CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical names:

a) *p*-cymene; 1-isopropyl-4-methylbenzene

and

b) 3-p-cumenyl-2-methylpropionaldehyde; 2-methyl-3-(4-isopropylphenyl)propanal [1];

3-(p-cumenyl)propionaldehyde; 3-(4-isopropylphenyl)propanal [2];

4-isopropylbenzaldehyde; cuminic aldehyde [3];

4-isopropylbenzoic acid; cuminic acid [4]

EC Numbers:

a) **202-796-7**
b) **203-161-7 [1]; 231-885-3 [2]; 204-516-9 [3]; 208-642-5 [4]**

CAS Numbers:

a) **99-87-6**
b) **103-95-7 [1]; 7775-00-0 [2]; 122-03-2 [3]; 536-66-3 [4]**

Index Numbers:

a) **601-094-00-1**
b) **TBD**

Index Number	Chemical name	EC Number	CAS Number
601-094-00-1	*p*-cymene; 1-isopropyl-4-methylbenzene	202-796-7	99-87-6
TBD	3-p-cumenyl-2-methylpropionaldehyde; 2-methyl-3-(4-isopropylphenyl)propanal [1]; 3-(p-cumenyl)propionaldehyde; 3-(4-isopropylphenyl)propanal [2]; 4-isopropylbenzaldehyde; cuminic aldehyde [3]; 4-isopropylbenzoic acid; cuminic acid [4]	203-161-7 [1]; 231-885-3 [2]; 204-516-9 [3]; 208-642-5 [4]	103-95-7 [1]; 7775-00-0 [2]; 122-03-2 [3]; 536-66-3 [4]

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0 BACKGROUND

The present CLH proposal includes a group of five structurally similar substances; four substances (*p*-cymene, 3-*p*-cumenyl-2-methylpropionaldehyde, 3-(*p*-cumenyl)propionaldehyde and 4-isopropylbenzaldehyde) that are, or are predicted to be metabolised to 4-isopropylbenzoic acid (4-iPBA), and the metabolite 4-iPBA itself. Except for 4-iPBA, all the substances have an indicated use as fragrance.

4-iPBA is not a registered substance under REACH, but it is notified in the CL Inventory. In a recent review, a short term toxicity test on rats exposed to 4-iPBA was described. Testicular toxicity was seen at the highest concentration of 150 mg/kg bw/day, and the observed testicular toxicity is in line with the toxicity demonstrated in studies on substances with related structures (Natsch et al. 2021), including several of the substances included in this proposal (*p*-cymene, 3-*p*-cumenyl-2-methylpropionaldehyde, 3-(*p*-cumenyl)propionaldehyde).

Formation of the metabolite 4-iPBA has been experimentally demonstrated in rats *in vivo* for two substances included in this proposal (*p*-cymene and 3-*p*-cumenyl-2-methylpropionaldehyde) and *in vitro* for two substances (3-*p*-cumenyl-2-methylpropionaldehyde and 3-(*p*-cumenyl)propionaldehyde).

For one substance included in this proposal, 4-isopropylbenzaldehyde, no reproductive toxicity data are available, however, the substance was identified as a precursor to 4-iPBA from a profiling scheme built in the OECD (Q)SAR Toolbox, and it was included in the current group based on its structure. No information on toxicokinetics or reproductive toxicity is thus available for 4-isopropylbenzaldehyde. The substance is registered at low tonnage and for intermediate use in the EU.

The substances included in this proposal are structurally similar to another group of substances forming the metabolite 4-*tert*-butylbenzoic acid, TBBA. The metabolites 4-iPBA and TBBA are only differing by a methyl group at the benzylic carbon (figure 1). A CLH proposal of substances forming the metabolite TBBA has been prepared by the DS in parallel to this proposal. Similar toxicity of the reproductive system is demonstrated for substances in both groups.

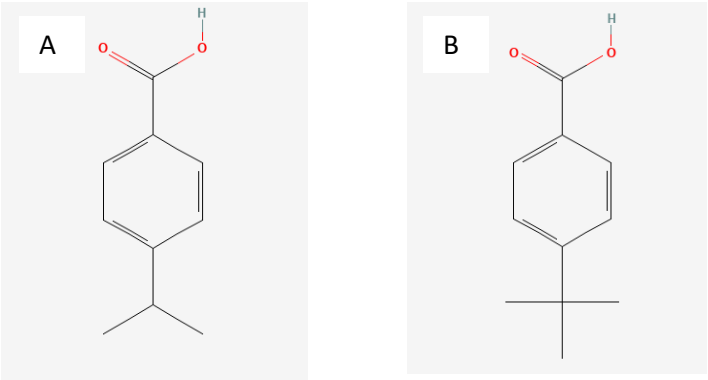


Figure 1. Structures of the metabolites 4-isopropylbenzoic acid (A; 4-iPBA) and 4-*tert*-butylbenzoic acid (B; TBBA).

CONTENTS

0 BACKGROUND ... 3

1 IDENTITY OF THE SUBSTANCES ... 1

1.1 NAME AND OTHER IDENTIFIERS OF THE SUBSTANCES ... 1
1.2 COMPOSITION OF THE SUBSTANCES ... 2

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ... 4

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING ... 7

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL ... 7

5 IDENTIFIED USES ... 7

6 DATA SOURCES ... 7

7 PHYSICOCHEMICAL PROPERTIES ... 8

8 EVALUATION OF PHYSICAL HAZARDS ... 9

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

9.1 SHORT SUMMARY AND OVERALL RELEVANCE OF THE PROVIDED TOXICOKINETIC INFORMATION ON THE PROPOSED CLASSIFICATION ... 12

10 IN THE PRESENT DOSSIER, ADVERSE EFFECTS ON TESTIS AND SPERM ARE INDICATED IN A SECOND SPECIES, I.E., RABBITS (DETAILS ARE GIVEN IN SECTION 10.10), THUS CONTRADICTING THE RAT SPECIFIC MECHANISM OF TOXICITY PROPOSED BY NATSCH AND CO-WORKERS (2021). EVALUATION OF HEALTH HAZARDS ... 13

10.1 ACUTE TOXICITY - ORAL ROUTE ... 19
10.2 ACUTE TOXICITY - DERMAL ROUTE ... 19
10.3 ACUTE TOXICITY - INHALATION ROUTE ... 19
10.4 SKIN CORROSION/IRRITATION ... 19
10.5 SERIOUS EYE DAMAGE/EYE IRRITATION ... 19
10.6 RESPIRATORY SENSITISATION ... 19
10.7 SKIN SENSITISATION ... 19
10.8 GERM CELL MUTAGENICITY ... 19
10.9 CARCINOGENICITY ... 19
10.10 REPRODUCTIVE TOXICITY ... 19
10.10.1 Adverse effects on sexual function and fertility ... 19
10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility ... 32
10.10.3 Comparison with the CLP criteria ... 45
10.10.4 Adverse effects on development ... 46
10.10.5 Short summary and overall relevance of the provided information on adverse effects on development 50
10.10.6 Comparison with the CLP criteria ... 56
10.10.7 Adverse effects on or via lactation ... 57
10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation 60
10.10.9 Comparison with the CLP criteria ... 64
10.10.10 Conclusion on classification and labelling for reproductive toxicity ... 64
10.11 SPECIFIC TARGET ORGAN TOXICITY-SINGLE EXPOSURE ... 64
10.12 ASPIRATION HAZARD ... 64

11 EVALUATION OF ENVIRONMENTAL HAZARDS ... 64

12 EVALUATION OF ADDITIONAL HAZARDS ... 64

13 ADDITIONAL LABELLING ... 64

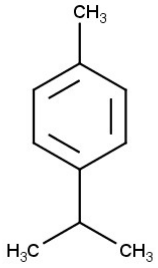
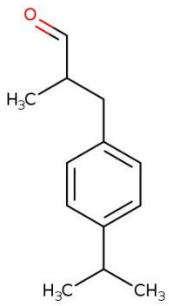
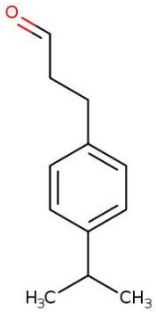
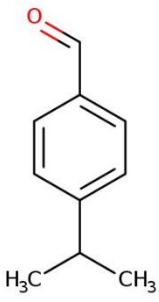
14 REFERENCES ... 64

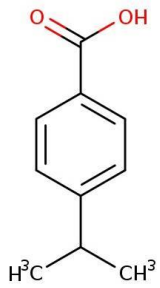
15 ANNEXES ... 65

1 IDENTITY OF THE SUBSTANCES

1.1 Name and other identifiers of the substances

Table 1: Substances identity and information related to molecular and structural formula of the substances

EC No.	CAS No.	Names in the IUPAC nomenclature or other international chemical names	Molecular formula	Structural formula	Molecular weight
202-796-7	99-87-6	*p*-cymene; 1-methyl-4-isopropylbenzene; benzene, 1-methyl-4-(1-methylethyl)-; 4-isopropyltoluene; *p*-isopropyltoluene	C10H14		134.22 g/mol
203-161-7	103-95-7	3-*p*-cumenyl-2-methylpropionaldehyde; 2-methyl-3-[4-(propan-2-yl)phenyl]propanal; benzenepropanal, .alpha.-methyl-4-(1-methylethyl)-; 2-methyl-3-(*p*-isopropylphenyl)propionaldehyde; cyclamal; cyclamen aldehyde; cyclamen aldehyde Extra	C13H18O		190.28 g/mol
231-885-3	7775-00-0	3-(*p*-cumenyl)propionaldehyde; 3-[4-(propan-2-yl)phenyl]propanal; benzenepropanal, 4-(1-methylethyl)-; 3-(4-isopropylphenyl)propanal; cuminylacetaldehyde; cyclemax	C12H16O		176.25 g/mol
204-516-9	122-03-2	4-isopropylbenzaldehyde; benzaldehyde, 4-(1-methylethyl)-; 4-(propan-2-yl)benzaldehyde; cuminaldehyde; cuminic aldehyde	C10H12O		148.20 g/mol

208-642-5	536-66-3	4-isopropylbenzoic acid; benzoic acid, 4-(1-methylethyl)-; 4-(propan-2-yl)benzoic acid; cuminic acid; cumic acid	C10H12O2		164.20 g/mol

1.2 Composition of the substances

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3 (CLP)**	**Current self-classification and labelling (CLP)**
p-cymene EC number: 202-796-7 CAS number: 99-87-6	CONFIDENTIAL	Flam. Liq. 3 H226 Acute Tox. 3 H331 Asp. Tox. 1 H301 Aquatic Chronic 2 H411	Flam. Liq.3 H226 Repr. 2 H361 Acute Tox. 3 H331 Acute Tox. 4 H302 Acute Tox. 4 H312 Acute Tox. 4 H332 Asp.Tox. 1 H304 STOT SE 3 H335 Skin Irrit. 2 H315 Eye Irrit. 2 H319 Skin Sens. 1A H317 Aquatic Chronic 1 H410 Aquatic Chronic 2 H411 Aquatic Chronic 3 H412 Not Classified
3-*p*-cumenyl-2-methylpropionaldehyde EC number: 203-161-7 CAS number: 103-95-7	CONFIDENTIAL	Not included in Annex VI	Repr. 2 H361 Skin Irrit. 2 H315 Eye Irrit. 2 H319 Skin Sens. 1H317 Skin Sens. 1B H317 Aquatic Acute 1 H400 Aquatic Chronic 1 H410 Aquatic Chronic 2 H411 Aquatic Chronic 3 H412 Not Classified
3-(*p*-cumenyl)propionaldehyde EC number: 231-885-3 CAS number: 7775-00-0	CONFIDENTIAL	Not included in Annex VI	Repr. 2 H361 Skin Irrit. 2 H315 Skin Sens. 1 H317 Skin Sens. 1B H317 Aquatic Acute 1 H400 Not Classified
4-isopropylbenzaldehyde EC number: 204-516-9 CAS number: 122-03-2	CONFIDENTIAL	Not included in Annex VI	Flam. Liq. 3 H226 Acute Tox. 4 H302 Asp. Tox. 1 H304 STOT SE 3 H335 Skin Irrit. 2 H315 Eye Irrit. 2 H319 Skin Sens. 1, 1B H317 Aquatic Chronic 2 H411

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3 (CLP)**	**Current self-classification and labelling (CLP)**
4-isopropylbenzoic acid EC number: 208-642-5 CAS number: 536-66-3	-	Not included in Annex VI	Acute Tox. 4 H302 Acute Tox. 4 H312 Acute Tox. 4 H332 Skin Irrit. 2 H315 Eye Irrit. 2 H319 Eye Irrit. 2A H319 STOT SE 3 H335

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

Table 3a: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Codes	Hazard statement Codes	Pictogram, Signal Word Codes	Hazard statement Codes	Suppl. Hazard statement Codes		
Current Annex VI entry	601-094-00-1	*p*-cymene; 1-isopropyl-4-methylbenzene	202-796-7	99-87-6	Flam. Liq. 3 Acute Tox. 3 Asp. Tox. 1 Aquatic Chronic 2	H226 H331 H304 H411	GHS02 GHS06 GHS08 GHS09 Dgr	H226 H331 H304 H411		inhalation: ATE = 3 mg/l (vapours)	
Dossier submitters proposal	601-094-00-1	*p*-cymene; 1-isopropyl-4-methylbenzene	202-796-7	99-87-6	**Add** Repr. 1B	**Add** H360FD		**Add** H360FD			**Add** **xxx**
Resulting Annex VI entry if agreed by RAC and COM	601-094-00-1	*p*-cymene; 1-isopropyl-4-methylbenzene	202-796-7	99-87-6	Flam. Liq. 3 Repr. 1B Acute Tox. 3 Asp. Tox. 1Aquatic Chronic 2	H226 H360FD H331 H304 H411	GHS02 GHS06 GHS08 GHS09 Dgr	H226 H360FD H331 H304 H411		inhalation: ATE = 3 mg/l (vapours)	**xxx**

Table 3b: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Codes	Hazard statement Codes	Pictogram, Signal Word Codes	Hazard statement Codes	Suppl. Hazard statement Codes		

Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	3-*p*-cumenyl-2-methylpropionaldehyde; 2-methyl-3-(4-isopropylphenyl)propanal [1]; 3-(*p*-cumenyl)propionaldehyde; 3-(4-isopropylphenyl)propanal [2]; 4-isopropylbenzaldehyde; cuminic aldehyde [3]; 4-isopropylbenzoic acid; cuminic acid [4]	203-161-7 [1]; 231-885-3 [2]; 204-516-9 [3]; 208-642-5 [4]	103-95-7 [1]; 7775-00-0 [2]; 122-03-2 [3]; 536-66-3 [4]	Repr. 1B	H360FD	GHS08 Dgr	H360FD			xxx
Resulting Annex VI entry if agreed by RAC and COM	TBD	3-*p*-cumenyl-2-methylpropionaldehyde; 2-methyl-3-(4-isopropylphenyl)propanal [1]; 3-(*p*-cumenyl)propionaldehyde; 3-(4-isopropylphenyl)propanal [2]; 4-isopropylbenzaldehyde; cuminic aldehyde [3]; 4-isopropylbenzoic acid; cuminic acid [4]	203-161-7 [1]; 231-885-3 [2]; 204-516-9 [3]; 208-642-5 [4]	103-95-7 [1]; 7775-00-0 [2]; 122-03-2 [3]; 536-66-3 [4]	Repr. 1B	H360FD	GHS08 Dgr	H360FD			xxx

Note xxx: The classification of mixtures as reproductive toxicant is necessary if the sum of the concentrations of individual substances, forming the same metabolite, in a mixture as placed on the market is equal to, or above, 0.3%.

Table 4: Reason for not proposing harmonised classification and status under consultation

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

Hazard class	Reason for no classification	Within the scope of consultation
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Hazard class not assessed in this dossier	No
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The substances, 3-*p*-cumenyl-2-methylpropionaldehyde, 3-(*p*-cumenyl)propionaldehyde, 4-isopropylbenzaldehyde and 4-isopropylbenzoic acid (4-iPBA) have no previous harmonised classification and labelling.

The substance *p*-cymene was added to CLP Annex VI on 11 March 2021 (RAC opinion from 15 March 2019). Current harmonised classification of *p*-cymene include Flam. Liq. 3 Acute Tox. 3, Asp. Tox. 1 and Aquatic Chronic 2. Reproductive toxicity has not previously been evaluated for harmonised classification. The DS proposes to add Repr. 1B (H360FD) to the existing classification.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level. All substances covered by this proposal are considered to fulfil the criteria for classification as toxic to reproduction (Repr. 1B, H360FD). Therefore, a harmonised classification is justified according to Article 36(1)(d) of the CLP Regulation.

5 IDENTIFIED USES

Based on information from the REACH registrations, the Spin[1] and PubChem[2] databases, four substances included in this proposal are used as fragrances/perfumes. No information on uses is found for 4-isopropylbenzoic acid (4-iPBA).

The substance 3-*p*-cumenyl-2-methylpropionaldehyde is used as a fragrance in a wide range of products. The uses include cosmetics, cleaning and washing agents, polishes and wax blends, rinsing agents for textiles, personal care products (deodorants), air care products, etc. Similar uses are indicated for 3-(*p*-cumenyl)propionaldehyde, *p*-cymene and 4-isopropylbenzaldehyde. *p*-cymene is also one of the ingredients of the active substance terpenoid blend QRD460. The terpenoid blend, consisting of *p*-cymene, d-limonene and α-terpinene, is approved as an active substance for plant protection products (until 10/08/25). Both *p*-cymene and 4-isopropylbenzaldehyde are additionally registered as intermediates under REACH.

According to the Swedish Products Register[3] there are products containing 3-*p*-cumenyl-2-methylpropionaldehyde, *p*-cymene and 4-isopropylbenzaldehyde at concentrations ranging from 0.00001 to 10%, median range from 0.005% to 0.093%.

6 DATA SOURCES

The registration dossiers in ECHA dissemination site is the main source of information. The full study reports of 3-*p*-cumenyl-2-methylpropionaldehyde (guideline studies according to OECD TG 415, OECD TG 414 and several repeated dose toxicity studies) were available to the dossier submitter.

[1] SPIN | Substances in Preparations in Nordic Countries (spin2000.net)

[2] PubChem (nih.gov)

[3] Products Register - Kemikalieinspektionen

In the registration dossier of *p*-cymene, there is one OECD TG study (TG 422) from 2019 available on the substance itself, which is the main source of information for reproductive toxicity of this substance. An opt-out registrant (JS member) has submitted several studies on reproductive toxicity, which were considered irrelevant by the DS for the purpose of harmonised classification since these additional studies were not conducted on *p*-cymene and no read-across justification was available.

7 PHYSICOCHEMICAL PROPERTIES

Table 5: Summary of physicochemical properties (M=measured, E=estimated)

Property	***p*-cymene**[4,5] **EC: 202-796-7** **CAS: 99-87-6**	**3-*p*-cumenyl-2-methylpropionaldehyde**[6] **EC: 203-161-7** **CAS: 103-95-7**	**3-(*p*-cumenyl)propionaldehyde**[7] **EC: 231-885-3** **CAS: 7775-00-0**	**4-isopropylbenzaldehyde**[8,9] **EC: 204-516-9** **CAS: 122-03-2**	**4-isopropylbenzoic acid**[10] **EC: 208-642-5** **CAS: 536-66-3**
Physical state at 20°C and 101,3 kPa	liquid	liquid	liquid	liquid	solid
Melting/freezing point	1: < - 20 °C (M) 2: -67.9 °C	< -50 °C (M)	<-20°C (M)	1: < -50 °C (M) 2: -50.5 °C (M)	117-118 °C (M)
Boiling point	1: 176 °C (M) 2: 177 °C	234 °C (M)	256.5 °C (M)	1: 242 °C (M) 2: 235 -236 °C	-
Relative density	1: 0.858 (M) 2: 0.86	0.948 (M)	0.966 (M)	1: 0.976 (M) 2: 0.9771 (M)	-
Vapour pressure	1: 211 Pa (20 °C) (M) 2: 145 Pa (20 °C)	0.3 Pa (20 °C) (M)	0.81 Pa (23 °C) (M)	3.5 Pa (20 °C) (M)	-
Surface tension	-	45.9 mN/m (M)	-	54 mN/m (M)	-
Water solubility	1: 15 mg/L (M) 2: nearly insoluble in water.	66 mg/L (M)	87.8 mg/L (M)	1: 243 mg/L (M) 2: < 1 g/L (M)	151 mg/L (M)
Partition coefficient n-octanol/water	1: 4.8 (20 °C) (M) 2: 4.1	3.4 (35 °C) (M)	3.5 (20 °C) (M)	2.8 (35 °C) (M)	3.4 (- °C) (M)

[4] Registration Dossier - ECHA (europa.eu)

[5] Registration Dossier - ECHA (europa.eu)

[6] Registration Dossier - ECHA (europa.eu)

[7] Registration Dossier - ECHA (europa.eu)

[8] Registration Dossier - ECHA (europa.eu)

[9] Registration Dossier - ECHA (europa.eu)

[10] PubChem. 4-Isopropylbenzoic acid | C10H12O2 - PubChem (nih.gov)

Property	*p*-cymene[4,5] EC: 202-796-7 CAS: 99-87-6	3-*p*-cumenyl-2-methylpropionaldehyde[6] EC: 203-161-7 CAS: 103-95-7	3-(*p*-cumenyl)propionaldehyde[7] EC: 231-885-3 CAS: 7775-00-0	4-isopropylbenzaldehyde[8,9] EC: 204-516-9 CAS: 122-03-2	4-isopropylbenzoic acid[10] EC: 208-642-5 CAS: 536-66-3
Flash point	1: 51.5 °C (M) 2: 47 °C	120 °C (M)	104 °C (M)	1: 104 °C (M) 2: 92-98°C	-
Flammability	-	-	-	-	-
Explosive properties	Non explosive (E)	Non explosive (E)	Non explosive (E)	-	-
Self-ignition temperature	1: > 400°C (M) 2: 435 °C	240 °C (M)	264 °C (M)	385 °C (M)	-
Oxidising properties	-	Non oxidising (E)	Non oxidising (E)	-	-
Granulometry	-	-	-	-	-
Stability in organic solvents and identity of relevant degradation products	-	-	-	-	-
Dissociation constant	-	-	-	-	-
Viscosity	1: 0.81 mm²/s (static) (M) 2: 7.1 mm²/s (static)	7.1 mm²/s (static) (M)	4.384 mPa · s (dynamic) (M)	-	-

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this CLH proposal.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 6: Summary table of toxicokinetic studies

