measured during the Prenatal Developmental Toxicity Study of Piperonal or Heliotropin by Oral Gavage in Rats

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

Organ weights and thyroid hormone levels

Mean total Triiodothyronine (T3) and total Thyroxine (T4) levels showed an apparent dose related trend towards a reduction across the dose groups, and mean Thyroid-Stimulating hormone (TSH) levels showed an apparent dose-related trend towards an increase. At 1000 mg/kg bw/day, the decrease in mean T3 and T4 was statistically significant (0.65x and 0.73x of control mean for total T3 and T4, respectively). The mean total T3 was below the historical control data range at 100, 300 and 1000 mg/kg bw/day; the possible adversity of this change could not be established within this study. Both mean total T4 and TSH remained within the historical control data range at these dose levels, and as such these changes were considered not to represent an adverse effect. In particular, there were no test item-related correlative alterations in thyroid gland weights or thyroid histopathology.

The liver weight of the dams was not recorded, which may have provided definitive evidence of the likely induction of liver metabolic enzymes.

However, based on the evidence of the 10-day range finding study, where increases in liver weight were observed at 500 and 1000 mg/kg bw/day, it may be assumed that liver hypertrophy was induced at 1000 mg/kg bw/day and probably also at 300 mg/kg bw/day in this study. The probable effects on liver weight may explain the effects on thyroid hormone levels because liver hypertrophy is associated with the induction of enzyme levels and the spectrum of induced enzymes can include UDP, which metabolizes thyroid hormones and is considered to be a non-adverse and readily reversible response to an overload of an organic xenobiotic chemical (Jahnke *et al.*, 2004)

Table 22 Thyroid Hormone level measured during the Prenatal Developmental Toxicity Study of Piperonal or Heliotropin by Oral Gavage in Rats

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

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

Pregnancy Data

Two females at 1000 mg/kg bw/day showed 100% fetal loss (post-implantation deaths) and one female in the 300 mg/kg bw/day dose group also showed 100% fetal loss. The Study Director indicates that occurrences of 100% fetal loss are rare in the HCD and therefore considers the single occurrence at 300 mg/kg bw/day as an adverse event, similar to the two occurrences in the high dose group. However, the animal with 100% fetal loss at 300 mg/kg bw/day was the subject of a deviation, "For one animal at 300 mg/kg bw/day (A052), the number of implantation sites and uterine content were not documented at necropsy. It was recorded that this animal had implantation sites but that it had no fetuses. Therefore, the animal was recorded as gravid and was concluded to have a 100% implantation loss. Although it could not be confirmed if this was due to early and/or late resorptions, it was considered that sufficient data was available for adequate evaluation of the study results." However, the possibility exists that the error was to record the animal as having implantation sites where there were none, that is, that the female was a non-pregnant animal. In this case the treatment could not have been the cause of absence of fetuses.

It is also noted that all four groups, including the control group, had several animals (5/21, 4/20, 5/20, 7/22; control to high dose respectively) with percentages of early resorptions per litter that exceeded the historical control range, so it is possible that the HCD was not fully representative for batch of animals used in this study.

Overall, the effect on early resorptions was considered to be adverse at 1000 mg/kg bw/day but not adverse at the two lower dose levels. Premature abortion and foetal loss have been reported to occur after diet restriction and reductions in maternal bodyweight gain in rabbits (Cappon *et al.*, 2005) but not in Sprague-Dawley rats (Fleeman *et al.*, 2005), although there may be a rat strain differences in this case (Han-Wistar rats used in the current study).

Table 23: Maternal reproduction parameters examined during the Prenatal Developmental Toxicity Study of Piperonal or Heliotropin by Oral Gavage in Rats

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

Developmental Findings

Adverse skeletal malformations were observed at 1000 mg/kg bw/day at a higher frequency than in the control group (15% vs 1.6%). These malformations were specific in that they were localized to the thoracic region of the body; no external fetal lesions were noted. In addition to these malformations there was an increase in several types of non-adverse variations and reduced ossifications that also affected the thoracic region of the body.

Table 24: Foetal skeletal malformations observed during the Prenatal Developmental Toxicity Study of Piperonal or Heliotropin by Oral Gavage in Rats

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

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

Conclusion

Under the test conditions of this OECD Guideline 414 Prenatal Developmental Toxicity Study, based on lower body weight gain and food intake, and clinical signs at 1000 mg/kg bw/day, the study authors considered the maternal NOAEL as 300 mg/kg bw/day. Based on two cases of 100% implantation loss at 1000 mg/kg bw/day, along with lower fetal body weights and skeletal malformations, the developmental NOAEL was considered as 300 mg/kg bw/day.

Ref.: Dassen Roland, 2021

Oral combined repeated dose toxicity study with the reproduction/developmental toxicity screening test (OECD TG 422) (see section 3.4.4.2 above)

In the OECD Guideline 422 Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test, 10 Wistar CrLI. WI (Han) rats per sex per dose were administered 0, 100, 300 and 1000 mg/kg bw/day of piperonal. 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 (Kruijssen, 2019).

Results

Effects on parental animals See section 3.4.4.2

Reproductive parameters

For effects related to reproduction toxicity, see section 3.4.4.2

Table 25: 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 Heliotropin by Oral Gavage in Rats

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

Effects in Offspring

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 ≤ 300 mg/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. At 1000 mg/kg bw/day, no pup survived past PND 1, so there is no body weight data available for this dose group. In female pups at 100 and 300 mg/kg bw/day, there was a statistically significant 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. No significant differences were observed in T4 levels, anogenital distance (AGD), areola/nipple retention in pups in either sex at 0, 100 and/or 300 mg/kg bw/day.

Conclusion

Under the test conditions of this OECD Guideline 422 Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test, the study authors considered the developmental NOAEL to be at least 300 mg/kg bw/day.

Ref: Kruijssen, 2019

Reproductive and developmental screening test (No Guideline)

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.

Results

Effects on parental animals

For effects related to parental toxicity, see section 3.4.4.2 above

Reproductive parameters

For effects related to reproduction toxicity, see section 3.4.4.2 above.

Effects in Offspring

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.

