CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical names:

a) 2-(4-*tert*-butylbenzyl)propionaldehyde

and

b) 4-*tert*-butylbenzoic acid

and

c) 3-(4-*tert*-butylphenyl)propionaldehyde [1];
4-*tert*-butyltoluene [2];
4-*tert*-butylbenzaldehyde [3];
methyl 4-*tert*-butylbenzoate [4]

EC Numbers:

a) 201-289-8
b) 202-696-3
c) 242-016-2 [1]; 202-675-9 [2]; 213-367-9 [3]; 247-768-5 [4]

CAS Numbers:

a) 80-54-6
b) 98-73-7
c) 18127-01-0 [1]; 98-51-1 [2]; 939-97-9 [3]; 26537-19-9 [4]

Index Numbers:

a) **605-041-00-3**
b) **607-698-00-1**
c) **TBD**

Index Number	Chemical name	EC Number	CAS Number
605-041-00-3	2-(4-*tert*-butylbenzyl)propionaldehyde	201-289-8	80-54-6
607-698-00-1	4-*tert*-butylbenzoic acid	202-696-3	98-73-7
TBD	3-(4-*tert*-butylphenyl)propionaldehyde [1]; 4-*tert*-butyltoluene [2]; 4-*tert*-butylbenzaldehyde [3]; methyl 4-*tert*-butylbenzoate [4]	242-016-2 [1]; 202-675-9 [2]; 213-367-9 [3]; 247-768-5 [4]	18127-01-0 [1]; 98-51-1 [2]; 939-97-9 [3]; 26537-19-9 [4]

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0 BACKGROUND

The present proposal includes a group of six substances. One of the substances in the group is the fragrance 2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral), which already has a harmonised classification as Repr.1B (H360Fd). Another substance included is 4-*tert*-butylbenzoic acid (TBBA). TBBA is used in the EU mainly at industrial sites as an intermediate, and it is formed during the metabolism of other substances, including e.g., lysmeral (Laue *et al.,* 2017). TBBA also has a harmonised classification as Repr.1B (H360F). The other four substances included in the proposal (3-(4-*tert*-butylphenyl)propionaldehyde, 4-*tert*-butyltoluene and 4-*tert*-butylbenzaldehyde and methyl 4-*tert*-butylbenzoate) are structurally similar to lysmeral and are also used as fragrances.

TBBA causes toxicity in male reproductive organs, including testicular lesions, spermatotoxic effects and infertility, at relatively low concentrations. Testes toxicity has been characterised by lower absolute and relative organ weights, testes atrophy from seminiferous tubular degeneration and destruction of the germinative epithelium resulting in disturbance of spermatogenesis including loss of late spermatids (CLH report, 4-*tert*-butylbenzoic acid, 2010).

There is evidence of formation of TBBA *in vivo* after administration of four of the substances included in this proposal; (3-(4-*tert*-butylphenyl)propionaldehyde forms TBBA in rats, 4-*tert*-butyltoluene and 4-*tert*-butylbenzaldehyde form TBBA in rats and dogs, and trace levels of TBBA have been demonstrated in mice and guinea pigs. Lysmeral forms TBBA *in vivo* in several species, including humans (Scherer *et al*., 2017). Two recent biomonitoring studies have detected TBBA in urine samples of German residents (Murawski et al. 2020, Scherer *et al*., 2021).

Data on reproductive toxicity are available for five of the substances included in the proposal (lysmeral, TBBA, 3-(4-*tert*-butylphenyl)propionaldehyde, 4-*tert*-butyltoluene and 4-*tert*-butylbenzaldehyde). The substance methyl 4-*tert*-butylbenzoate (EC 247-768-5) was identified as a precursor to TBBA based on the output from a profiling scheme built in the OECD (Q)SAR Toolbox and it was included in the group based on its structure. No other information on toxicokinetics or reproductive toxicity is available for this substance.

Lysmeral was classified as Repr. 1B (H360Fd) in 2020 (ATP 15 to CLP Annex VI). The substance causes effects on testes in rats and dogs, similar to the ones reported for TBBA, including reduced organ weights and degeneration, spermatotoxic effects, and reduced fertility. RAC used the harmonised classification of TBBA as supporting evidence in its opinion on lysmeral, as the metabolite was considered to be responsible for the testicular and sperm toxicity observed (ECHA, 2019). Lysmeral has been included in the present proposal with the main purpose to add a note to the existing entry in Annex VI of CLP, to account for additive mixture effects, as lysmeral forms the same metabolite (TBBA) as the other substances included in the proposal. Additionally, lysmeral is a member of the category formed for read-across purposes, and it is thus used as a source substance. The separate entry of lysmeral in Annex VI of CLP is retained for administrative reasons. In the present evaluation, the DS has assessed recent studies on lysmeral and TBBA that were not published at the time of their previous CLHassessment. These new studies are mainly of mechanistic character (*in vitro* and *ex vivo*) and is not considered by the DS to alter the conclusion by RAC to classify lysmeral as Repr.1B, H360Fd. The DS therefore decided to not open the current classification of lysmeral for reassessment. Similarily, no new experimental data is available for TBBA that question the current harmonised classification as Repr.1B for adverse effects on sexual function and fertility (H360F), however, the DS proposes to add Repr. 2 for developmental effects (H360d), based on the read-across approach used in the present dossier, described more in detail in section 10 below.

The substances included in the present proposal are structurally similar to another group of substances forming the metabolite 4-isopropylbenzoic acid, 4-iPBA. 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 4-iPBA has been prepared by the DS in parallel to this proposal. Similar toxicity to the reproductive system is demonstrated for substances in both groups.

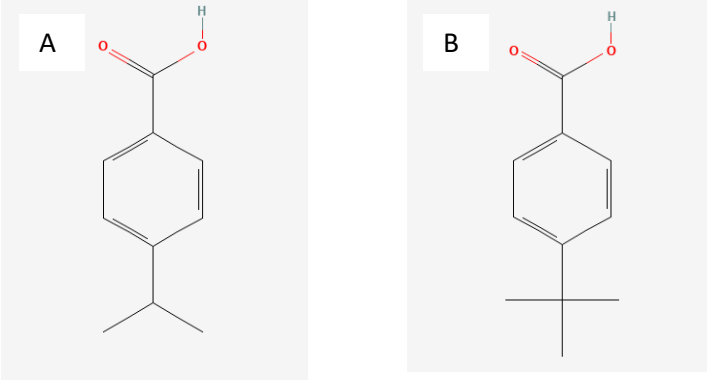


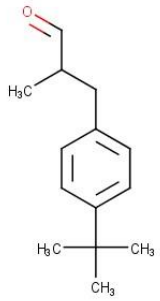
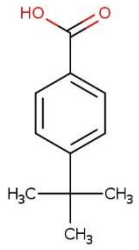
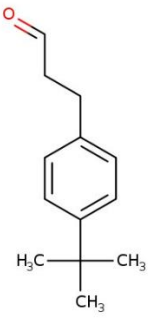
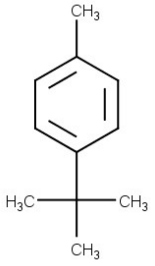
Figure 1. Structures of the metabolites 4-isopropylbenzoic acid (A; 4-iPBA) and 4-*tert*-butylbenzoic acid (B; TBBA).