Method	Results	Reference
p-cymene EC 202-796-7		
Experimental *in vivo* toxicokinetics study with *p*-cymene. Oral gavage: Male Dunkin-Hartley guinea pigs (n=3) and Wistar rats (n=3). Single dose of 100 mg/kg Inhalation: Male Dunkin-Hartley guinea pigs (n=2) and Wistar rats (n=2). Single dose of 100 mg/kg.	60-80% of the administered dose was recovered in urine in both species via both oral and inhalation exposure pathways. Around 20% (9%) formation of 4-isopropylbenzoic acid (4-iPBA) in rat urine after 48 hours, after oral exposure (inhalation). Only trace levels was detected in guinea pigs at the same timepoint. Conjugation with glycine of the iPBA formed was extensive in guinea-pigs.	Walde et al., 2009. Summarised in the registration dossier. Annex I, section 2.6 (guinea pig) and 2.7 (rat)

Method	Results	Reference
Metabolites were identified in urine (48 hours) by GLC.	The isopropyl group appears to be a site of metabolic activity in both rats and guinea pigs.	
3-*p*-cumenyl-2-methylpropionaldehyde EC 203-161-7		
In vivo repeated dose toxicity study with 3-*p*-cumenyl-2-methylpropionaldehyde. Male rats (n = 5) were treated for 28 days by oral gavage at 0, 30, 100, and 300 mg/kg bw/day. At day 28, blood samples were taken for metabolite analysis. One testis and the liver were snap-frozen in liquid nitrogen for later analysis. Metabolites in blood and tissue were determined by LC-MS.	The levels of 3-*p*-cumenyl-2-methylpropionaldehyde and cyclamen alcohol were below detection limit in all plasma samples including the non-diluted plasma samples. 4-isopropylbenzoic acid (4-iPBA) plasma concentrations were below detection limit in plasma samples collected from the control group and at 30 mg/kg/day. 4-iPBA was detected in all plasma samples at 100 and 300 mg/kg/day (dose-dependent). Cyclamen acid concentrations were below detection limit in plasma samples collected from the control group. Cyclamen acid was detected in all plasma samples of all test item groups (dose-dependent). Plasma levels of cyclamen acid were 27-fold lower (100 mg/kg/day) and 83-fold lower (300 mg/kg/day), respectively, compared to 4-iPBA. Testes and liver: In animals dosed with 30 mg/kg/day, trace amounts 4-iPBA-CoA were detected in the testes of only one individual. At 100 mg/kg/day, the conjugate was detectable at low levels in testes samples from all animals. At 300 mg/kg/day, 5-6 times higher levels than at 100 mg/kg/day were observed. The concentration in the liver was higher (> 500 fold). Metabolites: The key metabolic pathways observed are formation of 4-iPBA and subsequent conjugation mainly with glucuronic acid and glycine and/or hydroxylation and further oxidation. The major metabolite in the tissue samples and in the blood plasma is the acyl-glucuronide conjugate of 4-iPBA. The second most abundant peak is an unknown metabolite. Further abundant metabolites were 4-iPBA, hydroxylated 4-iPBA, hydroxylated 4-iPBA -acylglucuronide and the glycine conjugate of 4-iPBA.	Study report, 2019. Summarised in the registration dossier Reviewed in Laue *et al*., 2020. Annex I, section 2.1
Comparative *in vitro* metabolism study with 3-*p*-cumenyl-2-methylpropionaldehyde. Plated rat, rabbit and human hepatocytes were exposed to 5 or 50 µM of 3-*p*-cumenyl-2-methylpropionaldehyde for 0.5, 4, 8 (only rabbit hepatocytes) and 22 h in triplicate. Coenzyme A	Formation of 4-iPBA-CoA was observed in rabbit hepatocytes. The 4-iPBA-CoA conjugates decreased over the incubation period. At the lower test dose, the conjugates were close to detection limit after 22 h of exposure. Formation of 4-iPBA-CoA conjugates was observed in rat hepatocytes. The conjugate level remained stable over the incubation	Laue *et al*., 2020. Summarised in registration dossier Reviewed in Natsch et al. 2021. Annex I, section 2.3

Method	Results	Reference
conjugates were analysed by LC-HRMS. Phase I and phase II metabolites were determined in rat and human hepatocytes by GC-MS and LC-HRMS at 0.5, 4 and 22 hours of exposure to 50 µM 3-*p*-cumenyl-2-methylpropionaldehyde	period. The conjugates were about 20-fold higher at 22 h of exposure, compared to rabbit hepatocytes. Human hepatocytes exposed to 50 µM 3-*p*-cumenyl-2-methylpropionaldehyde, had lower levels of of 4-iPBA-CoA compared to rat and rabbit hepatocytes, whereas levels were similar compared to rabbit hepatocytes at the lower test concentration. A decrease of the 4-iPBA-CoA level was observed at 22 h. In plated rat hepatocytes, 3-*p*-cumenyl-2-methylpropionaldehyde was rapidly oxidized to the direct carboxylic acid (cyclamen acid) which was completely transformed to 4-iPBA as the major metabolite. The metabolism in human hepatocytes was different, with the reduction of the aldehyde to cyclamen alcohol as the key first phase I metabolite, a lower but more sustained formation of cyclamen acid and around six-fold lower formation of 4-iPBA.	
Comparative *in vitro* metabolism study with 3-*p*-cumenyl-2-methylpropionaldehyde. Cryopreserved primary hepatocytes from mice, rats, rabbits and humans. Incubation with 10 and 100 µM for 0, 1 and 4 hours, in duplicates. Metabolites were analysed with LC-MS. The study was conducted with non-labeled material.	Five main metabolite peaks were observed: the direct oxidation product (cyclamen acid) and several glucuronide conjugates, i.e. the glucuronide of cyclamen alcohol , the glucuronide of a desaturated cyclamen alcohol as well as the glucuronide of a hydroxylated cyclamen alcohol. Cyclamen alcohol itself was not detected by LC-MS. These metabolites occurred at high levels in all four species. In rats, cyclamen acid was also further degraded to 4-isopropylbenzoic acid (4-iPBA). Levels of this metabolite were below detection limit in rabbit, human and mouse hepatocyte incubations.	Natsch *et al*., 2021. Summarised in registration dossier Review of mechanistic and *in vivo* evidence to indicate relevancy to human health. Hence, only part of the publication that is not summarised elsewhere (see Laue et al. 2017: Laue et al. 2020) is given here. Annex I, section 2.4
3-*p*-cumenyl-2-methylpropionaldehyde EC 203-161-7 and 3-(*p*-cumenyl)propionaldehyde EC 231-885-3		
In vitro metabolism study with 3-*p*-cumenyl-2-methylpropionaldehyde and 3-(*p*-cumenyl)propionaldehyde (among other substances). Rat hepatocytes in suspension were incubated in the presence of 100 µM of the test chemicals for 4 h. Benzoic acid derivatives were determined by GC-MS at 0.5, 4 and 22 h. Formation of CoA conjugates in plated rat hepatocytes following 0.5, 4 and 22 hours of exposure to the chemicals at 5 and 50 µM was assessed by LC-HRMS.	3-*p*-cumenyl-2-methylpropionaldehyde and 3-(*p*-cumenyl)propionaldehyde exposure led to formation of *p*-alkyl-benzoic acids, including 4-iPBA, in rat hepatocytes in suspension. High and stable benzoic acid-CoA conjugates were detected in plated hepatocytes exposed to 3-*p*-cumenyl-2-methylpropionaldehyde, 3-(*p*-cumenyl)propionaldehyde and 4-iPBA.	Laue *et al*., 2017. Summarised in registration dossier Reviewed in Natsch et al. 2021. Annex I, section 2.2

Method	Results	Reference
Incubation of rat hepatocytes was also performed with 4-isopropylbenzoic acid (4-iPBA)		
3-(*p*-cumenyl)propionaldehyde EC 231-885-3		
Comparative *in vitro* metabolism of 3-(*p*-cumenyl)propionaldehyde. Hepatocytes from mouse, rat, rabbit and human. Incubations were conducted in triplicate at 1, 10 and 100 µM for 0, 1 and 4 h. Metabolite profiles were determined by HPLC-UV and LC-MS. LDH leakage was used to monitor cell viability after exposure of the test substance. GLP compliant.	A total of eight metabolites were detected, with similar results obtained for each species. Metabolite C6 (glucuronide conjugate of the alcohol) was the most common metabolite in most of the 1 h and 4 h hepatocyte incubations. Metabolite C8 (acid) was observed widely and was the second largest component in most mouse, rabbit and human hepatocyte incubations. Metabolite C5 (glucuronide conjugate) was also detected in most incubations and tended to be the second largest component in rat hepatocyte incubations. Most of the remaining metabolites were present at low levels and/or in a limited number of incubations, although metabolite C4 (hydroxylated substance) was detected in most incubations. 4-isopropylbenzoic acid (4-iPBA) (C7) was observed in mouse hepatocytes (at 100 µM, 1 and 4 hours) <5%, in rat hepatocytes <5% (100 µM, 4 hours), rabbit hepatocytes <5% (100 µM, 4 hours). 4-iPBA was not detected in human hepatocytes.	Study report, 2012. Summarised in the registration dossier Annex I, section 2.5

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

Toxicokinetic studies are available for four of the substances included in the CLH-dossier, i.e., *p*-cymene, 3-*p*-cumenyl-2-methylpropionaldehyde, 3-(*p*-cumenyl)propionaldehyde and 4-isopropylbenzoic acid (4-iPBA). The data is summarised below. No toxicokinetic studies are available for 4-isopropylbenzaldehyde.

From the toxicological studies showing systemic adverse effects after repeated exposure, it can be concluded that *p*-cymene, 3-*p*-cumenyl-2-methylpropionaldehyde, 3-(*p*-cumenyl)propionaldehyde and 4-iPBA are readily taken up *via* oral administration and distributed in the body. There is evidence from studies with guinea pig and rat that *p*-cymene is also taken up via inhalation (Walde *et al*. 2009).

Distribution of 3-*p*-cumenyl-2-methylpropionaldehyde has been demonstrated to liver and testis (Study report, 2019). Moreover, it could be assumed that *p*-cymene, 3-(*p*-cumenyl)propionaldehyde and 4-iPBA and/or metabolites are distributed to testis as demonstrated by observed testicular toxicity following exposure to the substances.

Metabolism of 3-*p*-cumenyl-2-methylpropionaldehyde and 3-(*p*-cumenyl)propionaldehyde to 4-iPBA has been demonstrated in rat hepatocytes *in vitro*. 4-iPBA is also formed by mouse and rabbit hepatocytes exposed to 3-(*p*-cumenyl)propionaldehyde *in vitro*.

Indirectly, 4-iPBA has been identified as a metabolite in mouse, rabbit and human hepatocytes exposed to 3-*p*-cumenyl-2-methylpropionaldehyde and 3-(*p*-cumenyl)propionaldehyde by detection and measurement of 4-iPBA-CoA conjugates (Laue *et al*. 2020). The study also indicated quantitative species differences in the formation of the conjugates after exposure to 3-*p*-cumenyl-2-methylpropionaldehyde, i.e., formation of 4-iPBA-CoA was higher in rats when compared to other rodent or human hepatocytes.

Administration of *p*-cymene and 3-*p*-cumenyl-2-methylpropionaldehyde has been shown to form 4-iPBA *in vivo* in rats (*p*-cymene and 3-*p*-cumenyl-2-methylpropionaldehyde) and guinea pigs (*p*-cymene) (Study report 2019; Walde *et al*. 2009).

Excretion has not been extensively studied within this group of substances. An oral *in vivo* study with *p*-cymene where 60-80% of the administered dose was recovered in urine in rats and guinea pigs *via* both oral and inhalation exposure pathways indicate that the substances are at least in part eliminated *via* the kidneys (Walde *et al*. 2009).

A mechanism of action for the testicular- and spermatotoxicity caused by the substances comprised in the present dossier was proposed by authors employed at a fragrance company (Natsch et al. 2021). It is hypothesised that the accumulation of stable 4-iPBA-coenzyme A (CoA) conjugates interferes with the metabolism of key lipids required for spermatogenesis, by e.g. depleting the pool of free CoA. *In vitro* experiments with 3-*p*-cumenyl-2-methylpropionaldehyde have demonstrated higher and stable levels of 4-iPBA-CoA conjugates in rat hepatocytes compared to rabbit and human hepatocytes. Therefore, the 4-iPBA-CoA conjugates are stated to be primarily formed in rats. This indicates, according to study authors, that the toxicity of 4-iPBA and hence substances forming 4-iPBA, is not relevant to humans.

Relevance to humans was discussed in the RAC opinion of the structurally similar substance 2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral) (ECHA, 2019), in which a similar mechanism of action was proposed by the DS (BASF SE) based on formation of 4-*tert*-butylbenzoic acid (TBBA) and stable CoA-TBBA conjugates. The species differences demonstrated *in vitro* were considered by RAC as quantitative rather than qualitative and hence that the proposed mode of action, although plausible, was not sufficient to preclude relevance for humans.

In the present dossier, adverse effects on testis and sperm are indicated in a second species, i.e., rabbits (details are given in section 10.10), thus contradicting the rat specific mechanism of toxicity proposed by Natsch and co-workers (2021).

10 EVALUATION OF HEALTH HAZARDS

Read-across Justification

Category approach - Chemical grouping

The substances included in the proposal are grouped in a category for the purpose of harmonised classification. For four of the substances in the category (*p*-cymene, 3-*p*-cumenyl-2-methylpropionaldehyde, 3-(*p*-cumenyl)propionaldehyde, 4-isopropylbenzoic acid (4-iPBA)) there are substance-specific data available on reproductive toxicity, however, for two of the substances (3-(*p*-cumenyl)propionaldehyde and 4-iPBA) the studies available are considered being of limited quality or relevance for the purpose of harmonised classification as stand-alone. For one of the substances, 4-isopropylbenzaldehyde, there are no reproductive toxicity studies available. The entire database across all substances, however, is a convincing literature demonstrating adverse effects on reproduction by structurally similar substances.

To support the data for individual substances for the purpose of harmonized classification, a chemical grouping approach was utilized. The method of chemical categories or grouping is supported in REACH Article 13 - *Information on intrinsic properties of substances may be generated by means other than tests, provided that the conditions set out in Annex XI are met. In particular for human toxicity, information shall be generated whenever possible by means other than vertebrate animal tests, through the use of alternative methods, for example, in vitro methods or qualitative or quantitative structure-activity relationship models or from information from structurally related substances (grouping or read-across).*

The REACH Guidance document on grouping of chemicals complies with the OECD principles for the validation of Chemical grouping and recommends a stepwise procedure to the formation of chemical categories. The reporting format is described below.

Identification of a structure-based category and its members

The substance 4-isopropylbenzoic acid (4-iPBA) is formed during the metabolism of other substances, some which are known fragrances.

The basis for grouping includes the following:

- structural similarity (benzene ring with a substituent that can degrade to a carboxylic acid group and an isopropyl group in *para* position) and;
- experimentally demonstrated or modelled formation of the metabolite 4-iPBA.

The members of the proposed category are slightly structurally dissimilar by the different substituents on the benzene ring. Common to all group members, however, is that the substituent can degrade into a carboxylic acid, thus forming 4-iPBA. Additionally, one member of the group is 4-iPBA (the metabolite) itself.

4-iPBA is structurally similar to *tert*-butylbenzoic acid (TBBA), which is known to cause toxic effects in male reproductive organs. TBBA has harmonised classification as Repr. 1B H360F (ATP 03 of CLP). Substances with similar structures have been demonstrated to metabolise to TBBA and 4-iPBA and thereby cause reproductive toxicity (Laue et al. 2017, Laue et al. 2020, Natsch et al. 2021). The available data on 4-iPBA is limited, but demonstrates similar toxicity on male reproductive organs as other, structurally similar substances, including TBBA.

The individual REACH registrants of the substances included in this category do not use read-across in this manner, but the DS considers this an appropriate approach since the available data for some of the substances in the group is lacking or is not sufficiently robust on its own, and read-across is considered justified.

Figure 2. Category members.

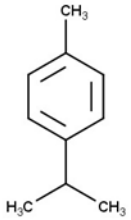
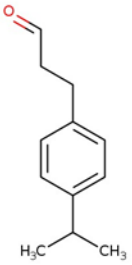
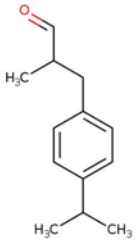
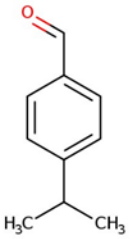
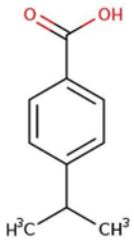
<table>
<tr><td>*p*-cymene
EC 202-796-7
  </td><td>3-(*p*-cumenyl)propionaldehyde
EC 231-885-3
  </td></tr>
<tr><td>3-*p*-cumenyl-2-methylpropionaldehyd
EC 203-161-7
  </td><td>4-isopropylbenzaldehyde
EC 204-516-9
  </td></tr>
<tr><td colspan="2">4-isopropylbenzoic acic
EC 208-642-5
  </td></tr>
</table>

Table 7: Data matrix.

Chemical name	*p*-cymene	3-*p*-cumenyl-2-methylpropionaldehyde	3-(*p*-cumenyl)propionaldehyde	4-isopropylbenzaldehyde	4-isopropylbenzoic acid (4-iPBA)
CAS	99-87-6	103-95-7	7775-00-0	122-03-2	536-66-3
EC	202-796-7	203-161-7	231-885-3	204-516-9	208-642-5
PHYSICO-CHEMICAL DATA					
Molecular weight	134.22	190.28	176.25	148.20	164.20
Melting Point	1: < - 20 °C 2: -67.9 °C	<-50 °C	<-20°C	1: < -50 °C 2: -50.5 °C	117-118 °C
Boiling Point	1: 176 °C 2: 177 °C	234 °C	257 °C	1: 242 °C 2: 235 -236 °C	-
Density	1: 0.858 2: 0.86	0.948	0.966	1: 0.976 2: 0.9771	-
Vapour Pressure	1: 211 Pa (20 °C) 2: 145 Pa (20 °C)	0.3 Pa (20 °C)	0.81 Pa (23 °C)	3.5 Pa (20 °C)	-
Partition Coefficient (log Kow)	1: 4.8 (20 °C) 2: 4.1	3.4 (35 °C)	3.5 (20 °C)	2.8 (35 °C)	3.4 (-)
Water Solubility	1: 15 mg/L 2: nearly insoluble in water.	66 mg/L	87.8 mg/L	1: 243 mg/L 2: < 1000 mg/L	151 mg/L
TOXICOKINETICS					
Data available on 4-iPBA formation *in vitro*	no	yes	yes	no	NA
Data available on 4-iPBA formation *in vivo*	yes	yes	no	no	NA
MAMMALIAN TOXICITY					
Reproductive toxicity - Fertility	LOAEL: 100-150 mg/kg bw/day (male rats reproductive organs)	LOAEL: 75-120 (male rats reproductive organs)	LOAEL: 75 mg/kg bw/day (male rats reproductive organs) LOAEL: 300 mg/kg bw/day (male rabbits reproductive organs)	-	LOAEL: 150 mg/kg bw/day (male rats reproductive organs)
Reproductive toxicity - Development	LOAEL: 100 mg/kg bw/day (pups weight)	LOAEL: 75-150 mg/kg bw/day (pups and fetal weight)	-	-	-

Reporting format for the category

1.1 Category definition

This category covers the substance 4-isopropylbenzoic acid (4-iPBA) and precursor substances that metabolise into 4-iPBA.

1.1.a Category hypothesis

The selected category members have similar structures, physicochemical, biological and (repro)toxicological properties that would be expected to behave in a predictably similar manner across the defined category spectrum. Reproductive toxicity is an intrinsic hazard of all the category members and read-across can be performed to fill data gaps of reproductive toxicity where data is lacking or not sufficiently robust.

1.1.b Applicability domain of the category

The category applies to the substance 4-isopropylbenzoic acid (4-iPBA) and substances that metabolise to 4-iPBA.

Criterion for selection of substances was primarily the structural similarity (a benzene ring with a substituent that can degrade to a carboxylic acid group and an isopropyl group in *para* position). Secondly, experimentally demonstrated or predicted formation of the metabolite 4-iPBA.

One member of the group is 4-iPBA, the metabolite itself. The four additional members of this category consist of a benzene ring with a substituent that can degrade to a carboxylic acid group and an isopropyl group in *para* position. The substituent differs between group members but has in common the formation into a carboxylic acid group.

1.1.1c. List of endpoints covered

For the purpose of harmonized classification and labelling the category approach was applied to the endpoint reproductive toxicity.

1.2 Category Members

Category members are four substances (*p*-cymene, 3-*p*-cumenyl-2-methylpropionaldehyde, 3-(*p*-cumenyl)propionaldehyde and 4-isopropylbenzaldehyde) that can form the metabolite 4-isopropylbenzoic acid (4-iPBA), and 4-iPBA itself. See figure 2.

1.3 Purity/impurities

The information regarding impurities is reported as confidential for all members of the group (see confidential Annex), except for 4-isopropylbenzoic acid (4-iPBA) which is not a registered substance under REACH (and no information on impurities is available).

2 Category justification

The category includes four substances that metabolise into 4-isopropylbenzoic acid (4-iPBA), and 4-iPBA itself.

Based on the output from a profiling scheme built in the OECD (Q)SAR Toolbox identifying precursors of 4-iPBA, three substances with a structure predicted to metabolise to 4-iPBA were identified:

p-cymene (EC 202-796-7)
3-(*p*-cumenyl)propionaldehyde (EC 231-885-3),
4-isopropylbenzaldehyde (EC 204-516-9).

The substance *p*-cymene is a predicted precursor to 4-iPBA. The simulated metabolism indicates other concomitant metabolic pathways, which gives an uncertainty as to whether 4-iPBA is the main metabolite. The formation of 4-iPBA has, however, been demonstrated experimentally *in vivo.*

The substance 3-(*p*-cumenyl)propionaldehyde is a predicted precursor to 4-iPBA and formation of the metabolite has also been demonstrated *in vitro*.

There are no experimental toxicokinetics data available for the substance 4-isopropylbenzaldehyde, but simulation demonstrates that 4-iPBA is a main metabolite of this substance, which is also indicated by its chemical structure, i.e., an aldehyde that can be oxidised to carboxylic acid.

For the substance 3-*p*-cumenyl-2-methylpropionaldehyde, formation of 4-iPBA was not predicted by (Q)SAR Toolbox, however, the metabolite has been demonstrated experimentally both *in vitro* and *in vivo*.

For four of the substances, *p*-cymene EC 202-796-7, 3-*p*-cumenyl-2-methylpropionaldehyde EC 203-161-7, 3-(*p*-cumenyl)propionaldehyde EC 231-885-3 and 4-iPBA EC 208-642-5, their similar reprotoxicological profile (section 10.10) supports the formation of the group. No reprotoxicological data is available for 4-isopropylbenzaldehyde (EC 204-516-9). Additional support for this grouping/category includes similar physicochemical and toxicological data (see further the data matrix, Table 7).