No clinical signs of toxicity were reported. An increase in pup mortality and a statistically significant decrease in viability of offspring were noted at 1000 mg/kg bw/day but further details were not reported.

A non-statistically significant decrease in body weight gain during PND 1-4 was reported at $\geq$ 500mg/kg bw/day but further details were not specified.

Conclusion

Considering the limited reporting of methods and results, the non-guideline study was considered not reliable and only used for supporting evidence. (Vollmuth *et al.*, 1990)

Overall conclusion on reprotoxicity from the Applicant:

Based on the effects observed in an OECD Guideline 422 Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test, the parental NOAEL was considered as 300 mg/kg bw/day. The NOAEL for reproductive toxicity was considered as 300 mg/kg bw/day. Based on the effects observed in an OECD Guideline 414 Prenatal Developmental Toxicity Study, the developmental NOAEL was considered as 300 mg/kg bw/day.

Taken from EFSA Journal (2012)**Table 26** (= IV.3 in EFSA) Developmental and Reproductive Toxicity Studies:**Table IV.3: Developmental and Reproductive Toxicity Studies**

Chemical Name	Study type Durations	Species/Sex No / group	Route	Dose levels	NOAEL (mg/kg bw/day). Including information of possible maternal toxicity	Reference	Comments
Picnoral [05.016]	Reproductive and developmental toxicity: 1 week before mating until 4 days post parturition	Rat; F 10	Gavage	0, 250, 500, 1000 mg/kg bw/day	Maternal: 500 Foetal: 500	(Vollmuth et al., 1990)	Unpublished study with limited report of results. Summary of study published as abstract. Validity of the study cannot be evaluated.

Taken from RAC (2024)

RAC considers the developmental effects not to be secondary non-specific consequences of other toxic effects. In addition, RAC concluded that there is no compound-specific mechanistic data to support that the effects on development are not relevant to humans. Additionally, the putative involvement of glycine depletion at high dose levels as the unique cause of the observed effects, as put forward by the trade association after the consultation, has not been substantiated. Furthermore, some developmental effects were already observed from the low dose level (see for further details in the section RAC general comment at the start of this opinion). Therefore, in line with the Dossier Submitter proposal, RAC concludes that classification in Category 1B for adverse effects on development is warranted.

Ref.: Committee for Risk Assessment-RAC, 2024

SCCS comment

The SCCS agrees with the parental NOAEL of 300 mg/kg derived from an OECD Guideline 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test. The NOAEL for reproductive toxicity is also considered as 300 mg/kg bw/day as well as the developmental NOAEL based on the effects observed in an OECD Guideline 414 Prenatal Developmental Toxicity Study.

In concordance with the observations made after repeated dose exposure (see section 3.4.4), 300 mg/kg bw/day will be used as the toxicological point of departure for the calculation of MoS.

3.4.6 Mutagenicity / genotoxicity**3.4.6.1 Mutagenicity / genotoxicity *in vitro*****From the Applicant:****Bacterial Reverse Mutation Test (Ames)**

Guideline: Similar to OECD Guideline 471
Test System: *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 and *E. coli* WP2 uvrA
Replicates: 3 to 5 plates per test
Test substance: Heliotropin
Vehicle: DMSO
Batch: Not specified
Purity: 100%

Test concentrations: 300, 600, 1200 and 2400 µg/plate in the presence or absence of external metabolic activation
Negative control: DMSO
Positive control: With S9-mix: Benzo(a)pyrene and 2-aminoanthracene, and
Without S9-mix: 9-aminoacridine, 2-nitrofluorene, sodium azide and furylfuramide
GLP: Not specified
Study period: 1982

Heliotropin was tested in a study performed similar to OECD Guideline 471 to evaluate its mutagenic potential in *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537, TA1538 and *E. coli* WP2 uvrA, with and without metabolic activation (S9-mix). The concentrations of the test substance ranged from 300 to 2400 µg/plate. Negative solvent control and positive controls were used in the study.

Results

The test substance did not show any mutagenic activity up to the highest concentration in the presence or absence of S9-mix.

Conclusion

Under the conditions of the study, Heliotropin was not mutagenic in the bacterial reverse mutation test (Ames test), either in the presence or absence of S9-mix.

Ref.: Sekizawa & Shibamoto, 1982 cited in Anonymous (REACH), 2021

SCCS comment on Bacterial Reverse Mutation Test (Ames)

Heliotropin was not mutagenic in the bacterial reverse mutation test (Ames test), in either the presence or absence of S9-mix.

In vitro Mammalian Gene Mutation Test

Guideline: Similar to OECD Guideline 476
Test system: Mouse lymphoma L5178Y cells
Replicates: Duplicates in first trail and triplicates in second trail under non activated conditions
Test substance: Heliotropin
Vehicle: DMSO
Batch: Not specified
Purity: Not specified
Test concentrations: With S9-mix: from 62.5 to 1000 µg/mL, and
without S9-mix: from 200 to 1000 µg/mL in duplicates in first trail
and from 600 to 1000 µg/mL in triplicates in second trail
Negative control: DMSO
Positive control: With S9-mix: Dimethylnitrosamine, and without S9-mix: Ethylmethanesulphonate
GLP: Yes
Study period: 1982

Heliotropin was tested in a study performed similar to OECD Guideline 476 to evaluate its mutagenic potential in mouse lymphoma L5178Y cells, with and without metabolic activation (S9mix). The concentrations of the test substance ranged from 62.5 to 1000 µg/mL with metabolic activation & from 200 to 1000 µg/mL in first trail and from 600 to 1000 µg/mL in second trail without metabolic activation. Negative solvent control and positive controls were used in the study.

Results

The test substance did not induce significant increase in the mutant frequency at the TK locus in mouse lymphoma L5178Y cells up to the highest concentration in the presence or absence of S9-mix.

Conclusion

Under the conditions of the study, Heliotropin was not mutagenic in the mammalian cell gene mutation test, either in the presence or absence of S9-mix.

Ref: Study 1982 cited in Anonymous (REACH), 2021

SCCS comment on *in vitro* mammalian gene mutation test

Heliotropin was not mutagenic in the mammalian gene mutation test, in either the presence or absence of S9-mix.

