SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

3,5,5-trimethylcyclohex-2-enone

EC No 201-126-0

CAS RN 78-59-1

Evaluating Member State(s): France

Dated: 12 April 2022

Evaluating Member State Competent Authority

French Agency for Food, Environmental and Occupational Health Safety (ANSES) on behalf French Ministry of Environment
Email: reach@anses.fr

Year of evaluation in CoRAP: 2013

Before concluding the substance evaluation a Decision to request further information was issued on 17 June 2015.

Furthermore, an Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.i test method: OECD TG 443) has been required by ECHA in a Decision on a compliance check dated 21 December 2018.

Member State concluded the evaluation without any further need to ask more information from the registrants under Article 46(1) decision.

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

The Substance, 3,5,5-trimethylcyclohex-2-enone (EC number 201-126-0; further designated as isophorone in the document) was originally selected for substance evaluation in order to clarify concerns about:

- Human health/CMR (initially focusing on carcinogenicity and mutagenicity);
- Exposure/Workers exposure;
- High aggregated tonnage;
- Wide dispersive use.

During the evaluation also additional concerns were identified:

- Risk for the environment;
- Endocrine disruption;
- Reproductive toxicity.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

A compliance check was initiated on 05 October 2017. Based on Article 41 of REACH, ECHA requested the registrants to submit by 4 January 2021 information on:

Extended one-generation reproductive toxicity study (Annex IX, Section 8.7.3.; test method OECD TG 443) in rats, oral route with the Substance specified as follows:

- At least two weeks premating exposure duration for the parental (P0) generation;
- Dose level setting shall aim to induce systemic toxicity at the highest dose level;
- Cohort 1A (Reproductive toxicity);
- Cohort 1B (Reproductive toxicity) with extension to mate the Cohort 1B animals to produce the F2 generation; and
- Cohorts 2A and 2B (Developmental neurotoxicity).

The final study report was not available when this Conclusion document was finalised in April 2022.

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in the table below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION	
Conclusions	
Need for follow-up regulatory action at EU level	X
Harmonised Classification and Labelling	
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	

No conclusion can be reached regarding reproductive toxicity and endocrine disruption, as the results of the reproductive toxicity study (EOGRTS) will provide relevant information for these properties (final study report not available).

The evaluating MSCA (eMSCA) intends to prepare a regulatory management option analysis (RMOA) after report finalisation of the requested EOGRTS study under CCH. It will further evaluate reproductive toxicity and endocrine disruption potential and the potential appropriate follow-up regulatory actions. Possible risks related to the presence of isophorone in drinking water will also be further considered.

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

4.1.1. Harmonised Classification and Labelling

Based on the current evidence, a classification as Acute toxicity 4 (without *) – H 302 and 312 (oral and dermal) as well as STOT SE 3 – H336 (may cause drowsiness or dizziness) is indicated as an amendment and addition to the current harmonised classification.

Revision of isophorone harmonised classification regarding reproductive toxicity may need further consideration after reception of the final report of the EOGRTS study.

The Acute toxicity and STOT SE proposals for classification will be addressed if a revision of the harmonised classification is justified for reproductive toxicity.

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

Not applicable

4.1.3. Restriction

Not applicable

4.1.4. Other EU-wide regulatory risk management measures

Not applicable

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

Not applicable

5.2. Other actions

Not applicable

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Indication of a tentative plan is not a formal commitment by the evaluating Member State. A commitment to prepare a REACH Annex XV dossier (SVHC, restrictions) and/or CLP Annex VI dossier should be made via the Registry of Intentions.

Table 2

FOLLOW-UP		
Follow-up action	**Date for intention**	**Actor**
Preparation of RMOA	2023	France

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

The Substance, 3,5,5-trimethylcyclohex-2-enone (EC number 201-126-0; further designated as isophorone in the document) was originally selected for substance evaluation in order to clarify concerns about:

- Human health/CMR (initially focusing on carcinogenicity and mutagenicity);
- Exposure/Workers exposure;
- High aggregated tonnage;
- Wide dispersive use.

During the evaluation also additional concerns were identified:

- Risk for the environment;
- Endocrine disruption;
- Reproductive toxicity.

Table 3

EVALUATED ENDPOINTS **Endpoint evaluated** **Initial and additional concerns**	**Outcome/conclusion**
Mutagenicity	**Concern refuted**. Contradictory findings *in vitro*; negative results *in vivo*. No further action.
Carcinogenicity	**Concern confirmed**. Isophorone has an harmonised classification as Carc. 2 – H351, and is classified as possibly carcinogenic to humans (group 2B) by the IARC (2021). There is no new data identified that suggest an update of current harmonised classification. No further action.
Toxicity for reproduction and development	**Concern unresolved**. Fertility: EOGRTS required under CCH process. Final report not yet available. Development: Prenatal developmental study, as required during SEV was submitted by the registrants. No firm conclusion can be reached on both since the required EOGRTS (under CCH process) is not available for now. Further action may be needed and be reviewed in a RMOA.
Endocrine disruption properties - Human health	**Concern unresolved**. No endocrine disruptor potential was raised in several *in vitro* and *in vivo* assays. However, in the male pubertal assay, there was an indication of anti-androgenic effect at the high dose of 800 mg/kg bw/day. Moreover, an absence of endocrine disruption of the thyroid cannot be excluded from this study. Assessment of the endocrine properties of isophorone should be reviewed at the light of the EOGRTS (final report awaited). This will be analysed in a RMOA.

Endocrine disruption properties - Environment	**Concern refuted**. No endocrine disruption potential was raised from two assays: a fish short-term reproduction assay (OECD TG 229) and an amphibian metamorphosis assay (OECD TG 231).
Human heath exposure and risk assessment	**Concern unresolved.** No unacceptable risk is anticipated based on the data updated (2020) in the registration dossier. The evaluating MSCA notes that DNELs derivation may be impacted by the results of the EOGRTS (final report awaited).
Risk for/via the environment	**Concern unresolved.** A potential risk of groundwater contamination is emphasized by the environmental risk assessment. It indicates a potential to contaminate drinking water and a possible risk on human health via drinking water. Risk assessment for human health via drinking water can also be impacted by the results of the EOGRTS and will be further characterised in a RMOA .
Additional endpoints	
Acute Toxicity	Isophorone has an harmonised classification as Acute Tox 4 - H302/312. Data indicate that a classification as STOT SE 3 – H336 is required.
Irritation	Isophorone is not irritant to skin. Isophorone has an harmonised classification as Eye Irrit. 2 – H319 and STOT SE 3 – H335. No further action.
Sensitisation	Isophorone is not a skin sensitizer. No data is available for respiratory sensitisation. No further action.
Repeated dose toxicity	By oral route: Subacute to subchronic studies: No specific target organ identified. The lowest NOAEL available is 102.5 mg/kg bw/day based on a decreased body weight gain in male rats. Chronic/carcinogenicity studies: main target organs for non-neoplastic effect in treated animals: kidney, liver (tested doses: 250 and 500 mg/kg bw/day). By dermal route: No reliable study. By inhalation: No fully reliable study. Isophorone induced respiratory and eye irritation. No further action.

7.2. Procedure

The Substance was included in the Community rolling action plan (CoRAP) for evaluation in 2013. On 30 March 2013, the CoRAP was published on ECHA website and the competent authority of France was appointed to carry out the evaluation.

All the endpoints and uses were evaluated by the eMSCA. The sources of information for the evaluation were the registration data and literature searches performed in Scopus and Pubmed databases (last in August 2021).

Based on the evaluation of the available data, the eMSCA concluded that there was a need to request further information to clarify the concerns regarding Human health, Workers exposure, General population exposure assessment, and Environment. Therefore, the

eMSCA prepared a draft decision to request further information. The draft decision was submitted to ECHA on 19 March 2014. Member State Committee adopted the final decision. The final decision was sent to the registrants on 17 June 2015. The Registrants were required to submit the study by 24 June 2016.

On 5 June 2020, the lead registrant updated its registration dossier to comply with the requests in the final decision.

During substance evaluation, a data gap was identified according to Annex X, Section 8.7.3 of REACH. A decision on compliance check under REACH was sent to the registrant on 21 December 2018 to request an EOGRTS, the results of which were expected to be submitted by 4 January 2021. However, the final study report was not available when this Conclusion document was finalised in March 2022.

The substance evaluation conclusion report was prepared based on the updated registration dossier in June 2020.

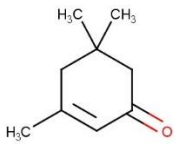
7.3. Identity of the substance

Table 4

SUBSTANCE IDENTITY	
Public name:	3,5,5-trimethylcyclohex-2-en-1-one
EC number:	201-126-0
CAS number:	78-59-1
Index number in Annex VI of the CLP Regulation:	606-012-00-8
Molecular formula:	C9H14O
Molecular weight range:	138.2069
Synonyms:	Isophorone; Isoacetophorone 1,1,3-Trimethyl-3-cyclohexene-5-one 1,5,5-Trimethyl-3-oxocyclohexene

Type of substance X Mono-constituent

Structural formula:



7.4. Physico-chemical properties

Table 5

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Melting point at 101.3 kPa	- 8.1°C
Boiling point at 101.3 kPa	215.2°C
Vapour pressure	0.4 hPa at 20°C
Water solubility	12 $g.L^{-1}$ at 20°C
Partition coefficient n-octanol/water (Log Kow)	1.66 at 23°C (OECD 107)

7.5. Manufacture and uses

7.5.1. Quantities

Table 6

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☐ 10 – 100 t	☒ 100 – 1000 t	☐ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

7.5.2. Overview of uses

The following uses were reported in ECHA dissemination site[2].

Table 7

USES	
	Use(s)
Manufacture	Manufacture of the substance
Formulation	PC 9a: Coatings and paints, thinners, paint removes PC 27: Plant protection products PC 35: Washing and cleaning products PC 18: Ink and toners
Uses at industrial sites	**Uses as intermediate** SU 8: Manufacture of bulk, large scale chemicals (including petroleum products) SU 9: Manufacture of fine chemicals Technical function of the substance: intermediate (precursor) **Use in ink products** PC 18: Ink and toners **Use in coatings** PC9a: coatings and paints, thinners, paint removes **Use in cleaning agents** PC35: washing and cleaning products

[2] https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/14527/3/1/4

Uses by professional workers	**Agrochemical use – transfer processes** PC27: Plant protection products SU1: agriculture, forestry and fishing **Use as a coformulant in plant protection products, spray applications by professionals** PC27: Plant protection products SU1: agriculture, forestry and fishing
Consumer Uses	/
Article service life	/
Uses advised against	**Professional workers:** Coating and cleaning use in public domain **Consumer uses:** Do-it-yourself application Cosmetic use

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

Table 8

HARMONISED CLASSIFICATION ACCORDING TO ANNEX VI OF CLP REGULATION (REGULATION (EC) 1272/2008)							
Index No	**International Chemical Identification**	**EC No**	**CAS No**	**Classification**		**Spec. Conc. Limits, M-factors**	**Notes**
				Hazard Class and Category Code(s)	**Hazard statement code(s)**		
606-012-00-8	3,5,5-trimethylcyclohex-2-enone isophorone	201-126-0	78-59-1	Acute Tox 4* Acute Tox 4* Eye Irrit. 2 STOT SE 3 Carc. 2	H302 H312 H319 H335 H351	STOT SE 3; H335: C ≥ 10 %	

7.6.2. Self-classification

- In the registration(s)*:* same classification as harmonized classification
- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory*:*

  Acute Tox. 3 – H331
  STOT SE 3 – H336

7.7. Environmental fate properties

7.7.1. Degradation

Isophorone is readily biodegradable. No other study on biodegradation is required according to REACH Annex VIII, 9.2.2.1, column 2.

The biodegradability of the Substance was shown in ready biodegradability and inherent biodegradability tests. In both tests, the substance was degraded by more than 95%.

Moreover, there are no appropriate functional groups and no experimental observations. Therefore isophorone can be assumed to be hydrolytically stable in aqueous media under environmental conditions.

Finally, the half-life for the O3 sensitized photo degradation of isophorone was calculated to be 23 minutes and the half-life for the OH sensitized photo degradation of isophorone was determined to be of 16 hours.

7.7.2. Environmental distribution

Using QSAR models of US EPA, the soil sorption coefficient (Koc) of isophorone was calculated to be 58.32. Based on its log Kow <3 and the QSAR result, isophorone is considered to have a low potential for adsorption. Therefore, a test is not required according to REACH Annex VIII, 9.3.1, column 2.

7.7.3. Bioaccumulation

According to column 2 of annex IX REACH regulation 1907/2006 and considering the low potential for bioaccumulation for isophorone with a log kow of 1.66, an experimental test was not necessary.

Nevertheless, the bioaccumulation potential of isophorone was evaluated by the registrant in a bioaccumulation study with bluegill sunfish (Lepomis macrochirus). The BCF after 14 days of exposure was calculated to be 7 indicating that isophorone has a very low bioaccumulation potential. The half-life which was derived from a 7-day depuration phase was calculated to be <1 day. The eMSCA can support this conclusion.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

7.8.1.1. **Fish**

Table 9 - Short-term toxicity to fish

Species	Guideline	Endpoints	Toxicity [mg/l]	Reliability Index	Reference
Pimephales promelas	Other	LC_{50} – 96h Mortality; Flow-through conditions - Freshwater	228[1]	RI 1	Geiger DL et al. (1990)
Cyprinodon variegatus	Acute Toxicity Test based on a procedure of the Committee on Methods for Toxicity Tests with Aquatic Organisms	LC_{50} – 96h Mortality; Flow-through conditions - Saltwater	140	RI 2	Ward GS et al. (1981)

[1] based on measured concentrations.

Table 10 - Long-term toxicity to fish

Species	Guideline	Endpoints	Toxicity [mg/l]	Reliability Index	Reference
Pimephales promelas	Early Life Stage Test	NOEC – 35d Weight Mortality LOEAC – 35d Weight Flow-through conditions - Freshwater	11[1] 56[1] 19[1]	RI 2	Cairns MA et al. (1982)
Pimephales promelas	Flow-Through Early Life Stage Test, US EPA proposal	NOEC – 32d Weight LOEAC – 32d Weight Flow-through conditions - Freshwater	4.2-15.6[1,2] 8.8-22.7[1]	RI 2	Lemke AE et al. (1983)

[1] Based on measured concentrations.
[2] Two Flow-through tests using the same species. Deviation of the results explained by differences in the feeding regime.

Discussion

Based on fish weight as the most sensitive endpoint the NOEC is determined as 11 mg/l. Similar results were obtained in 2 flow-through tests using the same species (Lemke et al., 1983). After 32 days of exposure, NOECs of 4.2 (2nd test) and 15.6 mg/l (1st test) were obtained for the endpoint fish weight. Deviation of the results are explained by differences in the feeding regime.

NOECs from three early life stage tests are in the same order of magnitude. Therefore, the geometric mean of these 3 NOECs is calculated resulting in a value of 8.9 mg/l. The studies were conducted according to accepted scientific principles and were assessed as "reliable with restrictions".

7.8.1.2. **Aquatic invertebrates**

7.8.1.2.1. **Short-term toxicity to aquatic invertebrates**

Table 11. Short-term effects on aquatic invertebrates

Method	Results	Remarks	Reference
Daphnia magna freshwater static Methods for acute toxicity tests with fish, macro invertebrates, and amphibians, US EPA (1975)	LC_{50} (48 h): 120 mg/l test mat. (nominal) based on: mortality (CL 72-170) LC_{50} (24 h): 430 mg/l test mat. (nominal) based on: mortality (CL 360-500)	2 (reliable with restrictions) key study experimental result **Test material (EC name):** 3,5,5-trimethylcyclohex-2-enone	LeBlanc GA (1980)
Daphnia magna freshwater static DIN 38412, part 11	EC_{50} (24 h): 254 mg/l test mat. (nominal) based on: mobility (CL 221-293)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name):** 3,5,5-trimethylcyclohex-2-enone	Unpublished study report 1996a

7.8.1.2.2. **Long-term toxicity to aquatic invertebrates**

Data waiving

Reason: other justification

Justification: The results of the chemical safety assessment according to Annex I indicate no need to investigate further the effects on aquatic organisms since all RCRs for the aquatic environment are below 1. Therefore according to column 2 of Annex IX of REACH regulation 1907/2006 the testing of long-term toxicity to aquatic invertebrates is not necessary.