3 Data matrix

The data matrix is constructed with some category endpoints versus members (Table 7). Data for physicochemical properties are included in the matrix, and information on 4-isopropylbenzoic acid (4-iPBA) formation, as well as reproductive toxicity studies are presented to indicate similar adverse effects on reproductive organs of the category members.

A more comprehensive review of fertility and developmental toxicity studies of the group members can be found in Section 10.10.

4 Conclusions per endpoint for classification and labelling

Based on available data across members of this category, similar systemic effects can be predicted. The available data on reproductive toxicity across the category members are in line, and reproductive toxicity is an intrinsic hazard of the category members. Hence, read-across can be performed to fill data gaps of reproductive toxicity where data is lacking or not sufficiently robust. The substances for which most data are available are *p*-cymene and 3-*p*-cumenyl-2-methylpropionaldehyde, while the information available for 3-(*p*-cumenyl)propionaldehyde and 4-isopropylbenzoic acid (4-iPBA) is more limited. No mammalian reproduction toxicity data is available for 4-isopropylbenzaldehyde. The available database permits an assessment of the reproductive toxicity of this category of substances.

Acute toxicity

10.1 Acute toxicity - oral route

Not evaluated in this CLH proposal.

10.2 Acute toxicity - dermal route

Not evaluated in this CLH proposal.

10.3 Acute toxicity - inhalation route

Not evaluated in this CLH proposal.

10.4 Skin corrosion/irritation

Not evaluated in this CLH proposal.

10.5 Serious eye damage/eye irritation

Not evaluated in this CLH proposal.

10.6 Respiratory sensitisation

Not evaluated in this CLH proposal.

10.7 Skin sensitisation

Not evaluated in this CLH proposal.

10.8 Germ cell mutagenicity

Not evaluated in this CLH proposal.

10.9 Carcinogenicity

Not evaluated in this CLH proposal.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 8: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
p-cymene EC 202-796-7			
OECD TG 422 screening for reproductive/developmental toxicity	*p*-cymene EC 202-796-7 Purity: assumed	***Parental generation*** **Mortality and Clinical Observations** *Females*	Study report, 2019. Robust

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
study. GLP compliant Rats Sprague Dawley Crl:CD®(SD). 10 animals/sex/group. Assigned reliability 1 by the Registrant (The information on statistical significances is not clear from the data provided).	100% Vehicle: corn oil The substance was administered via oral gavage at doses 0, 50, 100, 200 mg/kg bw/day once daily. Duration of treatment: P0 males: 2 weeks pre-cohabitation, during cohabitation (up to 2 weeks) and continuing during post-cohabitation until the day prior to termination (approximately 35 days). P0 females: 2 weeks pre-cohabitation, cohabitation (up to 2 weeks) and during gestation and lactation continuing until LD 13 (approximately 63 days	1, 1, 6, 9 animals at 0, 50, 100 and 200 mg/kg bw/day, respectively, were euthanized on GD 25 due to failure to become pregnant. One female at 200 mg/kg bw/day was euthanized for welfare reasons on GD 24 (not pregnant). **Body weights** No effects on body weights and body weight changes. In females at 200 mg/kg bw/day body weights could not be evaluated during gestation and lactation because none of the females were pregnant. **Behaviour** *Males* ↓ hindlimb grip strength at 200 mg/kg bw/day week 5 (-35%; p≤0.05). **Reproductive organs** *Males* ↓ absolute testes (-14%), epididymides (-14%), and levator ani-bulbocavernosus muscle (-14%) weights at 200 mg/kg bw/day. Possible substance-related differences in seminal vesicles and prostate (-22% and -24%, respectively, at 200 mg/kg bw/day but no dose-response). Two males had unilateral or bilateral small testis at 200 mg/kg bw/day, which correlated with microscopic findings of germ cell degeneration/depletion (7 animals vs. none of controls). One of these males had a small prostate. One additional male at 200 mg/kg bw/day had a small levator ani-bulbocavernosus muscle complex. Seminiferous tubular atrophy and reduced sperm in the epididymis at 50 mg/kg bw/day (1/10) and at 100 mg/kg bw/day (1/10). Sperm retention in testis at 100 mg/kg bw/day (7 males vs. none of controls). Two males with decreased sperm in the epididymis. Germ cell degeneration/depletion and sperm retention in testis at 200 mg/kg bw/day (in 7, 2 and 9 animals, respectively, vs. none of controls). Ten males with decreased sperm in the epididymis (vs none of controls). *Females* ↑ animals with at least one irregular cycle (cycles < 4 days or > 5 days duration) during the	study summary in Registration dossier, ECHA's dissemination site, 2022. Study 8 Annex I 3.10.1.8

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		pre-cohabitation period at 200 mg/kg bw/day. **Fertility** ↓ pregnant females at 100 and 200 mg/kg bw/day. Fertility index 90%, 90%, 40% and 0% at 0, 50, 100 and 200 mg/kg bw/day, respectively. ↑ pre-implantation loss at 50 mg/kg bw/day (5.9% vs. 1.8% in controls) and at 100 mg/kg bw/day (7.1% vs 1.8% in controls). **Thyroid Analysis** *Males* ↓ T4 values at 100 and 200 mg/kg bw/day (-56% to -37%). The majority of animals TSH values were below the level of detection in males at all doses and in females at 50 and 100 mg/kg bw/day. **Organ weights and histopathology** *Males* ↑ absolute and relative liver weight at 100 mg/kg bw/day (relative only; +8%,) and at 200 mg/kg bw/day (absolute only; +27% and +41%; respectively). Hepatocellular hypertrophy at 200 mg/kg bw/day (2/5). *Females* ↑ absolute and relative liver weight at 100 mg/kg bw/day (+26% and +22%, respectively). No organs weights were taken at 200 mg/kg bw/day as all females were euthanized prior to termination due to non-pregnancy. Hepatocellular hypertrophy at 50 mg/kg bw/day (1/6 females), in 2/5 males and at 200 mg/kg bw/day (2/5 males and in 1/10 females). NOAEL: 50 mg/kg bw/day (male reproduction). ***F1 generation*** **Clinical observations** 9, 9 and 4 litters appeared normal for most of the observation period at 0, 50 and 100 mg/kg bw/day, respectively. No offspring was produced at 200 mg/kg bw/day. ↓ live birth index at 100 mg/kg bw/day (94.3% vs. 100% in controls). ↓ post-implantation survival index at 100 mg/kg	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		bw/day (87.3% vs. 95% in controls). ↓ litters with less than 100% viability at 100 mg/kg bw/day (1 of 4 litters with 100% viability vs. 9 of 9 in controls). ↓ pup weight on PND 1 at 100 mg/kg bw/day dose group (-10% in males and -9% in females). NOAEL: 50 mg/kg bw/day (developmental toxicity)	
Short-term repeated dose toxicity (similar to OECD TG 407 Repeated Dose Oral Toxicity) Crl:CD(SD) IGS rats, males and females, 3 animals/sex/dose Assigned reliability 2 by the Registrant Not GLP compliant No information whether results reported were statistically significant compared to controls.	*p*-cymene EC 202-796-7 Purity: no information Vehicle: corn oil The substance was administered via oral gavage at doses 0, 50, 150 and 500 mg/kg bw/day daily for 14 consecutive days.	**Mortality and clinical observations** One female at 500 mg/kg bw/day was sacrificed on Day 13 due to welfare reasons. No other clinical signs were observed. **Body weight and food consumption** *Males* ↓ terminal body weight at 500 mg/kg bw/day (-12%). *Females* ↓ terminal body weight at 150 mg/kg bw/day (-9%) and at 500 mg/kg bw/day (-14%). ↓ food consumption at 500 mg/kg bw/day. **Organ weights** *Males* ↑ liver at 150 (+29%) and 500 (+50%) mg/kg bw/day. ↓ spleen at 500 mg/kg bw/day (-27%). *Females* ↑ liver at 150 (+29%) and 500 (+23%) mg/kg bw/day. ↓ spleen at 500 mg/kg bw/day (-22%). **Reproductive organs** *Males* Small epididymides and testes at 150 mg/kg bw/day (1/3 animals). Small, soft testes at 500 mg/kg bw/day (2/3). *Females* Small thymus, uterus and cervix at 500 mg/kg bw/day (1/3). NOAEL: 50 mg/kg bw/day (male reproduction)	Study report, 2018. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 9 Annex I 3.10.1.9
3-*p*-cumenyl-2-methylpropionaldehyde EC 203-161-7			
OECD TG 415 One-	3-*p*-cumenyl-2-	***Parental generation***	Study

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Generation Reproduction Toxicity Study No deviations GLP compliant Sprague Dawley rats, 25 males and females per dose group. Assigned reliability 1 by the Registrant.	methylpropionaldehyde EC 203-161-7 Purity: no information Vehicle: Corn oil Substance was administered orally (gavage) at doses 0, 25, 75, 150 mg/kg bw/day. Male P generation rats were gavaged once daily beginning 83 days prior to cohabitation, through cohabitation, continuing until day before sacrifice. Female P generation rats were gavaged once daily beginning 14 days before cohabitation, through cohabitation and until day 25 of gestation (rats that did not deliver) or day 22 postpartum (rats that delivered a litter). Treated rats were mated with untreated cohorts of male and female rats. F1 generation rats were not directly dosed but may have been exposed to the substance in utero during gestation and through maternal milk postpartum. F1 generation was followed until day 60 postpartum.	**Clinical observations** *Males* ↑ slight or moderate excess salivation at 150 mg/kg bw/day (p≤0.01). **Body weight and food consumption** *Males* ↓ body weight changes at 150 mg/kg bw/day (-10%, day 1- to termination; p≤0.05). ↑ relative food consumption at 150 mg/kg bw/day from day 50 to day 84. *Females* <u>Gestation</u> ↓ body weight at 150 mg/kg bw/day (-5% and -8%, GD 18 and 21, respectively; p≤0.01). <u>Lactation</u> ↓ body weight at 75 mg/kg bw/day (-4%, LD 8; p≤0.05) and at 150 mg/kg bw/day (-7%, -8.5%, -6%, LD 5, 8, 11, respectively; p≤0.01). Effects on body weights correlated with statistically significant lower feed consumption during lactation. **Reproductive organs and function** *Males* ↓ fertility index at 150 mg/kg bw/day (4.3%; p≤0.01) (when treated males mated with untreated females). ↑ white fibrous masses epididymis at 150 mg/kg bw/day (10 vs. 0; p≤0.01). <u>Sperm effects</u> No motile sperm at 150 mg/kg bw/day. No motile sperm in 13 of 25 rats at 75 mg/kg bw/day. ↓ sperm count (-36%) and sperm density (cauda epididymis, -30%) at 75 mg/kg bw/day (p≤0.05). *Females (when treated females were mated with untreated males)* ↓ implantation sites per delivered litter at 150 mg/kg bw/day (-11%; p≤0.01). ↑ dams with all pups dying between days 1 and 5 postpartum at 150 mg/kg bw/day (16.7% vs.	report, 2011a. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. DS had access to full study report Study 1 Annex I 3.10.1.1 Additional data in confidential Annex

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		0; p≤0.01). ↓ number of pups delivered/litter at 150 mg/kg bw/day (-15%; p≤0.01). ↓ number of liveborn pups/litter at 150 mg/kg bw/day (-17%; p≤0.01). ↑ number of stillborn pups/litter at 150 mg/kg bw/day (2.7% vs 0.8% in controls; p≤0.01). ↑ pup mortality at 25 mg/kg bw/day (4 vs. 0, days 12-15; p≤0.01) and at 150 mg/kg bw/day (18 vs. 1, day 1; p≤0.01 and 51 vs. 12, days 2-5; p≤0.01). ↓ viability index at 150 mg/kg bw/day (75.7% vs. 96.3%; p≤0.01). ↓ lactation index at 25 mg/kg bw/day (96.5 vs. 99.7%; p≤0.01). ↓ number of surviving pups/litter at 150 mg/kg bw/day (-17% day1 to -36% day 22; p≤0.01). ↓ live litter size at weighing at 150 mg/kg bw/day (-18% day 1 and -23% day 22; p≤0.01). **Organ weights** *Males* ↓ absolute cauda epididymis weight at 75 mg/kg bw/day (-10%; p≤0.05) (no-dose response). ↓ absolute brain weight at 75 mg/kg bw/day (-3%; p≤0.05) and at 150 mg/kg bw/day (-5%; p≤0.01). ↑ relative testis weight (left and right) at 150 mg/kg bw/day (+10-11%; p≤0.01). ↑ relative liver weight at 75 mg/kg bw/day (+10%; p≤0.01) and at 150 mg/kg bw/day (+19%; p≤0.01). ↓ absolute adrenal weight at 75 mg/kg bw/day (-11%; p≤0.05) and at 150 mg/kg bw/day (-14%; p≤0.01). ↑ relative kidney weights (left and right) at 150 mg/kg bw/day (+12 and +13%; p≤0.01). *Females* ↓ absolute and relative non-gravid uterus weight at 75 mg/kg bw/day (-18% and -19%, respectively; p≤0.05) and at 150 mg/kg bw/day (-20% and -22%, respectively; p≤0.01). ↓ left and right ovary weight at 150 mg/kg bw/day (-17%; p≤0.05). ↑ absolute and relative liver weight at 25 (+7%	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		and +7%, respectively; p≤0.05 and p≤0.01) and at 75 (+18% and +17%, respectively; p≤0.01) and 150 mg/kg bw/day (+20% and +19%, respectively; p≤0.01). ***F1 generation*** *Pups of treated males* ↓ no litters produced at 150 mg/kg bw/day (p≤0.01). ↓ relative brain weight in males at 25 (-6%) and 75 (-6%) mg/kg bw/day (p≤0.05). *Pups of treated females* ↑ litters with pups with lenticular opacities at 150 mg/kg bw/day (771 vs. 4 in controls; p≤0.01). ↓ mean pup body weight/litter at 75 mg/kg bw/day (ranging from -10% on day 1; p≤0.01 to -13% on day 22; p≤0.01) and at 150 mg/kg bw/day (ranging from -11% on day 1; p≤0.01 to -14% on day 22; p≤0.01). *Male pups* ↓ body weight gains at 75 mg/kg bw/day (-7% days 30-37; p≤0.05) and at 150 mg/kg bw/day (-12% days 23-30 and -9% days 30-37 postpartum; p≤0.01). ↓ mean body weight at 75 mg/kg (from day 23 to 51 ranging from -13%; p≤0.01 to -6%; p≤0.05) and at 150 mg/kg bw/day (ranging from -20% to -8%, days 23-57; p≤0.01). ↓ absolute feed consumption at 150 mg/kg bw/day (-17%; days 23 to 30; p≤0.01 and -8%, days 30-37; p≤0.05). ↑ relative feed consumption at 75 mg/kg bw/day (ranging from +9%; p≤0.01 to +5%, p≤0.05, days 30-44) and at 150 mg/kg bw/day (ranging from +8%; p≤0.01 to +6%; p≤0.05 days 30-57). ↓ anogenital distance at 75 and 150 mg/kg bw/day (-7% and -6%, respectively, day 22 postpartum; p≤0.05). ↓ absolute left epididymis weight (-10%) at 150 mg/kg bw/day (p≤0.01). ↑ relative testis weight at 75 mg/kg bw/day (left and right +5%; p≤0.05) and at 150 mg/kg bw/day (left +5% and right +6%; p≤0.05). ↓ absolute pituitary weight at 150 mg/kg (-17%; p≤0.01). ↓ absolute brain weight at 150 mg/kg (-4%;	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		p≤0.01). ↓ absolute and relative adrenals weight at 75 mg/kg bw/day (-15% and -16%, respectively; p≤0.01) and at 150 mg/kg bw/day (absolute only, -13%; p≤0.05). *Female pups* ↑ anogenital distance at 150 mg/kg bw/day (+4%, day 1 postpartum; p≤0.01 when covaried with fetal bw/litter). ↓ body weight gains at 150 mg/kg bw/day (-9%, days 23-30; p≤0.05). ↓ body weight at 75 mg/kg bw/day (ranging from -12% day 23; p≤0.01, to -6% day 51; p≤0.05) and at 150 mg/kg bw/day (from -18% day 23; p≤0.01, to -6% day 51; p≤0.05). ↓ absolute feed consumption at 150 mg/kg bw/day (-14% days 23 to 30; p≤0.01). ↑ relative feed consumption at 75 mg/kg bw/day (+6% days 23-57; p≤0.05) and at 150 mg/kg bw/day (+4% days 23-57; p≤0.01). ↓ absolute brain weight at 75 mg/kg bw/day (-3%; p≤0.05) and at 150 mg/kg bw/day (-6%; p≤0.01). ↓ absolute ovary (left) weight at 150 mg/kg bw/day (-17%; p≤0.05). NOAEL: 25 mg/kg bw/day (male reproduction) NOAEL: 25 mg/kg bw/day (pups weight)	
Short-term repeated dose toxicity study No guideline study Not GLP compliant Male Wistar rats, 5 animals per group Assigned reliability 2 by the Registrant	3-*p*-cumenyl-2-methylpropionaldehyde EC 203-161-7 Male Wistar rats were dosed daily via oral gavage at 0, 30, 100 and 300 mg/kg bw/day for 28 days.	**Mortality and Clinical Observations** No mortality or clinical signs of toxicity were noted. **Body weights** ↓ body weight at 300 mg/kg bw/day (-11%; day 28). **Male reproductive organs** ↑ gross lesions in the epididymis at 300 mg/kg bw/day. A focal nodule unilaterally in the tail of the epididymis in 3 of 5 males at 300 mg/kg bw/day. <u>Sperm effects</u> *100 mg/kg bw/day* ↓ motile sperm (-24%). ↓ progressive sperm (30%). ↓ cells with normal morphology (-13%).	Study report, 2020a. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 3 Annex I 3.10.1.3

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		↑ cells with detached head (+343%) and abnormal neck (+600%). ↓ cells with coiled tail (-61%). *300 mg/kg/day,* ↓ total sperm count in epididymis (-39%). ↓ motile sperm (-78%; statistically significant). ↓ progressive sperm (-90%; statistically significant). ↓ sperm with normal morphology (-96%). ↓ sperm cell count at (3/5 males, below 200 cells required for morphology analysis). ↓ cells with a coiled tail (-87%). ↑ cells with detached head (+146%) and abnormal head (+100%) and/or neck (+800%). ↑ degeneration of elongating spermatids (all animals). ↑ spermatid retention (all animals). ↑ degeneration of round spermatids (1 of 5 males). **NOAEL:** 30 mg/kg bw/day (male reproduction)	
Short-term repeated dose toxicity study No guideline study Not GLP compliant Male New Zealand White rabbits, 5 animals per dose group. Assigned reliability 2 by the Registrant Not clear whether the effects reported were statistically significantly different from controls.	3-*p*-cumenyl-2-methylpropionaldehyde EC 203-161-7 Male rabbits were dosed daily via oral gavage at 0, 30, 100 and 300 mg/kg bw/day for 14 days.	**Mortality and Clinical Observations** No mortality or adverse clinical signs. **Body weight** No effects on body weight. **Organ weights** ↑ absolute and relative liver weight at 300 mg/kg bw/day (+18% and +21%) (no information on statistical significance). ↑ absolute and relative kidneys weight at 300 mg/kg bw/day (+11% and +13%) (no information on statistical significance). **Sperm effects** ↓ trend in mean number motile sperm (-5%, -13% and -31% at 30, 100 and 300 mg/kg bw/day, respectively). ↓ trend in total sperm count (-9%, -12%, -27% at 30, 100 and 300 mg/kg bw/day, respectively).	Study report, 2011b. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 4 Annex I 3.10.1.4
OECD TG 408 Repeated Dose 90-Day Oral Toxicity	3-*p*-cumenyl-2-methylpropionaldehyde,	**Clinical observations** No marked clinical signs were observed (salivation of single animals at 30 and 120	Study report, 2020b.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Study in Rodents GLP compliant Male and Female Wistar-Han rats 10 animals per sex per dose	EC 203-161-7 Purity: ≥98% Vehicle: corn oil Substance was administered once daily via oral gavage at doses 0, 15, 30, 120 mg/kg bw/day. Duration of treatment: 90 days	mg/kg bw/day after dosing and some incidence of fur loss, scabs and incidental erected fur). **Body weights** *Males* ↓ terminal body weight at 120 mg/kg bw/day (-9%; p≤0.05). *Females* ↓ terminal body weight at 120 mg/kg bw/day (-7%; p≤0.05). **Clinical biochemistry** *Males* ↓ protein concentration at 120 mg/kg bw/day (-14%; p≤0.05). ↓ total cholesterol and HDL cholesterol at 120 mg/kg bw/day (-28% and -31%; p≤0.01). *Females* ↓ protein concentration at 120 mg/kg bw/day (-17%; p≤0.05). **Behaviour** *Males* ↓ hind grip strength at 120 mg/kg bw/day (-31%; p≤0.01). *Females* ↑ total movements and ambulations at 15 mg/kg bw/day (+58% and +64%; p≤0.01). ↑ hind grip strength at 15 mg/kg bw/day (+318%; p≤0.05). **Organ weights** *Males* ↑ relative liver weight at 120 mg/kg bw/day (+21%; p≤0.01). ↑ relative kidney weight at 120 mg/kg bw/day (+8%; p≤0.05). ↓ absolute and relative seminal vesicle gland weight at 30 mg/kg bw/day (relative only -19%; p≤0.05) and at 120 mg/kg bw/day (-28%; p≤0.01 and -20%; p≤0.05). ↓ absolute epididymis weight at 120 mg/kg bw/day (-13%, p≤0.01). ↑ relative testis weight at 120 mg/kg bw/day (+14%; p≤0.01).	Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Full study report was available to DS Study 5 Annex I 3.10.1.5

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Females ↑ absolute and relative liver weight at 15 mg/kg bw/day (+22%), at 30 mg/kg bw/day (+23% and +27%) at 120 mg/kg/day (+33% and + 44%) (p≤0.01). ↑ absolute and relative kidney weight at 30 mg/kg bw/day (+8%; p≤0.05, and + 11%; p≤0.01) at 120 mg/kg/day (+8%; p≤0.05, and +16%; p≤0.01). ↑ relative heart weight at 30 mg/kg bw/day (+13%; p≤0.01) at 120 mg/kg/day (+12%; p≤0.05). **Reproductive organs** *Males* A nodule on the tail of the epididymis (unilateral, left) in one male at 120 mg/kg bw/day (correlated microscopically to a sperm granuloma). Sperm effects ↓ percentage of motile sperm at 120 mg/kg bw/day (32% vs 71% in controls; p≤0.01). ↓ progressive sperm at 120 mg/kg bw/day (6% vs. 25% in controls; p≤0.01). ↓ number of cells with normal morphology at 15 mg/kg bw/day (163 vs. 181 in controls; p≤0.05) and at 120 mg/kg bw/day (48 vs. 181 in controls; p≤0.01). ↑ number of cells with detached head at 120 mg/kg bw/day (103 vs. 3 in controls; p≤0.01) and abnormal neck (46 vs. 2 in controls; p≤0.01). ↓ number of cells with a coiled tail at 120 mg/kg bw/day (2 vs. 13 in controls; p≤0.01). ↑ sperm granulomas in the epididymis at 120 mg/kg bw/day (3/10 animals). NOAEL: 30 mg/kg bw/day (male reproduction)	
3-(*p*-cumenyl)propionaldehyde EC 231-885-3			
Oral (gavage) 14-day repeated dose toxicity study in rats No Guideline Not GLP compliant male Crl:CD(SD) rats	3-(*p*-cumenyl)propional dehyde EC 231-885-3 Purity: no info Vehicle: corn oil Substance was	**Mortality and clinical observations** No clinical signs were observed. **Body weights** ↓ body weight at 250 mg/kg bw/day (first week). ↓ terminal body weights at 250 mg/kg bw/day. **Organ weights**	Study report A. Robust study summary in Registration dossier,