In vitro Chromosomal Aberration Test

Guideline:	Similar to OECD Guideline 473
Test system:	Chinese hamster Ovary (CHO) cells
Replicates:	Duplicate flasks/dose
Test substance:	Heliotropin
Vehicle:	DMSO
Batch:	1
Purity:	Not specified
Test concentrations:	Without S9-mix: 63, 125, 250 and 500 µg/mL, and with S9-mix: 625, 1250, 2500 and 5000 µg/mL
Negative control:	DMSO
Positive control:	With S9-mix: Cyclophosphamide, and without S9-mix: Triethylenemelamine
GLP:	Yes
Study period:	1988

Heliotropin was tested in a study performed similar to OECD Guideline 473 to evaluate its ability to induce chromosomal aberrations (clastogenic potential) in Chinese hamster Ovary (CHO) cells, with and without metabolic activation (S9-mix). The concentrations of the test substance ranged from 63 to 500 µg/mL without metabolic activation and from 625 to 5000 µg/mL with metabolic activation. Negative solvent control and positive controls were used in the study.

Results

In the S9 activated system, the test substance could not be evaluated for chromosome aberrations at 5000 µg/mL due to toxicity. The test substance did not induce significant increase in chromosome aberrations in Chinese hamster Ovary (CHO) cells in the presence or absence of S9 mix.

Conclusion

Under the conditions of the study, Heliotropin was not genotoxic in the chromosomal aberration test, either in the presence or absence of S9-mix.

Ref: Study, 1988 cited in Anonymous (REACH), 2021

SCCS comment on the *in vitro* chromosomal aberration test

After analysis of the data available in the ECHA website based on a report to which the SCCS did not have access, the SCCS considers the results of the study as inconclusive:

<https://echa.europa.eu/pt/registration-dossier/-/registered-dossier/5265/7/7/2/?documentUUID=ebbb91a2-602f-4548-9e5e-0ef9d9489495>.

The following experimental conditions used in the study did not meet recommendations of the current OECD TG473:

- exposure times used in the study were for 2 h and 14 hours, with and without S9-mix, respectively – according to the OECD TG 473 cells should be exposed to the test chemical **with and without metabolic activation** for 3-6 hours, and sampled at a time equivalent to about 1.5 normal cell cycle lengths after the beginning of treatment. Additionally, cells should be continuously exposed **without metabolic activation** until sampling at a time equivalent to about 1.5 normal cell cycle lengths. The doubling time of the CHO cells used in the study was not provided on the website, however according to literature data, the doubling time of the cells is around 16–22 hours. If so, the exposure times, both with and without S9-mix, do not meet the basic criteria recommended by the OECD TG473.
- fixation times (start of exposure up to fixation or harvest of cells) used in the study were: 12 and 16 hours, with and without S-9 mix, respectively – as mentioned above, according to OECD TG 473 the cell sampling should be performed after about 1.5 normal cell cycle, which means at least around 24 h (16+8 h). Therefore, the recommendation was also not fulfilled.
- only 100 metaphases were scored in the study – according to OECD TG 473 minimum 300 metaphases should have been scored.

From the Applicant:**Table 27:** Additional Micronucleus Test provided in February 2026

Guideline:	OECD TG 487 with 3 deviations identified by the Applicant: - for the condition of short exposure in presence of S9, due to high toxicity of two highest concentrations, only two valid concentrations (not 3) - toxicity of the highest concentration of Test item on short exposure condition in absence of S9 was 39% thus did not meet the minimum required toxicity level for the top concentration - Although within the Min-Max intervals of the historical control ranges, positive controls showed a lower response than expected according to the previous historical data (95th CI)
GLP:	In compliance with GLP but no certificate of GLP presented
Test system:	Human lymphoblast cell line TK6 (clone CRL-8015™)
Test substance:	Piperonal (alternative names 1,3-Benzodioxole-5-carboxaldehyde; 3,4-(Methylenedioxy)benzaldehyde; Heliotropin
Batch (Purity):	STBK4284 (99.9%, Sigma, Ref. P49104)
Vehicle:	DMSO
Assay medium:	RPMI 1640 (A10491) supplemented with 10% FBS (F9665), and 1% PS (P4333)
Concentrations:	Short exposure: C1: 2.35 mM; C2: 2.94 mM; C3: 3.68 mM; C4: 4.60 mM Extended exposure: C1: 0.86 mM; C2: 1.07 mM; C3: 1.34 mM; C4: 1.68 mM; C5: 2.13 mM

Opinion on the safety of Heliotropin (CAS No. 120-57-0, EC No. 204-409-7)

Exposure duration/ S9 use:	Short exposure: 4 h $\pm$ 30 minutes followed by 20 $\pm$ 1 h of recovery, with or without S9 Long Exposure: 24 $\pm$ 1 h
Cytochalasin B	Not used
Replicates	4 replicates
Positive controls:	Short exposure: - Mitomycin C (MMC, clastogen) 300 ng/mL - Vinblastine (VB, aneugen) 200 ng/mL - Cyclophosphamide (CYC, clastogen requiring metabolic activation) - 3610 ng/mL (S9+/-) Long Exposure: - MMC - 120 ng/mL - VB- 15 ng/mL
Negative control:	Medium
Study period:	December 11 th 2025 - January 7 th 2026
Results:	Negative
Reference:	OROXCELL 2026. AMENDMENT 1 TO FINAL STUDY REPORT, Oroxcell Study n° OXL-25-229, <i>In vitro</i> evaluation of the micronucleus formation of Piperonal by flow cytometry on TK6 cells, 2026.02.16

This study aimed to evaluate the effect of the test item "Piperonal" on micronucleus formation on TK6 cells (human lymphocyte cell line) using the flow cytometry. The proposed protocol is based on the original OECD Guideline 487 "In vitro Mammalian Cell Micronucleus Test".