Discussion

The lowest available 48h-LC_{50} was found to be 120 mg/l (LeBlanc, 1980) and will be considered as the relevant endpoint for the short-term toxicity of isophorone to aquatic invertebrates. Nevertheless, no analytical monitoring has been carried out, not permitting to conclude that the concentration was maintained throughout the test. Moreover and owing to lack of information on test conditions recorded in IUCLID dossier, all validity conditions of the test have not been checked. Consequently the study is assessed as reliable with restrictions.

At last and based on the results of the chemical safety assessment, the eMSCA can support the justification of data waiving for the long-term toxicity to aquatic invertebrates.

7.8.1.3. **Algae and aquatic plants**

Table 12. Effects on algae and aquatic plants

Method	Results	Remarks	Reference
Scenedesmus subspicatus (new name: Desmodesmus subspicatus) (algae) freshwater static Method: other: Growth Inhibition Test according to a proposal of the Umweltbundesamt, Germany	EC_{50} (72 h): 475 mg/l test mat. (nominal) based on: biomass EC_{10} (72 h): 64 mg/l test mat. (nominal) based on: biomass	2 (reliable with restrictions) key study experimental result **Test material (EC name):** 3,5,5-trimethylcyclohex-2-enone	Unpublished study report 1996b
other algae: Champia parvula (marine red macroalgae) (algae) saltwater semi-static Method: other: see Test Conditions	NOEC (14 d): 49.8 mg/l (nominal) based on: vegetative growth and sexual reproduction of females NOEC (14 d): 29.9 mg/l (nominal) based on: vegetative growth and reproduction of tetrasporophytes LOEC (14 d): 83.07 mg/l (nominal) based on: vegetative growth and sexual reproduction of females LOEC (14 d): 49.8 mg/l (nominal) based on: vegetative growth and sexual reproduction of tetrasporophytes	2 (reliable with restrictions) key study experimental result **Test material (EC name):** 3,5,5-trimethylcyclohex-2-enone	Thursby GB et al. (1985)

Discussion

Based on nominal concentrations, the 72h-EC_{10} value (based on biomass development) was determined as 64 mg/l and the EC_{50} as 475 mg/l (Huels AG, 1996). Nonetheless, it should be noted that some details on test conditions have not been recorded in IUCLID dossier. Therefore, all validity conditions of the test have not been checked. These missing points justified that this data is reliable with restrictions.

In the second study (Thursby et al., 1985), the most sensitive endpoints after a 14-day exposure period were found to be growth of tetrasporophytes and number of tetrasporangia exhibiting a LOEC of 49.8 mg/l. The next lowest test concentration was 60 % of this value, thus the NOEC is calculated to be 29.9 mg/l.

7.8.1.4. **Sediment organisms**

Data waiving

Justification: The results of the chemical safety assessment according to Annex I indicate no need to investigate the effects on sediment organisms since all RCRs for this compartment are below 1. Evaluation was done with a PNEC derived by EPM method as a first screening approach. Furthermore physico-chemical properties (log Koc 1.76) and ready biodegradability indicate a low potential for adsorption to sediment. Therefore according to column 2 of Annex X of REACH regulation 1907/2006 the testing of long-term toxicity to sediment organisms is not necessary.

Discussion

The results of the chemical safety assessment according to Annex I indicate no need to investigate the effects on sediment organisms since all RCRs for this compartment are below 1. Evaluation was done with a PNEC derived by EPM method as a first screening approach. Furthermore, physico-chemical properties (log Koc 1.76) and ready biodegradability indicate a low potential for adsorption to sediment. Therefore, according to column 2 of Annex X of REACH regulation 1907/2006 the testing of long-term toxicity to sediment organisms is not necessary.

7.8.1.5. **Other aquatic organisms**

No data available.

7.8.2. Terrestrial compartment

The results of the chemical safety assessment according to Annex I indicate no need to investigate the effects on soil organisms since all RCRs for this compartment are below 1. Evaluation was done with a PNEC derived by EPM method as a first screening approach. Furthermore, physico-chemical properties (log Koc 1.76) and ready biodegradability indicate a low potential for adsorption to soil. Therefore, and according to column 2 of Annex IX and X of REACH regulation 1907/2006 the testing of short and long-term toxicity to terrestrial organisms is not necessary.

7.8.2.1. **Toxicity to soil macro-organisms**

Data waiving

- **Toxicity to soil macro-organisms except arthropods**

Justification: The results of the chemical safety assessment according to Annex I indicate no need to investigate the effects on soil organisms since all RCRs for this compartment are below 1. Evaluation was done with a PNEC derived by EPM method as a first screening approach. Furthermore, physico-chemical properties (log Koc 1.76) and ready biodegradability indicate a low potential for adsorption to soil. Therefore, according

to column 2 of Annex IX and X of REACH regulation 1907/2006 the testing of short and long-term toxicity to terrestrial organisms is not necessary.

- **Toxicity to terrestrial arthropods**

Justification: The results of the chemical safety assessment according to Annex I indicate no need to investigate the effects on soil organisms since all RCRs for this compartment are below 1. Evaluation was done with a PNEC derived by EPM method as a first screening approach. Furthermore, physico-chemical properties (log Koc 1.76) and ready biodegradability indicate a low potential for adsorption to soil. Therefore, according to column 2 of Annex IX and X of REACH regulation 1907/2006 the testing of short and long-term toxicity to terrestrial organisms is not necessary.

7.8.2.2. Toxicity to terrestrial plants

Data waiving

Justification: The results of the chemical safety assessment according to Annex I indicate no need to investigate the effects on soil organisms since all RCRs for this compartment are below 1. Evaluation was done with a PNEC derived by EPM method as a first screening approach. Furthermore, physico-chemical properties (log Koc 1.76) and ready biodegradability indicate a low potential for adsorption to soil. Therefore, according to column 2 of Annex IX and X of REACH regulation 1907/2006 the testing of short and long-term toxicity to terrestrial organisms is not necessary.

7.8.2.3. Toxicity to soil micro-organisms

Data waiving

Justification: The results of the chemical safety assessment according to Annex I indicate no need to investigate the effects on soil organisms since all RCRs for this compartment are below 1. Evaluation was done with a PNEC derived by EPM method as a first screening approach. Furthermore, physico-chemical properties (log Koc 1.76) and ready biodegradability indicate a low potential for adsorption to soil. Therefore, according to column 2 of Annex IX and X of REACH regulation 1907/2006 the testing of short and long-term toxicity to terrestrial organisms is not necessary.

7.8.2.4. Toxicity to other terrestrial organisms

No relevant information available

7.8.3. Microbiological activity in sewage treatment systems

Table 13. Effects on aquatic micro-organisms

Method	Results	Remarks	Reference
Activated sludge of a predominantly domestic sewage freshwater static equivalent or similar to OECD Guideline 209 (Activated Sludge, Respiration Inhibition Test)	EC_{50} (3 h): 100 mg/l test mat. (nominal) based on respiration rate	2 (reliable with restrictions) key study experimental result **Test material (EC name): 3,5,5-trimethylcyclohex-2-enone**	Yoshioka Y et al. 1986
Pseudomonas putida freshwater static	EC_{10} (18 h): 340 - 530 mg/l test mat. (nominal) based on: growth inhibition	2 (reliable with restrictions) supporting study experimental result	Unpublished study report 1996c

Method	Results	Remarks	Reference
Bringmann and Kuehn, Z. Wasser Abwasser Forsch. 10, 87-98 (1977)		**Test material (EC name): 3,5,5-trimethylcyclohex-2-enone**	

Discussion

EC_{50} activated sludge: 100 mg/l.

EC_{10} *Pseudomonas putida*: 435 mg/l (mean value)

Both studies were assessed as "reliable with restrictions".

The following information is taken into account for effects on aquatic micro-organisms for the derivation of PNEC:
A respiration inhibition test according to the draft OECD guideline 209 was conducted (Yoshioka et al., 1986) with activated sludge from a domestic Sewage Treatment Plant fed with synthetic sewage feed. The respiration rate was measured after a contact time of 3 hours at 5 different concentrations. Another test was conducted with *Pseudomonas putida* based on a contact time of 18h. Whereas for the first test an EC50 values was derived an EC10 value was given for the second study (Unpublished study report, 1996c).

Value to be used:

EC_{50}/LC_{50} for aquatic micro-organisms: 100 mg/l

EC_{10}/LC_{10} or NOEC for aquatic micro-organisms: 435 mg/l

7.8.4. Non compartment specific effects relevant for the food chain (secondary poisoning)

7.8.4.1. Toxicity to birds

Data waiving

Justification: The risk of secondary poisoning for birds through fish and earthworm food chains is rather unlikely for isophorone. The bioaccumulation potential was proven to be low (BCF: 7). Furthermore the log Kow is well below 3 (log Kow: 1.67) and the substance is readily biodegradable (>60% in 10 day-window). The exposure to birds will therefore be low as well as the risk of secondary poisoning. Therefore a long-term study to birds would not be necessary.

7.8.4.2. Toxicity to mammals

No relevant information available

7.8.5. PNEC derivation and other hazard conclusions

Table 14. PNEC derivation and other hazard conclusions

PNEC DERIVATION AND OTHER HAZARD CONCLUSIONS		
Hazard assessment conclusion for the environment compartment	**Hazard conclusion**	**Remarks/Justification**
Freshwater	PNEC aqua (freshwater): 0.089 mg/l	Assessment factor: 100 Extrapolation method: assessment factor There are data available from acute aquatic toxicity tests at three different trophic levels

		(fish, daphnia and algae). Furthermore there are also long-term values for algae and fish available. Due to the fact that algae and fish are not the most sensitive species, the assessment factor of 100 is applied to the lowest of the two long-term tests covering two trophic levels.
Marine water	PNEC aqua (marine water): 0.0089 mg/l	Assessment factor: 1000 Extrapolation method: assessment factor Results from long-term tests are available for fish and algae. Both are not the acutely most sensitive species. For fish (*Pimephales promelas*) NOECs from three early life stage tests are available that are in the same order of magnitude. Therefore, the geometric mean of these 3 NOECs is calculated to be 8.9 mg/l.
Intermittent releases to water	PNEC aqua (intermittent releases): 1.2 mg/l	Assessment factor: 100 Extrapolation method: assessment factor There is no information available about the intermittent release of the substance. Nevertheless a PNEC for intermittent release was calculated from the acute aquatic data. The most sensitive organisms for short term aquatic toxicity are daphnids (EC_{50} (48h): 120 mg/l). Data for three species are available thus an assessment factor of 100 was applied.
Sediments (freshwater)	PNEC sediment (freshwater): 0.839 mg/kg $_{sediment}$ dw	Extrapolation method: partition coefficient There are no data for the toxicity of the substance to sediment dwelling organism available. A PNEC was derived with the equilibrium partitioning method according to the following formula: PNEC sed = $K_{susp-water}$/RHO_{susp} * PNEC water *1000 = (2.36/1150)*0.089*1000 Values used for $K_{susp-water}$ calculation: Koc: 58.3 L/kg RHO_{susp}: 1150 kg/m^3 Conversion factor (ww-dw): 4.6. According to the physico-chemical properties currently known and a calculated log Koc of 1.7, there are no indications that the substance accumulates in sediment. Therefore a quantitative risk assessment seems not to be necessary for this compartment.
Sediments (marine water)	PNEC sediment (marine water): 0.0839 mg/kg $_{sediment}$ dw	Extrapolation method: partition coefficient There are no data for the toxicity of the substance to sediment dwelling organism available. A PNEC was derived with the equilibrium partitioning method according to the following formula: PNEC sed = K susp-water/RHO susp * PNEC marine water *1000 = (2.36/1150)*0.0089*1000 Values used for K susp-water calculation: Koc: 58.3 L/kg RHO susp: 1150 kg/m3 Conversion factor (ww-dw). 4.6 . According to the physico-chemical properties currently known and a calculated log Koc of 1.7, there are no indications that the substance accumulates in sediment. Therefore a quantitative risk assessment seems not to be necessary for this compartment.
Sewage treatment plant	PNEC STP: 1 mg/l	Assessment factor: 100 Extrapolation method: assessment factor

		The EC50 was determined after 3 hours in a respiration inhibition test according to the draft OECD guideline 209 with activated sludge from a domestic Sewage Treatment Plant fed with synthetic sewage feed.
Soil	PNEC soil: 0.12 mg/kg $_{soil}$ dw	Extrapolation method: partition coefficient There are no valid data for the toxicity of the substance to soil organism available. The PNEC was derived with the equilibrium partitioning method according to the following formula: PNEC soil = K $_{soil-water}$/RHO soil* PNEC water *1000 = (1.95/1700)*0.089*1000 Values used for K soil-water calculation: Henry's law constant: 0.37 Pa.m^3.mol^{-1}; Koc: 58.3 L/kg RHO $_{soil}$: 1700 kg/m^3 Conversion factor (ww-dw). 1.13 According to the physico-chemical properties currently known and a calculated log Koc of 1.7, there are no indications that the substance accumulates in soil. Therefore a quantitative risk assessment seems not to be necessary for this compartment.
Air	-	-
Secondary poisoning	PNEC oral: 0.02 g/kg food	Assessment factor: 90 There are no data available for the assessment of secondary poisoning. Based on the NOAEL for 90 days repeated toxicity test in rats (102.5 mg/kg $_{bw}$/d) a PNEC oral can be derived with a conversion factor of 20 (rat, >6 weeks) and an assessment factor of 90. As the substance has only a low bioaccumulation potential, biomagnification via the food chain is not expected.

7.8.6. Conclusions for classification and labelling

Due to the low acute aquatic toxicity values and the ready biodegradability, the substance does not fulfil the criteria for classification as dangerous according to CLP Regulation 1272/2008.

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

The main following data are issued from rather old and/or limited studies.

7.9.1.1. **Absorption**

Isophorone is well absorbed via oral and inhalative route (Dutertre-Catella, 1976; ATSDR, 1989 & 2008). A dermal absorption of 6 % was estimated from an in vitro dermal absorption study after exposure of human skin disks to isophorone (200 µL applied an exposure area of approximately 63.6 mm2) for 24 hours (Unpublished study report, 2006) with application of EFSA guidance (2012) for setting the dermal absorption value. Representativity of testing a large quantity of pure substance for real conditions of dermal exposure can be questioned.

7.9.1.2. **Distribution**

Isophorone is well and rapidly distributed in the body. Maximum amount in blood was found 30 minutes after an oral administration of 1000 mg/kg bw of isophorone in rabbits. Within 5 hours after a single oral administration of 4000 mg/kg bw of isophorone the highest concentrations were found in the stomach, pancreas, adrenals, spleen and liver of rats (Dutertre-Catella, 1976). Preliminary results of a pharmacokinetics study in rats indicated that 24 hours after an oral administration of 14C-isophorone, highest levels were found in liver (3.7%), kidney (1.1%), preputial gland (0.7%), testes, brain and lungs (Strasser, 1988). The concentration of isophorone in the preputial gland may be due to the high concentration of alpha-2µ-globuline to which it could bind (ECETOC, 1989). Twenty four hours after a single oral administration of 500 mg radioactive labeled isophorone/kg to mice and rats, the highest concentrations were found in kidney, liver, lung, spleen and adrenals (Thier, R., 1991). Fourty eight hours after oral administration of 1000 mg/kg bw of isophorone to male and female rats, only traces of isophorone could be determined in the stomach and no isophorone was measured in the other organs (Dutertre-Catella, 1976). After a repeated oral administration (8 days) of 500 mg/kg bw/day of isophorone in rats, the highest concentrations were found in kidney, liver, lung, spleen and adrenals 24 hours after the last administration (Thier, R., 1991).