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
10 animals per group Detailed information was lacking in the study summary.	administered once daily via oral gavage at doses 0, 25, 75 and 250 mg/kg bw/day for 14 consecutive days.	↓ seminal vesicle weight at 250 mg/kg bw/day. ↓ prostate weight at 250 mg/kg bw/day. ↑ absolute and relative liver weights at 75 and 250 mg/kg bw/day. **Reproductive organs** ↑ microscopic lesions in testes, epididymides and seminal vesicles (including atrophy) at 250 mg/kg bw/day. <u>Sperm effects</u> Not possible to determine number and percent motile sperm, number of non-motile sperm, total sperm count from the vas deferens at 250 mg/kg bw/day. ↓ sperm count and sperm density from the cauda epididymis at 75 (statistically significant) and 250 mg/kg bw/day (not statistically significant). ↑ abnormal sperm, sperm with detached heads or no heads at 75 and 250 mg/kg bw/day. NOAEL: 25 mg/kg bw/day (male reproduction)	ECHA's disseminat ion site, 2022. Study 6 Annex I 3.10.1.6
Oral (gavage) 14 - day repeated dose toxicity study in rabbits No Guideline Not GLP compliant male New Zealand White (Hra:(NZW)SPF) rabbits 5 animals per group	3-(*p*-cumenyl)propional dehyde EC 231-885-3 Purity: no info Vehicle: corn oil Substance was administered once daily via stomach tube at doses 0, 10, 30 and 100 mg/kg bw/day for 14 consecutive days. Since no toxicity was observed, a dose of 300 mg/kg bw/day was assessed in a study extension as well.	**Clinical observations** No clinical signs were observed. **Body weights** No effects on body weights and body weight gains. **Reproductive organs** ↑ microscopic findings in testes and epididymis in all animals at 300 mg/kg bw/day. Three of 5 rabbits had mild or minimal depletion of spermatozoa in the epididymides and two of these rabbits had detachment of the seminiferous tubules of the testes. <u>Sperm effects</u> ↑ abnormal sperm at 300 mg/kg bw/day (38%). NOAEL: 100 mg/kg bw/day (male reproduction)	Study report B. Robust study summary in Registratio n dossier, ECHA's disseminat ion site, 2022. Study 7 Annex I 3.10.1.7
4-isopropylbenzaldehyde EC 204-516-9			
No data on reproductive or chronic toxicity are available.	4-isopropylbenzalde hyde	Read-across, see justification in section 9.1.1.	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	EC 204-516-9		
4-isopropylbenzoic acid (4-iPBA) EC 208-642-5			
Short term toxicity study (5 days) Six male CD rats/group	4-iPBA EC: 208-642-5 The substance was administered via oral gavage at doses 0, 15, 50, 150 mg/kg bw/day daily for 5 consecutive days.	**Clinical observations** No clinical signs were observed. **Food consumption** ↓ food consumption at 150 mg/kg bw/day (-19%). **Organ weights** ↑ absolute and relative weight of epididymidis at 150 mg/kg bw/day (+20%; p≤0.01). ↑ cauda epididymal weight at 150 mg/kg bw/day (+43%). **Histopathology** Microscopic changes of testes and epididymides, including slight interstitial inflammatory cells (5/6 animals), apoptotic epithelial cells (4/6), degenerate spermatogenic cells in the ducts (5/6) and epithelial hyaline droplets (4/6) and reduced numbers of spermatozoa (4/6;) in the epididymides at 150 mg/kg bw/day. In the testes, a dose-related increase in the incidence of seminiferous tubular vacuolation was observed in animals in all treated groups. ↑ degenerate spermatocytes (4/6 animals) and spermatid giant cells (3/6 animals) at 150 mg/kg bw/day. Seminiferous tubular vacuolation in 1 and 2 animals at 15 and 50 mg/kg bw/day, and in 4 of 6 animals at 150 mg/kg bw/day. LOAEL: 150 mg/kg bw/day (male reproduction)	Natsch et al. 2021

Table 9: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
-				

Table 10: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
-				

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

***p*-cymene**

Reproductive/developmental toxicity study OECD TG 422 in rats (Study report, 2019)

In an OECD TG 422 screening for reproductive/developmental toxicity study (GLP compliant) from 2019, male and female Sprague Dawley rats (10 per sex and group) were exposed to *p*-cymene via oral gavage at 0, 50, 100, 200 mg/kg bw/day once daily. Parental males were dosed 2 weeks pre-cohabitation, during cohabitation (up to 2 weeks) and continuing during post-cohabitation until the day prior to termination (approximately 35 days). Parental females were dosed 2 weeks pre-cohabitation, cohabitation (up to 2 weeks) and during gestation and lactation continuing until LD 13 (approximately 63 days).

Varying degrees of germ cell degeneration/depletion, depletion, and/or sperm retention were present in the testis at 200 mg/kg bw/day. In some animals, this bilateral change consisted of multifocal drop out of round spermatids in early-stage tubules (stages I to VIII), with germ cell degeneration, multinucleated giant cells, and vacuolation; general to multifocal depletion of elongated/mature spermatids in early-stage tubules; germ cell drop out and disorganization in late stage tubules (stages X to XIII); bizarre mitotic figures in stage XIV tubules. In some animals, the findings consisted of multifocal depletion of round spermatids or depletion/degeneration of elongated spermatids in early-stage tubules and occasional vacuolation. Most males also had sperm retention (retention of sperm in late-stage tubules beyond stage VIII, stage at which sperm are released) along the apical surface of the tubules. In concert with the testicular findings, varying degrees of luminal cell debris and reduced sperm with or without cribriform change were present bilaterally in the epididymis at 200 mg/kg bw/day.

At 100 mg/kg bw/day, some males had marginal sperm retention bilaterally in the testis with 2 of these males having decreased sperm in the epididymis with or without cribriform change. Unilateral or bilateral seminiferous tubular atrophy, with or without luminal cell debris and reduced sperm in the epididymis, was observed in 1 male at 50 mg/kg bw/day and 1 male at 100 mg/kg bw/day.

Lower mean testes (-14%, statistically significant), epididymides (-14%, statistically significant), and levator ani-bulbocavernosus muscle weights (-15%, not significant) were present in P0 males at 200 mg/kg bw/day compared to controls. In the testes and epididymides, these weight differences, respectively, correlated microscopically with germ cell degeneration/depletion and decreased sperm, respectively. Possible treatment-related differences were present in the seminal vesicles (absolute weight -22%, not statistically significant) and prostate (absolute weight -24%, statistically significant) in adult P0 males.

Treatment-related macroscopic findings were limited to males at 200 mg/kg bw/day. Two males had unilateral or bilateral small testis. These findings correlated with microscopic findings of germ cell degeneration/depletion. One of these males also had a small prostate; there was no correlating microscopic finding. One additional male at 200 mg/kg bw/day had a small levator ani-bulbocavernosus muscle complex; this tissue was not examined microscopically.

In females, there were treatment-related alterations of estrous cyclicity at 200 mg/kg bw/day. The number of animals with all regular cycles (cycles that have 4 day, 4/5 day and 5 day durations) during pre-cohabitation were reduced at 200 mg/kg bw/day. The number of animals with at least one irregular cycle

(cycles < 4 days or > 5 days duration) during the pre-cohabitation period were increased at 200 mg/kg bw/day.

There were reductions in fertility at 100 and 200 mg/kg bw/day. The number of pregnant females was statistically significantly at 100 and 200 mg/kg bw/day. The number of pregnant females were 9, 9, 4 and 0 at 0, 50, 100 and 200 mg/kg bw/day, respectively. Thus, the male and female fertility indices were 90%, 90%, 40% and 0% at 0, 50, 100 and 200 mg/kg bw/day, respectively.

The number of females paired with males were 10, 10, 10 and 10 at 0, 50, 100 and 200 mg/kg bw/day, respectively. The number of females that had a defined GD 0 were 10, 10, 9 and 8 at 0, 50, 100 and 200 mg/kg bw/day, respectively. The mean number of days to mating were 2.5, 2.4, 3.2 and 2.6, respectively. These mating values were comparable with the concurrent control values and were within normal historical data. Mating and fertility data at 50 mg/kg bw/day were comparable with the concurrent control values.

Pre-implantation loss was increased at 50 and 100 mg/kg bw/day (5.9% and 7.1%, respectively, vs. 1.8% in controls) but this increase was not statistically significant.

Other / general toxicity

There were no treatment-related reductions in the body weights and body weight changes at any dose. No effects on food consumption and compound intake were noted at any dose.

Eighteen females across all groups, including controls, were euthanized early. Seventeen of these females (1/10 control, 1/10 at 50 mg/kg bw/day, 6/10 at 100 mg/kg bw/day, and 9/10 at 200 mg/kg bw/day) were euthanized on GD 25 due to failure to become pregnant. One female at 200 mg/kg bw/day was euthanized for welfare reasons on GD 24; this female was not pregnant. Microscopic findings in this female were present in the liver, adrenal, and kidney and were considered the source of morbidity.

Reductions of hindlimb grip strength was observed in males at 200 mg/kg bw/day. Absolute and relative liver weights were statistically significantly increased in males at 200 mg/kg bw/day (+27% and +41%, respectively) and in females at 100 mg/kg bw/day (+26% and +22%, respectively). Females at 200 mg/kg bw/day were not included as all females at this dose were euthanized due to non-pregnancy. Hepatocellular hypertrophy was minimally present in 2/5 males and 1/10 females (micropscopic findings in female euthanised on GD 24) at 200 mg/kg bw/day and in 1/6 females at 50 mg/kg bw/day. The distribution pattern of hepatocellular hypertrophy was diffuse at 200 mg/kg bw/day and centrilobular at 50 mg/kg bw/day. There was a marginal increase in incidence and severity of hyaline droplet accumulation of kidneys in males at 200 mg/kg bw/day. Minimal tubular epithelial vacuolation was present in the renal medulla in 2/5 males at 200 mg/kg bw/day.

At study termination, T4 values in P0 males were lower (-66% to -37%) at 100 and 200 mg/kg bw/day. Also, most or all of the individual animal TSH values were below the level of detection in P0 males at all doses and in P0 females at 50 and 100 mg/kg bw/day. There were no thyroid weight changes and/or microscopic findings in the thyroid glands in these animals.

Haematological findings in males at 200 mg/kg bw/day included increases in reticulocytes (+30%) with corresponding increases in red cell distribution width (+7%).

Clinical biochemistry findings (at ≥ 100 mg/kg bw/day) included decreases in triglycerides (-50 to -52%; males only); increases in alkaline phosphatase activity (+79% in females at 100 mg/kg bw/day and +45% in males at 200 mg/kg bw/day); and decreases in albumin (-9% in females at 100 mg/kg bw/day). At 200 mg/kg bw/day, increases in blood urea nitrogen (+50%; males only) were considered treatment-related.

More details in Annex I section 3.10.1.8.

F1 generation

There were no pregnant animals at 200 mg/kg bw/day.

There were 9, 9 and 4 litters at 0, 50 and 100 mg/kg bw/day, respectively.

The live birth index and post-implantation survival index were statistically significantly reduced at 100 mg/kg bw/day (-5.7% and -7.7%, respectively). The number of litters with less than 100% viability was

increased at 100 mg/kg bw/day with only 1 of 4 litters having 100% viability versus 9 of 9 in the concurrent control group. The viability indices on PND 4, 7 and 13 were comparable with concurrent control values at 100 mg/kg bw/day dose group, respectively. All offspring survival index values at 50 mg/kg bw/day were comparable with the concurrent control values.

The mean litter body weight was reduced at 100 mg/kg bw/day on PND 1 (-10%, statistical significance unclear). On the remaining intervals (PND 4, 7, 11 and 13), the mean litter body weights at 50 and 100 mg/kg bw/day were comparable with the control group values. The mean sex ratio was comparable among groups.

There were no organ weight differences in thyroid/parathyroid glands in the F1pups. There were no microscopic findings in the thyroid/parathyroid glands in the F1 pups. There were no differences from controls for T4 or TSH on PND 4 in F1 female pups. On PND 13, there were no differences from control for T4 levels in F1 male pups. Many of the individual animal TSH values were below the level of detection in males at 100 mg/kg bw/day and females at 50 and 100 mg/kg bw/day. There were no thyroid weight changes and/or microscopic findings in the thyroid glands in these animals. No more detailed information is provided the robust study summary.

Conclusions

Clear evidence of testicular and spermatotoxicity was demonstrated at 200 mg/kg bw/day without observations of adverse general toxicity. Similar effects were seen in a few animals at 100 mg/kg bw/day and seminiferous tubular atrophy was observed in 1 male (of 10) at 50 mg/kg bw/day.

Lower weights of testes and epididymides at 200 mg/kg bw/day correlated microscopically with germ cell degeneration/depletion and decreased sperm, respectively.

In females, alterations of estrous cyclicity were observed at 200 mg/kg bw/day, with increased number of animals with irregular cycle during pre-cohabitation at 200 mg/kg bw/day.

There were reductions in fertility at 100 and 200 mg/kg bw/day, fertility indices were 90%, 90%, 40% and 0% at 0, 50, 100 and 200 mg/kg bw/day, respectively, thus no pregnant animals or litter produced in the highest dose group.

The live birth index and post-implantation survival were statistically significantly reduced at 100 mg/kg bw/day. The mean pup weights on PND 1 (-10%) were statistically significantly lower at this dose level.

Repeated dose toxicity study in rats (Study report, 2018)

In a short-term repeated dose toxicity study (not GLP compliant) from 2018, Crl:CD(SD) IGS male and female rats (3 animals/sex/dose) were exposed to *p*-cymene via oral gavage at doses 0, 50, 150 and 500 mg/kg bw/day, daily for 14 consecutive days.

Fourteen days of oral gavage dosing with *p*-cymene was associated with macroscopic abnormalities in the testes (small size/soft texture at 150 and 500 mg/kg bw/day). One male at 150 mg/kg bw/day demonstrated small epididymides and testes, and two of three males at 500 mg/kg bw/day had small, soft testes. A single female at 500 mg/kg bw/day had a small thymus, uterus and cervix.

Organ weight differences were also observed in the liver (higher weight at ≥ 150 mg/kg bw/day) and spleen (lower weight at 500 mg/kg bw/day). See Annex I section 3.10.1.9.

General toxicity

There were no adverse clinical signs observed at any dose level in male or female rats except for the one female at 500 mg/kg bw/day that was sacrificed on day 13 due to welfare reasons.

In females body weights and body weight changes were reduced at 150 mg/kg bw/day females (terminal body weight -8.6%). Body weights, body weight changes and food consumption (females only) were reduced in male and female rats at 500 mg/kg bw/day (terminal body weights were -12% and -14%, respectively). There were no information available whether differences in this study were statistically significant.

Conclusions

Abnormalities of the testes were seen at at 150 and 500 mg/kg bw/day. One female (of 3) at 500 mg/kg bw/day had a small thymus, uterus and cervix. Except for one high dose female sacrificed on day 13 and effects on terminal body weight in high dose males and females (up to -14%) there were no observations of adverse general toxicity.

3-*p*-cumenyl-2-methylpropionaldehyde

One-Generation Reproduction Toxicity Study OECD TG 415 in rats (Study report, 2011a)

In a One-Generation Reproduction Toxicity Study (OECD TG 415) from 2011, male and female rats (25 per sex/group) were exposed by gavage to 3-*p*-cumenyl-2-methylpropionaldehyde at 0, 25, 75 or 150 mg/kg bw/day in corn oil. Male P generation rats were gavaged once daily, beginning 83 days prior to cohabitation, through cohabitation, continuing through the day before sacrifice. Female P generation rats were gavaged once daily, beginning 14 days before cohabitation, through cohabitation and day of gestation 25 (rats that did not deliver) or day 22 postpartum (rats that delivered a litter). The mating procedure used a cross-over design where treated males were mated with untreated females, and treated females were mated with untreated males. F1 generation rats were not directly dosed but may have been exposed to the test material *in utero* during gestation and through maternal milk postpartum.

Treated males

Based on the individual data, motility of the sperm from the vas deferens could not be observed in any of the rats at 150 mg/kg bw/day and in 13 of 25 rats at 75 mg/kg bw/day. The motility values in each of the samples reflected the presence of drifting debris, headless sperm, detached heads and/or less than the required number of sperm for evaluation. The effects observed at 150 mg/kg bw/day correlated with the infertility that was observed in the treated male rats that were mated with the untreated cohort female rats. Only 12 rats at 75 mg/kg bw/day had enough viable sperm (at least 200 sperm in 20 fields) available for analysis. The sperm motility values from the 12 treated male rats at 75 mg/kg bw/day were comparable to the vehicle control group values and were also within the ranges observed historically at the testing facility.

At 75 mg/kg bw/day 3-*p*-cumenyl-2-methylpropionaldehyde statistically significantly reduced the sperm count and density from the cauda epididymis, compared to controls. (Each of these average values were within the ranges observed historically at the testing facility). All sperm parameters evaluated were unaffected at 25 mg/kg bw/day.

3-*p*-cumenyl-2-methylpropionaldehyde increased the absolute and relative weights of the epididymides (left, right and cauda) at 150 mg/kg bw/day. The increased epididymal weights generally reflected the presence of masses on the cauda epididymis and microscopic observations of moderate to marked sperm granulomas. In male rats at 150 mg/kg bw/day the gross epididymal masses correlated microscopically with moderate to marked sperm granulomas, associated with mild to moderate epithelial degeneration. The testes were not affected. In addition, these male rats had decreased adrenal weights that correlated microscopically with minimal adrenal cortical atrophy, affecting the zona fasciculate and zona reticularis. These adrenal and epididymal gross or microscopic changes were not seen in male rats at 25 and 75 mg/kg bw/day.

3-*p*-cumenyl-2-methylpropionaldehyde caused infertility following mating with untreated female rats at paternal treatment of 150 mg/kg bw/day. There was only one pregnancy (1/24) produced by matings at this dose level. The pregnant dam did not deliver a litter. Fertility index was 92%, 100%, 87.5% and 4.3% at paternal treatment of 0, 25, 75 and 150 mg/kg bw/day, respectively. Natural delivery and litter observations were unaffected up to 75 mg/kg bw/day.

Treated females

The number of estrous stages per 14 days was comparable among the four dosage groups before the start of administration and during the precohabitation period. All mating and fertility parameters [numbers of

days in cohabitation, rats that mated, the fertility index (number of pregnancies per number of rats that mated), rats with confirmed mating dates during the first or second week of cohabitation and number of pregnancies per number of rats in cohabitation] were unaffected up to 150 mg/kg bw/day.

Pregnancy occurred in 25 (100.0%), 24 (96.0%), 24 (96.0%) and 24 (96.0%) of the 25 mated female rats at 0, 25, 75 and 150 mg/kg bw/day, respectively. All pregnant dams delivered litters. At 150 mg/kg bw/day, the average number of implantation sites per delivered litter was statistically significantly reduced, in comparison to the vehicle control group value (13.8 implantation sites vs. 15.5 implantation sites in controls). (The average value was within the range observed historically at the testing facility).

The number of dams with all pups dying between days 1 and 5 postpartum was statistically significantly increased at 150 mg/kg bw/day. Reflecting the reduction in implantation sites, the average number of pups delivered per litter, as well as the average number of liveborn pups per litter, was significant reduced at this dose. In addition, there was a statistically significant increase in the number of stillborn pups that were delivered at 150 mg/kg bw/day (8 vs. 3 stillborn pups in controls).

Pup mortality (i.e., found dead, presumed cannibalized or unscheduled sacrifice) was statistically significantly increased) at 150 mg/kg bw/day on days 1 to 5 postpartum, compared to control group values. As a result of the increase in pup mortality, the overall viability index (i.e., number of live pups on day 5 postpartum/number of liveborn pups on day 1 postpartum) was statistically significantly reduced at 150 mg/kg bw/day compared to control group value (75.7% vs. 96.3% in controls). Viability index was decreased also at 75 mg/kg bw/day (not statistically significant). At 150 mg/kg bw/day, the averages for the number of surviving pups per litter and the live litter size at weighing was statistically significantly reduced on days 1 through 22 postpartum, compared to control group values (and decreased in a dose-related manner days 1-8). In addition, the average pup body weight per litter was statistically significantly reduced at 75 and 150 mg/kg bw/day at each tabulated interval between days 1 and 22 postpartum, compared to the control group.

No other natural delivery and litter observations were affected by 3-*p*-cumenyl-2-methylpropionaldehyde up to 150 mg/kg bw/day. Values for the numbers of dams delivering litters, the duration of gestation, the gestation index (number of dams with one or more liveborn pups/number of pregnant rats), the numbers of dams with stillborn pups, lactation index, and percent male pups per number of pups sexed per litter were comparable among the four dose groups.

Statistically significantly reduced absolute non-gravid uterus weight (with the cervix) was seen at 75 (-19%; $p \leq 0.05$) and 150 mg/kg bw/day (-20%; $p \leq 0.01$) and statistically significant decreases in individual (left and right) ovarian weights were observed at 150 mg/kg bw/day (-17%, $p \leq 0.05$). An increased number of primordial follicles was noted at 150 mg/kg bw/day. No microscopic correlates were reported for the histopathology of these rat uterus and ovaries. Corpora lutea were present in all rats evaluated.

General toxicity, body weight and food consumption – parental generation

The number of male rats with slight or moderate excess salivation was statistically significantly increased at 150 mg/kg bw/day, compared to controls. This observation occurred intermittently during the dosage period. Among male rats statistically significant reductions in body weight gain occurred at 150 mg/kg bw/day intermittently during the dosage period prior to cohabitation. The average body weight for males on day 134 was ±0%, -3% and -7% in the three respective treated groups. More details in Annex I (Section 3.10.1.1)

Among females, during the first week of the dosage period (precohabitation), body weight gains were reduced at 75 mg/kg bw/day (-36%) and statistically significantly reduced at 150 mg/kg bw/day (-56%). These reductions were transient and did not persist during the second week of the dosage period. Despite the rebound during the second week of the dosage period, body weight gains at 75 and 150 mg/kg bw/day remained reduced (75 mg/kg bw/day) or statistically significantly reduced 150 mg/kg bw/day) for the entire premating dosage period, compared to controls. The average body weight on DS 15 was -2%, -2% and -3% at 25, 75 and 150 mg/kg bw/day, respectively.