Three deviations to the OECD TG 487 were observed in the present study:

1. The OECD TG487 states that at least three test concentrations that meet the acceptability criteria should be evaluated, however only two concentrations met this acceptance criteria for the condition **of short exposure in presence of S9** (high toxicity of two highest concentrations). For this reason, the Study Director used the two valid concentrations (rather than three) to reach his conclusion about the test item. The decision is based on the fact that the conditions meeting the acceptance criteria (5 000 events/replicate; 4 replicates for C1 and 3 replicates for C2) provide enough data for a robust statistical analysis (One-Way ANOVA and Pearson's correlation). Besides, the MN scoring of these conditions is very similar of those from negative and solvent controls, with values within the range of C-Chart for negative controls. Finally, there is no evidence of concentration-dependent effect of the test item on MN formation, given the r^2 and the p values of Pearson's correlation are not biologically significant.

2. The toxicity of the highest concentration of Test item on **short exposure condition in absence of S9** did not meet the minimum required toxicity level for the top concentration recommended by the TG487. Indeed, the concentration of 4.60 mM showed a cytotoxicity slightly below the threshold of 55+/- 5% (39.00 %).

Although it consists in a deviation of method, the Study Director considers that the data with this concentration is enough to fundament his conclusions. The decision is based on the fact that there is no statistical significance with any concentration of test item and, although there is a moderate correlation (concentration-dependent response), the MN scoring is very close to the values obtained with the negative controls. Besides, the absence of MN induction on long term treatment (24 hours) provided enough evidence that the Test Item has no aneugenic or clastogenic activity.

3. Although within the Min-Max intervals of the historical control ranges, positive controls showed a lower response than expected according to the previous historical data (95th CI), except for the aneugenic reference compound (VB) in the extended exposure without metabolic activation. Although it was observed a **reduced performance of positive**

controls, the Study Director considers that the methodology is under control in the laboratory. Such affirmation is based on the fact that all positive controls produced a statistically significant increase in MN formation in comparison with the solvent control, with a consistent fold increase on MN formation. Besides, the negative controls are within the range of 95% confidence interval obtained with the historical data. Finally, as mentioned before, VB (vinblastine), i.e., the aneugenic reference compound, induced a robust response (slightly above the upper limit of the 95% confidence interval obtained with the historical data), indicating that the method can detect aneugenic events, while the cell cycle progression and scoring are adequate. For such reasons, the Study Director considers that the attenuation of the magnitude of MN induction by the positive controls is consistent with the normal biological variability and does not affect assay validity.

In a Concentration range finding (CRF) assay, the TK6 cells were exposed to the highest concentration recommended by the OECD 487 for simple ingredient (10 mM) following the protocol of short (4h, in presence or absence of S9) and extended (24 h in absence of S9) exposure. The concentrations tested were 0.000056, 0.00016, 0.0005, 0.0015, 0.0045, 0.014, 0.041, 0.12, 0.37, 1.11, 3.33, 10 mM. The test item induced a sudden drop in cell viability between the highest concentration (C12: 10.00 mM) and C11 (3.33 mM) for the short exposure.

The Study Director decided to use 6 concentrations for the first run of Main assay (rather than the usual 4 concentrations), using the highest concentration (C12: 10.00 mM) with a ratio of 1.25 dilution to generate 5 additional concentrations for cell treatment.

An additional CRF assay was performed for the condition of extended exposure (24 h) due to the sudden increase in cell toxicity observed between the concentrations C10 (6.84% of toxicity) and C11 (89.25% of toxicity). The second CRF run was carried on using narrower range concentration (0.69 to 3.33 mM using a ratio of 1.25 dilution of 3.33 mM to generate the additional 7 concentrations) to find the concentration falling in the range of toxicity recommended by the TG 487. Based on the results of CRF assay Run 2, the concentration of C6 (2.13 mM) was chosen as the highest concentration for the Main Assay to generate 4 additional concentrations for cell treatment using a 1.25 dilution factor.

Four concentrations of the test item were evaluated during the short exposure, both in the presence and absence of S9 metabolic activation: C1 (2.35 mM), C2 (2.94 mM), C3 (3.68 mM) and C4 (4.60 mM).

Under **conditions without S9**, the highest concentration resulted in a toxicity of 39%, which is below the minimum cytotoxicity recommended by OECD TG 487. Although this represents a deviation, the Study Director considers the data adequate to support the study conclusions, based on the absence of statistical significance at any concentration, MN frequencies remaining within the C-Chart limits and close to negative control values despite a moderate correlation ($r^2 = 0.5472$; $p = 0.0002945$), and a fold induction at the highest concentration close to 1. In addition, no effects were observed following extended exposure (24 h), which met all acceptance criteria (52.77% toxicity at the highest concentration).

The concentration C3 (65.32%) and C4 (84.49%) for the condition **with metabolic activation (S9+)** showed a toxicity higher than $55 \pm 5\%$, mentioned by the OECD TG 487, therefore such data were not considered for the analysis. Nevertheless, several scientific considerations support the use of the two valid concentrations for drawing conclusions on the test item:

- Micronucleus (MN) frequencies at C1 and C2 were comparable to those observed in the negative and vehicle control groups
- Concentrations C3 and C4, despite inducing cytotoxicity above the recommended threshold, yielded MN frequencies below the upper limit of the control chart, indicating that even highly cytotoxic concentrations did not induce MN formation.
- A substantially higher number of cells was scored per condition (20,000 cells for C1 and

15,000 cells for C2), greatly exceeding the minimum number recommended by OECD Test Guideline 487.

- The number of valid replicates meeting the acceptance criteria (four replicates for C1 and three for C2) provided sufficient data for robust statistical analyses (one-way ANOVA and Pearson's correlation).

- No evidence of a concentration-related increase in MN formation was observed, as the r^2 values and p-values obtained from Pearson's correlation were not biologically significant.

Based on these considerations, the study director concludes that repeating the entire experiment would not provide additional relevant information, since testing a lower concentration would be unlikely to induce MN formation that was not already observed at the higher, more cytotoxic concentrations.

The test item Piperonal did not show statistically significant induction of micronucleus formation in any tested condition in TK6 cells. It was observed a low correlation between concentration and MN formation with the short exposure (both S9-/S9+). However, even though the values of r^2 are above the threshold of 0.3 (but clearly below 0.7), the biological significance is negligible. Finally, the MN in all groups treated with Piperonal were under the limit of C-Chart obtained with the historical data of the laboratory for negative controls, indicating that Piperonal-treated cells present MN content similar to the negative and solvent controls.