After inhalation to saturated vapours of isophorone (2000 mg/m3) for 4 hours, isophorone is mainly distributed in all organs examined (kidney, adrenals, liver, pancreas, brain, lungs, heart, stomach, spleen, testicles and ovaries) of rats when sacrificed immediately after exposure or 1.5 or 3 hours after (Dutertre-Catella, 1976).

7.9.1.3. **Metabolism**

After a single oral application of 1000 mg/kg bw of isophorone, the following metabolites were identified in the urine of rabbits and rats (Dutertre-Catella et al., 1978; Truhaut et al., 1970):

- 3,5,5-trimethylcyclohexan-1-one (dihydroisophorone – from hydrogenation);
- 3,5,5-trimethyl-2-cyclohexen-1-ol (isophorol – from reduction into alcohol and eliminated as glucuronide);
- 3,5,5-trimethylcyclohexan-1-ol, cis and trans isomers (from hydrogenation and then reduction).
- 5,5-dimethyl-1-cyclohexene-3-one-1-carboxylic acid was also found in the urine of rabbits. This metabolite is formed by oxidation of the 3 -methyl group of isophorone and then glucuronidated.

After a 8-day oral exposure of 500 mg/kg bw/day of isophorone, the following metabolites were identified in the urine of rats (Thier R., 1991):

- 6-oxoisophorone (3,5,5-Trimethyl-2-cyclohexene-1,6-dione),
- dihydroisophorone,
- 4-oxoisophorone (3,5,5-Trimethyl-2-cyclohexene-1,4-dione),
- 4-hydroxyisophorone (4-hydroxy-3,5,5-trimethyl-2-cyclohexene-1,4-dione),
- 6 hydroxyisophorone (6-hydroxy-3,5,5-trimethyl-2-cyclohexen-1-one),
- Alcohols were not found.

Finally, it is suggested that the glutathion system is also involved in the metabolism of isophorone since depletion of glutathion was observed in liver, testes and epididymis after intraperitoneal injection of 500 mg/kg bw of isophorone (Gandy, 1990).

7.9.1.4. **Excretion**

After single as well as repeated-dose oral administration of isophorone to rats, 80 % of radioactivity was excreted within 96 hours, with 50 - 65 % were detected in the urine (Thier, R., 1991). No data on amount of isophorone found in the faeces was presented and biliary excretion was not assessed. Unreacted isophorone could be isolated in the urine and

exhaled air of orally treated rabbits and rats (Dutertre-Catella et al., 1978; Truhaut et al., 1970; Thier, R., 1991). Preliminary results of a pharmacokinetic study indicate that 93% of radioactivity was excreted in the urine, expired air and faeces of rats in 24 hours (Strasser, 1988). These results indicate rapid elimination of isophorone, thus bioaccumulation is not expected.

After inhalative administration of saturated vapours of isophorone to rats for 4 hours, a part of the isophorone was expired unchanged (Dutertre-Catella, 1976).

7.9.2. Acute toxicity - Irritation/ Corrosion - Sensitisation

7.9.2.1. **Acute toxicity**

Oral

LD_{50} values of isophorone in rats were 1500 mg/kg bw (Unpublished study report, 1968a), 2100 mg/kg bw (Dutertre-Catella, 1976) and 3450 mg/kg (Unpublished study report, 1964). The LD50 in mice was 2200 mg/kg bw (Dutertre-Catella, 1976). These results confirm that isophorone fulfils CLP criteria for classification as Acute Tox. 4 – H302 (current harmonized classification: Acute Tox. 4*).

Clinical signs such as general apathy, lateral position and irregular respiration occurred at doses > 1000 mg/kg bw. Depression, wearness (leading to coma), ptosis, lacrimation and laboured respiration occurred at doses ≥ 1450 mg/kg bw (Unpublished study report, 1968a; Dutertre-Catella, 1976; Unpublished study report, 1965a). At doses ≥ 5000 mg/kg bw, congestion of lungs, kidneys and pancreas were observed (Unpublished study report, 1964).

In dead animals, lesions in livers were found at necropsy (Dutertre-Catella, 1976). Increased secretion in stomach and small intestine, thickening and haemorrhagic erosions of proventiculus lining, urine retention, hyperaemia of liver, pulmonary emphysema, edema or hyperaemia and splenic enlargement were reported in animals that died (Günzel, 1968).

Inhalation

In acute inhalation studies, LC_{50} values of isophorone were high: LC_0 > 3500 mg/m^3 (rats, mice, guinea pigs), LC_{50} = 7000 mg/m^3 (rats), $LC_{10/30}$ = 40200 mg/m^3 (rats/rabbits) (Unpublished study report, 1964; Unpublished study report, 1965b; Dutertre-Catella, 1976). In most of these studies, there were uncertainties on the actual concentrations and the form (vapour or aerosol) of the test materials at which the animals were exposed to. However, low toxicity was reported at saturated atmospheres.

Clinical signs included slight ptosis, lacrimation (Unpublished study report, 1964), irritation of eyes and nose, accelerated breathing and narcosis (Dutertre-Catella, 1976). At concentrations higher than 5000 mg/m^3, ataxia and coma, dyspnea, piloerection, depression and decrease activity were reported (Unpublished study report, 1965). At necropsy of the high exposure concentrations, congestion or bleeding of the lungs was found (Unpublished study report, 1965; Dutertre-Catella, 1976).

The acute toxicity studies performed in 1940 by Smyth *et al.* were considered not acceptable by the eMSCA since the purity of the tested material was questionable and could contribute to the severity of the effects observed (Rowe and Wolf, 1962). This was also noted by NTP (1986) and ACGIH (1986).

No classification for acute toxicity by inhalation is needed for isophorone.

Dermal

The LD_{50} after dermal application was 1700 mg/kg bw in rats (Günzel and Richter, 1968) and 1200 mg/kg bw (Dutertre-Catella, 1976) to > 3160 mg/kg bw (Unpublished study report, 1964) in rabbits. These results confirm that isophorone fulfils CLP criteria for classification as Acute Tox. 4 – H312 (current harmonized classification: Acute Tox. 4*).

Clinical signs were general apathy, later on occasionally coma, cachexia, tremor, lacrimation (Günzel and Richter, 1968) and depression, accelerated/laboured respiration,

sprawling, prostration and narcosis (Dutertre-Catella, 1976). Some signs of dermal irritation were also reported: slight to moderate erythema, slight edema and slight or moderate desquamation (Dutertre-Catella, 1976); hyperaemia and bleedings of subcutis (Günzel, 1968). At necropsy, hyperaemia and bleedings of subcutis (application area), uniform thickening of the cutaneous stomach mucosa, urine retention and pulmonary emphysema, edema or hyperaemia were observed in animals that died.

7.9.2.2. **Other acute effects**

Based on clinical signs reported in experimental animals (narcosis, depression, ataxia, lethargy, prostration in acute and repeated dose toxicity studies) and in workers (dizziness, fatigue and malaise) (see animal and human information below in section 7.9.4 related to repeated-dose toxicity), a classification as STOT SE 3 – H336 (may cause drowsiness or dizziness) can be required.

7.9.2.3. **Skin Irritation/ corrosion**

No fully reliable skin irritation assay is available. Most of the studies do not mention the scores for edema and erythema. Isophorone induces no irritation to slight irritation to rabbit's skin.

No classification is required according to CLP Regulation based on the scores reported in the studies performed by Dutertre-Catella (1976) and Unpublished study report (1979a).

7.9.2.4. **Eye Irritation/ corrosion**

In experimental studies, it was reported a marked eye irritation which was fully reversible within 14 days but not in 7 days (Unpublished study report, 1964b; Unpublished study report, 1979b).

In humans, irritating effects in eyes were observed from 230 mg/m^3 when exposed for a few minutes (Smyth, 1940) and from 359 mg isophorone/m^3 after 7 minutes (Unpublished study report, 1965a). No eye irritation was reported at 199 mg/m^3 in this latter study. In a study with 12 volunteers, eye irritation was reported at concentrations of 144 mg/m^3 for 15 minutes (Silverman et al., 1946).

Isophorone has a harmonized classification as Eye. Irrit. 2 – H319.

7.9.2.5. **Respiratory irritation**

Respiratory irritation was reported in the acute and repeated toxicity studies by inhalation. Furthermore, according to the ATSDR report (1989 & 2008), a concentration of 27.8 ppm for 5 minutes caused a 50% decrease (RD50) in the reflex respiratory rate of mice (De Ceaurriz, 1981).

In humans, throat irritation was reported at ≥ 199 mg/m^3 after exposure to isophorone for a few minutes, with a NOAEL reported at 100 mg/m^3 (Unpublished study report, 1965a; Smyth et al., 1940). In a further investigation, 12 volunteers were exposed to isophorone vapours for 15 minutes. At 144 mg/m^3, nose and throat irritations were reported (Silverman et al., 1946). 40 % of the exposed subjects objected isophorone odour at a concentration of 58 mg/m^3 (10 ppm), and this concentration was judged to be the highest tolerable air level for 8 hour exposure.

Isophorone has a harmonized classification as STOT SE 3 – H335.

7.9.2.6. **Sensitisation**

In a guinea pig maximization test (according to OECD TG 406), skin sensitisation was not observed in any of the 20 tested animals (Unpublished study report, 1988a). Although the negative result from this test should be taken with caution due to the lack of positive

control (only recommended by the 1981 version), there is no alert found in the literature for this endpoint.

Assessment of respiratory sensitisation potential is not possible due to a lack of data.

7.9.3. Repeated dose toxicity

7.9.3.1. **Oral**

Table 15. Studies on repeated dose toxicity after oral administration

Method	Results	Remarks	Reference
Studies in rats			
rat (Fischer 344/N) male/female (5/sex/group) subacute (oral: gavage) 0, 125, 250, 500, 1000, or 2000 mg/kg bw/d Exposure: 16 days (5 days/week) Method: Repeated Dose Toxicity, NTP Limitations: parameters restricted to clinical observation, body weight, gross necropsy and histopathology (not for all animals)	NOAEL: 500 mg/kg bw/day (male/female) (≥ 1000 mg/kg bw: reduced body weight gains in male and female rats, mortalities)	2 (reliable with restrictions) key study experimental result **Test material (EC name): 3,5,5-trimethylcyclohex-2-enone** **Purity: 97%**	Bucher (1986) NTP (1986)
rat (Fischer 344/N) male/female (10/sex/group) subchronic (oral: gavage) 0, 62.5, 125, 250, 500, or 1000 mg/kg bw/d Exposure: 13 weeks (5 days/week) Method: Repeated Dose Toxicity, NTP Limitations: parameters restricted to clinical observation, body weight, gross necropsy and histopathology	NOAEL: 500 mg/kg bw/day (male/female) (1000 mg/kg bw/day: reduced body weight gain not clearly dose-related; 1/10 female died)	2 (reliable with restrictions) key study experimental result **Test material (EC name): 3,5,5-trimethylcyclohex-2-enone** **Purity: 97%**	Bucher (1986) NTP (1986)

Method	Results	Remarks	Reference
rat (Albino rats of CFE strain) male/female (20/sex/group) subchronic (oral: feed) 0, 750, 1500 and 3000 ppm in the diet: males 57.0, 102.5 and 233.8 mg/kg bw/d; females 78.9, 163.8 and 311.8 mg/kg bw/d (nominal) Exposure: 90 days (daily) Method: Repeated Dose Toxicity Limitations: histopathology restricted to 5/sex for 20 organs analysed in the high dose and control group and only liver and kidney for the low / medium dose groups.	NOAEL: 102.5 mg/kg bw/day (male) (reduced body weight gain) NOAEL: >= 311.8 mg/kg bw/day (female) (no effects)	2 (reliable with restrictions) key study experimental result **Test material (EC name): 3,5,5-trimethylcyclohex-2-enone** **Purity not stated**	Unpublished study report, 1972a U.S. EPA (1978)
Studies in mice			
mouse (B6C3F1) male/female (5/sex/group) subacute (oral: gavage) 0, 125, 250, 500, 1000, or 2000 mg/kg bw/d Exposure: 16 days (5 days/week) Method: Repeated Dose Toxicity, NTP Limitations: parameters observed restricted to clinical observation, body weight, gross necropsy and histopathology (not for all animals)	NOAEL: 125 mg/kg bw/day (female) (≥250 mg/kg bw: reduced body weight gain, mortality) NOAEL: 500 mg/kg bw/day (male) (≥ 1000 mg/kg: reduced bodyweight gain, mortality)	2 (reliable with restrictions) key study experimental result **Test material (EC name): 3,5,5-trimethylcyclohex-2-enone** **Purity: 97%**	Bucher (1986) NTP (1986)
mouse (B6C3F1) male/female (10/sex/group) subchronic (oral: gavage) 0, 62.5, 125, 250, 500, or 1000 mg/kg bw/d Exposure: 13 weeks (5 days/week) Method: Repeated Dose Toxicity, NTP Limitations: parameters observed restricted to clinical observation, body weight, gross necropsy and histopathology	NOAEL: 500 mg/kg bw/day (female) (1000 mg/kg bw/day: mortality) NOAEL: ≥ 1000 mg/kg bw/day (male) (no compound-related effects were observed)	2 (reliable with restrictions) key study experimental result **Test material (EC name): 3,5,5-trimethylcyclohex-2-enone** **Purity: 97%**	Bucher (1986) NTP (1986)
Studies in dogs			
dog (Beagle) male/female (4/sex/group) subchronic (oral gelatine capsules) 0, 35, 75 and 150 mg/kg bw/d	NOAEL: ≥ 150 mg/kg bw/day (male/female) (no toxicological findings)	2 (reliable with restrictions) key study experimental result **Test material (EC name): 3,5,5-**	Unpublished study report, 1972b U.S. EPA (1978)

Method	Results	Remarks	Reference
Exposure: 90 days (daily (7 days/week)) Method: Repeated Dose Oral Toxicity		**trimethylcyclohex-2-enone** **Purity not stated**	

The NTP-conducted repeated dose toxicity studies in male and female Fischer rats and B6C3F1 mice. Animals were administered 0, 125, 250, 500, 1000 and 2000 mg isophorone/kg bw/day in a 16-day (dose-finding) study and 0, 62.5, 125, 250, 500 and 1000 mg/kg bw/day in a 13-week study (NTP, 1986).

In the dose-finding study in rats, 4/5 females and 1/5 males that received 2000 mg/kg bw/day isophorone died before the end of the study. Body weight gain was reduced in both sexes at 1000 mg/kg bw/day (-13.9% in males and -6.7% in females) and in survival animals receiving 2000 mg/kg bw/day (-25.2% in males and -11.4% in females). All dosed animals were lethargic after dosing. No treatment-related effects were reported at gross necropsy and upon microscopic examination from 6 selected rats from the 2 highest dose groups.

In the 13-week study in rats, 1/10 females receiving 1000 mg/kg bw/day died. At this same dose, animals were sluggish and lethargic after dosing. There was no clear effect of the treatment on the final mean body weight. No treatment-related effects were reported at gross necropsy and upon microscopic examination.