During gestation, at 150 mg/kg bw/day, body weight gains remained reduced (-5% to -21%) at each tabulated interval within the gestation dosage period relative to the control group values (not statistically significantly). Maternal body weight gains at 25, 75 and 150 mg/kg bw/day were +2%, -5% and -15%,

respectively, on DGs 0 to 21. Average body weight at 150 mg/kg bw/day GD days 18 and 21 was statistically significantly lower compared to controls (-5% and -8%, respectively; $p \leq 0.01$). The average body weight on DG 21 was -1%, -3% and -8% at 25, 75 and 150 mg/kg bw/day, respectively.

During lactation, statistically significant body weight losses were observed at 150 mg/kg bw/day at the beginning of the lactation period (DLs 1 to 8). Thereafter, body weight gains were comparable to or statistically significantly increased when compared to the control group values.

The average maternal body weight was statistically significantly reduced at 150 mg/kg bw/day on DLs 5 through 11, compared to controls. Maternal body weight gains at 25, 75 and 150 mg/kg bw/day were +8%, +44% and +24%, respectively, on DLs 1 to 22. The average body weight on DL 22 was ±0%, +3% and +1% at 25, 75 and 150 mg/kg bw/day, respectively.

Among males, absolute and relative feed consumption values were unaffected by 3-*p*-cumenyl-2-methylpropionaldehyde up to 150 mg/kg bw/day (Annex I Section 3.10.1.1).

Absolute and relative feed consumption values among females at 150 mg/kg bw/day during precohabitation were statistically significantly reduced during the first week of the premating dosage period (DSs 1 to 8), compared to controls (-8% and -6%, respectively). Absolute and relative feed consumption values in females during the gestation period were unaffected up to 150 mg/kg bw/day. Absolute feed consumption values at 25, 75 and 150 mg/kg bw/day were -5%, -11% and -20%, respectively, on DLs 1 to 15. Absolute and relative feed consumption values during the lactation period were unaffected at 25 mg/kg bw/day (Annex I, Section 3.10.1.1).

F1 Generation Pups of Treated Male Rats Mated with Untreated Female Rats:

There were no litters produced at 150 mg/kg bw/day from the mating of treated P generation male rats with untreated female rats. There were no adverse clinical signs observed in the F1 generation male or female rats at 25 and 75 mg/kg bw/day.

Body weight gains and average body weight and feed consumption in the F1 generation male and female rats were unaffected by paternal treatment at 25 and 75 mg/kg bw/day. The relative brain weight was reduced at 25 and 75 mg/kg bw/day in male pups (-6% compared to controls).

There were no effects on sexual maturation (preputial separation or vaginal opening) at any paternal dosage level tested.

Anogenital distance on days 1 or 22 postpartum in F1 male and female pups was not affected by treatment of P generation male rats at 25 and 75 mg/kg bw/day. Nipple eruption did not occur in any male pup, and all female pups had nipples present on day 12 postpartum.

Terminal body weights in the F1 generation male and female rats were comparable among the three remaining dosage groups and did not statistically significantly differ. The relative brain weight was reduced at 25 and 75 mg/kg bw/day in male pups (-6%).

There were no gross lesions observed in the F1 generation pups that were stillborn or found dead or in the F1 generation pups that survived to scheduled necropsy on day 22 postpartum.

F1 Generation Pups of Treated Female Rats Mated with Untreated Male Rats

At 150 mg/kg bw/day, 20 of 24 litters (statistically significant) had one or more pups with a lenticular opacity in one or both eyes. This observation only occurred in one pup from one litter in the control group on days 19 through 22 postpartum. At 150 mg/kg bw/day, lenticular opacities were first observed on day 16 and generally persisted until day 22 postpartum. This observation was more prevalent in F1 generation male rats than in the female rats (18 male rats vs. 6 female rats). This clinical sign was confirmed during scheduled necropsy examination.

In F1 generation male rats, body weight gains were statistically significantly reduced at 150 mg/kg bw/day maternal dose on days 23 to 37 postpartum, compared to controls. Thereafter, body weight gains were comparable to controls during the remainder of the postweaning period. Reflecting the initial reductions in weight gain, body weight gains in the F1 generation male rats in the 150 mg/kg bw/day maternal dosage group were -6% (statistically significant) for the entire postweaning period (days 23 to 57 postpartum).

In F1 generation female rats, body weight gains were statistically significantly reduced at 150 mg/kg bw/day maternal dose on days 23 to 30 postpartum, compared to controls. However, this reduction was transient, and body weight gains were comparable to the control group values during the remainder of the postweaning period. Body weight gains at 25, 75 and 150 mg/kg bw/day maternal dose were +1%, -2% and ±0%, respectively, on days 23 to 57 postpartum. Reflecting statistically significant reductions in the average pup body weight per litter that occurred prior to weaning, the average body weight was also statistically significantly reduced in the F1 generation male and female rats during post-weaning period at 75 and 150 mg/kg bw/day. Terminal average body weight in male offspring was -8% ($p \leq 0.01$) on day 57-60 postpartum, and -4% in female offspring (not statistically significant).

Corresponding to statistically significant reductions in body weight gains, absolute feed consumption values in the F1 generation male rats were statistically significantly reduced at 150 mg/kg bw/day on days 23 to 37 postpartum, compared to controls. Relative to body weight, F1 generation male rats consumed statistically significantly more feed on days 30 to 44 postpartum at 75 mg/kg bw/day and days 23 to 57 postpartum at 150 mg/kg bw/day.

Similar observations occurred in the F1 generation female rats, in that, absolute feed consumption values were statistically significantly reduced at 150 mg/kg bw/day on days 23 to 30 postpartum, compared to controls. Relative to body weight, F1 generation female rats consumed statistically significantly more feed on days 23 to 57 postpartum at 75 and 150 mg/kg bw/day.

Absolute feed consumption values in the F1 generation male rats were -2%, -3% and -7% at 25, 75 and 150 mg/kg bw/day, respectively, on days 23 to 57 postpartum. In F1 generation female rats, absolute feed consumption values were -2%, -1% and -4% at 25, 75 and 150 mg/kg bw/day, respectively, during the same period.

There were no effects on sexual maturation (preputial separation or vaginal opening) at any maternal dosage level tested. The average day on which sexual maturation was achieved was comparable among the dose group. The average body weight of male rats on the day preputial separation occurred was statistically significantly reduced at 75 and 150 mg/kg bw/day compared to controls. These reductions in body weight reflect statistically significant reductions in the average pup body weight per litter that occurred prior to weaning. (Each of the average values for body weight on the day of sexual maturation were within the ranges observed historically at the testing facility).

In male pups, anogenital distance on day 1 postpartum was not affected by treatment of P generation female rats at any dose level tested. On day 22 postpartum, there was a statistically significant reduction in the anogenital distance of male pups at 75 and 150 mg/kg bw/day. When covaried with fetal body weights per litter, the statistically significant reduction in anogenital distance was not apparent. This developmental delay correlated with an overall reduction in pup body weights on day 22 postpartum. In female pups, anogenital distance on day 1 postpartum was not initially affected by treatment of P generation female rats at any dose level tested. However, when covaried with fetal body weights per litter, there was a statistically significant increase in anogenital distance at 150 mg/kg bw/day, compared to controls. This increase in anogenital distance was no longer apparent by day 22 postpartum. Nipple eruption did not occur in any male pup, and all female pups had nipples present on day 12 postpartum.

The absolute weight of (left) epididymis was statistically significantly reduced (-10%) in male pups at 150 mg/kg bw/day, as were absolute weights of the pituitary (-17%), brain (-4%) and adrenals (-15% at 75 and -13% at 150 mg/kg bw/day). Relative weights of testis were statistically significantly increased at 75 and 150 mg/kg bw/day. In female offspring, absolute brain weights were statistically significantly reduced at 75 (-3%) and 150 mg/kg bw/day (-6%), as were absolute ovary weights at 150 mg/kg bw/day (-17%).

The only gross lesion observed in the F1 generation pups was a tan area on the left kidney of one male pup at 150 mg/kg bw/day. There were no other gross lesions observed in the F1 generation pups that survived to scheduled necropsy on day 22 postpartum. In the pups that were stillborn, found dead or humanely euthanized, no milk was present in the stomach of 1, 5, 5 and 14 F1 generation pups at 0, 25, 75 and 150 mg/kg bw/day, respectively. The remaining pups that were stillborn, found dead or humanely euthanized appeared normal.

Conclusions

Among treated males, spermatotoxicity was demonstrated at 150 mg/kg bw/day, and sperm count and density were statistically significantly lower also at 75 mg/kg bw/day, compared to controls. Sperm effects at 150 mg/kg bw/day correlated with complete infertility observed in untreated females mated with treated males at this dose level.

When treated females mated with untreated males, the majority of females became pregnant (96% at all dose groups compared 100% of controls). The number of implantation sites per delivered litter was affected at 150 mg/kg bw/day, as well as reduced average number of pups delivered per litter, reduced number of liveborn pups per litter and increased number of stillborn pups.

Moreover, pup mortality was increased at 150 mg/kg bw/day the first days postpartum, thus viability index was reduced. The number of surviving pups per litter and the live litter size were statistically significantly reduced at the highest dose days 1 through 22 postpartum (in a dose-related manner day 1-8). Pup body weight per litter was statistically significantly reduced at 75 and 150 mg/kg bw/day. At 150 mg/kg bw/day, 83% of the litters had one or more pups with lenticular opacities in the eyes. Reduced weight of several organs was observed in both male and female pups, including statistically significantly reduced brain weight.

There was no marked toxicity among treated paternal animals. Among treated maternal animals the average body weight was statistically significantly lower at 150 mg/kg bw/day at the end of gestation (-8%) and beginning of lactation (up to -8.5%). The non-gravid uterus weight was statistically significantly reduced at 75 and 150 mg/kg bw /day (up to -20%) and ovaries weight was statistically significantly lower at 150 mg/kg bw/day compared to controls.

90 day toxicity study OECD TG 408 in rats (Study report, 2020b)

In a repeated dose 90 days toxicity study (OECD TG 408, GLP compliant), male and female Wistar Han rats (10 per sex per dose) were exposed to 3-*p*-cumenyl-2-methylpropionaldehyde at 0, 15, 30, 120 mg/kg bw/day.

A statistically significantly lower relative seminal vesicle gland weight was noted in males at 30 (-18.6%) and 120 (-20.1%) mg/kg bw/day. There was no microscopic correlate. A statistically significantly lower absolute epididymis weight was noted in males at 120 mg/kg bw/day (-12.5%), which was without a microscopic correlate. There was no difference in the absolute testis weight. A statistically significantly higher relative weight of the testis was noted in males at 120 mg/kg bw/day, which was secondary to the lower terminal body weights in that group.

A nodule was noted on the tail of the epididymis (unilateral, left) of one male treated at 120 mg/kg bw/day. This correlated microscopically to a sperm granuloma.

There were no effects on sperm motility, concentration and morphology observed in males up to 30 mg/kg bw/day. At 120 mg/kg bw/day, statistically significantly lower percentage of motile sperm (-55%), progressive sperm (-76%) and number of cells with a normal morphology (-73%) was recorded. In addition, a statistically significant increase in number of cells with a detached head (+343%) and abnormal neck (+230%) were observed. The sperm count in the epididymides was not statistically significantly reduced. A statistically significant decrease in number of cells with a coiled tail was observed at 120 mg/kg bw/day (-85%).

Sperm granulomas in the epididymis (examined unilaterally) were noted in 3/10 males at 120 mg/kg bw/day only, at mild degree. In one male this correlated to a nodule macroscopically.

Decreased triiodothyronine (T3) and thyroxine (T4) levels were seen in males at 120 mg/kg bw/day (-31% and -55% of controls respectively), reaching statistical significance for T4. T3 and T4 levels in females at 120 mg/kg bw/day were not statistically different from controls. TSH remained within the historical control data range at these dose levels.

Statistically significantly higher relative liver weight was noted in males at 120 mg/kg bw/day (+21.4%). Statistically significantly higher absolute and relative liver weight was also noted in females starting at

15 mg/kg bw/day (up to +33.4% and +43.8%, respectively, increase in a dose-related manner). Hepatocellular liver hypertrophy up to mild degree was noted in males starting at 30 mg/kg bw/day, and in females starting at 15 mg/kg bw/day, up to mild degree, with a dose-related increase in incidence and severity. The hypertrophy was located predominantly in the centrilobular area, and occasionally extending to panlobular distribution (generally associated with mild severity grade). Pigment in the liver was noted in females starting at 15 mg/kg bw/day, concurrent with hypertrophy, at minimal degree.

Statistically significantly higher absolute and relative kidney weight was noted in females only, at 30 +7.5% and 10.5%, respectively) and 120 (+8.2% and +16.4%, respectively) mg/kg bw/day. There was no microscopic correlate. In males, a statistically significantly higher relative kidney weight was noted at 120 mg/kg bw/day (+8.4%). A statistically significantly higher relative heart weight was noted in females at 30 and 120 mg/kg bw/day (+12.8% and +12.1%, respectively). There was no microscopic correlate.

Vacuolation of the urothelium in the urinary bladder was noted in males and females at 120 mg/kg bw/day only, up to mild degree.

More details in Annex I section 3.10.1.5.

General toxicity

Body weight in males and females at 120 mg/kg bw/day was statistically significantly lower (-9.3% and -7.1%, respectively). Clinical biochemistry findings demonstrated statistically significantly lower total protein concentration in males and females at 120 mg/kg bw/day (-4% and -7%, respectively). Furthermore, a statistically significantly lower total cholesterol and HDL cholesterol was observed in males at 120 mg/kg bw/day (-28% and -31%, respectively).

More details in Annex I section 3.10.1.5.

Conclusions

Lower seminal vesicle gland weight was noted in males at 30 and 120 mg/kg bw/day and statistically significantly lower epididymis weights were observed in high dose males in the absence of marked general toxicity. A nodule which correlated microscopically to a sperm granuloma was noted on the tail of the epididymis of one male at 120 mg/kg bw/day.

Spermatotoxicity was demonstrated at 120 mg/kg bw/day including lower percentage of motile sperm progressive sperm and number of cells with a normal morphology, and an increase of cells with a detached head and abnormal neck. Sperm granulomas in the epididymis were noted in 3/10 males at 120 mg/kg bw/day.

Testicular toxicity screening test in rats (Study report, 2020a)

In a short-term repeated dose toxicity study from 2020, male Wistar Han rats were dosed daily to 3-*p*-cumenyl-2-methylpropionaldehyde by oral gavage at doses 0, 30, 100 and 300 mg/kg bw/day for 28 days (5 males per group). No adverse clinical signs of toxicity were noted and no mortality occurred during the study period.

Gross lesions were observed in the epididymis in males at 300 mg/kg bw/day. A focal nodule (soft, yellow) was noted unilaterally in the tail of the epididymis in 3 out of 5 males at 300 mg/kg bw/day. No effects on sperm motility, concentration and morphology were observed in males at 30 mg/kg bw/day.

At 100 mg/kg bw/day, a lower percentage of motile sperm (-24%), progressive sperm (-30%) and number of cells with a normal morphology (-13%) was recorded. In addition, an increased number of cells with a detached head (+443%) and abnormal neck (+700%), and decreased number of cells with a coiled tail (-61%) were observed.

At 300 mg/kg bw/day, severe effects on the sperm motility, concentration and morphology were observed, which consisted of decreased total sperm count in the epididymis (-39%), percentage of motile sperm (-78%), percentage of progressive sperm (-90%) and number of sperm cells with a normal morphology (-

96%). The change in percentage of motile sperm and progressive sperm were statistically significant. The sperm cell morphology from 3 out of 5 males could not be determined as the sperm cell count for morphology was below the required 200 cells. In addition, a lower number of cells with a coiled tail (-87%), accompanied by an increase in number of cells with detached head (+246%), abnormal head (+200%) and/or neck (+900%) and combined cells were observed in the remaining 2 out of 5 animals.

The right testis was evaluated histologically from all males. Degeneration of elongating spermatids was noted in all animals at 300 mg/kg bw/day, up to moderate degree. This was most readily observed in early tubular stages, approximately IVIII (corresponding to Step 15 to Step 19 spermatids) and was characterized by both an abnormal shape and abnormal location within the seminiferous tubules for the given stage. Abnormal shape was variable and consisted of either condensed, round, darkly basophilic nucleus with a bent/squiggled 'neck' giving a tadpole-like appearance to the nucleus, large cytoplasmic droplets extending into the lumen, and/or round shape with pale eosinophilic cytoplasm as a small condensed darkly basophilic nucleus which was often dissociated from the adjacent cells near the luminal border or sloughing into the lumen. Normally shaped elongated spermatids were not uncommonly present in the same tubular profile.

Spermatid retention was noted in all animals at 300 mg/kg bw/day, up to moderate degree. This was characterized by the presence of elongated spermatids (both normal and abnormal shaped) at the luminal surface of the seminiferous epithelium beyond the expected point of release (i.e. Stage VIII), and affected of primarily Stage IX-XII tubules. Less often, elongated spermatids were observed in low numbers at the base of the seminiferous tubules in all tubular stages.

Degeneration of round spermatids was prominent in one male at 300 mg/kg bw/day and was characterized by condensed and hyper-eosinophilic round spermatids, often dissociated from the surrounding cells in the seminiferous tubule and sloughing into the lumen. Concurrent depletion of spermatocytes (round and elongated) and degeneration of elongated spermatids were noted in this animal. The few remaining histologic changes noted in the testis, including minimal Sertoli cell vacuolation, were considered by the study authors to be incidental findings and/or were within the range of background pathology encountered in the testis of rats of this age and strain. There was no test item-related alteration in the prevalence, severity, or histologic character of those incidental tissue alterations.

According to study authors, the combination of histologic changes of the testis of treated male rats is suggestive of an abnormality in spermiogenesis (transformation of round spermatids to mature, elongated spermatids) and spermiation (release of mature spermatids from the seminiferous epithelium).

Increased vacuolation of Sertoli cells can be a substance-related change. However, in the present study vacuolation was observed in the controls as well and severity was minimal in all groups. The changes noted by light microscopy correlate with the changes on sperm analysis including lower sperm concentrations, and morphologic abnormalities. The nodules noted macroscopically in the epididymis of 3 of 5 males at 300 mg/kg bw/day are suggestive of sperm granulomas.

General toxicity

Slightly lower body weight and body weight gain was observed in males at 100 mg/kg bw/day starting on day 15, with body weight being -7% at the end of treatment (day 28). Body weight and body weight gain in males at 300 mg/kg bw/day were moderately decreased starting on day 8 (mean body weight was -11%on day 28), achieving statistical significance for body weight on day 29. No test item-related effects on body weight and body weight gain were observed in males at 30 mg/kg bw/day. Food consumption was minimally lower at 100 and 300 mg/kg bw/day in week 1 but lacked a dose-related effect.

The metabolite 4-isopropylbenzoic acid (4-iPBA) was below detection limit in plasma samples from controls and at 30 mg/kg bw/day, but was detected in all plasma samples at 100 and 300 mg/kg bw/day (a dose-related increase). In animals at 30 mg/kg bw/day, trace amounts 4-iPBA-CoA were detected in the testes of only one individual. At 100 and 300 mg/kg bw/day, the conjugate was detectable at levels in testes samples from all animals (dose-related increase). The concentration of the metabolite was clearly higher in the liver than in testes (>500-fold).

Conclusions

Gross lesions were observed in the epididymis at 300 mg/kg bw/day. Clear signs of spermatotoxicity were demonstrated at 100 and 300 mg/kg bw/day including lower percentage of motile sperm, progressive sperm and number of cells with a normal morphology. There were no marked general toxicity among treated rats.

Testicular toxicity was demonstrated, including degeneration of elongating spermatids and spermatid retention in all animals at 300 mg/kg bw/day. The nodules noted macroscopically in the epididymis of 3 of 5 males at 300 mg/kg bw/day were suggestive of sperm granulomas.

The metabolite *p*-iPBA was detected in all plasma samples, in testes and in the liver at 100 and 300 mg/kg bw/day.

Testicular toxicity screening test in rabbits (Study report, 2011b)

In a short-term repeated dose toxicity study from 2011 (non-GLP compliant), male New Zealand White rabbits were dosed daily via oral gavage at 0, 30, 100 and 300 mg/kg bw/day for 14 days (5 animals per dose group).

The weight of the epididymides, left cauda epididymis, testes, seminal vesicles (with and without fluid) and prostate and the ratios of these organ weights to terminal body weight were unaffected by treatment up to 300 mg/kg bw/day.

The values for the number of motile sperm and total sperm count from ejaculated semen samples were highly variable across the dosage groups, including the control group. A trend in the mean number of motile sperm (596.6, 543.3 and 431.0 at 30, 100 and 300 mg/kg bw/day, respectively, vs. 627.0 in controls) and total sperm count (616.6, 595.5 and 496.2 at 30, 100 and 300 mg/kg bw/day, respectively, vs. 679.0 in controls) from ejaculated samples in the treated groups was observed. According to study authors, individual values were highly variable and the lowest reported individual values were within the range of the control group values. According to study authors, all values across all treated groups were within the range of the concurrent control group values and/or the historical control range at the testing facility.

Table 11: Summary of cauda epididymal sperm morphology (adapted from registration dossier).

Group	1	2	3	4
Dose material	Corn oil	3-*p*-cumenyl-2-methylpropionaldehyde	3-*p*-cumenyl-2-methylpropionaldehyde	3-*p*-cumenyl-2-methylpropionaldehyde
Dose level (mg/kg/day)[a]	0	30	100	300
Rabbits examined N	5	5	5	5
Normal (mean±SD)	141.6±17.2	121.4±30.5	102.2±25.6	128.2±31.3
Percent abnormal (mean±SD)	30.4±9.5	31.1±9.1	48.9±12.8	35.9±15.7
Detached head (mean±SD)	39.0±19.9	35.0±13.5	58.8±15.1	52.2±23.0
No head (mean±SD)	13.8±6.3	15.2±11.9	26.8±14.9	10.4±5.7
Amorphous (mean±SD)	1.0±1.0	0.2±0.4	0.8±1.8	0.0±0.0
Macrohead (mean±SD)	0.0±0.0	0.0±0.0	0.2±0.4	0.2±0.4
Microhead (mean±SD)	0.0±0.0	0.0±0.0	0.2±0.4	0.0±0.0
Broken flagellum (mean±SD)	4.3±2.3	2.2±1.1	2.4±1.7	3.8±4.8
Coiled flagellum (mean±SD)	3.6±2.1	5.0±5.8	8.2±4.1	4.8±3.0
Bent flagellum (mean±SD)	0.8±0.8	0.0±0.0	0.4±0.5	0.6±0.9

[a]dosage occurred on days 1 through 14 of study.