It is noteworthy that, despite the %MN for some conditions treated with the Test Item were slightly above the upper limit of 95% confidence interval of the historical negative control, all values obtained with Piperonal-treated cells were close to the negative controls and they were all within the min-max range of historical data for negative controls. Furthermore, and as mentioned before, the numbers of MN were lower than the frequency 16 per 1000 (1.6%) which is considered to be biologically normal values (Van Hummelen and Kirsch-Volders, 1992). Based on these arguments, the test item Piperonal is considered as negative for induction of micronucleus formation under the tested conditions.

Ref: OROXCELL 2026.

SCCS comment on the additional Micronucleus Test provided in February 2026

The SCCS considers the study negative, although with some limitations:

- although the Applicant claimed that the study was performed according to GLP principles, no certificate of GLP was provided. Instead, confirmation of supervision of the study by an internal Quality Control Unit was provided.

- the methods description was incomplete, and development and validation studies were not annexed (instead references to an internal SOP METHOD_PROC_0812-v2 and validation reports GEN-24-216; GEN-25-010; VAL-25-029 were included).

- the concentrations analysed in the short exposure were not fully in line with OECD TG 487.

In the 4h -S9 mix condition the highest concentration tested (at 4.6 mM) induced 39% cytostasis, while in the 4h +S9 condition 2 concentrations were tested (instead of minimum 3 recommended) inducing on average 41.33% (at 2.35 mM) and 55.36% (at 2.94 mM) cytostasis.

After analysis of the results, the SCCS is of the opinion that the identified limitations did not have a substantial bearing on the study's conclusions.

SCCS comment on other *in vitro* genotoxicity testing of Heliotropin

In addition to the studies provided by the Applicant, other studies relevant for *in vitro* genotoxicity of Heliotropin were found in the literature and in the EFSA (2012) report. These are summarised in the Table in Annex I.

The Applicant also provided arguments based on weight of evidence to demonstrate the absence of genotoxic concern for Heliotropin. Concerning the results on the gene mutation tests from published literature, based on two reliable negative Ames tests (Sekizawa and

Shibamoto, 1982; Haworth *et al.*, 1983) plus additional negative Ames studies with low reliability (Heck *et al.*, 1989; Kasamaki *et al.*, 1982; White *et al.*, 1977), and one negative mammalian gene mutation of low reliability (Heck *et al.*, 1989), it can be concluded that Heliotropin is not mutagenic *in vitro*. The *in vitro* mammalian gene mutation test in mouse lymphoma L5178Y cells, reported on ECHA website, further supports this conclusion (Study from 1982 cited in Anonymous (REACH), 2021).

Two other studies of low reliability from published literature suggest that Heliotropin can induce chromosomal aberrations in Chinese hamster cell line B241 (Kasamaki *et al.*, 1982; Kasamaki and Urasawa, 1985). Another study on chromosomal aberration test on CHO cells was considered acceptable and negative by ECHA (Reference Study from 1988 cited in Anonymous (REACH), 2021)). However, after analysis of the data available on the ECHA website, the SCCS considered the study results as inconclusive. A new study on *in vitro* micronucleus assay was provided by the Applicant with negative results although with some limitations.

3.4.6.2 Mutagenicity / genotoxicity *in vivo*

From the Applicant:

There are no relevant *in vivo* mutagenicity or genotoxicity data on Heliotropin since there is no concern for these endpoints from the comprehensive battery of *in vitro* study results. This is in accordance with ECHA and SCCS Notes of guidance strategies for mutagenicity and genotoxicity.

SCCS comment

One *in vivo* dominant lethal assay, with 2 routes of exposure, was identified (Epstein *et al.*, 1972) and is summarised in the following table.

Table 28. *In vivo* study on mutagenicity of Heliotropin (Epstein *et al.*, 1972)

Endpoint	Test system/concentrations	Result by the Author	Comments by SCCS
<i>In vivo</i> Dominant lethal assay	ICR/Ha Swiss mice i.p. injection 0, 124, 620 mg/kg bw Heliotropine Cas# 120-57-0	Negative	Limited reliability: - non-GLP, not fully in line with OECD TG 478, - only 2 dose levels - poor reporting of experimental observations
<i>In vivo</i> Dominant lethal assay	ICR/Ha Swiss mice Oral gavage 1000 mg/kg bw (repeated doses on 5 successive days) Heliotropine	Negative	Limited reliability: - non-GLP, not fully in line with OECD TG 478, - only 2 doses used - poor reporting of experimental observations

In this study Heliotropin induced an increased number of early fetal deaths (2 weeks after i.p. exposure, at the higher dose level of 620 mg/kg), however, as stated by the authors the increase was not significant. Heliotropin was classified by the authors as a substance which could meet screening criteria but was judged non-significant by analysis of variance. Importantly, as stated by the authors themselves, the non-significance of effects of any agents in this category did not preclude effects at other dose levels and under other conditions of test.

The *in vivo* study was considered by the SCCS to be of limited reliability and not providing conclusive evidence of lack of chromosomal aberration potential of Heliotropin.

Overall SCCS comment on genotoxicity of Heliotropin

Heliotropin tested in gene mutations tests in bacteria and mammalian cells consistently showed negative results. Heliotropin showed positive results in chromosomal aberration tests *in vitro* of low reliability on Chinese hamster cell line B241. One study on CHO cells available from the ECHA website was considered by the SCCS as inconclusive. Additionally, one *in vivo* dominant lethal test did not provide conclusive evidence for the lack of mutagenic potential of Heliotropin. A new study on *in vitro* micronucleus assay on TK-6 cells was provided by the Applicant with negative results.

Based on all the available data, the SCCS is of the opinion that Heliotropin does not pose genotoxicity potential.