The NOAEL can be set at 500 mg /kg bw/day for male and female rats, considering effects observed in both subacute and subchronic studies (NTP, 1986)

In the dose-finding study in mice, all animals died at 2000 mg/kg bw/day. At lower dosages, reduced body weight gains were reported in male (-7.8% at 1000 mg/kg bw/day) and female mice (between -7.3% and -9.3% at 250, 500 or 1000 mg/kg bw/day). Mice receiving 1000 mg/kg bw/day staggered after dosing. No treatment-related effects were reported at gross necropsy and upon microscopic examination from 2 selected males and 2 selected females from the 1000 mg/kg bw/day group.

In the 13 week study in mice, 3/10 females that received 1000 mg/kg bw/day died before the end of the study. There was no dose related effect on the final mean body weights. No treatment-related effects were reported at gross necropsy and upon microscopic examination.

Considering both studies, the NOAEL can be set at 125 mg /kg bw/day in females and 500 mg/kg bw/day in males based on the decreased body weight gain reported in the 16-day study.

Other studies are also available in the registration dossier:

Male and female CFE rats were dosed with 750, 1000, or 3000 ppm isophorone (in corn oil) via diet - corresponding to 57.0, 102.5, 233.8 mg/kg bw/day for males and to 73.9, 163.8 and 311.8 mg/kg bw/day for females - for 13 weeks. The only observed effect was a reduced body weight gain (-12 to -13 %) in male rats at the highest dose. There was no treatment related effect on mortality, food consumption, hematology and biochemistry dosages, urinalysis, organ weight, gross necropsy and histopathology.

The NOAEL derived from this 13-week study is 102.5 mg/kg bw/day (1000 ppm) for males based on the decreased body weight and 311.8 mg/kg bw/day (3000 ppm) for females based on no treatment-related effect (Unpublished study report, 1972a).

In a further 90-day study, beagle dogs were given orally gelatine capsules containing doses of 35, 75, or 150 mg isophorone/kg bw. There was no treatment related effects on mortality, body weight, food consumption, on biochemistry, hematology and urinalysis parameters, on organ weight, at gross necropsy and histopathology. As the only minor clinical sign, incidence of soft stool was noted in the two upper levels.

The NOAEL can be set at ≥ 150 mg/kg bw/day for male and female beagle dogs from this 90-day study (Unpublished study report, 1972b).

7.9.3.2. **Dermal**

Table 16. Studies on repeated dose toxicity after dermal administration

Method	Results	Remarks	Reference
rat (Wistar) male/female (5/sex/group) subchronic 0.1 and 0.2 ml (isophorone pure) Exposure: 8 weeks (Daily) Limitation: level of details insufficient for proper interpretation	Fully reversible skin lesions Body weight gain was decreased in females	4 (not assignable) weight of evidence experimental result **Test material (EC name): 3,5,5-trimethylcyclohex-2-enone** **Purity not clearly stated**	Dutertre-Catella (1976)

In rats exposed to 0.1 or 0.2 ml of isophorone for 8 weeks, decrease of body weight gain (approximately -8%) in females and dermal irritation (erythema and crust on skin after 5-6 weeks of treatment) in both sexes were reported. This latter effect was completely reversed during recovery period (duration not reported) (Dutertre-Catella, 1976).

7.9.3.3. **Inhalation**

Table 17. Studies on repeated dose toxicity after inhalation exposure

Method	Results	Remarks	Reference
Subacute exposure			
mouse (Swiss OF1) male (10 animals/group) subacute (inhalation: vapour) (whole body) 0, 164 +/- 21 mg/m^3 (28 ppm) or 513 +/- 36 mg/m^3 (90 ppm) Exposure: 6 hours/day 4 consecutive days 9 days: 5 consecutive days the first week and 4 consecutive days the second week 14 days: 5 consecutive days the first and second weeks and 4 consecutive days the third week Method: Study designed to specifically detect sensory irritating properties of different substances, including isophorone. Limitation: only clinical observations and histopathological changes in the respiratory tract.	NOAEC: ≥ 513 mg/m^3 air (male) No respiratory effects were observed	2 (reliable with restrictions) weight of evidence experimental result **Test material (EC name): 3,5,5-trimethylcyclohex-2-enone** **Purity: 98%**	Zissu (1995)

Method	Results	Remarks	Reference
rat (Charles River Caesarian-derived) male/female (10/sex/group) subacute (inhalation: vapour) (whole body) 0, 250 mg/m^3 air (nominal conc.) 0, 208 +/- 10 mg/m^3 air (corresponds to about 36 ppm) (analytical conc.) Exposure: 28 days (6 hours/day; 5 days/week) Method: Repeated Dose Inhalation Toxicity; limit test Limitations: only one tested concentration, examinations restricted to clinical observation, body weight, hematology and for some organs: gross necropsy and histopathology (lung, liver, kidneys, adrenal, spleen for 3 animals/sex/group)	NOAEC: < 208 mg/m³ air (male/female) Clinical signs, reduced body weight (males) and changes in haematological parameters and reduced liver weights in both sexes.	3 (not reliable) weight of evidence experimental result **Test material (EC name): 3,5,5-trimethylcyclohex-2-enone** **Purity not stated.**	Unpublished study report (1968b)
Subchronic exposure			
rat and guinea pig (rat: Wistar; guinea pig: not specified) male/female (10 animals/dose) subchronic (inhalation: vapour) (whole body) 0, 144, 287, 575, 1150 and 2874 mg/m^3 air (nominal conc.) Vehicle: Air Exposure: 6 weeks (8 hours/day; 5 days/week) with a post-exposure of 14 days Method: Repeated Dose Inhalation Study Limitations: examinations restricted to body weight, hematology, urinalysis, gross necropsy and histopathology (liver, kidney, spleen, adrenals, heart muscle in 97/170 exposed animals and 12 controls and for some animals: voluntary muscle, pancreas, testicles and small intestine). No distinction was made between results for rats and guinea pigs. Purity of the tested material questionable (several highly volatile impurities?) leading to possible overestimated concentrations.	At 287 mg/m^3: kidney injury From 575 mg/m^3: mortality, poor growth, lung injury, blood cell changes. At 2874 mg/m^3: eye and nose irritation, clinical changes, pulmonary inflammation	3 (not reliable) disregarded study experimental result **Test material (EC name): 3,5,5-trimethylcyclohex-2-enone** **Commercial grade**	Rowe (1962) Smyth (1942)
Chronic exposure			
rat (Wistar) male/female (20/sex/group) rabbit (New Zealand White) male/female (2/sex/group)	NOAEC: < 1436 mg/m³ air (male/female) Slight conjunctivitis, slight irritation of nasal mucosa; micro vacuolization of livers	3 (not reliable) weight of evidence experimental result	Dutertre-Catella (1976)

Method	Results	Remarks	Reference
chronic (inhalation: vapour) (whole body) 0, 1436 mg/m^3 (analytical conc.) Vehicle: Air Exposure: 18 months (6 hours/day; 5 days/week) Method: Chronic Inhalation Toxicity Study Limitations: only one tested concentration, examinations limited to clinical signs, body weight and urinalysis once per week, hematology for the 10th first months, pathological examination (organs not specified: information only reported for liver and lungs). No distinction was made between results for rats and guinea pigs.	more pronounced in exposed animals.	**Test material (EC name): 3,5,5-trimethylcyclohex-2-enone** **Purity not clearly stated.**	

Experimental data

Studies are available for mice, rats, rabbits, and guinea pigs.

No effect was found at histopathological examination of the respiratory tract (nasal passages, trachea and lungs) of Swiss mice after exposure to 164 and 513 mg/m^3 for up to 14 days (Zissu, 1995). **A NOAEC (mice, 14 days) ≥ 513 mg/m^3 was set by the registrants**. As this study only focused on respiratory tract, other effects cannot be anticipated from this study.

Other studies by inhalation were performed with only one concentration associated with adverse effects and with limited examinations.

Rats exposed for 4 weeks (6 hours/day, 5 days/week) to 208 mg isophorone/m^3 presented clinical signs (slight nasal bleeding on day 3, reddish-brown discoloration of the fur from day 6 to 8 of exposure), reduced body weight gain (in males only, no further information), changes in hematology parameters when compared before and after exposure to isophorone and decreased absolute and relative liver weights (in males only, no further information). According to the registrants, there was no "clear-cut compound-related abnormalities" at gross pathology examination and "no unequivocal changes" at histopathological examination which was performed in 3 animals/sex/group. **A NOAEC (rat, 28 days) < 208 mg/m^3 was set by the registrants** (Unpublished study report, 1968b). The ATSDR (1989) considered that the concentration tested of 208 mg/m^3 can be considered as a NOAEC for haematological findings since the variations of these effects were slight in treated groups (increased percentage of lymphocytes of max. 5.8%, decreased percentage of neutrophils of max. 5% and increased haemoglobin content at 1.2-1.4 g/100 ml) and similar to unexposed animals (ATSDR, 1989).

Rats and guinea pigs were exposed for 6 weeks to isophorone vapour at concentrations between 287 mg/m^3 and 2874 mg/m^3. Kidney injury (congestion, dilatation of Bowman's capsule, cloudy swelling of convoluted tubular epithelium) was reported from 287 mg/m^3. Mortality, reduced body weight gain, blood cell changes (not further specified) and lung injury (irritation, congestion capillary leakage and desquamation of epithelium) reported from 575 mg/m^3. At 2874 mg/m^3, were also reported: albumin in the urine, chronic conjunctivitis and nasal irritation. Results from rats and guinea pigs, males and females were not distinct (Smyth et al., 1942). This study was criticized by Rowe and Wolf (1963) concerning the purity of the tested material. Given the uncertainties on the level of impurities that could contribute to the severity of the effects, this study was considered as not acceptable for the determination of a NOAEC. This deviation has also been noted by NTP (1986) and ACGIH (1986).

Eye and nose irritations were observed in Wistar rats and New Zealand rabbits at 1436 mg/m^3 after 18 months (6 hours/day, 5 days/week) exposure. In addition at this concentration, microvacuolization of the livers was more severe and more constant in treated animals (Dutertre-Catella, 1976). The level of information available in this publication does not allow proper assessment of the study.

Human information

After 1 month exposure to isophorone, workers exposed to 28.7-46 mg/m^3 (5-8 ppm) complained of malaise; complaints ceased when concentrations were decreased to 5.7-23 mg/m^3 (1-4 ppm) (Unpublished study report, 1973).

Justification for classification or non classification

After repeated oral exposure to isophorone in rodents, effects were restricted to reduced body weight at doses from about 102.5 mg/kg bw/day and mortality at high dose (from 1000 mg/kg bw/day).

Dermal irritation was reported in a limited dermal study in rats.

After inhalation, no respiratory effect was reported at concentration up to 513 mg/m^3. Effects (liver and kidney injury, biochemistry changes and respiratory irritation) were reported in other studies at concentrations of about 200 mg/m^3; however, the level of details available does not allow proper assessment of these studies.

The data available does not justify a classification of isophorone as STOT RE. However, it can be noted that the quality of several studies, in particular by dermal and inhalation routes, precludes adequate conclusion regarding hazard identification and characterisation.

7.9.4. Mutagenicity

7.9.4.1. *In vitro* studies

Several Ames tests with *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 with and without S9 were negative (NTP, 1986; Unpublished study report, 1978a; Unpublished study report, 1988b).

Positive results were observed in an umu-test using *S. typhimurium* TA 1335/pSK1002 (Ono *et al.,* 1991) and in a *Bacillus subtilis*/microsome rec-assay (Matsui et al., 1988).

Contradictory results were found in mouse lymphoma tests (O´Donoghue *et al.,* 1988; Honma *et al.,* 1999a&b; McGregor *et al.,* 1988). In the presence of metabolic activation, Honma *et al.* (1999b) reported inconclusive results. Without metabolic activation, negative results were reported by O'Donoghue *et al.* (1988) and Honma *et al.* (1999b) in test system using short treatment period (3-6h). In contrast, McGregor *et al.* (1988) and the NTP (1986) observed significant increases in mutation frequency in particular at concentrations producing some degree of cytotoxicity (reduced relative total growth). Isophorone was positive in one of two trials after 24-hour incubation (Honma *et al.* (1999a)).

Contradictory results were also found in chromosomal aberration tests. No significant increase in chromosomal aberrations was observed in Chinese Hamster Ovary (CHO) cells with and without metabolic activation (Gulati *et al.,* 1989; NTP, 1986). In a chromosomal aberration assay performed on Chinese hamster lung (CHL) cells, isophorone induced structural aberrations when cells are treated for 6 hours and then cultured in fresh medium for another 18 hours, only at the highest dose, i.e. 1.5 and 1.25 mg/ml with and without S9 mix, respectively (Matsuoka *et al.,* 1996).

A significant increase in SCE frequency was induced by isophorone in the absence of S9 mix at concentrations of 500 - 1000 mg/l (no increase in the presence of Aroclor 1254 - induced rat liver S9 mix). As these concentrations were cytostatic, increased SCE frequencies could only be detected after delayed harvest (6-13 h additional culture time) (Gulati *et al.,* 1989; NTP 1986).

Isophorone was negative for the induction of unscheduled DNA synthesis (UDS) in rat primary hepatocytes when tested up to toxic doses (O´Donoghue *et al.,* 1988; Unpublished study report, 1984a).

Isophorone was found positive in a transformation assay using A31-1-13 clone of BALB/c-3T3 cells culture without metabolic activation (Matthews *et al.* 1993).

Isophorone did not induce micronuclei in a hen's egg test within an inter-laboratory trial assessing this method as an alternative test system to the *in vivo* micronucleus test (Greywe *et al.,* 2012).

7.9.4.2. ***In vivo*** **studies**

In a micronucleus assay, 498 mg/kg of isophorone was administered intraperitoneally to CD-1 mice (5 animals/sex). This dose was chosen as to be the LD_{20} of the test substance. Significant increases in the number of micronucleated polychromatic erythrocytes were not observed (O´Donoghue et al., 1988). Negative results were also obtained in a micronucleus assay in CFLP mice (5 animals/dose/sex), 6 hours after gavage of 450, 900, 1800 mg/kg isophorone given in 2 equal parts separated by an interval of 24 hours (Unpublished study report, 1978b).

The NTP reported negative results in a chromosome aberration assay in which male mice (8 animals/dose) were administered isophorone once by intraperitoneal injection at doses up to 500 mg/kg bw (NTP, 1990).

No indication of a mutagenic effect was reported in a Sex Linked Recessive Lethal (SLRL) test with *Drosophilia melanogaster* at doses of 2000 mg isophorone/kg (feeding exposure) or 12,500 mg isophorone/l (by injection) (Foureman et al., 1994).

There was no covalent binding of isophorone or its metabolites to DNA of livers and kidneys (target organs in the NTP carcinogenicity study) in F344 rats (5 animals/sex/group) and B6C3F1 mice (25 animals/sex/group) that were administered 500 mg/kg ^{14}C-isophorone by gavage (Thier et al., 1990). Another DNA binding assay yielded no evidence of isophorone binding to DNA of kidney and preputial gland from rats gavaged with 500 mg/kg ^{14}C-isophorone (Morishita et al., 1997 cited by EFSA).

In its evaluation based on the above dataset, EFSA Panel concluded that there is no concern with respect to genotoxicity of isophorone (EFSA, 2015).

Based on these data, the eMSCA considers that there is no genotoxicity concern yet for isophorone.

Justification for classification or non classification: No classification is justified for isophorone regarding mutagenicity.

7.9.5. Carcinogenicity

The NTP conducted 2-year toxicology and carcinogenesis studies with isophorone. F344/N rats and B6C3F1 mice (50 animals/sex/species/group) were exposed to isophorone by gavage at dosage of 0, 250 or 500 mg/kg bw/day in corn oil, 5 days per week for 103 weeks (NTP, 1986).

According to the NTP and under the conditions of the studies:

There was ***some evidence of carcinogenicity*** **of isophorone in male F344/N rats** based on the occurrence of neoplastic lesions of the kidneys at both tested doses (tubular cell adenoma: 0/50; 0/50; 2/50 and tubular cell adenocarcinoma: 0/50; 3/50; 1/50; with positive significant trend) and increased incidence of carcinomas of the preputial gland (0/50; 0/50; 5/50; significant at the highest dose and with significant positive trend) in male rats given 500 mg/kg bw/day.