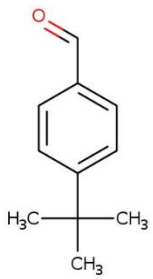
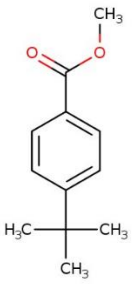
CONTENTS

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 or molecular weight range
201-289-8	80-54-6	2-(4-*tert*-butylbenzyl) propionaldehyde (lysmeral); 3-(4-(*tert*-butyl)phenyl)-2-methylpropanal; benzenepropanal, 4-(1,1-dimethylethyl)-.alpha.-methyl-	C14H20O		204.31 g/mol
202-696-3	98-73-7	4-*tert*-butylbenzoic acid (TBBA); benzoic acid, 4-(1,1-dimethylethyl)-	C11H14O2		178.23 g/mol
242-016-2	18127-01-0	3-(4-*tert*-butylphenyl)propionaldehyde; benzenepropanal, 4-(1,1-dimethylethyl)-; 3-(4-*tert*-butylphenyl)propanal; *p*-*tert*-butyldihydrocinnamaldehyde; bourgeonal	C13H18O		190.28 g/mol
202-675-9	98-51-1	4-*tert*-butyltoluene; benzene, 1-(1,1-dimethylethyl)-4-methyl-; *p*-*tert*-butyltoluene	C11H16		148.24 g/mol

213-367-9	939-97-9	4-*tert*-butylbenzaldehyde; benzaldehyde, 4-(1,1-dimethylethyl)-; *p*-*tert*-butylbenzaldehyde	C11H14O		162.23 g/mol
247-768-5	26537-19-9	methyl 4-*tert*-butylbenzoate; benzoic acid, 4-(1,1-dimethylethyl)-, methyl ester	C12H16O2		192.25 g/mol

1.1 Composition of the substances

Table 2a: Constituents

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)**
2-(4-*tert*-butylbenzyl) propionaldehyde (lysmeral) EC 201-289-8 CAS 80-54-6	>99 - <= 99.5 % (w/w)	Repr. 1B H360Fd	Acute Tox.4 H302 Acute Tox. 5 H313 Skin Irrit. 2 H315 Skin Sens. 1B H317 Skin Sens. 1 H317 Repr. 1B H360Fd Repr. 2 H361 Aquatic Chronic 3 H412 Aquatic Chronic 2 H411
4-*tert*-butylbenzoic acid (TBBA) EC 202-696-3 CAS 98-73-7	CONFIDENTIAL	Acute Tox.4 H302 STOT RE 1 H372 Repr. 1B H360F	Acute Tox. 3 H311 Acute Tox.4 H302 Acute Tox.4 H312 Acute Tox.4 H332 Eye Irrit. 2 H319 Repr. 1B H360 Repr. 2 H361 STOT RE 1 H372 STOT RE 2 H373 Aquatic Chronic 2 H411 Not Classified
3-(4-*tert*-butylphenyl)propionaldehyde EC 242-016-2 CAS 18127-01-0	CONFIDENTIAL	Not included in Annex VI	Repr. 2 H361 (fertility) Acute Tox. 3 H301 Acute Tox. 4 H302 STOT RE 2 H373 (Stomach, Liver) (oral) Skin Irrit. 2 H315 Skin Sens. 1 H317 Skin Sens. 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)**
			Aquatic Chronic 3 H412 Not classified
4-*tert*-butyltoluene EC 202-675-9 CAS 98-51-1	CONFIDENTIAL	Not included in Annex VI	Flam. Liq. 3 H226 Repr. 1B H360 Repr. 2 H361 Acute Tox. 2 H330 Acute Tox. 4 H302 Acute Tox. 4 H312 Acute Tox. 4 H332 Skin Irrit. 2 H315 Eye Irrit. 2 H319 STOT SE 3 H335 STOT SE 3 H336 STOT SE 1 H370 Aquatic Acute 2 H401 Aquatic Chronic 2 H411
4-*tert*-butylbenzaldehyde EC 213-367-9 CAS 939-97-9	CONFIDENTIAL	Not included in Annex VI	Repr. 2 H361 Acute Tox. 3 H301 Acute Tox. 4 H302 Resp. Sens. 1 H334 Skin Sens.1 H317 Aquatic Acute 1 H400 Aquatic Chronic 1 H410
methyl 4-*tert*-butylbenzoate EC 247-768-5 CAS 26537-19-9	CONFIDENTIAL	Not included in Annex VI	Acute Tox. 3 H301 Acute Tox. 4 H302 Acute Tox 4 H312 Acute Tox. 4 H332

Table 2b: Impurities if relevant for the classification of the substance

Substance (Name and numerical identifier)	**Impurity (Name and numerical identifier)**	**The additive contributes to the classification and labelling**
2-(4-*tert*-butylbenzyl) propionaldehyde (lysmeral) EC 201-289-8 CAS 80-54-6	CONFIDENTIAL	The impurities do not contribute to the classification and labelling
4-*tert*-butylbenzoic acid (TBBA) EC 202-696-3 CAS 98-73-7	CONFIDENTIAL	The impurities do not contribute to the classification and labelling
3-(4-*tert*-butylphenyl)propionaldehyde EC 242-016-2 CAS 18127-01-0	CONFIDENTIAL.	The impurities do not contribute to the classification and labelling
4-*tert*-butyltoluene EC 202-675-9	CONFIDENTIAL.	The impurities do not contribute to the classification and labelling

Substance (Name and numerical identifier)	**Impurity (Name and numerical identifier)**	**The additive contributes to the classification and labelling**
CAS 98-51-1		
4-*tert*-butylbenzaldehyde EC 213-367-9 CAS 939-97-9	CONFIDENTIAL.	The impurities do not contribute to the classification and labelling
methyl 4-*tert*-butylbenzoate EC 247-768-5 CAS 26537-19-9	CONFIDENTIAL.	The impurities do not contribute to the classification and labelling

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	605-041-00-3	2-(4-*tert*-butylbenzyl) propionaldehyde	201-289-8	80-54-6	Repr. 1B	H360Fd	GHS08 Dgr	H360Fd			
Dossier submitters proposal	605-041-00-3	2-(4-*tert*-butylbenzyl) propionaldehyde	201-289-8	80-54-6	Repr. 1B	H360Fd	GHS08 Dgr	H360Fd			**Add** xxx
Resulting Annex VI entry if agreed by RAC and COM	605-041-00-3	2-(4-*tert*-butylbenzyl) propionaldehyde	201-289-8	80-54-6	Repr. 1B	H360Fd	GHS08 Dgr	H360Fd			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	607-698-00-1	4-*tert*-butylbenzoic acid	202-696-3	98-73-7	Acute Tox. 4 STOT RE 1 Repr. 1B	H302 H372 H360F	GHS07 GHS08 Dgr	H302 H372 H360F			
Dossier submitters proposal	607-698-00-1	4-*tert*-butylbenzoic acid	202-696-3	98-73-7		**Add** H361d		**Add** H361d			**Add** xxx

Resulting Annex VI entry if agreed by RAC and COM	607-698-00-1	4-*tert*-butylbenzoic acid	202-696-3	98-73-7	Repr. 1B Acute Tox. 4 STOT RE 1	H360Fd H302 H372	GHS07 GHS08 Dgr	H360Fd H302 H372			xxx