3.4.7 Carcinogenicity

The carcinogenic potential of Heliotropin was tested in a study performed similar to OECD 453 in rats. Groups of 10-30 male and female rats received a diet containing 0, 0.1 or 0.5% (equivalent to 0, 1000 or 5000 ppm, respectively or approximately 0, 50 or 250 mg/kg bw/day, respectively) of Heliotropin for period of 2 years. The animals were checked for appearance, behaviour and weight increase. All animals which died before the termination of the test as well as those killed at the end of the testing period were dissected and subjected to postmortem and histological examination. Histopathological evaluation was conducted on liver, kidneys, suprarenal glands, heart, spleen, pancreas, cerebellum, and any identified tumours.

Results

Survival at the 2-year timepoint was 6/20 at the low-dose level and 25/60 at the high-dose level (data were not presented by gender). No effects were noted on clinical signs and growth over the course of the study and no histopathological changes or carcinogenicity were reported.

Conclusion

Under the conditions of the study, Heliotropin was not carcinogenic when administered in diet at 50 or 250 mg/kg bw/day for 2 years.

Ref: Bar & Griepentrog, 1967 cited in Anonymous (REACH), 2021

SCCS comments on Carcinogenicity

After analysis of the available data, the SCCS considers that Heliotropin is not likely to be a carcinogen.

3.4.8 Photo-induced toxicity

The UV/VIS spectra demonstrate significant absorbance between 290 and 700 nm, with peak absorbance at 310 nm and returning to baseline by 350 nm. The peak molar absorption coefficient (6370 L/mol/cm) within this range is above the benchmark of concern for photoirritating effects, 1000 L/mol/cm.

3.4.8.1 Phototoxicity / photo-irritation and photosensitisation

Heliotropin has been shown not to have photo-irritating potential in Guinea-pigs at 25% nor in humans at 0.1% (Api *et al.*, 2025).

The photosensitizing potential of Heliotropin has not been evaluated.

According to the Applicant, no concern regarding photo-induced toxicity is expected.

SCCS comment on Phototoxicity

In the absence of clinical reports, SCCS considers that photo-induced toxicity is unlikely.

3.4.8.2 Photomutagenicity / photoclastogenicity

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SCCS comments on photomutagenicity/photoclastogenicity

The SCCS considers that Heliotropin is not likely to be a photomutagen/photoclastogen.

3.4.9 Human data

/

3.4.10 Special investigations

/

3.5 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MOS)

The most relevant toxicity data for systemic toxicity are those of the combined 90 day and screening reproductive toxicity study on rats, by oral gavage. In this study, adverse effects were observed on the parental generation and the F1 generation at the highest dose level of 1000 mg/kg bw/day but no adverse effects were observed at the two lower dose levels of 300 and 100 mg/kg bw/day. The adverse effects on the P0 generation were limited to the observation of trabecular bone (increased bone density) in the femur and sternum. Trabecular bone may be considered as an adaptive response. In humans the bone density can increase and decrease in response to various factors, exercise, diet, pregnancy, etc.

The parental NOAEL by oral route is 300 mg/kg bw/day. The other effects of concern were a reduction in the fertility index and post-implantation survival of the P1 generation at 1000 mg/kg bw/day. The NOAEL by oral route for reproduction and developmental NOAEL are both 300 mg/kg bw/day.

Based on studies on oral absorption in mice and rats showing more than 90% of absorption, the oral NOAEL determined are converted in a systemic NOAEL of 270 mg/kg bw/d which will be considered as the Point of Departure (PoD) for further margin of safety calculation:

$$POD_{sys} = 270 \text{ mg/kg bw/day}$$

The derogation requested by the applicant is limited to the fine fragrances (adult) category at the maximum current concentration of use (1.8%).

Heliotropin systemic exposure has been assessed for different consumer products and different routes of exposure. For dermal exposure, as recommended by the 12th SCCS Notes of Guidance, in absence of data, a default value of 50% has been used to assess the systemic exposure; for oral exposure 90% of oral absorption has been considered based on the experimental study in rat; finally, 100% of bioavailability has been considered of inhalation route.

Taking this consideration into account, the SED and the MOS for adult is recalculated as described in the Table below.

Systemic exposure dose (SED) from Fine Fragrances = 0.079 mg/kg bw

No observed adverse effect level (NOAEL) = 300 mg/kg bw/d
(90 day and screening reproductive toxicity study on rats, oral, rat)

Bioavailability 90% = 270 mg/kg bw/d

Margin of Safety	adjusted NOAEL/SED = 3417
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MoS based on overall exposure considering other sources**Table 29:** MoS based on overall exposure to Heliotropin, taking into account a market presence rate for e-cigarettes (as used by the Applicant)

Product category	Systemic Exposure Dose (SED) for Adults (mg/kg bw/day)	NOAEL Adjust (mg/kg bw/day)	MoS
Fine fragrances	0.079	270	3417
Food	0.073	270	3699
Household products	0.061	270	4426
e-cigarettes and tobacco products (only exposure to e-cigarettes is considered as explained above; a correction for market presence rate is applied as suggested by the applicant)	0.076	270	3553
Total SED (µg/kg bw/d)	0.289	270	934

Table 30: MoS based on overall exposure to Heliotropin, not taking into account a market presence rate for e-cigarettes (as calculated by the SCCS)

Product category	Systemic Exposure Dose (SED) for Adults (µg/kg bw/day)	NOAEL Adjust (mg/kg bw/day)	MoS
Fine fragrances	0.079	270	3417
Food	0.073	270	3699
Household products	0.061	270	4426
e-cigarettes and tobacco products (only exposure to e-cigarettes is considered as explained above; no correction for market presence rate is applied as suggested by the applicant)	0.918	270	294
Total SED (µg/kg bw/d)	1.13	270	239

Adult exposure to Heliotropin in Fine Fragrances separately or in combination with other products is safe, whether a correction for occurrence of Heliotropin in e-cigarettes is applied as suggested by the Applicant or not, as recommended by SCCS.

3.6 DISCUSSION

Heliotropin (CAS No. 120-57-0, EC No. 204-409-7), also known as piperonal, is an aromatic compound commonly used in cosmetic products for its sweet, floral scent. Naturally found in some essential oils but mostly produced synthetically, Heliotropin is a fragrance ingredient in perfumes, lotions, creams, shampoos, and deodorants. In addition, Heliotropin can function as a fixative, helping to stabilize and prolong other scents in a formulation, as well as a masking agent, softening the odour of other ingredients to enhance overall product appeal.