The Registrants attributed the kidney tumours to an alpha2µ-globulin associated mechanism (Saito et al. 1992; Lehman-McKeeman et al. 1990; Dietrich et al. 1991; Swenberg et al. 1989). Thus, they considered the observed nephropathy in male rats is therefore irrelevant to other species. However, if we consider the seven criteria established by the IARC in order to conclude that an agent induces tumours of the kidney by an alpha2µ-globulin-associated response (IARC, 1999), not all are fulfilled with isophorone.

Considering the carcinomas of the preputial gland, it should be noted that true tumor incidences of this lesion is not known since the preputium was only investigated

histopathologically when gross lesions were found. Nonetheless, the NTP considered that this finding should not be discounted.

Survival (33/50, 33/50 and 13/50) and mean body weight was reduced at the highest tested dose. Non neoplastic effects consisted in various effects on the kidneys (tubular cell hyperplasia: 0/50; 1/50; 4/50; epithelial hyperplasia of the renal pelvis: 0/50; 5/50; 5/50; increased mineralization of the medullary collecting ducts: 1/50; 31/50; 20/50 and greater severity of the nephropathy in the low dose group) and increased incidence of fatty metamorphosis of adrenal cortex (7/50, 21/50, 26/50).

There was ***no evidence of carcinogenicity*** **of isophorone in female F344/N rats** at doses up to 500 mg/kg bw/day.

As non neoplastic lesions, treated females presented reduced mean body weight in the high dose group and an increased incidence of nephropathy (21/50; 39/50; 32/50) and of focal hyperplasia of anterior pituitary in dosed females (3/49, 6/48, 13/47).

For male B6C3F1 mice, there was an *equivocal evidence of carcinogenicity* of isophorone as shown by increased incidences of hepatocellular adenomas and carcinomas (18/48; 18/50; 29/50 [statistically significant at the highest dose]) and of mesenchymal tumors of the integumentary system (fibroma, fibrosarcoma, neurofibrosarcoma, or sarcoma: 6/48; 8/50; 14/50 [statistically significant at the highest dose]). A statistically significant increased incidence of lymphomas or leukemias was noted in low dose male mice (8/48; 18/50; 5/50).

Coagulative necrosis (3/48; 10/50; 11/50) and hepatocytomegaly (23/48; 39/50; 37/50) were observed more frequently in the livers of dosed male mice than in vehicle controls. Increased incidence of hyperkeratosis of forestomach (0/47, 5/49, 4/49) and of chronic focal inflammation of the kidney (7/48, 18/50, 21/50) were also reported.

There was ***no evidence of carcinogenicity*** **of isophorone in female B6C3F1 mice** at doses up to 500 mg/kg bw/day.

Hyperkeratosis of forestomach was observed at increased incidences in the high dose group of females (1/50, 0/50, 5/49). Focal hyperplasia of the pituitary gland also occurred at increased incidence (5/47, 7/41, 13/44).

Isophorone is currently classified as Carc. 2 – H351 that seems consistent with the results of the NTP study.

In addition, a case control study of brain and other contral nervous system cancer among workers at semiconductor and storage device manufacturing facilities was performed by Rodrigues *et al.* (2020). Isophorone was only cited in one table of the publication without any statistically significant association with CNS cancers.

Carcinogenicity of isophorone was assessed by the IARC in its monograph volume 130 (1,1,1-Trichloroethane, Hydrazobenzene, N-Methylolacrylamide, Diphenylamine, and Isophorone) in October 2021. Isophorone was classified in group 2B based on inadequate evidence in humans and sufficient evidence in experimental animals (IARC, 2021).

The eMSCA considers that isophorone is a suspected carcinogen.

Justification for classification or non classification: The available data confirm that isophorone fulfils CLP criteria for classification as Carc. 2 (current harmonised classification).

7.9.6. Toxicity to reproduction (effects on fertility and developmental toxicity)

Table 18. Studies on fertility

Method	Results	Remarks	Reference
rat (Wistar) male/female one-generation study inhalation (whole body) 500 ppm = 2873 mg/m^3 (saturation) (analytical conc.) Exposure: Exposure period: 6 hours/day Males and females treated for 3 months and then mated (treated/treated, treated/untreated, untreated/untreated). Exposure continued in females until they delivered and in males for a total of 6 months Limitations: one test concentration, examination restricted in parents to clinical signs, body weight and mortality and in pups to number and vitality. Necropsy performed in parents and pups (information only reported for lung and liver). No details or numerical value.	NOAEC (P): < 2873 mg/m^3 (male/female) Irritation of eyes and nose in treated group. Histopathological findings on liver and lungs in control and treated groups. NOAEC (F1): > 2873 mg/m^3 (male/female) No treatment-related effect on mortality and at pathological examination.	3 (not reliable) experimental result **Test material (EC name): 3,5,5-trimethylcyclohex-2-enone** Purity not clearly stated	Dutertre-Catella (1976)
Rats CD male/ female; 10/sex/group Reproduction/developmental toxicity screening test Oral; gavage (in corn oil) 0, 100, 300, 1000 (terminated after 3 treatments in males and 1 treatment in females), 750 mg/kg bw/day, once daily Duration: 43 days in males (beginning 2 weeks prior mating) and 64-68 days in females (2 weeks prior mating, during mating, gestation and until LD13) According to OECD TG 421 GLP compliant	P0: Salivation in all dosed animals, reduced motility and discolouration of the urine from 300 mg/kg bw/day. Increased severity with dose. Decreased body weight and BW gain in the high dose group of males during the whole treatment. Effect also reported in females during lactation (-6.4 to -13%) and lactation day 1 (-9.3%). No effect thereafter. No information on corrected BW. No effect on reproduction function. F1: Reduced BW at 750 mg/kg bw/day on both sexes on LD1, 4 and 13 (-15.8%, 21.4%, 18.9%). Effect also found on litter basis.	1 (reliable) experimental result **Test material (EC name): 3,5,5-trimethylcyclohex-2-enone** Purity not provided.	Unpublished study report (2020a)

7.9.6.1. Effects on fertility

In a very limited one generation study, Wistar rats were exposed to 0 or 2873 mg/m^3 isophorone in air (Dutertre-Catella, 1976). After 3 months of exposure, animals were mated (5 treated males with 5 treated females; 5 control males with 5 control females; 5 control males with 5 females treated; 5 treated males with 5 control females). Treatment

was stopped at parturition for females and after a total exposure duration of 6 months for males. Examinations were limited to clinical signs and body weight of parents and to number and vitality of pups. Necropsy was also performed in parents and pups but it is not indicated what were exactly the organs examined (only information available for liver and lung). In parents, irritation of nose and eye was reported. The number and vitality of pups were similar among groups. There was no abnormalities at necropsy for pups. There was no treatment-related effect on liver and lung. However, the level of information is insufficient to have an adequate interpretation of this study. The design of the study is not appropriate to conclude on effects of isophorone on fertility and development.

A reproduction/developmental toxicity screening test (OECD TG 421) was performed as a dose range-finding study for an EOGRTS. Information below was available from the disseminated dossier (ECHA website, accessed in 2021). In this study, CD rats were gavaged with isophorone at doses of 0, 100, 300 or 1000 mg/kg bw/day with treatment beginning 2 weeks prior mating until exposure day 43 for males and until lactation day (LD) 13 for females. The high dose group of 1000 mg/kg bw/day was terminated after 3 treatments for males and 1 treatment for females due to toxicity. A group of animals tested with 750 mg/kg bw/day was thus included. Salivation was reported in all dosed groups and also reduced motility and discolouration of the urine and faeces from 300 mg/kg bw/day. The severity of these clinical signs increased with increasing dose. Two females from the 750 mg/kg bw/day group were found dead during gestation (one considered treatment-related). The body weight and body weight gain (+7.2% versus +15.8% in control) were reduced in males dosed with 750 mg/kg bw/day from exposure day 15 until sacrifice. At this same dose, in females, the body weight was decreased during gestation (max. -13%) and until lactation day 1 (-9.3%), associated with a reduced body weight gain (+32.2% versus +53.2% in the control). There is no information on corrected body weight in order to discriminate a direct effect of the test substance on dams from reduced litter weight. Food consumption was decreased only in males on week 3 for the low and intermediate dose groups. There was a slightly not statistically significant increase of T4 in males at 100 mg/kg bw/day. This effect was not dose-related and values from the control group seems rather low compared to historical control range. The registrants concluded to a NOAEL for parental toxicity at 100 mg/kg bw/day. There was no effect on reproduction function (histological examination of testis, ovary and epididymis, fertility and gestation index). Although registrants considered that there is no effect on number of oestrus cycle, a dose-related increase of mean oestrus cycle length was noted (4.08, 4.19, 4.30, 4.46 days in each treated dose, respectively). The registrants concluded to a NOAEL for reproductive toxicity ≥ 750 mg/kg bw/day. In pups, the only observed effect considered as treatment-related was a decreased body weight on lactation day 1, 4 and 13 in the 750 mg/kg bw/day group (-15.8%, -21.4%, -18.9%). This effect was also observed on litter basis (-15.8%, -20.8%, -19.4% compared to control group on LD1, 4 and 13, respectively). Other parameters examined were not statistically affected by the treatment (clinical sign, viability, gross examination, T4, nipple retention). The absolute anogenital distance was reduced in males and females (-14% and -11.9%, respectively) but not when adjusted with body weight. The registrants concluded to a NOAEL for F1 toxicity at 300 mg/kg bw/day.

An EOGRTS in rats, by oral route with cohort 1A and 1B, with extension to mate the cohort 1B to produce the F2 generation and with cohort 2A and 2B (developmental neurotoxicity) was required by ECHA under compliance check, in December 2018 (decision number: CCH-D-2114453561-52-01/F). Registrants have had to submit the requested information by 4 January 2021. For the time being, the final study report is not available.

Table 19. Studies on developmental toxicity

Method	Results	Remarks	Reference
Range-finding teratogenicity study Rats and mice (Fischer 344 rats and CD-1 mice) inhalation (whole body)	**Rats** Decreased body weight (<10%) and food consumption at 150 ppm (861 mg/m^3).	2 (reliable with restrictions) key study **Test material (EC name): 3,5,5-**	Unpublished study report (1983)

Method	Results	Remarks	Reference
0, 50, 100, 150 ppm (nominal conc.)(equiv. to 0, 287, 574, 861 mg/m^3) 0, 52, 100, 144 ppm (analytical conc. (GC analysis)) 0.09, 57, 110, 162 ppm (analytical conc. (UV analysis)) Exposure: 6 hours per day, GD6-15	1 exencephaly in one late resorption at 150 ppm. **Mice** Decreased body weight gain (<10%), decreased spleen weight at 150 ppm. 3 exencephalies (one late resorption in one litter and 2 live fetuses in another litter) at 150 ppm.	**trimethylcyclohex-2-enone**	
Main teratogenicity Test rat (Fischer 344) inhalation (whole body) 0, 156, 300 and 640 mg/m^3 (corresponds to 0, 27, 52 and 111 ppm) (analytical conc.) 0, 144, 289 and 664 mg/m^3 (corresponds to 0, 25, 50 and 115 ppm) (target concentration) Exposure: GD6-15 (6 h/d)	NOAEC (maternal toxicity): 300 mg/m^3 based on < 7% reduction in body weight gain NOAEC (teratogenicity): ≥ 640 mg/m^3 (111 ppm) Female crown-rump distance was decreased at 111 ppm	2 (reliable with restriction) key study experimental result **Test material (EC name): 3,5,5-trimethylcyclohex-2-enone**	Unpublished study report (1984b)
Main teratogenicity Test mouse (CD-1) inhalation (whole body) 0, 156, 300 and 640 mg/m^3 (corresponds to 0, 27, 52 and 111 ppm) (analytical conc.) 0, 144, 289 and 664 mg/m^3 (corresponds to 0, 25, 50 and 115 ppm) (target concentration) Exposure: GD6-15 (6 h/d)	NOAEL (maternal toxicity): 300 mg/m^3 based on < 6% reduction in bw gain NOAEL (teratogenicity): ≥ 640 mg/m^3 (111 ppm) Extra point or area of ossification between the frontal bones in the head was mainly reported in all groups (not dose-related).	2 (reliable with restriction) key study **Test material (EC name): 3,5,5-trimethylcyclohex-2-enone**	Unpublished study report (1984b)
Preliminary teratogenicity study Rats (Fischer 344(F344/HanZtn Rj) and Wistar (Crl:Wi(Han)) 6 pregnant females/group Inhalation (nose only) 0, 150, 220 ppm Corresponding to 790 and 1330 mg/m^3 [analytical conc.]) (groups A, B, C for F344 strain and D, E, F for Wistar strain) Due to unscheduled mortality and toxicity on GD6-GD7, groups exposed to 220 ppm were reduced to 150 ppm from GD8 (groups C and F).	F344 strain: Only 2/6 females survived in the group C (GD6-GD7). Incidence of clinical signs slightly higher in treated groups. Reduction of BW (-23% and -16.4% on GD21 for group B and group C, resp.) and BW gain from GD12. Post-implantation losses in treated groups mostly due to " empty implantation sites", leading to a decreased number of viable fetuses. No identification of test-item related effect on fetal weight, on external, visceral or skeletal abnormalities. Some skeletal	3 (not reliable) Supporting study **Test material (EC name): 3,5,5-trimethylcyclohex-2-enone** Low relative humidity (about 20%) No statistics performed in dams in the high dose group of F344 rats. Foetuses from the treated groups were pooled (groups B and C, E and F) for pathological	Unpublished study report (2016)

Method	Results	Remarks	Reference
Vehicle: air (sham filtered air) Exposure: 6 hours per day, Days 6-20 of gestation. Not GLP	variations/ ossification stage noted among groups. Wistar strain: Group F: only 2/6 females survived (GD6-8), slightly higher incidence of clinical signs. No maternotoxicity in group E. No test-item related effect on fetal weight, on external, visceral or skeletal abnormalities. Some skeletal variations noted among groups.	examination analysis. The very low number of fetuses available prevents adequate assessment of developmental toxicity.	
Main teratogenicity study Rat Fischer 344; pregnant females; 24/group 5 groups: one control group exposed to air (group A); 3 exposed groups (groups B, C, D); one reference group not exposed (group E). Nose-only (vapour); exposure from GD5-19; 6h/day Target concentrations: 17, 53, 150 ppm (corresponding to 100, 300, 850 mg/m^3) According to OECD TG 414 GLP	Dams: No treatment related mortality. Loss of stability and bleeding of the vagina at higher incidence in group D. Adjusted BW similar among groups. Food consumption decreased in group D between day 5-8 of exposure. Serum level of total T3 elevated in groups C and D; no effect on T4, TSH and thyroid gland. Not stat. signif. increase of post-implantation loss (6.13%, 5.18%, 9.37%, 15.82%, 8.18% in groups A, B, C, D, E) with impact on live foetuses: 93.87%; 94.83%; 90.63%; 84.18%; 91.83%. NOAEL maternal as reported by registrants = 850 mg/m^3. Foetuses: Fetal BW per litter significantly reduced in all exposed groups (< 5% in groups B and C; 13.6% in group D). No external, visceral or skeletal abnormalities related to treatment. NOAEL teratogenicity as reported by registrants = 850 mg/m^3. NOAEL embryo/foetotoxicity as reported by registrants = 300 mg/m^3.	2 (reliable with restrictions) Key study **Test material (EC name): 3,5,5-trimethylcyclohex-2-enone** Low relative humidity due to technical error (6.25-7.23% compared to 30-70% as recommended in the OECD guideline) The highest tested dose is higher than that associated with exencephalies in previous studies (144 ppm). However, there was no significant maternotoxicity in this present study. This questions the reliability of the study.	Unpublished study report (2020b)

7.9.6.2. **Developmental toxicity**

In a preliminary teratogenicity study by inhalation in rats, one instance of exencephaly (1/12 litters) was noted in one late resorption at 150 ppm (corresponding to 861 mg/m^3) (Exxon, 1964). In dams, decrease of body weight on day 12 (-6%) and body weight gain (days 0-16; -20%) was reported at this dose and clinical signs (alopecia, excessive lacrimation, staining) from 100 ppm (574 mg/m^3). Increased relative weights of liver, spleen and kidneys were noted in all treated groups. However, in the absence of histopathological analysis, the relevance of these changes is unknown. In the main study with rats, isophorone elicited minor effects in the pregnant dams: decreased food consumption (days 6-20 and 0-20), lower body weights (on days 12 and 15 of gestation (< 7%)) and increases in alopecia and staining of the cervical and anogenital areas at 115 ppm. No developmental effect was reported except a decrease of crown-rump distance in females at 111 ppm (640 mg/m^3) that could indicate a growth retardation. However, this was mainly due to two foetuses from two different litters.