Table 3c: 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 Code(s)	**Hazard statement Code(s)**	**Pictogram, Signal Word Code(s)**	**Hazard statement Code(s)**	**Suppl. Hazard statement Code(s)**		
Current Annex VI entry	No current Annex VI entry										
Dossier submitter's proposal	TBD	3-(4-*tert*-butylphenyl)propionaldehyde [1]; 4-*tert*-butyltoluene [2]; 4-*tert*-butylbenzaldehyde [3]; methyl 4-*tert*-butylbenzoate [4]	242-016-2 [1]; 202-675-9 [2]; 213-367-9 [3]; 247-768-5 [4]	18127-01-0 [1]; 98-51-1 [2]; 939-97-9 [3]; 26537-19-9 [4]	Repr. 1B	H360Fd	GHS08 Dgr	H360Fd			xxx
Resulting Annex VI entry if agreed by RAC and COM	TBD	3-(4-*tert*-butylphenyl)propionaldehyde [1]; 4-*tert*-butyltoluene [2]; 4-*tert*-butylbenzaldehyde [3]; methyl 4-*tert*-butylbenzoate [4]	242-016-2 [1]; 202-675-9 [2]; 213-367-9 [3]; 247-768-5 [4]	18127-01-0 [1]; 98-51-1 [2]; 939-97-9 [3]; 26537-19-9 [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	No: (2-(4-*tert*-butylbenzyl)propionaldehyde) Yes (H360d): 4-*tert*-butylbenzoic acid (TBBA) Yes (H360Fd): 3-(4-*tert*-butylphenyl)propionaldehyde; 4-*tert*-butyltoluene; 4-*tert*-butylbenzaldehyde; methyl 4-*tert*-butylbenzoate
Specific target organ toxicity-single exposure	hazard class not assessed in this dossier	No

Hazard class	Reason for no classification	Within the scope of consultation
Specific target organ toxicity-repeated exposure	hazard class not assessed in this dossier	No
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	hazard class not assessed in this dossier	No
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The substance 2-(4-*tert*-butylbenzyl) propionaldehyde (lysmeral) has a harmonsied classification as Repr. 1B H360Fd. The DS proposes no change to the current harmonised classification for lysmeral. Lysmeral is included in the proposal with the purpose to add a note for additive mixture effects. Additionally, the substance is part of the category formed for read-across purposes in the present proposal, and as a source substance (see Background section above). The DS does not propose reassessment of the current harmonised classification of lysmeral. The substance 4-*tert*-butylbenzoic acid (TBBA) has harmonised classification as Repr. 1B H360F, Acute Tox.4 H302 and STOT RE 1 H372. The DS proposes to add classification as Repr. 2 for developmental effects to the current classification.

There are no previous harmonised classification and labelling for the substances 3-(4-*tert*-butylphenyl)propionaldehyde, 4-*tert*-butyltoluene, 4-*tert*-butylbenzaldehyde and methyl 4-*tert*-butylbenzoate.

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 the PubChem[2] databases all substances included is this proposal are used as fragrances/perfumes and/or for masking. The reported uses in the Registration dossier for the substance *tert*-butylbenzoic acid (TBBA) include use as binding agent in paints and coatings and intermediate use. The use for masking is reported in PubChem database.

3-(4-*tert*-butylphenyl)propionaldehyde (EC 242-016-2) is used as a fragrance in a wide range of industries. The uses include cosmetics, cleaning and washing agents, polishes and wax blends, biocides, air care products, etc. Similar uses are reported for the substance 2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral).

The substances with EC numbers 202-675-9, 213-367-9, and 247-768-5 are all registered as intermediates under REACH, but reported use categories in PubChem database include use as fragrance, in flavouring and masking.

According to the Swedish Products Register there are products containing (some of these) fragrances between 0.0001-1%, median range from 0.1% to 0.2%.

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

[2] PubChem (nih.gov)

6 DATA SOURCES

Registered data available at ECHA dissemination site is the main source of information. Moreover, the full study report (OECD TG 422) for 3-(4-*tert*-butylphenyl)propionaldehyde was available to the DS. Some additional information/summarised data on 4-*tert*-butyltoluene from the OECD TG 421 (not full study report) was also available to the DS. The PubMed database has been used to search for additional information on reproductive toxicity for the substances.

For 2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral) and *tert*-butylbenzoic acid (TBBA) which already have harmonised classifcation as Repr. 1B, the main sources of information include the previous CLH reports and RAC opinions. The DS has also assessed more recent studies on lysmeral and TBBA that were not published at the time of the previous CLH assessments. These studies are mainly of mechanistical character (*in vitro, ex vivo*) and are summarised in the current proposal.

7 PHYSICOCHEMICAL PROPERTIES

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

Property	**2-(4-*tert*-butylbenzyl)propionaldehyde[3] EC 201-289-8**	**4-*tert*-butylbenzoic acid[4] EC 202-696-3**	**3-(4-*tert*-butylphenyl)propionaldehyde[5] EC 242-016-2**	**4-*tert*-butyltoluene[6] EC 202-675-9**	**4-*tert*-butylbenzaldehyde[7,8] EC 213-367-9**	**methyl-4-*tert*-butylbenzoate[9] EC 247-768-5**
Physical state at 20°C and 101,3 kPa	liquid	solid	liquid	liquid	liquid	-
Melting/freezing point	< -20°C (M)	165 - 167 °C (M)	-10.7 °C (M)	-52 °C (M)	< -20 °C (M)	-
Boiling point	279.5°C (M)	280 °C (M)	207 °C (M)	193 °C (M)	1: 248.7 °C (M) 2: 107 °C	-
Relative density	0.94 (M)	1.142 (M)	0.959 (M)	0.86 (M)	1: 0.97 (M) 2: 0.97	-
Vapour pressure	0.0025 hPa (20°C) (M)	0.00057 hPa (20°C) (M)	0.002 hPa (20°C) (M)	1.3 hPa (30°C) (M)	0.04 hPa (20°C) (M)	1.36 hPa (20°C) (M)
Surface tension	based on chemical structure, no surface activity is predicted	-	48.7 mN/m (M)	28.55 mN/m (M)	21.6 mN/m (M)	-
Water solubility	33 mg/L(M)	47.1 mg/L (pH 4.3) (M) 12600 mg/L (pH 7) (M)	132 mg/L (M)	4 mg/L (M)	120 mg/L (M)	35 mg/L (M)
Partition coefficient n-octanol/water	4.2 (M)	3.4 (M)	3.2 (M)	4.4 (M)	3.1 (M)	4.3 (M)
Flash point	79°C (M)	-	73.5 °C (M)	61.5 °C (M)	112 °C (M)	132.5 °C (M)

[3] ECHA, 2017, Proposal for Harmonised Classification and Labelling,2-(4-tert-butylbenzyl)propionaldehyde

[4] ECHA 2010, ANNEX VI REPORT: 4-TERT-BUTYLBENZOIC ACID (CAS: 98-73-7)

[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)

Property	2-(4-*tert*-butylbenzyl)propionaldehyde[3] EC 201-289-8	4-*tert*-butylbenzoic acid[4] EC 202-696-3	3-(4-*tert*-butylphenyl)propionaldehyde[5] EC 242-016-2	4-*tert*-butyltoluene[6] EC 202-675-9	4-*tert*-butylbenzaldehyde[7,8] EC 213-367-9	methyl-4-*tert*-butylbenzoate[9] EC 247-768-5
Flammability	Flammability upon ignition derived from flash point. The substance has no pyrophoric properties and does not liberate flammable gases on contact with water (E)	non flammable (M)	-	-	non flammable (E)	-
Explosive properties	non explosive (E)	non explosive (E)	non explosive (E)	non explosive (E)	-	non explosive (M)
Self-ignition temperature	257°C (M)	no selfignition up to the melting point (M)	350 °C (M)	452 °C (M)	400 °C (M)	-
Oxidising properties	No oxidizing properties (E)	No oxidizing properties (E)	No oxidizing properties (E)	No oxidizing properties (E)	No oxidizing properties (E)	-
Granulometry	Substance is marketed or used in a non solid or granular form	-	-	-	-	-
Stability in organic solvents and identity of relevant degradation products	Stability of the substance is not considered as critical	-	-	-	Test substance is stable (E)	-
Dissociation constant	Substance does not contain any ionic structure	PKa 4.36 at 25 °C (M)	-	-	No dissociating properties (E)	-
Viscosity	12.3 mPa*s at 20°C (M)	-	-	1.677 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
3-(4-*tert*-butylphenyl)propionaldehyde EC 242-016-2		
14 days dose range finding study for the OECD TG 422 study on 3-(4-*tert*-butylphenyl)propionaldehyde. 3-(4-*tert*-butylphenyl)propionaldehyde	On study day 1, levels of 3-(4-*tert*-butylphenyl)propionaldehyde acid and 4-*tert*-butylbenzoic acid (TBBA) were below the limit of detection in all male and female predose plasma samples and in samples from males and females administered 5 mg/kg bw/day. Mean 3-(4-*tert*-	Study report, 2019. Summarized in registration dossier. Annex I, section 3.10.1.1