Physicochemical properties

Heliotropin is soluble in different solvents, including ethanol, and is slightly soluble in water. Detailed data on the stability of the test substance under the conditions of use should be provided.

Toxicokinetics

In the absence of any proper *in vitro* study, **the default value of 50% is used for the dermal absorption** and the safety assessment.

Based on the data provided, a **bioavailability by oral route of 90%** is selected for use in safety assessment in this dossier.

Heliotropin can be metabolised by oxidation to piperonylic acid followed by glycine- or glucuronide conjugation. Alternatively, it can be reduced to piperonylic alcohol and conjugated to glucuronic acid or glutathione. As a third option, the methylenedioxy-ring might be cleaved leading to a catechol.

Exposure

As Heliotropin is proposed to be classified as Repr.1B, the overall exposure has to take into account cosmetic products, but also other sources: food, household cleaning products, and e-cigarettes and tobacco products. Three routes are then considered: the dermal route, the oral route and the inhalation route.

The derogation requested by the applicant is limited to the fine fragrances for adults at the maximum current concentration of use (1.8%).

In this opinion the exposures for individual product types (cosmetic and non-cosmetic sources) are calculated. The overall exposure of Adults to Heliotropin is estimated to **289 µg/kg bw/d** if a market presence rate for e-cigarettes is applied (as proposed by the Applicant) and **1130 µg/kg bw/d** if not (as calculated by the SCCS, see Table 11 and 12 above).

Toxicological Evaluation

Irritation and corrosivity

Based on data made available to SCCS, Heliotropin is considered as non-irritant for the skin and for mucous membrane.

Skin sensitisation

Based on results from the *in silico*, *in chemico* and *in vitro* data Heliotropin should be considered as a skin sensitiser. The LLNA results in an EC3 value of 25%, which points to a weak to moderate skin sensitising potency. The validity of this study could not be evaluated by the SCCS, since it had no access to the full study report.

The SCCS could not evaluate the assessment through QRA2, since the Applicant did not provide details on several aspects of the approach as applied to Heliotropin. The SCCS could therefore not evaluate if Heliotropin could induce skin-sensitisation when used up to 1.8% in fine fragrance products. For future submissions, the SCCS encourages Applicants to provide a more detailed description on how QRA2 was applied to support a conclusion on this aspect.

Taking into consideration all the available evidence, which indicates that Heliotropin is a weak to moderate skin sensitiser, the SCCS considers the risk of induction of skin sensitisation in consumers to be low.

Acute toxicity

The SCCS considers Heliotropin a low acute toxicant via any route of exposure.

Repeated dose toxicity

Based on available data, A NOAEL of 300 mg/kg can be derived from the OECD Guideline 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test.

Reproductive toxicity

The European Risk Assessment Committee (RAC) of ECHA issued in September 2024 an opinion recommending among others a classification for Heliotropin as 'Reprotoxic of Category 1B (H360Fd)'. Following the RAC opinion, the European Commission may propose a classification for Heliotropin as a 'Repr.1B' (CLP Regulation Annex VI entry).

Based on available data, a NOAEL of 300 mg/kg can be derived from an OECD Guideline 422 Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test. The NOAEL for reproductive toxicity is also considered as 300 mg/kg bw/day as well as the developmental NOAEL based on the effects observed in an OECD Guideline 414 Prenatal Developmental Toxicity Study.

In concordance with the observations made in after repeated dose exposure (see section 3.4.4), 300 mg/kg bw/day will be used as the toxicological a point of departure for the calculation of the MoS.

Mutagenicity / genotoxicity

Heliotropin was tested in gene mutations tests in bacteria and mammalian cells consistently showing negative results. Heliotropin showed positive results in chromosomal aberration tests *in vitro* of low reliability on Chinese hamster cell line B241. One study on CHO cells available from ECHA website was considered by the SCCS as inconclusive. Additionally, one *in vivo* dominant lethal test did not provide conclusive evidence for the lack of mutagenic potential of Heliotropin. A new study on *in vitro* micronucleus assay on TK-6 cells was provided by the Applicant with negative results.

Based on all the available data, the SCCS is of the opinion that Heliotropin does not pose a genotoxic potential.

Carcinogenicity

After analysis of the available data, the SCCS considers that Heliotropin is not likely to be a carcinogen.

Photo-induced toxicity

In the absence of clinical reports, SCCS considers that photo-induced toxicity is unlikely.

The SCCS considers that Heliotropin is not likely to be a photomutagen/photoclastogen.

Human data

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Special investigation

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Safety assessment

The SCCS has concluded that adult exposure to Heliotropin in Fine Fragrances when used separately or in combination with other products is safe.

4. CONCLUSION

1. In light of the data provided and taking under consideration the possible classification as 'Repr.1B' under Regulation (EC) No 1272/2008 (CLP Regulation) and the conditions laid out in Article 15 (2) (d) of the Regulation (EC) No 1223/2009, does the SCCS consider Heliotropin, safe when used as an ingredient in fine fragrances for adults only up to 1.8%?

Based on the assessment of data provided and taking into consideration the concerns related to the possible classification as 'Repr.1B' under Regulation (EC) No 1272/2008 (CLP Regulation), the SCCS considers Heliotropin safe when used up to the maximum concentration of 1.8% only in fine fragrances for adults.

2. Alternatively, what is, according to the SCCS, the maximum concentration considered safe for use of Heliotropin in cosmetic products?

/

3. Does the SCCS have any further scientific concerns regarding the use of Heliotropin in cosmetic products?

/

5. MINORITY OPINION

None

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7. GLOSSARY OF TERMS

See SCCS/1647/22, 12th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – Appendix 15 - from page 158.

8. LIST OF ABBREVIATIONS

See SCCS/1647/22, 12th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – Appendix 15 - from page 158.