According to study authors, the values for percent motile sperm, number of nonmotile sperm from the semen ejaculate sample and cauda epididymal sperm count and density were comparable among the four dosage groups. More details in Annex I Section 3.10.1.4.

General toxicity
No adverse clinical signs were observed. Body weights and body weight gains were unaffected by exposure up to 300 mg/kg bw/day. Terminal body weights were comparable among the four dosage groups. More details on body weight and food consumption in Annex I Section 3.10.1.4.

Conclusions

A dose-related trend of the mean number of motile sperm and total sperm count from ejaculated samples in treated groups was observed in rabbits in absence of adverse general toxicity.

3-(*p*-cumenyl)propionaldehyde

Short term repeated dose toxicity test in rats (Study report A)

In a 14-day repeated dose toxicity study (no guideline, not GLP-compliant) male Crl:CD(SD) rats (10 per group) were exposed daily to 3-(*p*-cumenyl)propionaldehyde at doses 0, 25, 75 and 250 mg/kg bw/day.

At 250 mg/kg bw/day the weights of the epididymides, caudal epididymis, testes and paired kidneys and the ratios of these organ weights to terminal body weight were unaffected. The weights of the seminal vesicle and prostate were reduced in this group. At this dose microscopic examination showed microscopic lesions in testes, epididymides and seminal vesicles including atrophy in the latter. At 250 mg/kg bw/day, values for the number and percent motile sperm, the number of non-motile sperm and the total sperm count from the vas deferens were unable to be determined.

At 250 and 75 mg/kg bw/day, the average sperm count and sperm density from the cauda epididymis was reduced or statistically significantly reduced. In addition, the percentage of abnormal sperm, specifically sperm with detached heads or no heads, was statistically significantly increased at 75 and 250 mg/kg bw/day. In these groups, also a slight increase in the number of sperm that had a broken flagellum was noted. Few details were provided in the robust study summary.

General toxicity

All rats survived until scheduled sacrifice and no treatment related clinical observations were recorded. At 250 mg/kg bw/day, terminal body weights were statistically significantly reduced in the high dose group (no detailed information available). At 250 and 75 mg/kg bw/day statistically significant increase in absolute and relative weights of the liver were seen, without related microscopic changes. See Annex I Section 3.10.1.6.

Conclusions

The weights of the seminal vesicle and prostate were reduced at 250 mg/kg bw/day. At this dose, microscopic examination showed microscopic lesions in testes, epididymides and seminal vesicles including atrophy in the latter. The number and percent motile sperm, number of non-motile sperm and the total sperm count from the vas deferens could not be determined at 250 mg/kg bw/day.

At 250 and 75 mg/kg bw/day, the average sperm count and sperm density from the cauda epididymis was reduced and the percentage of abnormal sperm was statistically significantly increased. There were no adverse general toxicity observed.

Short term repeated dose toxicity test in rabbits (Study report B)

In a 14-day repeated dose toxicity study (no guideline, not GLP compliant) male New Zealand White rabbits (5 animals/group) were exposed daily to 3-(*p*-cumenyl)propionaldehyde via stomach tube at doses

0, 10, 30 and 100 mg/kg bw/day. Since no toxicity was observed, a dose of 300 mg/kg bw/day was assessed in a study extension.

No treatment related clinical observations were recorded and no gross lesions were revealed. Body weights and body weight gains were unaffected at 300 mg/kg bw/day. Absolute and relative food consumption values were unaffected and terminal body weights and organ weights were comparable to the control group. Relative liver and kidney weights were slightly increased at 300 mg/kg bw/day (+13% and 9%, respectively) without reaching statistical significance (Annex I section 3.10.1.7).

Microscopic findings were noted in the testes and epididymis of rabbits given 300 mg/kg bw/day. All five rabbits in this group had minimal or mild increases in residual bodies in the testes. Three of these rabbits also had mild or minimal depletion of spermatozoa in the epididymides and of these three rabbits, two also had detachment of the seminiferous tubules of the testes. The detachment in the seminiferous tubules was noted when large clear areas were present within the seminiferous epithelium separating the germ cells. Values for number and percent motile sperm, number of non-motile sperm and total sperm count were comparable to the control group. The values for sperm count and concentration from ejaculated semen samples were highly variable across the dose groups. The percentage of abnormal sperm on DS 15 was highest at 300 mg/kg bw/day (38.0%), compared to controls, as well as the values in the initial study (ranged from 15.4% to 29.2%). The abnormal sperm consisted primarily of sperm with detached heads. According to study authors, the increase for the group average could be attributed to two rabbits (of 5), which had 49.5% and 60.0% abnormal sperm, respectively. The percentage of abnormal sperm before dose administration was also highest in the 300 mg/kg/day dose group (23.7%), as compared to the concurrent control (17.8%), and as compared to the pre-dose values for the rabbits in the other groups (ranged from 12.9% to 17.7%).

Conclusions

Microscopic findings were noted in the testes and epididymis of rabbits at 300 mg/kg bw/day in the absence of adverse general toxicity. All five rabbits in this group had minimal or mild increases in residual bodies in the testes. Three of these rabbits also had mild or minimal depletion of spermatozoa in the epididymides and of these three rabbits, two also had detachment of the seminiferous tubules of the testes. The percentage of abnormal sperm on DS 15 was highest at 300 mg/kg bw/day. The abnormal sperm consisted primarily of sperm with detached heads.

4-isopropylbenzaldehyde

There are no data available on reproductive toxicity for the substance 4-isopropylbenzaldehyde. However, Api et al. published a safety assessment of this substance in 2020. Developmental and reproductive toxicity was not assessed in that study, since data on these endpoints were lacking.

4-isopropylbenzoic acid (4-iPBA)

Short term toxicity study in rats reviewed in (Natsch et al. 2021)

The metabolite 4-isopropylbenzoic acid (4-iPBA) was studied in a 5 day short-time study in male CD rats (6 per group) at 0, 15, 50 and 150 mg/kg bw/d by oral gavage. There were no clinical signs observed. Food consumption was reduced at high dose (-19%).

The absolute and relative weights of the epididymides were increased at 150 mg/kg bw/d (+20%). In particular, a statistically significant increase in cauda epididymal weight was demonstrated at 150 mg/kg bw/day (+43%). Microscopic examination of the testes and epididymides demonstrated a spectrum of changes in epidydimis and testes. In the epididymides, there were minimal to slight interstitial inflammatory cells (5/6 animals), apoptotic epithelial cells (4/6), degenerate spermatogenic cells in the ducts (5/6) and epithelial hyaline droplets (4/6) and reduced numbers of spermatozoa (4/6). In the testes,

a dose-related increase in the incidence of seminiferous tubular vacuolation was observed in animals across all treated groups. Degenerate spermatocytes (4/6) and spermatid giant cells (3/6) were observed at 150 mg/kg bw/day. The seminiferous tubular vacuolation was only seen in one and two animals in the low and intermediate dose groups, and in four of six animals in the high dose group. The sperm analysis did not indicate adverse treatment-related effects on sperm motility, concentration or morphology.

Conclusions

Testicular toxicity in the form of seminiferous tubular vacuolation was demonstrated in 4 of 6 animals at 150 mg/kg bw/day without observations of adverse general toxicity.

Structurally similar substances

The substance *p*-tert-butylbenzoic acid, TBBA, has a harmonised classification as toxic to reproduction (category 1B, H360F) based on toxicity in reproductive organs, including testicular lesions, spermatotoxic effects and infertility. Testes toxicity was characterised by lower organ weights, testes atrophy from seminiferous tubular degeneration and destruction of the germinative epithelium resulting in disturbance of spermatogenesis, including loss of late spermatids.

Another substance, 2-(4-tert-butylbenzyl)propionaldehyde (lysmeral) has a harmonised classification as toxic to reproduction (category 1B, H360Fd) based on testes effects (reduced organ weights and degeneration), spermatotoxicity and reduced fertility. Developmental effects included post-implantation loss and decreased pup body weights.

10.10.3 Comparison with the CLP criteria

The criteria for classification as Repr. 1B for adverse effects on sexual function and fertility are considered fulfilled for *p*-cymene, 3-*p*-cumenyl-2-methylpropionaldehyde, 3-(*p*-cumenyl)propionaldehyde, 4-isopropylbenzaldehyde and 4-isopropylbenzoic acid (4-iPBA), based on a weight of evidence approach and read-across within this category. The substances show clear effects on male reproductive system, including testicular toxicity and toxicity to the sperm. The majority of available studies are conducted in rats, but two studies of limited quality demonstrate similar findings in one additional species (rabbit).

Infertility was demonstrated in rats in the OECD TG 422 study (males and females exposed to *p*-cymene) and in the OECD TG study 415, at paternal exposure to 3-*p*-cumenyl-2-methylpropionaldehyde.

Effects related to maternal exposure included reduced number of implantation sites and increased pup mortality, as well as statistically reduced uterus- and ovaries weights (in the OECD TG 415 study following exposure to 3-*p*-cumenyl-2-methylpropionaldehyde). Statistically significant smaller uterus weights were also observed in rats in a short term repeated dose toxicity study on *p*-cymene. Additionally, irregular cycles during pre-cohabitation and increased pre-implantation loss were noted among exposed female rats in the OECD TG 422 study on *p*-cymene. No marked general toxicity in terms of reduced body weight or clinical condition was noted in parental animals.

The available data provide in a weight of evidence approach, and using a read-across approach, clear evidence of an adverse effect on sexual function and fertility for this category of five substances. There is no mechanistic evidence to indicate that the observed effects are not relevant for humans. Classification in Repr. 1B, H360F is therefore warranted.

Classification in Repr. 1A is not appropriate as it should be based on human data and no human data are available.

Classification in Repr. 2 is not appropriate as the evidence for adverse effects on sexual function and fertility from existing experimental data on these substances is considered as clear evidence and not some evidence.

10.10.4 Adverse effects on development

Table 12: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
p-cymene EC 202-796-7			
OECD TG 422 screening for reproductive/developmental toxicity study. GLP compliant Deviations from test guideline were considered by the Registrant not to have compromised the validity or integrity of the study Rats Sprague Dawley Crl:CD®(SD). 10 animals/sex/group. Assigned reliability 1 by the Registrant	*p*-cymene EC 202-796-7 Purity: assumed 100% Vehicle: corn oil The substance was administered via oral gavage at doses 0, 50, 100, 200 mg/kg bw/day once daily Duration of treatment: P0 males: 2 weeks pre-cohabitation, during cohabitation (up to 2 weeks) and continuing during post-cohabitation until the day prior to termination (approximately 35 days). P0 females: 2 weeks pre-cohabitation, cohabitation (up to 2 weeks) and during gestation and lactation continuing until LD 13 (approximately 63 days).	***Maternal animals*** **Mortality and Clinical Observations** 1 control animal, 1 animal at 50 mg/kg bw/day, 6 animals at 100 mg/kg bw/day, and 9 animals at 200 mg/kg bw/day were euthanized on GD 25 due to failure to become pregnant. One female at 200 mg/kg bw/day was euthanized for welfare reasons on GD 24 (not pregnant). **Body weights** No effects on body weights and body weight changes. **Clinical biochemistry** ↑ alkaline phosphatase activity at 100 mg/kg bw/day (+79% p≤0.05). ↓ albumin at 100 mg/kg bw/day (-9%; p≤0.05). ↓ ALT at 50 (-32%; p≤0.01) and 100 mg/kg bw/day (-49%; p≤0.01). ↓ CHOL at 100 mg/kg bw/day (-29%; p≤0.05). **Reproductive organs and function** ↑ animals with at least one irregular cycle (cycles < 4 days or > 5 days duration) during pre-cohabitation at 200 mg/kg bw/day. **Fertility** ↓ fertility at 100 mg/kg bw/day and 200 mg/kg bw/day. ↓ pregnant females at 100 and 200 mg/kg bw/day. Fertility index 90%, 90%, 40% and 0% at 0, 50, 100 and 200 mg/kg bw/day, respectively. ***F1 generation*** **Clinical observations** There were 9, 9 and 4 litters at 0, 50 and 100 mg/kg bw/day, respectively. There were no offspring produced at 200 mg/kg bw/day. ↓ live birth index at 100 mg/kg bw/day 94.3% vs 100%; p≤0.01). ↓ post-implantation survival index at 100 mg/kg	Study report, 2019. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 8 Annex I section 3.10.1.8

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		bw/day (87.3% vs. 95% in controls). ↓ litters with less than 100% viability at 100 mg/kg bw/day (1 of 4 litters with 100% viability vs. 9 of 9 in controls). ↓ pup weight on PND 1 at 100 mg/kg bw/day. NOAEL: 50 mg/kg bw/day (developmental toxicity)	
3-*p*-cumenyl-2-methylpropionaldehyde EC 203-161-7			
OECD TG 414 Prenantal Developmental Toxicity Study GLP compliant Female Wistar-Han rats 22 females per dose group	3-*p*-cumenyl-2-methylpropionaldehyde EC 203-161-7 Purity: no information Vehicle: corn oil Substance was administered once daily via oral gavage at doses 0, 25, 75, 150 mg/kg bw/day Duration of treatment: gestations days 6-20.	***Maternal animals*** **Mortality and clinical observations** No clinical signs or mortality were observed. **Body weight and food consumption** ↓ body weight loss at 150 mg/kg bw/day (-0.9 vs. +18.1 g; p≤0.01) ↑ food consumption at 25 mg/kg bw/day (GD 5-6 +18%; p≤0.05), at 75 mg/kg bw/day (GD 5-6 + 21%; p≤0.01, GD 10-11 +17%; p≤0.05) and at 150 mg/kg bw/day (GD 5-6 +17%; p≤0.05). ↓ food consumption at 150 mg/kg bw/day (GD 6-7, -12%; p≤0.01). **Thyroid hormone analysis** ↓ T3 and T4 levels at 75 mg/kg bw/day (-34% and -23% respectively; p≤0.01) and at 150 mg/kg bw/day (-44%; p≤0.05, and -4%; p≤0.01, respectively). ↑ TSH levels at 25 mg/kg bw/day (+49%; p≤0.01), at 75 mg/kg bw/day (+44%; p≤0.05). **Organ weights** ↑ absolute liver weights at 75 mg/kg bw/day and at 150 mg/kg bw/day. ↑ microscopic liver changes at 25, 75, and 150 mg/kg bw/day (consisting of minimal hepatocellular single-cell necrosis). **Maternal developmental toxicity** ↑ number of corpora lutea at 25 mg/kg bw/day (12.3 vs. 11.5; p≤0.05) and 150 mg/kg bw/day (12.8 vs 11.5 p≤0.05). At 75 mg/kg bw/day the number of corpora lutea was 12.6 but not statistically significant according to study report. ***Fetuses*** ↓ fetal body weight (males, females, and combined) at 75 (-5% combined; p≤0.05) and 150 mg/kg bw/day (-9% combined, p≤0.01).	Study report, 2021. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. DS had access to full study report Study 2 Annex I 3.10.1.2

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		At 150 mg/kg bw/day, a malformation of bent humerus was observed for a single fetus and variations of bent scapula, wavy ribs, thoracolumbar supernumerary ribs, and incomplete ossification of the frontal, parietal, squamosal, and supraoccipital bones of the skull were also observed for this fetus. At 25 mg/kg bw/day a discolored liver lobe was noted for one fetus. NOAEL: 75 mg/kg bw/day (fetal weight)	
One-Generation Reproduction Toxicity Study OECD TG 415 No deviations GLP compliant Sprague Dawley rats, 25 males and females per dose group. Assigned reliability 1 by the Registrant.	3-*p*-cumenyl-2-methylpropionaldehyde EC 203-161-7 Purity: Vehicle: Corn oil The substance was administered orally (gavage) at doses 0, 25, 75, 150 mg/kg bw/day. Male P generation rats were gavaged once daily beginning 83 days prior to cohabitation, through cohabitation, continuing through the day before sacrifice. Female P generation rats were gavaged once daily beginning 14 days before cohabitation, through cohabitation and on day 25 of gestation (rats that did not deliver) or day 22 postpartum (rats that delivered a litter). Treated rats were mated with untreated cohorts of male and female rats. F1 generation rats were not directly dosed but may have been exposed to the substance in utero during gestation and through maternal milk postpartum. F1 generation was followed until day 60 postpartum.	*Maternal animals* **Clinical observations** No clinical signs were observed. **Body weight** <u>Gestation</u> ↓ mean body weight at 150 mg/kg bw/day (-5% and -8%, GD 18 and 21, respectively; p≤0.01). <u>Lactation</u> ↓ mean body weight at 75 mg/kg bw/day (-4%, LD 8; p≤0.05) and at 150 mg/kg bw/day (-7%, -8.5%, -6%, LD 5, 8, 11, respectively; p≤0.01). Effects on body weights correlated with statistically significant lower feed consumption during lactation. **Reproductive organs and function** ↓ implantation sites per delivered litter at 150 mg/kg bw/day (-11%; p≤0.01). ↑ dams with all pups dying between days 1 and 5 postpartum at 150 mg/kg bw/day (16.7% vs. 0; p≤0.01). ↓ mean number pups delivered/litter at 150 mg/kg bw/day (-15%; p≤0.01). ↓ number liveborn pups/litter at 150 mg/kg bw/day (-17%; p≤0.01). ↑ number stillborn pups/litter at 150 mg/kg bw/day (2.7% vs 0.8% in controls ; p≤0.01). ↑ pup mortality at 25 mg/kg bw/day (4 vs. 0, days 12-15; p≤0.01) and at 150 mg/kg bw/day (18 vs. 1, day 1; p≤0.01, 51 vs. 12, and days 2-5; p≤0.01). ↓ viability index at 150 mg/kg bw/day (75.7% vs. 96.3%; p≤0.01). ↓ lactation index at 25 mg/kg bw/day (96.5 vs. 99.7%; p≤0.01). ↓ number surviving pups/litter at 150 mg/kg bw/day (-17% to -36%, day 1-22; p≤0.01).	Study report, 2011a. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. DS had access to full study report Study 1, Annex I section 3.10.1.1 Additional data in Confidential Annex

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		↓ live litter size at weighing at 150 mg/kg bw/day (-18% to -23%, day 1-22; p≤0.01). ***F1 generation*** *Pups of treated males* ↓ no litters produced at 150 mg/kg bw/day (p≤0.01). ↑ absolute feed consumption in males at 25 mg/kg bw/day (days 30-37; p≤0.01, days 23-57; p≤0.05). ↓ relative brain weight in males at 25 and 75 mg/kg bw/day (-6%; p≤0.05). *Pups of treated females* ↑ litters with pups with lenticular opacities at 150 mg/kg bw/day (771 vs. 4 in controls; p≤0.01). ↓ mean pup body weight/litter at 75 mg/kg bw/day (ranging from -19% day 8; p≤0.01, to -10% day 18, p≤0.05) and at 150 mg/kg bw/day (ranging from -19% day 8, p≤0.01 to -9%, day 18; p≤0.05). *Male pups of treated females* ↓ body weight gains at 75 mg/kg bw/day (-7%, days 30-37; p≤0.05) and at 150 mg/kg bw/day (-12%, days 23-30 and -9%, days 30-37; p≤0.01). ↓ mean body weight at 75 mg/kg (ranging from -13% day 23; p≤0.01, to -6%, day 50; p≤0.05) and at 150 mg/kg bw/day (ranging from -20%, day 23 to -8%, day 57; p≤0.01). ↓ absolute feed consumption at 150 mg/kg bw/day (-17%, days 23 to 30; p≤0.01 and -8%, days 30-37; p≤0.05). ↑ relative feed consumption at 75 mg/kg bw/day (+9% days 30-37; p≤0.01, +5% days 37-44; p≤0.05) and at 150 mg/kg bw/day (+8% days 30-37; p≤0.05 and +8% days 37-44, +6% days 44-51, +6% days 51-57; p≤0.01). ↓ anogenital distance at 75 and 150 mg/kg bw/day (-7% and -6%, respectively, day 22; p≤0.05). ↓ absolute terminal body weight at 150 mg/kg (-8%; p≤0.01). ↓ absolute left epididymis weight at 150 mg/kg bw/day (-10%; p≤0.01). ↑ relative testis weight at 75 mg/kg bw/day (+5%; p≤0.05) and at 150 mg/kg bw/day (+5% and +6%; p≤0.050). ↓ absolute pituitary weight at 150 mg/kg (-17%; p≤0.01). ↓ absolute brain weight at 150 mg/kg (-4%; p≤0.01).	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		↓ absolute and relative adrenals weight at 75 mg/kg bw/day (-15% and -16%, respectively; p≤0.01) and at 150 mg/kg (absolute only, -13%; p≤0.05) *Female pups of treated females* ↑ anogenital distance at 150 mg/kg/day (+4% at LD 1; p≤0.01 when covaried with fetal bw/litter). ↓ body weight gains at 150 mg/kg/day (-9% days 23-30; p≤0.05). ↓ body weight at 75 mg/kg (ranging from -12%, day 23; p≤0.01, to -6%, day 51; p≤0.05) and at 150 mg/kg bw/day (ranging from -18%, day 23; p≤0.01, to -6%, day 51; p≤0.05). ↓ absolute feed consumption at 150 mg/kg/day (-14% days 23 to 30; p≤0.01). ↑ relative feed consumption at 75 mg/kg bw/day (+6% days 23-57; p≤0.05) and at 150 mg/kg bw/day (+4% days 23-57; p≤0.01). ↓ absolute brain weight in females at 75 mg/kg bw/day (-3%; p≤0.05) and at 150 mg/kg (-6%; p≤0.01). ↓ absolute ovary (left) weight in females at 150 mg/kg bw/day (-17%; p≤0.05). NOAEL 25 mg/kg bw/day (pups weight)	

Table 13: Summary table of human data on adverse effects on development

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
-				

Table 7: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
-				

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

***p*-cymene**

Reproductive/developmental toxicity study OECD TG 422 (Study report, 2019)

In an OECD TG 422 screening for reproductive/developmental toxicity study (GLP compliant) from 2019, male and female Sprague Dawley rats were exposed to *p*-cymene via oral gavage at 0, 50, 100, 200 mg/kg bw/day once daily. Parental males were dosed 2 weeks pre-cohabitation, during cohabitation (up to 2 weeks) and continuing during post-cohabitation until the day prior to termination (approximately 35 days). Parental females were dosed 2 weeks pre-cohabitation, cohabitation (up to 2 weeks) and during gestation and lactation continuing until LD 13 (approximately 63 days).

There were treatment-related reductions in fertility at 100 and 200 mg/kg bw/day. The number of pregnant females was statistically significantly reduced (4 and 0 females pregnant and the male and female fertility indices of 40% and 0%) at 100 and 200 mg/kg bw/day. The male and female fertility indices were 90%, 90%, 40% and 0% at 0, 50, 100 and 200 mg/kg bw/day, respectively.