Method	Results	Reference
was administered to 20 male and 20 female Sprague Dawley rats orally by gavage three times (approximately 6 hours apart) daily for 14 consecutive days at 0, 5, 25, 50 mg/kg bw/day. No information on sampling intervals or analytical method.	butylphenyl)propionaldehyde acid concentrations were below the level of detection, or generally lower than TBBA concentrations from 25 and 50 mg/kg bw/day samples and tended to be higher in females. Mean TBBA concentrations were slightly higher in females at 25 mg/kg bw/day but were similar to males at 50 mg/kg bw/day. On study day 14, TBBA plasma concentrations quickly increased and maintained steady state from 0.5 to 24 hr in males and females at all dose levels. At doses ≥ 25 mg/kg bw/day, TBBA concentrations in females were almost twice that of males and increased in a nearly dose proportional manner.	
5 day study with 3-(4-*tert*-butylphenyl)propionaldehyde dosed daily (oral gavage) at 0, 25, 100 and 250 mg/kg bw. Species/strain/sex/no. of animals per dose: Rat/CD/male/6 Urine was collected after administration of the fifth dose for a maximum of 22 hours. There is no information on sampling intervals. Urine samples were analysed for 4-*tert*-butylbenzoic acid (TBBA), 4-*iso*-butylbenzoic acid and 4-*iso*-propylbenzoic acid. There is no information on analytical method.	The metabolite 4-*tert*-butylbenzoic acid (TBBA) was detected in the urine (in a dose-dependent manner) of animals treated with 3-(4-*tert*-butylphenyl)propionaldehyde for 5 days. The mean concentration of TBBA was 35.75 µg/ml in urine from animals exposed to 25 mg/kg bw/day and 274.8 µg/ml in urine of animals treated at 100 mg/kg bw/day. 3 animals treated with 250 mg/kg bw/day and 1 animal treated with 100 mg/kg bw/day were killed for welfare reasons on day 1. The three remaining animals in the high dose group were killed before dosing on day 2.	Givaudan, 2009. Summarized in registration dossier Annex I section 2.1
4-*tert*-butyltoluene EC 202-675-9		
In vivo toxicokinetic study of 4-*tert*-butyltoluene in rats and guinea pigs. 2 experimental studies. Intragastric and inhalational single administration of 100 mg/kg bw. Male Wistar rats and Dunkin Hartley guinea pigs. No information on the number of animals. No information on analytical method.	4-*tert*-butyltoluene was well absorbed through the gastro-intestinal and respiratory tract and was quickly distributed. In rats, 73% of the administered dose was recovered in the urine and feces within 3 days. After 10 days, 83% of the administered dose was recovered; the ratio of urinary/fecal radioactivity was ca. 3.5 : 1. Elimination was biphasic with the slower elimination phase beginning on day 6 after dosing. At day 3 after dosing, urinary excretion of radioactivity was 45% and 25% after oral and inhalational exposure, respectively, in rats and 42% and 41% after oral and inhalational administration, respectively, in guinea pigs.	Ingebrigtsen and Walde. 1982; Walde and Scheline. 1983. Summarized in registration dossier. Annex I section 2.2
In vivo study of the elimination of 4-*tert*-	4-*tert*-butylbenzoic acid (TBBA) was identified as metabolite in the urine in rats treated with 4-*tert*-butyltoluene. TBBA was	Study report, 1982 Summarized in registration

Method	Results	Reference
butyltoluene. Male SPF albino rats (males) were gavaged daily with 0, 25 and 100 mg/kg bw for 5 consecutive days. Control: 4 animals, dose groups: 8 animals/dose. After the last application urine was collected for 24 hours. Urinary metabolites were detected by GC.	detected in a dose-related way in urine of all treated rats. GC/MS revealed additional peaks which were tentatively assigned to the trimethylsilyl derivatives of p-*tert*-hydroxybutylbenzoic acid and p-*tert*-carboxybutylbenzoic acid. These results suggested that p-*tert*-butyltoluene was metabolized by rats to a considerable degree to TBBA , which was eliminated in the urine (probably as glucuronide). Minor amounts of TBBA were further oxidized at the *tert*iary butyl group and then excreted in the urine. The oxidation reaction was likely to be a result of microsomal enzymes in the liver.	dossier. Annex I, section 2.3
Metabolism study of 4-*tert*-butyltoluene in rats. Route of administration: unspecified.	A metabolic pathway of the test substance was postulated. No change in the urinary sulfate ratio (inorganic/total) was observed in rats after dosing with 4-*tert*-butyltoluene. This result was taken as evidence that *in situ* oxidation of the aromatic ring system was not a pathway for the metabolism of 4-*tert*-butyltoluene. Thus, it was concluded that the *p*-methyl group or one of the methyl groups of the *tert*iary butyl moiety was oxidized in the liver to hydroxy- and carboxyl derivatives. These compounds were presumed to be eliminated as glucuronide or glycine conjugates.	Gerarde, 1960 Summarized in Registration dossier. Annex I section 2.4
Toxicokinetic study of 4-*tert*-butyltoluene in male outbred albino NMRI mice by inhalation. No information on the number of animals. 8 hours inhalation to 1000 ppm. Analysis by GC.	Uptake data in mesenterial fat and brain, as well as elimination data from these organs, do not suggest a marked tendency towards an accumulation of 4-*tert*-butyltoluene in fat or nervous tissue.	Rasmussen *et al*. 1980. Summarized in Registration dossier. Annex I section 2.5
Study of the elimination of 4-*tert*-butyltoluene in mice, dog and guinea pig. 6 male SPF albino mice, 2 male Beagle dogs, and 5 male Himalayan guinea pigs were exposed orally by gavage (capsule for dogs) daily to 100 mg/kg bw/day for 5 consecutive days. Urine was collected up to 24 hours after last application and analysed with GC.	Both metabolites 4-*tert*-butylbenzoic acid (TBBA) and the glycine conjugate of TBBA, TBHA were identified in urine of mice, guinea pigs and dogs. TBHA was the main metabolite in urine samples of mice and guinea pigs, whereas TBBA levels were below the detection limit and in very low levels, respectively. In dogs, TBBA was the main metabolite found in urine.	Study report, 1985 Summarized in Registration dossier. Annex I section 2.6; 2.7 and 2.8
4-*tert*-butylbenzaldehyde EC 213-367-9		
Toxicokinetic study of 4-*tert*-butylbenzaldehyde in rats. 8 male SPF albino rats were	4-*tert*-butylbenzoic acid (TBBA) was identified as metabolite in urine of rats treated with 4-*tert*-butylbenzaldehyde. The glycine	Study report, 1982. Summarized in Registration