Annex I – Genotoxicity data from literature related to current submission

Endpoint	Test system/concentrations	Result	Comments by SCCS	Ref.
Gene Mutation (Ames test)	<i>S. typhimurium</i> TA98; TA100;TA1535; TA1537; TA1538 <i>E. coli</i> WP2uvrAtrp- 0, 300, 600, 1200, 2400 µg/plate Piperonal [05.016]), Cas# 120-57-0 Plate incorporation method -S9; preincubation method +S9	Negative	Reliable study compliant with OECD TG 471.	(Sekizawa and Shibamoto, 1982)
Gene Mutation (Ames test)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537; TA1538 10,000 µg/plate Piperonal Cas# 120-57-0 +/-S9 Plate incorporation method	Negative	Low reliability: -Non-GLP study -No data on cytotoxicity nor on the concentrations tested. -poor presentation of data	(Heck <i>et al.</i> , 1989)
Gene Mutation (Ames test)	<i>S. typhimurium</i> TA98; TA100 0.05 to 5000 µg/plate Piperonal Cas# 120-57-0 +/-S9	Negative	Low reliability: -Non-GLP study -only 2 strains - poor presentation of and data	(Kasamaki <i>et al.</i> , 1982)
Gene Mutation (Ames test)	<i>S. typhimurium</i> TA98; TA100; TA1537 0.02, 0.2, 2.0, 20.0, 200, 1000 and 5000 µg/plate Piperonal Cas# 120-57-0 +/-S9	Negative	Low reliability: -Non-GLP study -only 2 strains - no presentation of the data	(White <i>et al.</i> , 1977)
Gene Mutation (Ames test)	<i>S. typhimurium</i> TA98; TA100; TA1535; TA1537 0, 10, 33, 100, 333, 1000 µg/plate	Negative Also Piperonyl butoxide and Piperonyl sulfoxide were negative	Reliable study -Report with NTP results on 250 compounds tested in various laboratories	(Haworth <i>et al.</i> , 1983)

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	Piperonal Cas# 120-57-0 +/-S9 preincubation method		-aligned with OECD TG471	
Gene Mutation (Rec assay)	<i>B. subtilis</i> M45 (rec-), H17 (rec+) 20 µg/disc Piperonal Cas# 120-57-0	Negative	Low reliability -Study in Japanese -No TG on this test	(Oda <i>et al.</i> , 1979) <i>cit.</i> EFSA 2012
Gene Mutation (Rec assay)	<i>B. subtilis</i> M45 (rec-), H17 (rec+) 5000 µg/disc Piperonal Cas# 120-57-0	Positive (-S9) No data (+S9)	Limited Relevance and Reliability: -tests in the presence of S9 were not successful -No TG on this test	(Sekizawa and Shibamoto, 1982)
Gene Mutation (Mammalian cell gene mutation)	Mouse lymphoma L5178Y cells 1000 µg/ml Piperonal Cas# 120-57-0 +/-S9	Negative	Low reliability: -non-GLP study. - poor presentation of data	(Heck <i>et al.</i> , 1989)
Gene Mutation (Mammalian cell gene mutation)	Mouse lymphoma L5178Y cells + S9-mix: from 62.5 to 1000 µg/mL, and - S9-mix: from 200 to 1000 µg/mL in first trail and from 600 to 1000 µg/mL in second trail Piperonal Cas# 120-57-0, purity not specified	Negative (data not accessible)	Limited reliability according ECHA: -well documented - meets generally accepted scientific principles -acceptable for assessment	Study 1982 cited in (Anonymous (REACH), 2021)
Chromosomal aberration	Chinese hamster cell line B241 50 nM (0.0075 µg/mL) Piperonal CAS# 120-57-0 +/-S9	Positive	Low Reliability: - non-GLP study - low number of cells scored (137) - Data shown only for one concentration -Dose-dependent increase of total aberrations reported - the result for Heliotropin after excluding gaps is one of the lowest among the test substances. Historical negative control would be needed to be able to	(Kasamaki <i>et al.</i> , 1982)

Opinion on the safety of Heliotropin (CAS No. 120-57-0, EC No. 204-409-7)

			assess the result, and this has not been provided.	
Chromosomal aberration	Chinese hamster cell line B241 10, 50, 100nM (0.0015, 0.0075, 0.015 µg/mL) Piperonal CAS# 120-57-0 24h exposure Frequencies of structural and numerical chromosome aberrations were calculated from analysis of about 200 and more than 500 metaphase spreads, respectively.	Positive Weak but significant induction of structural aberrations and no induction of numerical aberration	Low reliability: - no S9 used - unclear number of cells scored - poor presentation of data	(Kasamaki and Urasawa, 1985)
Chromosomal aberration	Chinese hamster Ovary (CHO) cells + S9-mix: 625, 1250, 2500 and 5000 µg/mL - S9-mix: 63, 125, 250 and 500 µg/mL Exposure duration: 14 hours in the non-activated study; 2 hours in the activated study - Fixation time (start of exposure up to fixation or harvest of cells): 16-hour harvest in the non-activated study; 12-hour harvest in the activated study Heliotropin, purity not specified	Negative	Limited reliability according ECHA: - GLP study - Study without detailed documentation; The following conditions of OECD TG473 not met: - 2 hr exposure +S9 instead of recommended 3-6 -/+S9, - Not clear how the fixation time relates to real cell cycle duration of the CHO cells used. For example, 12-hour harvest may indicate that the cells had doubling time =8h. However this was not confirmed. - according to ECHA website: - at least 300 metaphases per concentration were not examined, "a minimum of 100 metaphase spreads (50 per duplicate flask)	Reference Study 1988 cited in (Anonymous (REACH), 2021) https://echa.europa.eu/registration-dossier/-/registered-dossier/5265/7/7/2/?documentUUI=D=ebbb91a2-602f-4548-9e5e-0ef9d9489495

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			were examined” instead of 300 recommended.	
DNA repair (Unscheduled DNA synthesis) <i>in vitro</i>	Isolated rat hepatocytes 10 to 502 µg/ml Piperonal [05.016]), Cas# 120-57-0	Positive	Limited reliability: - non-GLP study - poor reporting of data	(Heck <i>et al.</i> , 1989)

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