In a preliminary teratogenicity study by inhalation in mice, there were three instances of exencephaly noted in mouse fetuses: in a late resorption in one litter and in two live fetuses in a second litter (3/12 litters) at 150 ppm (861 mg/m^3). In dams, decreased body weight gain (days 6-16; -9%) and decreased spleen weight were reported at this concentration. However, in the absence of histopathological analysis, the relevance of this latter effect is unknown. In the main teratogenicity study with mice, isophorone elicited very minor effect in the pregnant dams in the form of lower body weights (on day 18 of gestation < 6%) at 111 ppm (640 mg/m^3). No developmental effect was reported at the higher concentration of 111 ppm.

Overall, the choice of the concentrations tested in the main studies in rats and mice (max tested dose of 111 ppm) is questionable since no major toxicity was noted in dams at 150 ppm in the range-finding studies and this does not justify lowering the concentrations. The observation of exencephalies, which is a rare and serious teratogenic effect, in two species at 150 ppm in a context of low maternal toxicity raises a concern for teratogenicity. Because this concentration was not tested in the main test, it cannot be excluded that this lesion is related to treatment. In this context, a new developmental study was required in the Substance Evaluation Decision (2015). According to this decision, the study had to be performed in Fischer 344 rats by inhalation using the maximum tolerable and attainable concentration based on a suitably designed sighting study.

In response to this request, the registrant decided to performed a preliminary prenatal developmental toxicity study using two different strains of rats (F344 and Wistar) (Unpublished study report, 2016).

Six females rats per group of each strain were exposed nose-only to isophorone (as vapour) at 150 or 220 ppm (analytical concentrations: 790 and 1330 mg/m^3) or sham filtered air from gestation day 6 to 20 for 6 hours/day. Due to unscheduled mortalities (4/6 Fischer 344 and 4/6 Wistar females) on GD6-GD7, the exposure to 220 ppm was reduced to 150 ppm from GD8. It can be noted that the deaths occurred very early after the beginning of exposure (before the second day of exposure for F344 females and before the 3rd day of exposure for Wistar females). The role of isophorone in these deaths (67% of treated females) occurring at 1330 mg/m^3 can be questioned considering the low acute toxicity of the substance (e.g. LC0 > 3500 mg/m^3 in rats, mice and guinea pigs).

Fischer 344 dams that survived in the group treated to 220/150 ppm presented reduction of body weight (no statistics performed since there were only 2 females). Females from the 150 ppm group showed statistically significant reduction of body weight on GD16-21 (-23% on GD21) and of body weight gain from GD12. However, body weight corrected to gravid uterine weight was not different from control. Clinical signs mainly consisted in alopecia, piloerection, dirty fur and chromodacryorrhea and were more frequent in treated dams than in controls. There was no macroscopical findings in dams.

Pre-implantation losses were increased in treated groups (4.7%, 42.4%, 30.5% in control group, 150 ppm group, 220/150 ppm group, respectively). Pre-implantation losses were not considered related to treatment since isophorone exposure began after implantation. There was also an increase of post-implantation losses in treated groups (84.4%, 63.7% versus 17.52% in controls). This effect seems mostly related to "empty implantation sites"

(4/6 females in the 150 ppm group and 1/2 females in the 220/150 ppm group). Consequently, the number of viable fetuses was reduced with only one dams with live fetuses in each of the treated groups (53 foetuses / 6 litters in control group and 5 foetuses/ 1 litter in the 150 ppm group, 8 foetuses / 1 litter in the 220/150 ppm group). The study did not identify test-item related effects on fetal body weight, fetal external observation and visceral and skeletal abnormalities/variations.

Wistar dams that survived in the group treated to 220/150 ppm presented a slightly incidence of clinical signs (e.g. piloerection, chromodacryorrhea, dirty fur). Females treated to 150 ppm did not show difference in clinical signs, body weight, body weight gain, food consumption and macroscopic findings from controls. Mean corporea lutea, implantation sites and pre and post-implantation loss were similar in treated and control groups. The study did not identify test-item related effects on fetal body weight, fetal external observation and visceral and skeletal abnormalities/variations.

Overall, this study did not identify exencephalies as concern raised from the previous preliminary prenatal developmental studies. However, this study is not adequate to detect potential developmental toxicity due to major deficiencies: very few foetuses available, foetuses from both treated groups were pooled for pathological examination analysis, no characterisation of dose-response relationship. In contrast, a high incidence of post-implantations losses was reported in this study.

A main prenatal developmental toxicity study was submitted by the registrants in 2020 (Unpublished study report, 2020b). Fischer 344 pregnant females (24/group) were exposed nose-only to isophorone (as vapour) at concentrations of 17, 53 or 150 ppm (corresponding to 100, 300, 850 mg/m^3) from gestation day (GD) 5 to 19, 6 hours per day. In addition, were included a pregnancy reference group with not exposed animals in order to identify possible findings related to the procedure itself and a control group exposed to filtered compressed fresh air 6 hours/day from GD5-19.

No mortality of dams occurred during the course of the study. Clinical signs related to nose-only procedure were reported among groups (e.g. chromorhinorrhea, dacryorrhea, wet fur, piloerection). In addition, loss of stability was observed particularly in the high dose group, in 11 animals affected on day 5 of exposure. The incidence of this effect clearly regressed from day 9 of exposure until the end of the study (between 0-2 dams affected). Bleeding of the vagina was observed from GD12-16 with a higher number of females affected in the high dose group (max. 13 females affected on GD15; 1-3 occurrences in a same female mostly on consecutive days). At 150 ppm, there was transient reduction of body weight (approximately -3.5% compared to control group; only statistically significant on day 17 of exposure) and body weight gain (from GD14-17; not statistically significant). However, adjusted body weight was similar among groups. Food consumption was decreased at 150 ppm between day 5 to 8 of exposure (approximately -15%). Serum levels of total T3 was elevated in medium and high dose groups (3.52 ng/ml and 3.73 ng/ml, respectively, versus 3.05 ng/ml in control group), with great variability in a same group. There was no effect on T4 and TSH values and not correlated effects on weight and/or histopathology of the thyroid. Laboratory reported that the values were comparable to historical controls (however, these controls were not available to eMSCA). Uterus weight was slightly (but not significantly) reduced (about 52 g in the medium and high dose groups versus 54.5 g in the control group). There was also a slight (not statistically significant) increase of post-implantation loss in the medium and high dose groups (9.37% and 15.82%, respectively, versus 6.13% in the control group). Two total resorptions was found at 150 ppm. Laboratory reported that the values were comparable to historical controls (however, these controls were not available to eMSCA). By consequence, the percentage of live foetuses was: 93.87%, 94.83%, 90.63%, 84.18% (for control group, low, medium and high dose groups). There was no effect on sex ratio and anogenital distance of foetuses. No statistical differences were found in litter weight, although a decrease of 9% was observed at 150 ppm. Fetal body weight per litter was significantly reduced in all exposed groups (< 5% at 17 and 53 ppm and at 13.6% at 150 ppm). At external examination, mostly hematoma reported. Some variations were reported at higher incidence in exposed groups at visceral examination (e.g. thymus long, testis malpositioned, kidney (adrenal absent) and/or uterer absent) and at skeletal examination (e.g. incomplete ossification of supraoccipital bone, non-ossified caudal vertebrae,

incidence of sternebrae offset ossification sites). According to the study report, there was no test-item related effect on external, visceral and skeletal abnormalities or variations.

Based on this study (Unpublished study report, 2020b), there was no exencephaly reported. However, it should be noted that the doses were not adequately chosen since there was no sign of maternotoxicity up to the highest tested dose.

Overall conclusion: Exencephalies occurred in rats and mice at 150 ppm (861 mg/m^3) of isophorone in old studies. No similar findings were reported at doses up to 150 ppm in the range-finding and main studies submitted. Therefore, it can be considered that the concern is clarified. In contrast, the two recent studies reported some increases of post-implantation loss and lower pup body weight in Fischer rats.
In-depth analysis of the EOGRTS can allow whether to confirm the relevance of this effect.

7.9.6.3. **Justification for classification or non classification**

Some developmental effects were reported in the studies (post-implantation loss, decreased foetal/pup body weight), which may require classification. However, analysis of the expected EOGRTS is needed before drawing any firm conclusion on toxicity for fertility and development.

7.9.7. Hazard assessment of physico-chemical properties

Not evaluated.

7.9.8. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

7.9.8.1. **For workers**

Dermal systemic effects – long term

No reliable dermal toxicity study was available for DNEL derivation. In this context, a route to route extrapolation to convert oral NOAEL in rat to an equivalent dermal NOAEL was applied by the registrants. In this context, they have proposed the following DNEL derivation:

NOAEL = 102.5 mg/kg bw/day based on decreased body weight (90 day oral study; rats).

Oral absorption = 100% and dermal absorption = 5%

Corrected dermal NOAEL = 102.5 x 100/5 = 2050 mg/kg bw/day

Assessment factors:

- Intraspecies differences: 5
- Interspecies differences: 10 (4 x 2.5)
- Subchronic – chronic extrapolation: 2

DNEL set by the registrants = 20.5 mg/kg bw/day.

The eMSCA notes that a lower NOAEL is available from the OECD TG 421 study (NOAEL = 100 mg/kg bw/day for parental animals). Moreover, the dermal absorption was recalculated and should be considered at 6% instead of 5%.

This would lead to a DNEL of 16.7 mg/kg bw/day.

It has also to be noted that the point of departure for DNEL derivation may be modified depending on the results of the EOGRTS.

Dermal systemic effects - acute

According to R8 guidance document, DNEL for acute toxicity should be derived if an acute toxicity hazard (leading to C&L) has been identified and there is a potential for high peak exposures. Isophorone fulfils criteria for Acute Tox 4 – H332.

An acute DNEL of 41 mg/mg/kg bw (2x DNEL long term) was derived by the registrants, considering that this dose does not lead to systemic dermal effects in the acute dermal studies (without further justification).

The eMSCA notes that there is no adequate study for deriving an acute dermal DNEL. When looking at the effects reported in the oral repeated dose toxicity studies and considering that this acute DNEL corresponds to 2x long term DNEL, the registrant's approach seems sufficiently conservative. Anyway, considering the R8 guidance document, acute DNEL is more relevant for inhalation than for dermal route. Moreover, no acute risk assessment was provided in the CSR for dermal exposure.

Inhalation systemic and local effects – long term

The registrants have proposed the current MAK value of 11 mg/m^3 (2 ppm) for long term systemic and local DNEL.

They consider this value in line with other DNEL values derived from inhalation studies and presented in the registration dossiers. Different points of departure were considered, the lowest being the LOAEC of 208 mg/m3 from a 4 week study (Unpublished study report, 1968b) where isophorone induced reduced body weights, decreased liver weight and haematological changes. The corrected LOAEC was set at 104.52 mg/m^3 (208 x 6/8 x 6.7, according to R8 guidance). The selected overall assessment factor was 37.5 (2.5 for interspecies difference, 5 for intraspecies difference and 3 for LOAEC/NOAEC).

The DNEL would thus be 2.78 mg/m^3 according to the registration dossiers.

The eMSCA notes the following:

- There is a certain inconsistency in the approach taken by the registrants regarding the DNELs by inhalation. Indeed, in contrast to the DNEL for workers, the DNEL for general population was based on this LOAEC of 208 mg/m^3.
- A DNEL derived for workers from the LOAEC of 208 mg/m^3 results in a lower value than the MAK value (2.78 versus 11 mg/m^3). However, the effects reported at the LOAEC of 208 mg/m^3 were very slight, leading the ATSDR (1989) to consider this value as a NOAEC. In addition, several methodological limitations were noted in this study which was assigned with a Klimisch score of 3 by the eMSCA.
- Most of the inhalation studies were only performed with one concentration and/or did not report adverse effects. Some information can be bring by the prenatal developmental toxicity studies suggesting that the LOAEC of 208 mg/m^3 may be conservative: e.g. NOAEC of 300 mg/m^3 (based on a decreased body weight < 10% at 640 mg/m^3) from Unpublished study report (1984b) or NOAEC of 850 mg/m3 from the recent Unpublished study report (2020b) study (based on no adverse effect).
- Another option could have been to derive the inhalatory DNEL from oral studies using route to route extrapolation.

Overall, the eMSCA notes that the MAK value seems in line with available human data: after 1 month, workers exposed to 39-46 mg/m^3 (5-8 ppm) complained of malaise; complaints ceased when concentrations were decreased to 6-23 mg/m^3 (1-4 ppm) (Unpublished study report, 1973).

Inhalation systemic and local effects - acute

According to R8 guidance document, DNEL for acute toxicity should be derived if an acute toxicity hazard (leading to C&L) has been identified and there is a potential for high peak exposures. Isophorone is classified as STOT SE 3 (irritation for respiratory tract). High peaks of exposure can be anticipated considering the volatility of isophorone and the use pattern (spray application).

An acute DNEL of 22 mg/m^3 (2x DNEL long term) was derived by the registrants, considering that local irritation was only reported for higher concentrations.

The eMSCA notes that there is no detailed justification on the choice of the factor of 2 in the registration dossiers. Remarks raised on the long term DNEL regarding the experimental dataset by inhalation also apply here.

However, the eMSCA notes that the DNEL set by the registrants seems conservative considering human data: throat irritation was reported at ≥ 199 mg/m^3 after exposure to isophorone for a few minutes, with a NOAEL reported at 100 mg/m^3 (Unpublished study report, 1965a; Smyth and Seaton, 1940). In a further investigation, nose and throat irritations were reported in volunteers exposed to isophorone vapours for 15 minutes (Silverman et al., 1946). 40 % of the exposed subjects objected isophorone odour at a concentration of 58 mg/m^3 (10 ppm), and this concentration was judged to be the highest tolerable air level for 8 hour exposure.

7.9.8.2. **For general population**

Inhalation systemic and local effects – acute and long term

The registrants set a DNEL of 0.7 mg/m^3 based on the LOAEC of 208 mg/m^3 from a 4 week study (Exxon, 1968) where isophorone induced reduced body weights, decreased liver weight and haematological changes.

Corrected LOAEC = LOAEC x 6/24 = 52 mg/m^3.

Selected assessment factors:

- Intraspecies differences: 10
- Interspecies differences: 2.5
- Subacute – chronic extrapolation: 1 (other observed LOAEC values from studies of longer duration were > 208 mg/m^3)
- LOAEC/NOAEC = 3

Remarks raised by the eMSCA on inhalatory DNEL for workers also apply here.

Dermal route of exposure

No DNEL was set by the registrants considering that isophorone is not intended for consumer use and that a general population DNEL for dermal route is not relevant for humans exposed via the environment.