Method	Results	Reference
gavaged with 4-*tert*-butylbenzaldehyde at 12.5 and 50 mg/kg bw/day for 5 consecutive days. Control: 4 animals. Urine was collected 24 hours after last application. Detection of metabolites by GC-MS	conjugate of TBBA, TBHA was not found.	dossier. Annex I section 2.9
Toxicokinetic study of 4-*tert*-butylbenzaldehyde in mice, dog and guinea pig. 6 male SPF albino mice, 2 male Beagle dogs, and 5 male Himalayan guinea pigs were exposed orally by gavage (capsule for dogs) daily to 100 mg/kg bw/day for 5 consecutive days. Urine was collected up to 24 hours after last application and analysed with GC.	The glycine conjugate of 4-*tert*-butylbenzoic acid, TBHA was found to be the main metabolite in urine samples of mice and guinea pig, whereas TBBA was found in the urine in low concentrations only. In urine samples of dogs, 4-*tert*-butylbenzoic acid (TBBA) was found to be the main metabolite, whereas TBHA was found in the urine in low concentrations only.	Study report, 1985. Summarized in Registration dossier. Annex I section 2.10; 2.11 and 2.12.
3-(4-*tert*-butylphenyl)propionaldehyde EC 242-016-2, *4-tert*-butyltoluene EC 202-675-9 and 2-(4-*tert*-butylbenzyl)propionaldehyde EC 201-289-8		
In vitro metabolism study with 3-(4-*tert*-butylphenyl)propionaldehyde, *p-tert*-butyltoluene and 2-(4-*tert*-butylbenzyl)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 following 0.5, 4 and 22 hours of exposure to the chemicals at 5 and 50 µM was also assessed by LC-HRMS. Incubation of rat hepatocytes was also performed with 4-*tert*-butylbenzoic acid (TBBA).	Incubation with 3-(4-*tert*-butylphenyl)propionaldehyde, p-*tert*-butyltoluene and 2-(4-*tert*-butylbenzyl)propionaldehyde resulted in formation of *p*-alkyl-benzoic acids, including 4-*tert*-butylbenzoic acid (TBBA), in suspended rat hepatocytes. High and stable TBBA-CoA conjugates were detected in plated hepatocytes incubated with 3-(4-*tert*-butylphenyl)propionaldehyde, p-*tert*-butyltoluene, 2-(4-tert-butylbenzyl)propionaldehyde and TBBA.	Laue *et al*., 2017. Annex I section 2.13
2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral) EC 201-289-8		
Metabolism study with 2-(4-*tert*-butylbenzyl)propionaldehyde Plasma kinetics Rats, n=4/dose Doses: 25 and 100 mg/kg bw	There was a rapid absorption of the radioactive compound for both doses applied and proportionate plasma maximum concentration (Cmax) has been observed. In contrast, the AUC was found to increase disproportionate to the dose applied which is	Study report, 1995. Summarized in registration dossier. Annex I section 2.14

Method	Results	Reference
Single oral dose	interpreted to be indicative for a saturation of the renal clearance. Cmax was 14.3 µg/mL after oral administration of 25 mg/kg bw 2-(4-*tert*-butylbenzyl)propionaldehyde. $Cmax_{25}$ = 14.3 µg equivalents/ml $Tmax_{25}$ = 3.5 hours $T1/2_{25}$ = 8 hours $Cmax_{50}$ = 52 µg equivalents/ml $Tmax_{50}$ = 1.8 hours $T1/2_{50}$ = 9.8 hours	
Metabolism study with 2-(4-*tert*-butylbenzyl)propionaldehyde Plasma kinetics Rats, n=5 Dose: 50 mg/kg bw Single oral dose	The metabolite lysmerylic acid was detected in all plasma samples. Cmax = 8.7 µg /g $AUC_{0\text{-}24h}$ = 8.7 µg *h/g Tmax = 4 hours T1/2 = 5.8 hours	Study report, 2006 Summarized in registration dossier. Annex I section 2.15
Metabolism study with 2-(4-*tert*-butylbenzyl)propionaldehyde, 5 days Rats, n= 8(4)/dose(control) Doses: 0; 100; 400 mg/kg bw/d Oral gavage	The main urinary metabolite in orally treated rats was found to be 4-*tert*-butylbenzoic acid, TBBA.	Study report, 1982 Summarized in registration dossier. Annex I section 2.16
Metabolism study with 2-(4-*tert*-butylbenzyl)propionaldehyde Plasmakinetics Mice, n=10 Dose: 50 mg/kg bw Single oral dose	The metabolite lysmerylic acid was detected in all plasma samples Cmax = 18.4 µg/g $AUC_{0\text{-}24h}$ = 85.1 µg *h/g Tmax = Directly after application T1/2 = 3.3 hours	Study report, 2006 Summarized in registration dossier. Annex I section 2.17
Metabolism study with 2-(4-*tert*-butylbenzyl)propionaldehyde, 5 days Mice (n=5), rats (n=8), guinea pigs (n=5), dogs (n=6) and monkeys (n=2) Doses: 100 (mice), 50, 100, 200, 400 (rat), 100 (guinea pigs), 100 (monkeys), 44.6 (dogs). Oral administration	The main urinary metabolite in rats, dogs and rhesus monkeys was found to be 4-*tert*-butylbenzoic acid (TBBA), whereas in the guinea pig and mouse TBHA resulting from glycine conjugation predominates. Urinary TBHA amounts in the rat were low compared to other rodent species, thus glycine conjugation or urinary TBHA excretion might not occur in the same rate as it does in other rodents. The urinary TBBA amounts in one of the two rhesus monkeys were found to be comparable to amounts in rats, whereas the other monkey showed 2-3 fold lower TBBA amounts than the rat.	Study report, 1985 Summarized in registration dossier. Annex I section 2.18
Metabolism study with 2-(4-*tert*-butylbenzyl)propionaldehyde *in vitro.* Rat hepatocytes	In rat hepatocytes 2-(4-*tert*-butylbenzyl)propionaldehyde was metabolized to 4-*tert*-butylbenzoic acid (TBBA) and to an unidentified metabolite up to 50% and 7%, respectively, during the period of 27 to 45.5 hours after plating.	Study report, 1982 Summarized in registration dossier. Annex I section 2.19
Metabolism study with 2-(4-*tert*-butylbenzyl)propionaldehyde *in vitro.*	In liver microsomes, oxidation of 2-(4-*tert*-butylbenzyl)propionaldehyde to lysmerylic acid or reduction to lysmerol, further oxidized at the *tert*-butyl group to form a hydroxy-metabolite, was observed. In hepatocytes, oxidation to lysmerylic acid was confirmed and its further dehydrogenation to (E)-3-(4-	Study report, 2010 Summarized in registration dossier. Annex I section 2.20