General toxicity – maternal animals

There were no treatment-related reductions in the body weights and body weight changes at any dose. No effects on food consumption and compound intake were noted at any dose.

Eighteen females across all groups, including controls, were euthanized early. Seventeen of these females (1/10 control, 1/10 at 50 mg/kg bw/day, 6/10 at 100 mg/kg bw/day, and 9/10 at 200 mg/kg bw/day) were euthanized on GD 25 due to failure to become pregnant. One female at 200 mg/kg bw/day was euthanized for welfare reasons on GD 24; this female was not pregnant. Microscopic findings in this female were present in the liver, adrenal, and kidney and were considered the source of morbidity.

Absolute and relative liver weights were statistically significantly increased in females at 100 mg/kg bw/day (+26% and +22%, respectively). Females at 200 mg/kg bw/day were not included as all females at this dose were euthanized due to non-pregnancy. Hepatocellular hypertrophy was minimally present in 1/10 females (micropscopic findings in female euthanised on GD 24) at 200 mg/kg bw/day and in 1/6 females at 50 mg/kg bw/day.

Clinical biochemistry findings (at ≥ 100 mg/kg bw/day) included increases in alkaline phosphatase activity (+79% in females at 100 mg/kg bw/day) and decreases in albumin (-9% in females at 100 mg/kg bw/day). More details in Annex I section 3.10.1.8.

F1 generation

There were 9, 9 and 4 litters that appeared normal for most of the observation period at 0, 50 and 100 mg/kg bw/day, respectively. There were no pregnant animals at 200 mg/kg bw/day.

The live birth index was statistically significantly reduced (94.3% vs. 100%) at 100 mg/kg bw/day. The post-implantation survival index was reduced at 100 mg/kg bw/day (87.3% vs. 95%). The number of litters with less than 100% viability was increased at 100 mg/kg bw/day with only 1 of 4 litters having 100% viability versus 9 of 9 in the control group. The viability indices on PND 4, 7 and 13 were comparable with control values at 100 mg/kg bw/day, respectively. All offspring survival index values at 50 mg/kg bw/day were comparable with controls.

The mean pup weights on PND 1 were reduced at 100 mg/kg bw/day (-11% in males, -9% in females, no information on statistical significance). On the remaining intervals (PND 4, 7, 11 and 13), the mean litter body weights at 50 and 100 mg/kg bw/day were comparable with the control group values.

The mean sex ratio was comparable among groups.

There were no organ weight differences in thyroid/parathyroid glands in the F1 pups, and no microscopic findings. There were no differences from controls for thyroxine (T4) or thyroid stimulating hormone (TSH) on PND 4 in F1 female pups. On PND 13, there were no differences from control for T4 levels in F1 male pups. Many of the individual animal TSH values were below the level of detection in males at 100 mg/kg/day and females at 50 and 100 mg/kg/day.

Conclusions

There were no pregnant animals at 200 mg/kg bw/day, thus no offspring was produced at the highest dose level. There were 9, 9 and 4 litters at control, low- and mid- dose levels, respectively.

Several findings are suggestive of effects on pups *in utero*. The live birth index and the post-implantation survival were statistically significantly reduced at the mid dose level. The average pup weight on PND 1 (-10%) was statistically significantly lower at this dose level. There were no marked general toxicity among maternal animals.

3-*p*-cumenyl-2-methylpropionaldehyde

Prenatal developmental toxicity study OECD TG 414 in rats (Study report, 2021)

In an OECD TG 414 prenatal developmental toxicity study (GLP compliant) from 2021, female Wistar-Han rats (22 females per group) were exposed to 3-*p*-cumenyl-2-methylpropionaldehyde once daily via oral gavage at doses 0, 25, 75, 150 mg/kg bw/day. Maternal animals were dosed during gestation days 6-20.

General toxicity – maternal animals

There were no clinical observations noted at any dose level at the daily examinations or approximately 1-hour post dose. No mortality was observed. At 150 mg/kg bw/day, a slight mean body weight loss with correspondingly lower mean food consumption were noted following the initiation of dosing (GD 6–7) compared to the control group. Mean body weight gains and food consumption were generally comparable to the control group for the remainder of the study and when the entire treatment period (GD 6–21) was evaluated and the mean absolute body weights in this group were unaffected. Mean body weights and body weight gains at 25 and 75 mg/kg bw/day and mean corrected body weights, corrected body weight gains at 25, 75, and 150 mg/kg bw/day were unaffected (Annex I 3.10.1.2).

Higer mean (absolute) liver weights were observed at 75 and 150 mg/kg bw/day. Higher mean liver weights were not dose-responsive, but multiple individual absolute liver values at 75 and 150 mg/kg bw/day were out of the concurrent control group range. There were no microscopic correlates. Microscopic liver changes were present at 25, 75, and 150 mg/kg bw/day and consisted of minimal hepatocellular single-cell necrosis. Single-cell necrosis was perivascular and/or random, and was characterized as individual eosinophilic rounded hepatocytes with pyknotic nuclei. Single-cell necrosis was dose-responsive in incidence rate. More details in Annex I Section 3.10.1.2.

Maternal developmental toxicity

Intrauterine survival was unaffected by test substance administration at 25, 75 and 150 mg/kg bw/day. Parameters evaluated included mean litter proportions of post-implantation loss, mean number of live fetuses, and fetal sex ratios.

Mean numbers of corpora lutea and implantation sites and the mean litter proportions of pre-implantation loss were similar across all groups.

Fetuses

Mean fetal body weights (males, females, and combined) at 75 and 150 mg/kg bw/day were lower than the concurrent control group (-4 % to -6%; $p \leq 0.05$ and -9%; $p \leq 0.01$, respectively) and below the mean values in the lab historical control data. Intrauterine growth at 25 mg/kg bw/day was unaffected by test substance administration.

There were no effects observed on number of live offspring, changes in sex ratio, changes in litter size and weights.

Mean anogenital distances (absolute and relative to the cube root of the fetal weight) were unaffected at all dose levels. Differences from the control group were slight and not statistically significant.

No external developmental malformations were observed for fetuses in treated groups. In the control group, one fetus was noted with a thread-like tail which consisted skeletally of absent sacral and caudal vertebrae. No external developmental variations were observed in fetuses in this study.

No skeletal developmental malformations were noted. At 150 mg/kg bw/day a malformation of bent humerus was observed for a single fetus; variations of bent scapula, wavy ribs, thoracolumbar supernumerary ribs, and incomplete ossification of the frontal, parietal, squamosal, and supraoccipital bones of the skull were also observed for this fetus.

No test substance-related visceral developmental variations were noted.

A discoloured liver lobe was noted for one fetus at 25 mg/kg bw/day.

Conclusions

The mean fetal body weight (males, females, and combined) at 75 and 150 mg/kg bw/day was statistically significantly reduced in dose-related manner (up to -9%). At 150 mg/kg bw/day, a malformation of bent humerus was observed for a single fetus. There was no marked general toxicity observed in maternal animals.

3-*p*-cumenyl-2-methylpropionaldehyde

One-Generation Reproduction Toxicity Study OECD TG 415 in rats (Study report, 2011)

In a One-Generation Reproduction Toxicity Study (OECD TG 415) from 2011, male and female rats were exposed by gavage to 3-*p*-cumenyl-2-methylpropionaldehyde at 0, 25, 75 or 150 mg/kg bw/day in corn oil. The mating procedure used a cross-over design where treated males were mated with untreated females, and treated females were mated with untreated males. F1 generation rats were not directly dosed but may have been exposed to the test material *in utero* during gestation and through maternal milk postpartum. F1 generation was followed until day 60 postpartum.

Treated males

3-*p*-cumenyl-2-methylpropionaldehyde caused infertility following mating with untreated female rats at paternal treatment of 150 mg/kg bw/day. Natural delivery and litter observations were unaffected by 3-*p*-cumenyl-2-methylpropionaldehyde up to 75 mg/kg bw/day.

Treated females

Pregnancy occurred in 25 (100%), 24 (96%), 24 (96%) and 24 (96%) of the 25 mated female rats at 0, 25, 75 and 150 mg/kg bw/day, respectively. All pregnant dams delivered litters. At 150 mg/kg bw/day, the average number of implantation sites per delivered litter was statistically significantly reduced, compared to controls (13.8 implantation sites vs. 15.5).

The number of dams with all pups dying between days 1 and 5 postpartum was statistically significantly increased at 150 mg/kg bw/day (16.7% vs 0 in controls). Reflecting the reduction in implantation sites (-11%; $p \leq 0.01$), the average number of pups delivered per litter (-15%) as well as the average number of liveborn pups per litter (-17%) was statistically significantly reduced at 150 mg/kg bw/day compared to controls. In addition, there was a statistically significant increase in the number of stillborn pups that were delivered at 150 mg/kg bw/day (2.7% vs. 0.8% in controls).

Pup mortality (i.e., found dead, presumed cannibalized or unscheduled sacrifice) was statistically significantly increased at 150 mg/kg bw/day on days 1 to 5 postpartum, compared to controls. As a result of the increase in pup mortality, the overall viability index (i.e., number of live pups on day 5 postpartum/number of liveborn pups on day 1 postpartum) was statistically significantly reduced at 150 mg/kg bw/day compared to controls (75.7% vs. 96.3%). Viability index was decreased also at 75 mg/kg bw/day (not statistically significant). At 150 mg/kg bw/day, the averages for the number of surviving pups per litter and the live litter size at weighing was statistically significantly reduced on days 1 through 22 postpartum, compared to controls (and decreased in a dose-related manner days 1-8). In addition, the average pup body weight per litter was statistically significantly reduced at 75 and 150 mg/kg bw/day at each tabulated interval between days 1 and 22 postpartum, compared to controls.

No other natural delivery and litter observations were affected by treatment up to 150 mg/kg bw/day. Values for the numbers of dams delivering litters, the duration of gestation, the gestation index (number of dams with one or more liveborn pups/number of pregnant rats), the numbers of dams with stillborn pups, lactation index, and percent male pups per number of pups sexed per litter were comparable among the four dosage groups.

General toxicity, body weight and food consumption – parental generation

Among females, during the first week of the dosage period (precohabitation), body weight gains were reduced at 75 mg/kg bw/day (-36%) and statistically significantly reduced at 150 mg/kg bw/day (-56%), as compared to controls. These reductions were transient and did not persist during the second week of the dosage period. Despite the rebound during the second week of the dosage period, body weight gains at 75 and 150 mg/kg bw/day remained reduced (75 mg/kg bw/day) or statistically significantly reduced (150 mg/kg bw/day) for the entire premating dosage period, compared to controls. The average body weight on day 15 was -2%, -2% and -3% at 25, 75 and 150 mg/kg bw/day, respectively.

During gestation, at 150 mg/kg bw/day, body weight gains remained reduced (-5% to -21%) at each tabulated interval within the gestation dosage period relative to control group values (not statistically significant). Maternal body weight gains at 25, 75 and 150 mg/kg bw/day were +2%, -5% and -15%, respectively, on DG 0 to 21. The average body weight on DG 21 was -1%, -3% and -8% at 25, 75 and 150 mg/kg bw/day, respectively.

During lactation, statistically significant body weight losses were observed at 150 mg/kg bw/day at the beginning of the lactation period (DL 1 to 8). Thereafter, body weight gains were comparable to or statistically significantly increased when compared to control group values.

The average maternal body weight was statistically significantly reduced at 150 mg/kg bw/day on DL 5 through 11, compared to controls. Maternal body weight gains at 25, 75 and 150 mg/kg bw/day were +8%, -44% and -24%, respectively, on DL 1 to 22. The average body weight on DL 22 was ±0, +3% and +1% at 25, 75 and 150 mg/kg bw/day, respectively.

Absolute and relative feed consumption values among females at 150 mg/kg bw/day during pre-cohabitation were statistically significantly reduced during the first week of the premating dosage period (day 1 to 8), compared to controls (-8% and -6%, respectively). Absolute and relative feed consumption values in females during the gestation period were unaffected up to 150 mg/kg bw/day. Absolute feed consumption values at 25, 75 and 150 mg/kg bw/day were -5%, -11% and -20%, respectively, on DL 1 to 15. Absolute and relative feed consumption values during the lactation period were unaffected at 25 mg/kg bw/day (Annex I, Section 3.10.1.1).

F1 generation pups of treated male rats mated with untreated females

There were no litters produced at 150 mg/kg bw/day from the mating of treated P generation male rats with untreated female rats. Therefore, these parameters were not evaluated at 150 mg/kg bw/day. There were no adverse clinical signs observed in the F1 generation male or female rats at 25 and 75 mg/kg bw/day (Annex I section 3.101.1).

Body weight gains and average body weight and feed consumption in the F1 generation male and female rats were unaffected by paternal treatment at 25 and 75 mg/kg bw/day. The relative brain weight was reduced at 25 and 75 mg/kg bw/day in male pups (-6%).

There were no effects on sexual maturation (preputial separation or vaginal opening) at any paternal dose level tested.

Anogenital distance on days 1 or 22 postpartum in F1 male and female pups was not affected by treatment of P generation male rats at 25 and 75 mg/kg bw/day. Nipple eruption did not occur in any male pup, and all female pups had nipples present on day 12 postpartum.

There were no gross lesions observed in the F1 generation pups that were stillborn or found dead or in the F1 generation pups that survived to scheduled necropsy on day 22 postpartum.

F1 generation pups of treated female rats mated with untreated male rats

At 150 mg/kg bw/day, 20 of 24 litters had one or more pups with a lenticular opacity in one or both eyes. This observation only occurred in one pup from one litter in the vehicle control group on days 19 through 22 postpartum. At 150 mg/kg bw/day, lenticular opacities were first observed on day 16 and generally persisted until day 22 postpartum. This observation was more prevalent in F1 generation male rats than in the female rats (18 male rats vs 6 female rats). This clinical sign was confirmed during scheduled necropsy examination.

In F1 generation male rats, body weight gains were statistically significantly reduced at 150 mg/kg bw/day maternal dose on days 23 to 37 postpartum, compared to controls. Thereafter, body weight gains were comparable to the control group values during the remainder of the postweaning period. Reflecting the initial reductions in weight gain, body weight gains in the F1 generation male rats at 150 mg/kg/day were -6% (statistically significant) for the entire postweaning period (days 23 to 57 postpartum). In F1 generation female rats, body weight gains were statistically significantly reduced at 150 mg/kg bw/day on days 23 to 30 postpartum, compared to controls. However, this reduction was transient, and body weight gains were comparable to the control group values during the remainder of the postweaning period. Body weight gains at 25, 75 and 150 mg/kg bw/day were +1%, -2% and ±0, respectively, on days 23 to 57 postpartum. Reflecting statistically significant reductions in the average pup body weight per litter that occurred prior to weaning, the average body weight was also statistically significantly reduced in the F1 generation male and female rats during post-weaning period at 75 and 150 mg/kg bw/day. Terminal average body weight in male offspring was -8% ($p \leq 0.01$) on day 57-60 postpartum, and -4% in female offspring (not statistically significant).

Corresponding to statistically significant reductions in body weight gains, absolute feed consumption values in the F1 generation male rats were statistically significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) at 150 mg/kg bw/day on days 23 to 30 postpartum and days 30 to 37 postpartum, compared to controls. Relative to body weight, F1 generation male rats consumed statistically significantly more feed on days 30 to 44 postpartum at 75 and 150 mg/kg bw/day and days 23 to 57 postpartum at 150 mg/kg bw/day.

Similar observations occurred in the F1 generation female rats, in that, absolute feed consumption values were statistically significantly reduced at 150 mg/kg bw/day on days 23 to 30 postpartum, compared to controls. Relative to body weight, F1 generation female rats consumed statistically significantly more feed on days 23 to 57 postpartum at 75 and 150 mg/kg bw/day.

Absolute feed consumption values in the F1 generation male rats were -2%, -3% and -7% at 25, 75 and 150 mg/kg bw/day, respectively, on days 23 to 57 postpartum. In F1 generation female rats, absolute feed consumption values were -2%, -1% and -4% at 25, 75 and 150 mg/kg bw/day, respectively, during the same period.

There were no effects on sexual maturation (preputial separation or vaginal opening) at any maternal dosage level tested. The average day on which sexual maturation was achieved was comparable among the dosage group and within the ranges observed historically at the Testing Facility. The average body weight of male rats on the day preputial separation occurred was significantly reduced at 75 and 150 mg/kg bw/day compared to controls. These reductions in body weight reflect statistically significant reductions in the average pup body weight per litter that occurred prior to weaning.

In male pups, anogenital distance on day 1 postpartum was not affected by treatment of P generation female rats at any dosage level tested. On day 22 postpartum, there was a statistically significant reduction in the anogenital distance of male pups at 75 and 150 mg/kg bw/day compared to controls. When covaried with fetal body weights per litter, the statistically significant reduction in anogenital distance was not apparent. This developmental delay correlated with an overall reduction in pup body weights on day 22 postpartum. In female pups, anogenital distance on day 1 postpartum was not initially affected by treatment of P generation female rats at any dose level tested. However, when covaried with fetal body weights per litter, there was a statistically significant increase in anogenital distance at 150 mg/kg bw/day, compared to controls. This increase in anogenital distance was no longer apparent by day 22 postpartum. Nipple eruption did not occur in any male pup, and all female pups had nipples present on day 12 postpartum.

The absolute weight of (left) epididymis was statically significantly reduced (-10%) in male pups at 150 mg/kg bw/day, as were absolute weights of the pituitary (-17%), brain (-4%) and adrenals (-15% at 75

and -13% at 150 mg/kg bw/day). Relative weights of testis were statistically significantly increased at 75 and 150 mg/kg bw/day. In female offspring, absolute brain weights were statistically significantly reduced at 75 (-3%) and 150 mg/kg bw/day (-6%), as were absolute ovary weights at 150 mg/kg bw/day (-17%).

One gross lesion observed in the F1 generation pups was a tan area on the left kidney of one male pup at 150 mg/kg bw/day. There were no other gross lesions observed in the F1 generation pups that survived to scheduled necropsy on day 22 postpartum. In the pups that were stillborn, found dead or humanely euthanized, no milk was present in the stomach of 1, 5, 5 and 14 F1 generation pups at 0, 25, 75 and 150 mg/kg bw/day, respectively. There was no correlation between dams with low weight and pups found dead.

Conclusions

Several findings are suggestive of effects on pups *in utero*. At LD 1, at the highest dose, litter size (-15%) and mean pup weight (-11%, a dose-related decrease) was statistically significantly reduced. There was a significant increase of pups dying the first days of lactation at the highest dose.

In stillborn pups and pups found dead or humanely euthanized, no milk was present in the stomach in a dose related manner, up to 14 pups at the highest dose. An assessment of individual maternal animals showed no indications of marked toxicity, including lower body weight in the maternal animals of these pups, indicating that the effects observed were not secondary to maternal toxicity.

Weight gains in male and female pups were reduced at 150 mg/kg bw/day after the lactation period. In males, the reduced body weight gains persisted for the entire postweaning period. Corresponding to the reductions in body weight gains, the absolute feed consumption was reduced, but relative to body weight pups consumed statistically significantly more feed. Reflecting the statistically significant reductions in the average pup body weight per litter that occurred prior to weaning, the average body weight was also statistically significantly reduced in the F1 generation male and female rats during the entire postweaning period at 75 and 150 mg/kg bw/day. Terminal average body weight in male offspring was -8% ($p \leq 0.01$) and -4% in female offspring (not statistically significant).

In pups of high dosed female rats mated with untreated male rats, the majority of litters had pups with a lenticular opacity in the eyes. There were first observed on day 16 and persisted until day 22 postpartum. This clinical sign was also confirmed during scheduled necropsy examination.

Absolute brain weight was statistically significantly reduced in both male (-4% high dose) and female pups (-3% and -6% in mid and high dose, respectively), as were weights of epididymis, testis, pituitary, adrenal gland in male pups, and ovaries in female pups.

In maternal animals there were slight but statistically significant effects on body weight at 150 mg/kg bw/day during gestation and beginning of lactation.

Structurally similar substances

The substance 2-(4-tert-butylbenzyl)propionaldehyde (lysmeral) has a harmonised classification as toxic to reproduction (category 1B, H360Fd) based on testes effects (reduced organ weights and degeneration), spermatotoxicity and reduced fertility. Developmental effects included post-implantation loss and decreased pup body weights.

10.10.6 Comparison with the CLP criteria

The criteria for classification in Repr. 1B for adverse effects on development is considered fulfilled for *p*-cymene, 3-*p*-cumenyl-2-methylpropionaldehyde, 3-(*p*-cumenyl)propionaldehyde, 4-isopropylbenzaldehyde and 4-isopropylbenzoic acid (4-iPBA), based on a weight of evidence approach and read-across within this group. Statistically significant effects on pup mortality were observed in rats in two OECD test guideline studies (OECD TG 415; 3-*p*-cumenyl-2-methylpropionaldehyde, OECD TG 422; *p*-cymene) at 150 and 100 mg/kg bw/day, respectively. Evidence included reduced post-implantation

survival, reduced number of live pups at birth and reduced pup survival at the beginning of lactation. Additionally, a consistent reduction of fetal and offspring weight at birth (up to -11%) was demonstrated in rats in three OECD guideline studies on 3-*p*-cumenyl-2-methylpropionaldehyde and *p*-cymene (OECD TG 414, OECD TG 415 and OECD TG 422).

In the one-generation study (OECD TG 415) on 3-*p*-cumenyl-2-methylpropionaldehyde, a statistically significant increased number of lens opacities was observed in pups of treated females mated with untreated males. This effect first appeared on day 16 postpartum and was confirmed at necropsy examination. Reduced weight of several organs was observed in both male and female pups, including statistically significantly reduced brain weight. Death of the developing organism, structural abnormality and altered growth are listed among the major manifestation of developmental toxicity (ECHA, 2017). The effects seen on pups are not considered secondary to maternal toxicity since there were no marked toxicity observed in parental animals.

The available data provide, in a weight of evidence approach and using a read-across approach, clear evidence of an adverse effect on development and there is no mechanistic evidence to indicate that the observed effects are not relevant for humans. Classification in Repr. 1B, H360D is therefore warranted.

Classification in Repr. 1A is not appropriate as it should be based on human data and no human data are available.

Classification in Repr. 2 is not appropriate as the evidence for adverse effects on development from existing experimental data is considered as clear evidence and not some evidence.