Oral systemic effects – acute and long term

The lregistrants have proposed the following DNEL derivation:

NOAEL = 102.5 mg/kg bw/day based on decreased body weight (90 day oral study; rats).

Selected assessment factors:

- Intraspecies differences: 10
- Interspecies differences: 10 (4 x 2.5)
- Subchronic – chronic extrapolation: 2

DNEL set by the registrants = 0.51 mg/kg bw/day.

The eMSCA notes that a lower NOAEL is available from the OECD TG 421 study (NOAEL = 100 mg/kg bw/day for parental animals). However, this does not impact significantly the DNEL (0.5 versus 0.51 mg/kg bw/day). It has also to be noted that the point of departure for DNEL derivation may be modified depending on the results of the EOGRTS.

7.9.9. Conclusions of the human health hazard assessment and related classification and labelling

According to CLP Regulation the Substance is currently classified as:
- Acute Tox 4* - H302/312,
- Eye Irrit. 2 – H319,
- STOT SE 3 – H335 and
- Carc. 2 – H351.

The above harmonised classification is consistent with available data.

However, in addition :

- For acute toxicity, the current minimal classification Acute Tox 4* can be modified to Acute Tox. 4;
- a classification as STOT SE 3 – H336 seems justified based on experimental and human data;
- No classification is needed for repeated dose toxicity and mutagenicity;
- The ongoing EOGRTS is needed to conclude firmly on effects on reproduction and development.

7.10. Assessment of endocrine disrupting (ED) properties

7.10.1. Endocrine disruption – Environment

7.10.1.1. Fish Short –term Reproduction assay (OECD TG 229) – Level 3

The test was conducted under flow-through conditions with fathead minnows (*Pimephales promelas*). The study met the requirements for test validity in accordance with the OECD 229 (RI 1). Fish were exposed to isophorone at nominal concentrations of 0 (control), 0.8, 8.0 and 80 mg/L. During the exposure, measured isophorone concentrations ranged from 88.6 to 103 % of nominal concentrations. The maximum tolerated concentration (MTC) of 80 mg/L corresponds to the mean of 2 endpoints. The first endpoint is equal to 1/3 of the arithmetic mean of all acute values available (LC_{50}) for fathead minnows (82 mg/L). The second endpoint of 79 mg/L is based on chronic toxicity of isophorone to early life stage fathead minnows and where a NOEC and LOEC for survival are reported (56 and 112 mg/L).

The eMSCA consider that the MTC may be overestimated. The NOEC value (mortality) could have been used, corresponding to a concentration causing < 10% mortality.

Table 20a. Summary of the Fish Short –term Reproduction assay

Method	Results	Remarks	Reference
Fish short-term reproduction assay using the fathead minnow, pimephales promelas OPPTS 890.1350 (2009) - OECD 229 (2009) Nom. Conc.= 0.8, 8.0 and 80 mg/L Meas. Conc. = 0.748, 7.62, 79.8 mg/L	No significant difference in mortality, GSI and SCC. Signs of toxicity at 80 mg/L. In females at 80 mg/L: -significant decrease in fecundity, weight, VTG - significant oocyte atresia	Effect at 80 mg/L could be interpreted to be secondary to the overt general toxicity	Unpublished study report, 2011j

Biological Results

Mortality, Behavior and Appearance

No fish mortality was observed during the exposure; however, signs of generalized toxicity were observed at the high treatment level of 80 mg isophorone/L such as a marked reduction in feeding and the appearance of emaciation in some male fish.

Fecundity and Fertility

Median fecundity values showed a monotonic decrease with increasing concentration of isophorone. Only statistically significant at the highest dose level of 80 mg/L isophorone.

Weight

- ✓ *Male*: No monotonic dose response with increasing concentrations of isophorone. The Dunnett's test was statistically significant (α = 0.05) for the difference between the control and 80 mg/L treatment group.
- ✓ *Female*: a monotonic decrease with increasing concentration of isophorone was reported. Only statistically significant at the highest dose level of 80 mg/L isophorone.

Gonado-somatic Index (GSI)

No change in median GSI values for both male and female fish is observed.

Secondary Sex Characteristics (Tubercle Score)

Tubercle score was not found to be significantly different among control and isophorone-exposed fish.

Vitellogenin

- ✓ *Male*: The median male VTG data as measured by ELISA did not exhibit a monotonic response with increasing isophorone concentration. No significant differences in VTG among the treatment groups.
- ✓ *Female*: Median female VTG data showed a monotonic decrease with increasing concentration of isophorone. Only statistically significant at the highest dose level of 80 mg/L isophorone.

Gonad Histopathology

Female fathead minnows exposed to 80 mg/L isophorone had increased incidence of mild to moderate (grades 2 and 3) oocyte atresia as compared to controls.

In addition, the incidence of ovaries containing a preponderance of pre-vitellogenic oocytes, i.e. stage 1 ovaries (predominantly composed of perinucleolar and cortical alveolar oocytes) was also increased in female fathead minnows exposed to 80 mg/L, likely reflecting the loss of mature vitellogenic oocytes.

7.10.1.2. **Amphibian Metamorphosis Assay (OECD TG 231) – Level 3**

The test was conducted under flow-through conditions on amphibian metamorphosis of *Xenopus laevis*. The study met the requirements for test validity in accordance with the OECD guideline (RI 1). Amphibian larvae at stage 51 were exposed to isophorone at nominal concentrations of 0 (control), 0.6, 6.0 and 60 mg/L. During the exposure, measured isophorone concentrations in the test vessels ranged from 88.7 to 106% of nominal concentrations. The maximum tolerated concentration (MTC) of 60 mg/L corresponds to 1/3 of a 96h-LC_{50} of 182 mg/L for *Xenopus laevis* tadpoles (study conducted in the framework of this AMA assay as literature search revealed no pre-existing isophorone toxicity studies conducted with amphibians).

Table 20b. Summary of the Amphibian Metamorphosis Assay

Method	Results	Remarks	Reference
Amphibian metamorphosis assay with Xenopus laevis OPPTS 890.110 (2009) - OECD 231 (2009) Nom. Conc.= 0.6, 6.0 and 60 mg/L	No significant difference in mortality, behaviour, appearance, wet weight, HLL, development stage and thyroid histopathology. SVL slightly but significantly shorter for 60 mg/L on day 21 only.	The decrease of SLV indicates some generalized toxicity	Unpublished study report (2011k)

Biological Results

Mortality, Behavior and Appearance

No abnormal behaviors were noted among control or isophorone exposed tadpoles. No statistically significant differences in tadpole mortality between the various treatment groups and controls.

Wet weight

- ✓ On day 7 of exposure, mean wet weights among control and isophorone exposed tadpoles were not statistically different.
- ✓ On day 21 of exposure, median wet weights among control and isophorone exposed tadpoles were not statistically different.

Snout-Vent Length (SVL)

- ✓ On day 7 of the exposure, SVL among control and isophorone exposed tadpoles were not statistically different.
- ✓ On day 21 of the exposure, tadpoles in the 60 mg/L isophorone treatment group were found to have SVL that were statistically shorter than controls (with a difference of 1.3226 mm, the minimum significant difference for the Dunnett's test was 1.2553 mm).

Hind Limb Length (HLL)

- ✓ On day 7 of the exposure, HLL among control and isophorone exposed tadpoles were not statistically different.
- ✓ On day 21 of the exposure, HLL among control and isophorone exposed tadpoles were not statistically different.

Table 21: Mean ± SD Wet weight, SVL, and normalized HLL of *X. laevis* exposed to Isophorone for 21 days (Median Values in Parentheses)[a]

Mean Measured Isophorone Concentration (mg/L)	N[b]	Wet weight (g)	SVL (mm)	Mean HLL[c]
< LLQ[d] (Control)	4	2.00 ± 0.221 (1.98)	26.6 ± 0.856 (26.7)	0.681 ± 0.019 (0.673)
0.584	4	1.94 ± 0.161 (1.96)	26.3 ± 0.449 (26.4)	0.684 ± 0.071 (0.721)
5.63	4	1.94 ± 0.121 (1.95)	26.4 ± 0.743 (26.7)	0.706 ± 0.051 (0.719)
59.0	4	1.70 ± 0.106 (1.73)	25.3* ± 0.514 (25.4)	0.710± 0.053 (0.711)

[a] LLQ (lowest level quantified) = 0.199 mg $_{isophorone}$/L
b Indicates number of replicate test vessels per treatment group, not total number of tadpoles
c Hind limb length is normalized by snout-vent length
d LLQ (lowest level quantified) = 0.199 mg Isophorone/L
* indicates a significant difference from controls (Dunnett's test)

Developmental Stage

- ✓ There was no significant difference in developmental stage among day 7 control and isophorone-exposed tadpoles (range of NF = 53-55, 70% of tadpoles at NF = 54).
- ✓ There was no significant difference in developmental stage among day 21 control and isophorone-exposed tadpoles (range of NF = 56-62, ,USEPA guideline with NF ≥ 57).

Table 22: Stage distribution of *X. laevis* exposed to Isophorone for 21 days

Mean Measured Isophorone Concentration (mg/L)	Stage 56	Stage 57	Stage 58	Stage 59	Stage 60	Stage 61	Stage 62
< LLQ[a] (Control)	0	7	16	10	19	5	3
0.584	2	9	12	10	23	3	2
5.63	0	8	10	13	22	4	2
59.0	1	4	7	17	20	5	6

[a] LLQ (lowest level quantified) = 0.199 mg $_{isophorone}$/L

Thyroid Histopathology

Compared to thyroid glands from controls, there were no significant histopathological effects observed among thyroid glands from isophorone exposed tadpoles.

7.10.1.3. **Summary –Environment**

Table 23: Summary of assay investigating endocrine disrupting properties on fish and amphibians

Method	Results	Remarks
Fish short-term reproduction assay using the fathead minnow, *pimephales promelas* OPPTS 890.1350 (2009) - OECD 229 (2009) Nom. Conc.= 0.8, 8.0 and 80 mg/L	No significant difference in mortality, GSI and SCC. Signs of toxicity at 80 mg/L. In females at 80 mg/L: -significant decrease in fecundity, weight, VTG - significant oocyte atresia	Effect at 80 mg/L could be interpreted to be secondary to the overt general toxicity
Amphibian metamorphosis assay with *Xenopus laevis* OPPTS 890.110 (2009) - OECD 231 (2009) Nom. Conc.= 0.6, 6.0 and 60 mg/L	No significant difference in mortality, behaviour, appearance, wet weight, HLL, development stage and thyroid histopathology. SVL slightly but significantly shorter for 60 mg/L on day 21 only.	The decrease of SLV indicates some generalized toxicity

7.10.2. Endocrine disruption - Human health

Isophorone was listed on the Endocrine Disruptor Screening Program (EDSP) of US-EPA. Tier 1 *in vitro* and *in vivo* studies were performed. The submission of the study reports was required in the Substance Evaluation Decision (2015) and obtained in 2016.

In an *in vitro* androgen receptor binding assay performed according to US EPA OPPTS 890.1150 guideline, the potential binding of isophorone to the androgen receptor (AR) was evaluated (Unpublished study report (2011a)). The rat prostate cytosol was used as a source of AR without further purification. The assay consisted of two sets of experiments: a saturation binding experiment and a competitive binding experiment. Each experiment (saturation and competitive binding) consisted of three runs and each run contained three

replicates at each concentration, ranging from 10^{-10} to 10^{-3} M. Isophorone had no significant effect on specific binding of the [^{3}H]-ligand. A logIC_{50} cannot be calculated since isophorone did not displace 50% of the radioligand at any test concentration. Based on these results, isophorone can be considered as non-binder to AR *in vitro*.

Isophorone was tested in an *in vitro* human recombinant aromatase assay according to US EPA OPPTS 890.1200 guideline (Unpublished study report (2011b)). Four independent runs were performed with concentrations ranging from 10^{-10} to 10^{-3} M. Run #1 did not fulfill validity criteria and thus was not included in the interpretation of the study. The average aromatase inhibition was 20% at the highest dose of isophorone tested, while no effect was seen at any other concentrations. Based on these results, isophorone can be considered as non-inhibitor of aromatase activity *in vitro*.

Isophorone was tested in an *in vitro* estrogen receptor transcriptional activation assay in human cell line hERα-HELA-990 according to US EPA OPPTS 890.1300 guideline (Unpublished study report (2011c)). This test identifies chemicals that bind to and activate the estrogen receptor α (ERα). Three independent runs were performed with concentrations ranging from 10^{-9} to 10^{-3} M. Based on the RPCmax (maximum relative percentage of positive control) < 10%, isophorone was considered negative for ERα-mediated agonism in this test system.

In an *in vitro* estrogen receptor binding assay according to US EPA OPPTS 890.1250 guideline, potential of isophorone to displace a known synthetic radiolabeled ligand (^{3}H-17β-estradiol) from the estrogenic receptor was assessed (Unpublished study report (2011d)). Cytosol from SD rats uterus was used as the source of receptor. The competitive binding assays consisted in 3 independent repetitions performed in triplicate at each concentration, ranging from 10^{-10} to 10^{-3} M. Isophorone had no significant effect on specific binding of the [^{3}H]-ligand. A logIC_{50} cannot be calculated since isophorone did not displace 50% of the radioligand at any test concentration. Based on these results, it was not observed an interaction of isophorone with estrogen receptors *in vitro*.

In an *in vitro* steroidogenesis assay performed according to US EPA OPPTS 890.1550 guideline (Unpublished study report (2011e)), the potential positive or negative effect of isophorone on testosterone and estradiol production was assessed. In 5 independent runs, H295R cells were incubated with isophorone at concentrations ranging from 10^{-10} to 10^{-4} M (or solvent or positive control) for 48 hours. Only 3 assays fulfilled the quality criteria and were used to analyze the eventual effects of isophorone. Isophorone did not affect testosterone production at any concentration. Slightly decreased estradiol levels (0.9-fold and 0.8-fold) were observed at 2 mid-level in one run and a slight increase (1.2-fold) was noted at the highest concentration in another run. These punctual changes in estradiol production are of questionable biological relevance and do not support a positive response to isophorone. Therefore, isophorone, even at high concentration did not alter testosterone and estradiol productions, under the conditions of this study.

In an uterotrophic assay performed according to OECD TG 440, ovariectomized female SD rats (6/group) were exposed via subcutaneous (sc) injection to isophorone at dose levels of 0 (untreated control and vehicle control), 5, 15 or 50 mg/kg bw/day for 3 consecutive days (Unpublished study report (2011f)). On test day 4, animals were weighted, euthanized and the uteri were excised and weighted before and after blotting. All animals survived and no clinical signs of toxicity was observed in treated groups. Isophorone had no effect on body weight but body weight gain was statistically significantly decreased at the highest tested dose (- 32%). No treatment-related increase in mean uterine weight was found. In contrast, positive control induced expected response. Therefore, there is no indication of estrogenicity at doses ≤ 50 mg/kg bw/day (sc injection) in this test system for isophorone.