Method	Results	Reference
Microsomes and hepatocytes of rats, mice, rabbits and humans.	*tert*-Butyl-phenyl)-2-methyl-acrylic acid was observed. Putative decarboxylation of lysmerylic acid, followed by oxidation to the propanoic acid derivative and beta-oxidation led to the identified metabolite *p-tert*-butyl-benzoic acid (TBBA). This metabolite was conjugated with glycine to form *p-tert*-butyl-hippuric acid (TBHA) in rodents. Qualitative evaluation of the metabolic profiles of different species largely confirmed *in vivo* findings. 2-(4-*tert*-butylbenzyl)propionaldehyde was metabolized nearly completely in the hepatocytes of all species whereas lysmerylic acid was quantitatively the main metabolite. The metabolite (E)-3-(4-*tert*-Butyl-phenyl)-2-methyl-acrylic acid was more pronounced in hepatocytes of rats than in hepatocytes of mice or humans (not detected in hepatocytes of rabbits). In line with findings *in vivo*, species differences in metabolic profiles were seen for TBHA, which was more pronounced in mice in rats. TBHA was not detectable in incubates of hepatocytes of rabbits and humans. When compared to other rodent or non-rodent animal species, rats showed the highest concentration of TBBA, whereas it was lower in rabbits and humans.	
Study of metabolism and excretion of 2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral). Humans, n = 1 (pilot), n = 5 (follow-up) Dermal (pilot) and single oral dose (follow-up).	Pilot: Peak amounts of metabolites lysmerol and lysmerylic acid were excreted into the urine about 3-6 h after, whereas 4-*tert*-butylbenzoic acid (TBBA) and the glycine conjugate of TBBA, TBHA appeared about 12 h after dermal application. TBBA represented 0.67% of the applied dermal dose, followed by TBHA (0.04 %), lysmerol (0.02 %), and lysmerylic acid (0.012 %). In total, the lysmeral-related analytes represented 0.75% of the dermally applied dose. Follow-up: Oral administration resulted in peak amounts of the 4 metabolites between 3 and 6 h after application with lysmerol and lysmerylic acid appearing slightly earlier in the urine than the secondary metabolites hydroxyl-lysmerylic acid and TBBA. A rapid urinary excretion was observed, since more than 90% of all measured lysmeral metabolites were excreted after 12 h, and the excretion was found to be complete by 48 h after the oral intake. The sum of the 4 metabolites assessed in urine reflected about 16.5% of the applied dose. TBBA represented about 14.3% of the administered dose, followed by lysmerol, yielding 1.82% of the dose. The urinary fraction of hydroxy-lysmerylic acid and lysmerylic acid was 0.20% and 0.16% of the applied dose, respectively.	Scherer M *et al*., 2017 ECHA, 2017a Annex I section 2.21

Method	Results	Reference
OECD 428 Absorption study with 2-(4-*tert*-butylbenzyl)propionaldehyde, *in vitro*, human skin.	The percentage of dermally absorbed 2-(4-*tert*-butylbenzyl)propionaldehyde was calculated to be between 5 and 7% with the highest values obtained for the hydroalcoholic vehicle.	Study report, 2016 Summarized in registration dossier. Annex I section 2.22
Dermal absorption study with 2-(4-*tert*-butylbenzyl)propionaldehyde, *in vivo.* Rat, n=18	The mean total proportion of the dose in excreta and tissues was about 19%, which represents the apparent level of absorption of radioactivity into the systemic circulation.	Study report, 1995 Summarized in registration dossier. Annex I section 2.23
Dermal absorption study with 2-(4-*tert*-butylbenzyl)propionaldehyde, *in vivo.* Humans, n=3 Dose: 14.7 µCi or 11.37 mg Vehicle: 70% ethanol Exposed area: 10 cm2 back skin, semi-occlusive Duration: 6 hours	A mean of 1.4% (range 0.8 - 2.4%) of the applied dose was excreted in urine within 24 hours, whereas radioactivity was below the detection limit in urine samples of later time points and in all faeces and blood plasma samples.	Huntingdon Research Centre, 1994 Summarized in registration dossier. Annex I section 2.24
Hepatic lipogenesis and gluconeogenesis study with 2-(4-*tert*-butylbenzyl)propionaldehyde. Rat hepatocytes , *in vitro*	Addition of glycine, which represents a relevant substrate to form the respective hippurate (TBHA), did not affect 4-*tert*-butylbenzoic acid (TBBA) inhibition of lipogenesis in the rat cells. Furthermore, coenzyme A (CoA), acetyl-CoA and citrate levels were decreased in these cells.	McCune *et al*., 1982 Summarized in registration dossier. Annex I section 2.25
Study of CoA conjugate formation with 2-(4-*tert*-butylbenzyl)propionaldehyde. Rat and human hepatocytes, *in vitro.*	After 2-(4-*tert*-butylbenzyl)propionaldehyde addition, 4-*tert*-butylbenzoic acid (TBBA) was rapidly transformed to *p*-*tert*-butyl-benzoyl-CoA and accumulated to stable levels within 0.5 – 4 h. The concentration of the TBBA-CoA conjugate remained stable over time. Around 5-times lower TBBA-CoA levels were detected after 0.5 h incubation with 2-(4-*tert*-butylbenzyl)propionaldehyde in plated human versus rat hepatocytes. In contrast to the stable levels of TBBA-CoA in rats, a decrease over time was observed in human hepatocytes.	Givaudan, 2017 Summarized in registration dossier. Annex I section 2.26
Comparative *in vitro* metabolism study with 2-(4-*tert*-butylbenzyl)propionaldehyde. Plated rat, rabbit and human hepatocytes were exposed to 5 or 50 µM of 2-(4-*tert*-butylbenzyl)propionaldehyde for 0.5, 4, 8 (only rabbit hepatocytes) and 22 h in triplicate. Coenzyme A conjugates were analysed by LC-HRMS.	In rat hepatocytes, TBBA-CoA conjugate levels increased from about 1.4 µM at 0.5h to about 2 µM (50 µM test dose) over the time period observed. In rabbit hepatocytes initial formation of TBBA-CoA was observed at a lower level (about 1 µM) than in rat hepatocytes. The TBBA-CoA conjugates decreased over the 22 h incubation period and at 22 h incubation, 0.09 µM TBBA-CoA was detected in rabbit hepatocytes exposed to 50 µM of the test substances. In human hepatocytes initial levels of TBBA-	Laue et al. 2020. Annex I section 2.27

Method	Results	Reference
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 2-(4-*tert*-butylbenzyl)propionaldehyde	CoA were about 0.25 µM which decreased to levels close to the limit of detection at 22 h. The same major metabolites, including TBBA, were detected in rat and human hepatocytes after exposure to 2-(4-*tert*-butylbenzyl)propionaldehyde although the levels and/or time-concentration profiles of the metabolites differed. For example, lower concentrations of TBBA were detected in human hepatocytes compared to rat hepatocytes. During the study, the levels of two endogenous C8-CoA conjugates were monitored in rat, rabbit and human hepatocytes. It was observed that the two conjugates were suppressed in rat but not in rabbit and human hepatocytes. The two conjugates were considered potential intermediates in lipid metabolism.	
4-tert-butylbenzoic acid (TBBA) EC 202-696-3		
Biomonitoring study. Urine samples were collected in the population-representative German Environmental Survey for Children and Adolescents 2014-2017 from German residents aged 3-17 years (N=2133) with the aim to. analyse urine metabolites of the fragrance 2-(4-*tert*-butylbenzyl)propionaldehyde.	Four metabolites: 4-*tert*-butylbenzoic acid (TBBA), lysmerol, lysmerylic acid and hydroxy-lysmerylic acid were found in quantifiable amounts in 100, 99, 40 and 23% of the samples, respectively. Girls had higher urinary concentration of 2-(4-*tert*-butylbenzyl)propionaldehyde metabolites than boys. Use of fragrances, fabric softner and personal care products, especially perfumes, was positively associated with urinary concentrations of 2-(4-*tert*-butylbenzyl)propionaldehyde metabolites.	Murawski et al. 2020. Annex I section 2.28
Biomonitoring study. In total 329 urine samples from the Environmental Specimen Bank collected between 2000 and 2018 were analysed for metabolites of the fragrance 2-(4-*tert*-butylbenzyl)propionaldehyde.	Two major metabolites, 4-*tert*-butylbenzoic acid (TBBA) and lysmerol, were found in quantifiable concentrations in almost all samples in the study and correlated significantly. A significant decline was found for TBBA and lysmerol for the monitored years with the most pronounced decrease from 2012 to 2015.	Scherer et al. 2021. Annex I section 2.29

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

Toxicokinetic studies are available for 4 of the substances included in the CLH-dossier, i.e., 3-(4-*tert*-butylphenyl)-propionaldehyde, 4-*tert*-butyltoluene, 4-*tert*-butylbenzaldehyde and 2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral). The data is summarized below. No toxicokinetic studies are available for methyl 4-*tert*-butylbenzoate and *tert*-butylbenzoic acid (TBBA).