10.10.7 Adverse effects on or via lactation

Table 15: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
p-cymene EC 202-796-7			
OECD TG 422 screening for reproductive/developmental toxicity study. GLP compliant Deviations from test guideline were considered by the Registrant not to have compromised the validity or integrity of the study. Rats Sprague Dawley Crl:CD®(SD). 10 animals/sex/group. Assigned reliability 1 by the Registrant	*p*-cymene EC 202-796-7 Purity: assumed 100% Vehicle: corn oil The substance was administered via oral gavage at doses 0, 50, 100, 200 mg/kg bw/day once daily. Duration of treatment: P0 males: 2 weeks pre-cohabitation, during cohabitation (up to 2 weeks) and continuing during post-cohabitation until the day prior to termination (approximately 35 days). P0 females: 2 weeks pre-cohabitation,	***Maternal animals*** **Body weights** No effects on body weights and body weight changes. ***F1 generation*** **Clinical observations** There were 9, 9 and 4 litters at 0, 50 and 100 mg/kg bw/day, respectively. There were no offspring produced at 200 mg/kg bw/day. ↓ live birth index at 100 mg/kg bw/day 94.3% vs 100%; p≤0.01). ↓ post-implantation survival index at 100 mg/kg bw/day (87.3% vs. 95% in controls). ↓ litters with less than 100% viability at 100 mg/kg bw/day (1 of 4 litters with 100% viability vs. 9 of 9 in controls). ↓ pup weight on PND 1 at 100 mg/kg bw/day. NOAEL: 50 mg/kg bw/day (developmental	Study report, 2019. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 8 Annex I section 3.10.1.8

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	cohabitation (up to 2 weeks) and during gestation and lactation continuing until LD 13 (approximately 63 days).	toxicity)	
3-*p*-cumenyl-2-methylpropionaldehyde EC 203-161-7			
OECD TG 415 One-Generation Reproduction Toxicity Study No deviations GLP compliant Sprague Dawley rats, 25 males and females per dose group. Assigned reliability 1 by the Registrant.	3-*p*-cumenyl-2-methylpropionaldehyde EC 203-161-7 Purity: Vehicle: Corn oil Substance was administered orally (gavage) at doses 0, 25, 75, 150 mg/kg/day Male P generation rats were gavaged once daily beginning 83 days prior to cohabitation, through cohabitation, continuing through the day before sacrifice. Female P generation rats were gavaged once daily beginning 14 days before cohabitation, through cohabitation and on day 25 of gestation (rats that did not deliver) or day 22 postpartum (rats that delivered a litter). Treated rats were mated with untreated cohorts of male and female rats. F1 generation rats were not directly dosed but may have been exposed to the substance in utero during gestation and through maternal milk postpartum. F1 generation was followed until day 60 postpartum.	***Maternal animals*** **Clinical observations** No clinical signs were observed. **Body weight** Gestation ↓ body weight at 150 mg/kg bw/day (-5% and -8%, GD 18 and 21, respectively; p≤0.01). Lactation ↓ body weight at 75 mg/kg bw/day (-4%, LD 8; p≤0.05) and at 150 mg/kg bw/day (-7%, -8.5%, -6%, LD 5, 8, 11, respectively; p≤0.01). Effects on body weights correlated with statistically significant lower feed consumption during lactation. **Reproductive organs and function** ↓ implantation sites per delivered litter at 150 mg/kg bw/day (-11%; p≤0.01). ↑ dams with all pups dying between days 1 and 5 postpartum at 150 mg/kg bw/day (16.7% vs. 0; p≤0.01). ↓ mean number pups delivered/litter at 150 mg/kg bw/day (-15%; p≤0.01). ↓ number liveborn pups/litter at 150 mg/kg bw/day (-17%; p≤0.01). ↑ number stillborn pups/litter at 150 mg/kg bw/day (2.7% vs 0.8% in controls ; p≤0.01). ↑ pup mortality at 25 mg/kg bw/day (4 vs. 0, days 12-15; p≤0.01) and at 150 mg/kg bw/day (18 vs. 1, day 1; p≤0.01, 51 vs. 12, and days 2-5; p≤0.01). ↓ viability index at 150 mg/kg bw/day (75.7% vs. 96.3%; p≤0.01). ↓ lactation index at 25 mg/kg bw/day (96.5 vs. 99.7%; p≤0.01). ↓ number surviving pups/litter at 150 mg/kg bw/day (-17% to -36%, day 1-22; p≤0.01). ↓ live litter size at weighing at 150 mg/kg bw/day (-18% to -23%, day 1-22; p≤0.01).	Study report, 2011. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 1, Annex I section 3.10.1.1

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		F1 generation *Pups of treated females* ↑ litters with pups with lenticular opacities at 150 mg/kg bw/day (771 vs. 4 in controls; p≤0.01). ↓ mean pup body weight/litter at 75 mg/kg bw/day (ranging from -19% day 8; p≤0.01, to -10% day 18, p≤0.05) and at 150 mg/kg bw/day (ranging from -19% day 8, p≤0.01 to -9%, day 18; p≤0.05). *Male pups of treated females* ↓ body weight gains at 75 mg/kg bw/day (-7%, days 30-37; p≤0.05) and at 150 mg/kg bw/day (-12%, days 23-30 and -9%, days 30-37; p≤0.01). ↓ mean body weight at 75 mg/kg (ranging from -13% day 23; p≤0.01, to -6%, day 50; p≤0.05) and at 150 mg/kg bw/day (ranging from -20%, day 23 to -8%, day 57; p≤0.01). ↓ absolute feed consumption at 150 mg/kg bw/day (-17%, days 23 to 30; p≤0.01 and -8%, days 30-37; p≤0.05). ↑ relative feed consumption at 75 mg/kg bw/day (+9% days 30-37; p≤0.01, +5% days 37-44; p≤0.05) and at 150 mg/kg bw/day (+8% days 30-37; p≤0.05 and +8% days 37-44, +6% days 44-51, +6% days 51-57; p≤0.01). ↓ anogenital distance at 75 and 150 mg/kg bw/day (-7% and -6%, respectively, day 22; p≤0.05). ↓ absolute terminal body weight at 150 mg/kg (-8%; p≤0.01). ↓ absolute left epididymis weight at 150 mg/kg bw/day (-10%; p≤0.01). ↑ relative testis weight at 75 mg/kg bw/day (+5%; p≤0.05) and at 150 mg/kg bw/day (+5% and +6%; p≤0.050). ↓ absolute pituitary weight at 150 mg/kg (-17%; p≤0.01). ↓ absolute brain weight at 150 mg/kg (-4%; p≤0.01). ↓ absolute and relative adrenals weight at 75 mg/kg bw/day (-15% and -16%, respectively; p≤0.01) and at 150 mg/kg (absolute only, -13%; p≤0.05) *Female pups of treated females* ↑ anogenital distance at 150 mg/kg/day (+4% at	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		LD 1; p≤0.01 when covaried with fetal bw/litter). ↓ body weight gains at 150 mg/kg/day (-9% days 23-30; p≤0.05). ↓ body weight at 75 mg/kg (ranging from -12%, day 23; p≤0.01, to -6%, day 51; p≤0.05) and at 150 mg/kg bw/day (ranging from -18%, day 23; p≤0.01, to -6%, day 51; p≤0.05). ↓ absolute feed consumption at 150 mg/kg/day (-14% days 23 to 30; p≤0.01). ↑ relative feed consumption at 75 mg/kg bw/day (+6% days 23-57; p≤0.05) and at 150 mg/kg bw/day (+4% days 23-57; p≤0.01). ↓ absolute brain weight in females at 75 mg/kg bw/day (-3%; p≤0.05) and at 150 mg/kg (-6%; p≤0.01). ↓ absolute ovary (left) weight in females at 150 mg/kg bw/day (-17%; p≤0.05). NOAEL 25 mg/kg bw/day (pups weight)	

Table 16: Summary table of human data on effects on or via lactation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
-				

Table 17: Summary table of other studies relevant for effects on or via lactation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
-				

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

***p*-cymene**

Reproductive/developmental toxicity study OECD TG 422 (Study report, 2019)

In an OECD TG 422 screening for reproductive/developmental toxicity study (GLP compliant) from 2019, male and female Sprague Dawley rats were exposed to *p*-cymene via oral gavage at 0, 50, 100, 200 mg/kg bw/day once daily. Parental males were dosed 2 weeks pre-cohabitation, during cohabitation (up to 2 weeks) and continuing during post-cohabitation until the day prior to termination (approximately 35

days). Parental females were dosed 2 weeks pre-cohabitation, cohabitation (up to 2 weeks) and during gestation and lactation continuing until LD 13 (approximately 63 days).

There were treatment-related reductions in fertility at mid- and high doses. The male and female fertility indices were 90%, 90%, 40% and 0% at 0, 50, 100 and 200 mg/kg bw/day, respectively.

General toxicity – maternal animals

There were no treatment-related reductions in the body weights and body weight changes at any dose. No effects on food consumption and compound intake were noted at any dose.

Eighteen females across all groups, including controls, were euthanized early due to failure to become pregnant.

Absolute and relative liver weights were statistically significantly increased in females at 100 mg/kg bw/day (+26% and +22%, respectively). Females at 200 mg/kg bw/day were not included as all females at this dose were euthanized due to non-pregnancy.

Clinical biochemistry findings (at ≥ 100 mg/kg bw/day) included increases in alkaline phosphatase activity (+79% in females at 100 mg/kg bw/day) and decreases in albumin (-9% in females at 100 mg/kg bw/day). More details in Annex I section 3.10.1.8.

F1 generation

There were 9, 9 and 4 litters that appeared normal for most of the observation period at 0, 50 and 100 mg/kg bw/day, respectively. There were no pregnant animals at 200 mg/kg bw/day.

The live birth index was statistically significantly reduced (94.3% vs. 100%) at 100 mg/kg bw/day. The post-implantation survival index was reduced at 100 mg/kg bw/day (87.3% vs. 95%). The number of litters with less than 100% viability was increased at 100 mg/kg bw/day with only 1 of 4 litters having 100% viability versus 9 of 9 in the control group. The viability indices on PND 4, 7 and 13 were comparable with control values at 100 mg/kg bw/day, respectively. All offspring survival index values at 50 mg/kg bw/day were comparable with controls.

The mean pup weights on PND 1 were reduced at 100 mg/kg bw/day (-11% in males, -9% in females, no information on statistical significance). On the remaining intervals (PND 4, 7, 11 and 13), the mean litter body weights at 50 and 100 mg/kg bw/day were comparable with the control group values.

The mean sex ratio was comparable among groups.

There were no organ weight differences in thyroid/parathyroid glands in the F1 pups, and no microscopic findings. There were no differences from controls for thyroxine (T4) or thyroid stimulating hormone (TSH) on PND 4 in F1 female pups. On PND 13, there were no differences from control for T4 levels in F1 male pups. Many of the individual animal TSH values were below the level of detection in males at 100 mg/kg/day and females at 50 and 100 mg/kg/day.

Conclusions

There were 9, 9 and 4 litters at control, low- and mid- dose levels, respectively. The live birth index and the post-implantation survival were statistically significantly reduced at the mid dose level. The average pup weight on PND 1 (-10%) was statistically significantly lower at this dose level. On the remaining intervals (PND 4, 7, 11 and 13), the mean litter body weights at 50 and 100 mg/kg bw/day were comparable with the control group values.

There were no marked general toxicity among maternal animals.

3-*p*-cumenyl-2-methylpropionaldehyde

One-Generation Reproduction Toxicity Study in rats OECD TG 415 (Study report, 2011)

In the One-Generation Reproduction Toxicity Study (OECD TG 415) from 2011, male and female rats (25 per sex and group) were exposed to 3-*p*-cumenyl-2-methylpropionaldehyde at 0, 25, 75 and 150 mg/kg bw/day. Male P generation rats were gavaged once daily beginning 83 days prior to cohabitation, through cohabitation, continuing through the day before sacrifice. Female P generation rats were gavaged once daily beginning 14 days before cohabitation, through cohabitation and on day 25 of gestation (rats that did not deliver) or day 22 postpartum (rats that delivered a litter). Treated rats were mated with untreated cohorts of male and female rats. F1 generation rats were not directly dosed but may have been exposed to the substance *in utero* during gestation and through maternal milk postpartum. Pups were terminated at day 57, 58 or 60 postpartum.

F1 Generation Pups of Treated Female Rats Mated with Untreated Male Rats

Pup mortality was statistically significantly increased at 150 mg/kg bw/day on days 1 to 5 postpartum, compared to controls (viability on day 5 postpartum was 75.7% vs. 96.3% for controls). Viability index was decreased also at 75 mg/kg bw/day (not statistically significant). At 150 mg/kg bw/day, the average number of surviving pups per litter and the live litter size at weighing was statistically significantly reduced on days 1 through 22 postpartum, compared to controls (and decreased in a dose-related manner days 1-8). The average pup body weight per litter was statistically significantly reduced at 75 and 150 mg/kg bw/day at each tabulated interval between days 1 and 22 postpartum, as compared to controls.

At 150 mg/kg bw/day, 20 of 24 litters had one or more pups with a lenticular opacity in one or both eyes (statistically significant). This observation only occurred in one pup from one litter in the vehicle control group on days 19 through 22 postpartum. At 150 mg/kg bw/day, lenticular opacities were first observed on day 16 and generally persisted until day 22 postpartum. This observation was more prevalent in F1 generation male rats than in the female rats (18 male rats vs. 6 female rats). This clinical sign was confirmed during scheduled necropsy examination. As this effect appeared in pups at the end of lactation period it may be related to exposure of the test substance via lactation. In female offspring, (not statistically significantly) increased corneal opacities was observed (68 vs. 0 in controls).

The average pup body weight per litter was statistically significantly reduced at 75 (e.g. -10% day 1, -19% day 8 and -13% day 22) and 150 mg/kg bw/day (e.g. -11% day 1, -19% day 8, and -14% day 22) at each tabulated interval between days 1 and 22 postpartum, compared to controls. In F1 generation male rats, body weight gains were statistically significantly reduced at 150 mg/kg bw/day on days 23 to 37 postpartum (-7% to -12%), compared to controls. Thereafter, body weight gains were comparable to the control group during the remainder of the postweaning period. Reflecting the initial reductions in weight gain, body weight gains in the F1 generation male rats at 150 mg/kg bw/day were reduced for the entire postweaning period.

In F1 generation female rats, body weight gains were statistically significantly reduced at 150 mg/kg bw/day on days 23 to 30 postpartum (-9%), compared to control group. However, this reduction was transient, and body weight gains were comparable to control group values during the remainder of the postweaning period. Body weight gains at 25, 75 and 150 mg/kg bw/day were +1%, -2% and ±0%, respectively, on days 23 to 57 postpartum.

Reflecting the statistically significant reductions in average pup body weight per litter that occurred prior to weaning (i.e. during lactation), the average body weight was statistically significantly reduced in the F1 generation male and female rats during post-weaning period at 75 and 150 mg/kg bw/day. The lower body weight of pups during lactation period indicates a potential effect by the test substance via lactation. The reduced body weight persisted throughout the study. Terminal average body weight in male offspring was -8% ($p \leq 0.01$) and -4% in female offspring (not statistically significant).

Corresponding to statistically significant reductions in body weight gains, absolute feed consumption values in the F1 generation male rats were statistically significantly reduced at 150 mg/kg bw/day on days 23 to 37 postpartum, compared to controls. Relative to body weight, F1 generation male rats consumed statistically significantly more feed on days 30 to 44 postpartum at 75 mg/kg bw/day and days 23 to 57 postpartum at 150 mg/kg bw/day.

Similar observations occurred in the F1 generation female rats, absolute feed consumption values were statistically significantly reduced at 150 mg/kg bw/day on days 23 to 30 postpartum, compared to controls.

Relative to body weight, F1 generation female rats consumed statistically significantly more feed on days 23 to 57 postpartum at 75 and 150 mg/kg bw/day.

Absolute feed consumption values in the F1 generation male rats were -2%, -3% and -7% at 25, 75 and 150 mg/kg bw/day, respectively, on days 23 to 57 postpartum. In F1 generation female rats, absolute feed consumption values were -2%, -1% and -4% at 25, 75 and 150 mg/kg bw/day, respectively, during the same period.

In the pups that were stillborn, found dead or humanely euthanized, no milk was present in the stomach of 1, 5, 5 and 14 F1 generation pups at 0, 25, 75 and 150 mg/kg bw/day, respectively. An assessment of individual maternal animals showed no indications of marked toxicity, including lower body weight in the maternal animals of these pups, indicating that the effects observed were not secondary to maternal toxicity. The remaining pups that were stillborn, found dead or humanely euthanized appeared normal.

General toxicity, body weight and food consumption – maternal animals

Among females, during the first week of the dosage period (precohabitation), body weight gains were reduced at 75 mg/kg bw/day (-36%) and statistically significantly reduced at 150 mg/kg bw/day (-56%). However, these reductions were transient and did not persist during the second week of the dosage period. Despite the rebound during the second week of the dosage period, body weight gains at 75 and 150 mg/kg bw/day remained reduced (75 mg/kg bw/day) or statistically significantly reduced (150 mg/kg bw/day) for the entire premating dosage period, compared to controls. The average body weight on day 15 was -2%, -2% and -3% at 25, 75 and 150 mg/kg bw/day, respectively.

During gestation, at 150 mg/kg bw/day, body weight gains remained reduced (-5% to -21%) at each tabulated interval within the gestation dosage period relative to controls (not statistically significantly). Maternal body weight gains at 25, 75 and 150 mg/kg bw/day were +2%, -5% and -15%, respectively, on DGs 0 to 21. The average body weight on DG 21 was -1%, -3% and -8% at 25, 75 and 150 mg/kg bw/day, respectively.

During lactation, statistically significant body weight losses were observed at 150 mg/kg bw/day at the beginning of the lactation period (DLs 1 to 8; -2.8 g vs. +19.6 g in controls). However, thereafter, body weight gains were comparable to or statistically significantly increased when compared to the control group values.

The average maternal body weight was statistically significantly reduced at 150 mg/kg bw/day on DLs 5 (-7%) through 11 (-6% on DL 11), compared to controls. Additionally, a statistically significant reduction ($p \leq 0.05$) in the average maternal body weight at 75 mg/kg bw/day on DL 8 was observed (-4%). This was considered by the study authors to be unrelated to test substance because the reduction was transient and did not persist. Maternal body weight gains at 25, 75 and 150 mg/kg bw/day were +8%, +44% and +24%, respectively, on DLs 1 to 22. The average body weight on DL 22 was ±0%, +3% and +1% at 25, 75 and 150 mg/kg bw/day, respectively.

Absolute feed consumption values at 25, 75 and 150 mg/kg bw/day were -5%, -11% and -20% respectively, on DLs 1 to 15. Absolute and relative feed consumption values during the lactation period were unaffected at 25 mg/kg bw/day. More details in Annex I, Section 3.10.1.1.

Conclusions

In pups of treated dams that mated with untreated males, at 150 mg/kg bw/day, the majority of litters had pups with lenticular opacities in the eyes. There were first observed on day 16 postpartum and persisted until day 22 postpartum. These effects thus appeared during the lactation period, indicating effect by the substance via lactation (although not disproven being a result from exposure *in utero* manifested post-natally).

Reflecting the statistically significant reductions in average pup body weight per litter that occurred prior to weaning, the average body weight was statistically significantly reduced in the F1 generation male and female rats during post-weaning period at 75 and 150 mg/kg bw/day. The consistently reduced body weight of pups of animals treated at these doses during lactation period indicates a potential effect by the test substance via lactation, which persisted throughout the study. Effects caused by *in utero* exposure can, however, not be excluded.

In maternal animals there were slight but statistically significant effects on body weight at 150 mg/kg bw/day during gestation and beginning of lactation, but no marked general toxicity was observed.

There are no studies available on the quantity, quality, or composition of the milk.

10.10.9 Comparison with the CLP criteria

According to CLP Annex I classification of substances for effects on or via lactation can be assigned on the:

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

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

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

Although there is some information indicating an effect of 3-*p*-cumenyl-2-methylpropionaldehyde via lactation, it cannot be ruled out that these findings are due to exposure *in utero* that are manifested post-natally. The evidence is considered insufficient to justify classification on or via lactation.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Classification of this group of five substances for adverse effects on sexual function and fertility and development is warranted: Repr. 1B H360FD.

A specific concentration limit for adverse effects on sexual function and fertility is not considered justified since the ED10 values for both 3-*p*-cumenyl-2-methylpropionaldehyde and *p*-cymene (effects on fertility) are above 5 mg/kg bw/day, thus within the medium potency group (4 mg/kg bw/day < ED10 value < 400 mg/kg bw/day) which does not justify a specific concentration limit. Similarly, for developmental effects, no SCL is proposed, since effects are seen only at doses well within the dose range of the medium potency group.

10.11 Specific target organ toxicity-single exposure

Not evaluated in this CLH proposal.

10.12 Aspiration hazard

Not evaluated in this CLH proposal.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in this CLH proposal.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this CLH proposal.

13 ADDITIONAL LABELLING

Not relevant.

14 REFERENCES

Api et al. 2020 RIFM fragrance ingredient safety assessment, cuminic aldehyde, CAS Registry Number 122-03-2, Food and Chemical Toxicology 144 (2020).

ECHA, 2008. Reach guidance on grouping of chemicals Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of chemicals May 2008

ECHA, 2017. Guidance on the Application of the CLP Criteria Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, Version 5.0 July 2017.

ECHA, 2019. Committee for Risk Assessment RAC Opinion proposing harmonised classification and labelling at EU level of 2-(4-tert-butylbenzyl)propionaldehyde.

Laue et al. 2017. p-Alkyl-benzoyl-CoA conjugates as relevant metabolites of aromatic aldehydes with rat testicular toxicity—studies leading to the design of a safer new fragrance chemical. Toxicological Sciences, 160(2), 2017, 244–255

Laue et al. 2020. Benzoyl-CoA conjugate accumulation as an initiating event for male reprotoxic effects in the rat? Structure–activity analysis, species specificity, and in vivo relevance. Archives of Toxicology (2020) 94:4115–4129.

Natsch et al. 2021. A species specific metabolism leading to male rat reprotoxicity of Cyclamen aldehyde: in vivo and in vitro evaluation. Review in Food and Chemical Toxicology 153 (2021).

PubChem database available at PubChem (nih.gov)

PubChem 4-isopropylbenzoic acid available at 4-Isopropylbenzoic acid | C10H12O2 - PubChem (nih.gov)

Registration dossier for 3-*p*-cumenyl-2-methylpropionaldehyde available at Registration Dossier - ECHA (europa.eu)

Registration dossier for 3-(*p*-cumenyl)propionaldehyde available at Registration Dossier - ECHA (europa.eu)

Registration dossier for *p*-cymene available at Registration Dossier - ECHA (europa.eu), Registration Dossier - ECHA (europa.eu)

Registration dossier for 4-isopropylbenzaldehyde available at Registration Dossier - ECHA (europa.eu), Registration Dossier - ECHA (europa.eu)

REGULATION (EC) No 1907/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.

REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

Spin database available at SPIN | Substances in Preparations in Nordic Countries (spin2000.net)

Swedish Product Register available at Products Register - Kemikalieinspektionen

15 ANNEXES

Annex I to the CLH report.

Confidential Annex II to the CLH report.