In a Hershberger assay performed according to OECD TG 441, isophorone was administered for 10 consecutive days via oral gavage to castrated male SD rats (7/group) at dose levels of 0, 100, 400 or 800 mg/kg bw/day to test androgenic activity (Unpublished study report (2011g)). To screen anti-androgenic activity, additional groups of 7 castrated SD rats received isophorone by oral gavage at the same dose levels for 10 days in conjunction with a daily subcutaneous injection of testosterone propionate (TP) at 0.4 mg/kg/day. Contrary to the uterotrophic assay, isophorone was administered by oral route. However, according to OECD guideline, subcutaneous injection is recommended to

model inhalation or dermal absorption (relevant routes of exposure for isophorone). The choice of the high dose level was based on a dose range finding assay showing high toxicity at 1000 mg/kg bw/day. Animals were euthanized approximately 24 hours after the final dose administration. The five androgen-dependent tissues, adrenals, kidneys and liver were weighted and macroscopically examined. Two animals died on test day 5 due to gavage error in the 800 mg/kg bw/day dose group without TP. There was no significant effect on body weight and body weight gain at any dose of isophorone with and without TP. Absolute liver weights were significantly increased in both androgenic and anti-androgenic portions of the study by 27-28% and 32-34% at 400 and 800 mg/kg bw/day of isophorone, respectively. There was no significant treatment-related effect on accessory sex tissues (seminal vesicles, ventral prostate, levator ani-bulbocavernosus muscle, Cowper's glands or glans penis), adrenal or kidney weights at any dose of isophorone. Positive effects were observed as expected with positive controls for both androgenic and anti-androgenic portions of the study. Coefficient variations (CV) meet the performance criteria except the %CV levels for ventral prostate at 800 mg/kg bw/day (55.2% versus 45% in the OECD guideline) in the androgen agonist portion of the study. Isophorone was negative for androgenicity and anti-androgenicity in this Hershberger assay at doses ≤ 800 mg/kg bw/day (oral gavage).

In a female pubertal assay performed according to US EPA OPPTS 890.1450 guideline, SD rats/dose group (16/group) were treated daily via oral gavage with isophorone in corn oil at doses of 0, 50, 200 or 800 mg/kg bw/day from post-natal day (PND) 22 to 42 (Unpublished study report (2011h)). The choice of the high dose level was based on a dose range finding assay showing high toxicity at 1000 mg/kg bw/day. However, the second dose is lower than recommended in the guideline ("*second dose should be one half of the highest dose level*") although no justification was provided. One animal in control group and one at 200 mg/kg bw/day died prior to study termination. One animal at 50 mg/kg bw/day was removed from study due to accidental injury. No treatment-related effect was observed on clinical signs and body weight over the dosing period. Mean age and body weight at vaginal opening were similar across groups. There was no difference in mean age at first vaginal estrous and in mean cycle length. The percent cycling (86% versus 100%) and percent of regularly cycling animals (73.3% versus 93.8%) was decreased at the highest dose but the differences were not statistically significant. According to the study report: *Given the minimal difference in estrous cycles, the lack of significance, the inherent variability in estrous cycle initiation in peripubertal female rats and the lack of effect on other endocrine-sensitive endpoint in this study, effects on cycle were deemed incidental and unrelated to treatment*. There was no effect on serum T4 or TSH level at any dose of isophorone. Various changes in biochemical parameters were noted. There were a significant decrease in serum urea nitrogen (UN) at all doses tested (-17%, -25%, -25%) and a significant increase in creatinine levels (+200%, +500%) in animals given 200 and 800 mg/kg bw/day. According to the study report, this finding was judged as a false positive since UN and creatinine show opposite trends, there was no histopathological correlate and the Jaffe method used is known to be subject to interferences from different classes of compounds including ketones. At 800 mg/kg bw/day, chloride was significantly reduced (but still within expected biological ranges), ALP levels was significantly decreased (-22%) and cholesterol was significantly elevated (+14%). Organ weight analysis revealed increased absolute (+18%), adjusted to PND22 body weight (+17%) and relative to terminal body weight (+16%) liver weights. Relative adrenal weights were statistically identified by ANOVA analysis but subsequent Dunnett's test failed to identify significant differences. There was no other significant treatment-related effects on organ weights at any doses of isophorone (kidney, ovaries, uterus, thyroid and pituitary). Gross necropsy and histopathology on uterus, ovaries, kidneys or thyroid did not show any effect related to isophorone-treatment. All mean and CV values for control animals met the performance criteria with the exception of the CV for liver weight (13.29 versus 13.13 specified in test guideline). There was no evidence of endocrine activity for isophorone in the female pubertal assay at doses up to 800 mg/kg bw/day.

In a male pubertal assay performed according to US EPA OPPTS 890.1500 guideline, 16 SD rats per dose group were treated daily via oral gavage with isophorone in corn oil at doses of 0, 50, 200 or 800 mg/kg bw/day from post-natal day (PND) 23 to 53 (Unpublished study report (2011i)). The choice of the high dose level was based on a dose range finding assay showing high toxicity at 1000 mg/kg bw/day. However, the second dose is lower than

recommended in the guideline ("*second dose should be one half of the highest dose level*") although no justification was provided. No treatment-related effects were observed on mortality, clinical signs and body weight. The mean age at preputial separation was slightly delayed at 800 mg/kg bw/day when data were adjusted for PND 23 body weights (+ 1.8 days). In contrast, unadjusted and absolute difference in age at preputial separation (+ 1.7 days) were not statistically significantly modified. There was no significant difference in body weight at preputial separation. There was a dose-related decrease in serum testosterone levels across isophorone doses (-28%, -41%, -58%). Only the increase at 800 mg/kg bw/day was identified as statistically significant. There was no treatment-related effects on serum T4 or TSH at any dose of isophorone. Significant changes (similar as those reported in the female pubertal assay) were observed at biochemistry: reduced chloride (at all doses but values still within expected biological ranges), dose-dependent decrease of ALP levels (-15%, -18%, -25%), decreased urea nitrogen (UN) (-25% and -42% at 200 and 800 mg/kg bw/day, respectively) and increase in creatinine (+ 400% at 800 mg/kg bw/day). There was a significant increase in absolute (31.7%), adjusted (31.5%) and relative (32.7%) liver weights at 800 mg/kg bw/day. These 3 parameters were also significantly increased (16-20%) at 200 mg/kg bw/day. At 50 mg/kg bw/day, only the relative liver weight was significantly increased (6.9%). At 800 mg/kg bw/day, absolute and adjusted seminal vesicle weights (plus coagulating glands, with or without fluid) were significantly decreased (24.3-25.6% less than control). There were no other significant treatment-related effects on organ weights at any dose of isophorone (kidneys, adrenals, testes, epididymides, thyroid, pituitary, ventral prostate, dorsolateral prostate, LABC). Gross necropsy and histopathology on testis, epididymis, kidneys or thyroid did not show any effect related to isophorone-treatment. Results from this study were generally within acceptable ranges for mean and coefficient of variation (CV) performance criteria as specified for control animals in the test guideline. Some deviations were noted but they can be considered to have little or no impact on the outcome of the study. In summary, based on these results, the study report exclude an effect of isophorone on thyroid development and/or function because statistical analysis did not demonstrate significant differences in both serum hormonal levels and on thyroid histology. However, there was a trend to an increase in follicular cell height and a reduction in colloid area. In addition, although it was not statistically significant, the mean serum TSH concentrations was increased by around 30% in 50 and 200 mg/kg/day groups. Furthermore, mean values of thyroid weight in controls did not met the performance criteria outlined in the test guideline (12.4 mg compared to 14 to 26 mg as acceptable range values). Lastly, CV in TSH level (64.7) was higher than the top acceptable range (58.3). Taken together, there are some doubts when the authors of the study claim that isophorone does not act on thyroid development and/or function. Regarding evaluation of the reproductive sphere, there was anti-androgenic effect of isophorone at 800 mg/kg bw/day.

7.10.3. Conclusion on endocrine disrupting properties (combined/ separate)

Environment

Endocrine disrupting properties of Isophorone was not an initial concern of the evaluation dossier. Regarding the current dataset for isophorone provided on dashboard EPA, QSARs models do not indicate a concern and isophorone is considered as negative in all *in vitro* assays investigating endocrine activity. In addition, two OECD CF level 3 assays was provided in the dossier. A Fish short-term reproduction assay OECD TG 229 with EAS specific endpoints and a Amphibian metamorphosis assay with *Xenopus laevis* OECD TG 231 with T specific endpoints. Results of these two tests do not demonstrate endocrine disruption by Isophorone on non target organisms.

Human Health

No endocrine disruptor potential was raised in several assays (steroidogenesis assay, estrogen receptor transactivation assay, androgen and estrogen binding assays, aromatase enzyme activity assay, uterotrophic assay, Hershberger assay and female pubertal assay). However, in the male pubertal assay, there was an anti-androgenic effect (decreased serum testosterone levels, decreased seminal vesicle weights, mean adjusted age at

preputial separation slightly delayed) at the high dose of 800 mg/kg bw/day. Moreover, an absence of endocrine disruption of the thyroid cannot be excluded from this study. Assessment of the endocrine properties of isophorone should be reviewed in a RMOA in light of the upcoming EOGRTS results.

7.11. PBT and VPVB assessment

7.11.1. Persistence

The biodegradability of the test item was studied in a "DOC-Die-Away Test" in accordance with EU method C4 -A. 95% of the test item degraded within this period and >60% in the 10 day-window.

Considering this data, the substance does not fulfill the P criteria.

7.11.2. Bioaccumulation

The bioaccumulation potential of isophorone was evaluated in a bioaccumulation study with bluegill sunfish (*Lepomis macrochirus*) performed according to accepted scientific principles. The BCF after 14 days of exposure was calculated to be 7 l/kg with a half-life (derived from a 7-day depuration phase) <1 day. Moreover, the potential for bioaccumulation can be estimated from the value of the n-octanol/water partition, log Kow (1.67). A BCF calculated value of 6.1 l/kg is given using Episuite.

Considering these data and a Log Kow < 4.5, the substance does not fulfill the B criteria.

7.11.3. Toxicity

Based on acute effects data, none L(E)C_{50} values to aquatic organisms are less than 0.1 mg/L (LC_{50} value for Daphnia = 120 mg/l and LC_{50} value for Fish = 140 mg/l). Consequently the screening criterion for toxicity is not fulfilled.

Based on chronic effects data, none NOEC values to aquatic organisms are less than 0.01 mg/L (with the lowest NOEC value of 8.9 mg/l for fish).

Furthermore the substance is not classified as STOT RE, carcinogenic or mutagenic 1A/1B or toxic for reproduction 1A/1B/2 according to CLP.

Considering these data, the substance does not fulfill the T criteria.

7.11.4. Overall conclusion

None of the P, B and T criteria are currently fulfilled. Therefore, the substance can be regarded as being not PBT/vPvB.

7.12. Exposure assessment

7.12.1. Human health

7.12.1.1. Worker

In the Decision on Substance Evaluation, the following request related to worker exposure was made :

- Perform a refined exposure assessment for PROC 11 using adequate models

This request related in particular to the inadequate use of ECETOC TRA for predicting vapour phase exposure and for agrochemical uses.

eMSCA conclusion:

Process categories and conditions of use are detailed in a confidential annex. Some remarks can be made on the exposure estimation for the agrochemical use:

- It is anticipated that operators should be exposed to higher levels of isophorone indoor than outdoor. Moreover, different methods of application, including hand-held spray equipment for high-level targets which is expected to be associated with higher

exposure than vehicle-mounted boom sprayer. Considering these elements, it seems that the scenario considering vehicle-mounted boom sprayer outdoor is not the most conservative one.

- As noted above, the eMSCA considers that the value for dermal absorption should be 6% based on the available dermal absorption study on isophorone. Moreover, it is generally recognised that dermal absorption of diluted product is higher than for concentrate. Thus, in the absence of dermal absorption study for diluted product, default value should be used according to EFSA guidance on dermal absorption (2017).

7.12.1.2. **Consumer**

No consumer use.

7.12.2. Environment

Environmental exposure assessment was carried out for the following emission scenarios:

- Manufacture of Isophorone (ES 1):
- Formulation of of product (Coatings and Paints, Thinners, paint removers, plant Protection Products, washing and Cleaning Products) (ES 2);
- Use at industrial sites – Use as intermediate (ES 3);
- Use at industrial sites - Use in Coatings (ES 4);
- Use at industrial sites - Cleaning agents (ES 5);
- Widespread use by professional workers - Use as a co-formulant in plant protection products, spray applications by professionals (ES 6-7).

7.13. Risk characterisation

7.13.1. Human health

In the Decision on Substance Evaluation, the following requests related to risk characterisation for human health were made :

- Perform an acute risk assessment

An acute risk assessment was required considering the classification of isophorone and the fact that high peaks of exposure can be anticipated considering the volatility of isophorone and the use pattern (spray application).

- Provide a risk assessment for bystanders and residents for agrochemical uses
- Provide a risk assessment for secondary exposure after coating and cleaning uses.

This risk assessment was required since isophorone is used in coating and cleaning agents in sector of use "SU22 public domain".

The RCR as calculated by the lead registrant are summarized in the confidential annex I.

eMSCA conclusion:

Considering the eMSCA comments on the DNELs set by the registrants and on the exposure estimations, no unacceptable risk is anticipated based on the scenarios presented in the lead registration dossier. This may change if the EOGRTS results impact the DNELs.

7.13.2. Environment

Environmental risk assessment shows acceptable risk for Manufacture of Isophorone (ES 1) considering the Risk mitigation measures listed below:

- No application of the STP sludge on agricultural soil (incineration)

Environmental risk assessment shows a contamination of groundwater (predicted values)

above the maximum allowable concentration of 0.1 µg/L given for pesticides by the Drinking Water Directive 98/83/EC for the following uses:

- Formulation of of product (Coatings and Paints, Thinners, paint removers, plant Protection Products, washing and Cleaning Products) (ES 2);
- Use at industrial sites – Use as intermediate (ES 3);
- Use at industrial sites - Use in Coatings (ES 4);
- Use at industrial sites - Cleaning agents (ES 5);
- Widespread use by professional workers - Use as a co-formulant in plant protection products, spray applications by professionals (ES 6-7).

It indicates a potential to contaminate drinking water and a possible risk on human health via drinking water.

Risk assessment for human health via drinking water can also be impacted by the results of the awaited EOGRTS studies and will be further characterised in a RMOA to follow.

7.14. References

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7.15. Abbreviations

AC	Article category
ACGIH	American Conference of Governmental Industrial Hygienists
ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail [French agency for food, environmental and occupational health & safety]
ATSDR	Agency for toxic substances and disease registry
BW	Body weight
CA	Chromosomal aberration
CCH	Compliance check
CHL	Chinese hamster lung cells
CHO	Chinese hamster ovary cells
CLP	Classification, labelling, packaging
CMR	Carcinogen, Mutagen, toxic for Reproduction
CoRAP	Community rolling action plan
CV	Coefficient variation
DD	Draft decision
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNEL	Derived no effect level
ED	Endocrine disrupting
EOGRTS	Extended one-generation reproductive toxicity study
ERC	Environmental release categories
eMSCA	evaluating Member state competent authority
EPM	Equilibrium partitioning method
GD	Gestation day
GLP	Good laboratory practice
IARC	International Agency for Research on Cancer
IP	intraperitoneal
JS	Joint submission
LABC	Levator Ani/bulbocavernosus
LD	Lactation day
LD50	Median lethal dose
LLNA	Local lymph node assay
LOAEL/C	Low observed adverse effect level/concentration
MF	Mutation frequency
MMAD	Median mass aerodynamic diameter
MN	Micronucleus
MSCA	Member state competent authority
MTD	Maximum tolerable dose
n.a.	Not available
NCE	Normochromatic erythrocytes
NOAEL/c	No observed adverse effect level/concentration
OECD	Organisation for economic co-operation and development
NTP	National Toxicology Program
PBT	Persistent, bioaccumulative and toxic
PC	Product categories
PCE	Polychromatic erythrocytes
PNEC	Predicted no-effect concentration

PROC	Process categories
QSAR	Quantitative structure-activity relationship
SCE	Sister chromatid exchange
SD	Sprague-Dawley
SLS	Sodium lauryl sulfate
STOT RE	Specific target organ toxicity, repeated-exposure
SU	Sector of end-use
SVHC	Substance of very high concern
TG	Technical guidance
UDS	Unscheduled DNA synthesis
vPvB	Very persistent very bioaccumulative
WHO	World health organisation
WOE	Weight of evidence