From the numerous toxicological studies showing systemic adverse effects after repeated exposure, it can be concluded that 3-(4-*tert*-butylphenyl)-propionaldehyde, 4-*tert*-butyltoluene, 4-*tert*-butylbenzaldehyde, lysmeral and TBBA are readily taken up *via* oral administration and distributed in the body. There is experimental evidence that some substances are taken up by other routes of exposure, such as skin (lysmeral) and lung (4-*tert*-butyltoluene), although dermal uptake was indicated to be lower compared to the oral route.

Distribution of lysmeral is predominantly to liver. Moreover, the substances and/or metabolites are distributed to testis as demonstrated by the testicular toxicity observed with administration of five substances in the group.

Metabolism of 3-(4-*tert*-butylphenyl)-propionaldehyde, 4-*tert*-butyltoluene and lysmeral to TBBA has been demonstrated in rat hepatocytes *in vitro*. TBBA is also formed by mouse and rabbit hepatocytes exposed to lysmeral.

Administration of 3-(4-tert-butylphenyl)propionaldehyde, 4-*tert*-butyltoluene, 4-*tert*-butylbenzaldehyde and lysmeral has been shown to form TBBA *in vivo* in rats (3-(4-tert-butylphenyl)propionaldehyde, 4-*tert*-butyltoluene, 4-*tert*-butylbenzaldehyde and lysmeral), mice, guinea pigs and dogs (4-*tert*-butyltoluene, 4-*tert*-butylbenzaldehyde and lysmeral) and in rhesus monkeys (lysmeral).

TBBA has been detected as a metabolite in human hepatocytes exposed to lysmeral (Laue *et al*., 2020). TBBA has also been detected in urine after dermal and oral exposure of human volunteers (ECHA 2017a, Scherer *et al*., 2016). Moreover, TBBA has been detected in human urine in two biomonitoring studies (Murawski *et al*., 2020 and Sherer *et al.*, 2021). The levels of TBBA in human urine correlated positively with use of fragranced fabric softeners and personal care products (Murawski *et al*., 2020).

Quantitative species differences in the formation of TBBA have been reported after exposure to lysmeral, i.e. formation of TBBA is higher in rats when compared to other rodent, non-rodent animal or human hepatocytes (Laue *et al*., 2020).

Rapid urinary excretion has been observed in rats after dermal administration of lysmeral. The excretion of 3-(4-*tert*-butylphenyl)-propionaldehyde, 4-*tert*-butyltoluene and 4-*tert*-butylbenzaldehyde has not been extensively studied but oral studies indicate that the substances are at least in part eliminated *via* the kidneys. Elimination of 4-*tert*-butyltoluene was primarily *via* the kidney (ratio 3,5:1, urine:feces) in rats (Ingebrigtsen and Walde, 1982; Walde and Scheline, 1983).

Some registrants have proposed a mechanism of action for the testicular- and spermatotoxicity caused by the substances comprised in the present dossier (Laue et al. 2020, ECHA 2017a). The mechanism of action is considered to be caused by the formation of stable TBBA-coenzyme A (CoA) conjugates, which would disrupt lipid synthesis by for example depletion of physiological CoA. This would interfere with other cellular processes and lead to cellular toxicity. CoA-TBBA is stated to be primarily formed in rats. *In vitro* experiments with lysmeral have demonstrated higher levels of stable CoA-TBBA conjugates in rat hepatocytes compared to human hepatocytes. This indicates, according to study authors, that the toxicity of TBBA, and hence substances forming TBBA during metabolism, is not relevant to humans (Laue *et al*., 2017).

The proposed mechanism of action and the relevance to humans was discussed in the RAC opinion of lysmeral (ECHA, 2019) in which the species differences demonstrated *in vitro* were considered quantitative rather than qualitative and hence that the proposed MoA, although plausible, was not sufficient to preclude relevance for humans. RAC furthermore stated that “It is not clear how relevant mechanistic findings from in vitro tests in hepatocytes are for the effects seen on testes tissue. For example, severe atrophy was seen already after only 24 hours after exposure. Although TBBA-CoA-conjugates were also formed in rat testes tissue ex vivo, concentrations were approximately 100-fold lower than in hepatocytes. Therefore, a direct effect of Lysmeral on this tissue cannot be ruled out.”

In Laue *et al*., 2020, it was demonstrated that exposure to lysmeral and several structural analogues (also considered to be toxic to reproduction by the authors) resulted in the formation of TBBA in suspended hepatocytes from rat, rabbit, and human. Moreover, that stable TBBA-CoA levels in plated hepatocytes were quantitatively species-dependent, with lower levels in rabbit and human hepatocytes and higher levels in rat hepatocytes. The levels of two prominent CoA-conjugates (octanoyl-CoA and octenoyl-CoA), identified by the authors as potential intermediates in lipid metabolism were measured in parallel. Results indicated suppression of those conjugates in rat, while no effects on the levels of the conjugates were detected in rabbit and human hepatocytes.

10 EVALUATION OF HEALTH HAZARDS

Read-across justification

Category approach - Chemical grouping

The substances included in this proposal are grouped in a category for the purpose of harmonised classification. Two substances, (2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral), EC 201-289-8 and *tert*-butylbenzoic acid, EC 202-696-3, already have harmonised classification as Repr. 1B (H360Fd and H360F, respectively). Lysmeral has been included in the present proposal with the main purpose to add a note to the existing entry in Annex VI of CLP, to account for additive mixture effects, as lysmeral forms the same metabolite (TBBA) as the other substances included in the proposal. Additionally, lysmeral is a member of the category formed for read-across purposes, and it is thus used as a source substance. The DS has decided not to open the current classification of lysmeral for reassessment. No new experimental data is available for TBBA that question the current harmonised classification as Repr.1B for adverse effects on sexual function and fertility (H360F), however, the DS proposes to add Repr. 2 for developmental effects (H360d), based on the read-across approach described herein.

For one of the substances in the category (methyl 4-*tert*-butylbenzoate, EC 247-768-5), there are no mammalian fertility or developmental toxicity studies available. For two of the substances in the category (3-(4-*tert*-butylphenyl)propionaldehyde EC 242-016-2 and 4-*tert*-butylbenzaldehyde, EC 213-367-9), the studies available are considered to be of limited quality or relevance for the purpose of harmonised classification as stand-alone. The entire database across all substances, however, is a convincing literature demonstrating adverse effects of structurally similar substances on reproduction.

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 is complying 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 *tert*-butylbenzoic acid (TBBA) is known to cause toxic effects in male reproductive organs, and this substance has a harmonised classification as Repr. 1B (H360F) (ATP 03 to CLP Annex VI). Substances of similar structures have been demonstrated to metabolise to TBBA and thereby cause reproductive toxicity (Laue et al. 2017, Laue et al. 2020). One additional member of the category, 2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral) has harmonised classification as Repr. 1B (H360Fd) (ATP 15 to CLP Annex VI).

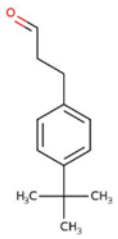
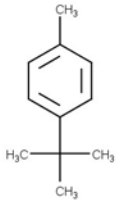
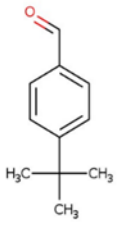
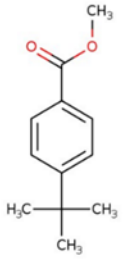
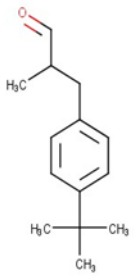
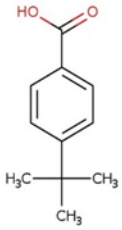
The basis for grouping includes the following:

- structural similarity: a benzene ring with a substituent that can degrade to a carboxylic acid group *and* a *tert*-butyl-group in *para* position and;
- experimentally demonstrated or predicted formation of the metabolite TBBA.

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 TBBA. Additionally, one member of the group is TBBA itself.

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, and read-across is considered justified.

Figure 2. Category members.

3-(4-*tert*-butylphenyl) propionaldehyde EC 242-016-2 	4-*tert*-butyltoluene EC 202-675-9 
4-*tert*-butylbenzaldehyde EC 213-367-9 	methyl 4-*tert*-butylbenzoate EC 247-768-5 
2-(4-*tert*-butylbenzyl) propionaldehyde EC 201-289-8 	4-*tert*-butylbenzoic acid EC 202-696-3 

Reporting format for the category

1.1 Category definition

This category covers the substance *tert*-butylbenzoic acid (TBBA) and precursor substances that metabolise into TBBA.

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 *tert*-butylbenzoic acid (TBBA) and substances that metabolise to TBBA.

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 a *tert*-butyl-group in *para* position). Secondly, experimentally demonstrated or predicted formation of the metabolite TBBA.

One member of the group is TBBA, the metabolite itself. The five additional members of this category consist of a benzene ring with a substituent that can degrade to a carboxylic acid group and a *tert*-butyl-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 five substances (2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral); EC 201-289-8, 3-(4-*tert*-butylphenyl)propionaldehyde; EC 242-016-2, 4-*tert*-butyltoluene; EC 202-675-9, 4-*tert*-butylbenzaldehyde; EC 213-367-9, methyl 4-*tert*-butylbenzoate; EC 247-768-5) that can form the metabolite *tert*-butylbenzoic acid (TBBA), and TBBA 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).

2 Category justification

The category includes five substances that metabolise into *tert*-butylbenzoic acid (TBBA), and TBBA itself.

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

3-(4-*tert*-butylphenyl)propionaldehyde (EC 242-016-2);

4-*tert*-butyltoluene (EC 202-675-9);

4-*tert*-butylbenzaldehyde (EC 213-367-9);

methyl 4-*tert*-butylbenzoate (EC 247-768-5).

The formation of the metabolite TBBA has been demonstrated *in vitro* and *in vivo for all these substances, except* 4-*tert*-butylbenzaldehyde where the formation of TBBA has only been tested and demonstrated *in vivo.*

4-*tert*-butyltoluene is one of the predicted precursors to TBBA. The simulated metabolism indicates, however, other concomitant metabolic pathways which gives an uncertainty as to whether TBBA is the main metabolite. However, as indicated above, the formation of TBBA has been demonstrated experimentally *in vitro* and *in vivo,* and data on reproductive toxicity (section 10.10) are in line with the toxicity shown for TBBA.

For methyl 4-*tert*-butylbenzoate there are no experimental data available but simulation indicates that TBBA is a main metabolite of this substance, which is also indicated by its chemical structure, i.e., a methylester that can be hydrolysed to a carboxylic acid.

The substance 2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral) was not identified as a precursor of TBBA by OECD QSAR Toolbox, however, the formation of TBBA has been demonstrated both *in vitro* and *in vivo*.

For five of the substances (3-(4-*tert*-butylphenyl)propionaldehyde (EC 242-016-2), 4-*tert*-butyltoluene (EC 202-675-9), 4-*tert*-butylbenzaldehyde (EC 213-367-9), lysmeral (EC 201-289-8) and TBBA (EC 202-696-3), their similar profile on reproductive toxicity (section 10.10) supports the formation of the category. No experimental data is available for methyl 4-*tert*-butylbenzoate.

Other support for this grouping includes similar physicochemical and toxicological properties (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 TBBA formation, as well as reproductive toxicity studies are presented to indicate similar adverse effects on reproductive organs and developmental toxicity 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 an intrinsic hazard of the category members. Hence, read-across can be performed to fill data gaps of reproductive toxicity where data is lacking of not sufficiently robust. Two substances, 2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral) and 4-*tert*-butylbenzoic acid (TBBA) already have harmonised classification as Repr. 1B for adverse effects on fertility, and lysmeral also has a classification in Repr. 2 for developmental toxicity. Additionally, lysmeral is included in the Candidate List pursuant to REACH article 57c. Except for the two substances already classified as Repr. 1B, the substance for which most data are available is 4-*tert*-butyltoluene. Less information is available for 3-(4-*tert*-butylphenyl)propionaldehyde and 4-*tert*-butylbenzaldehyde. No mammalian toxicity data is available for methyl 4-*tert*-butylbenzoate. The available database permits an assessment of the reproductive toxicity of this category of substances.

Table 7: Data matrix

Chemical name	2-(4-*tert*-butylbenzyl) propionaldehyde (lysmeral)	4-*tert*-butylbenzoic acid (TBBA)	3-(4-*tert*-butylphenyl)propionaldehyde	4-*tert*-butyltoluene	4-*tert*-butylbenzaldehyde	methyl 4-*tert*-butylbenzoate
CAS	80-54-6	98-73-7	18127-01-0	98-51-1	939-97-9	26537-19-9
EC	201-289-8	202-696-3	242-016-2	202-675-9	213-367-9	247-768-5
PHYSICO-CHEMICAL DATA						
Molecular weight	204.31	178.23	190.28	148.24	162.23	192.25
Melting Point	< -20°C	165 - 167 °C	-10.7 °C	-52 °C	< -20 °C	-
Boiling Point	279.5°C	280 °C	207 °C	193 °C	1: 248.7 °C 2: 107 °C	-
Density	0.94	1.142	0.959	0.86	0.97	-
Vapour Pressure	0.0025 hPa (20°C)	0.00057 hPa (20°C)	0.002 hPa (20°C)	1.3 hPa (30°C)	0.04 hPa (20°C)	1.36 hPa (20°C)
Partition Coefficient (log Kow)	4.2	3.4	3.2	4.4	3.1	4.3
Water Solubility	33 mg/L	47.1 mg/L (pH 4.3) 12600 mg/L (pH 7)	132 mg/L	4 mg/L	120 mg/L	35 mg/L
TOXICOKINETICS						
Data available on TBBA formation *in vitro*	yes	NA	yes	yes	no	no
Data available on TBBA formation *in vivo*	yes	NA	yes	yes	yes	no
MAMMALIAN TOXICITY						

Included in Annex VI	Repr. 1B (H360Fd)	Acute Tox. 4 (H302) STOT RE 1 (H372) Repr. 1B (H360F)	-	-	-	-
Reproductive toxicity - Fertility	LOAEL (oral) : 25-100 mg/kg bw/day (male rats reproductive organs) LOAEL (dermal) : 2000 mg/kg bw/day (male rats fertility)	LOAEL (oral): 6-7.9 mg/kg bw/day (male rats reproductive organs) LOAEL (dermal) : 60 -70 mg/kg bw/day (male rats reproductive organs) LOAEL (inhalation) : 12.5-495 mg/m3 (male rats reproductive organs)	LOAEL: 5-100 mg/kg bw/day (male rat reproductive organs)	LOAEL 15-50 mg/kg bw/day (male rat reproductive organs)	LOAEL 25 mg/kg bw/day (male rat reproductive organs)	-
Reproductive toxicity - Development	LOAEL: 10-15 mg/kg bw/day (pups weight, post-implantation loss)	-	LOAEL: 5 mg/kg bw/day (pups weight)	LOAEL 5 mg/kg bw/day (pups weight)	-	-

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**
3-(4-*tert*-butylphenyl)propionaldehyde EC 242-016-2			
OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test) No deviations GLP compliant Test animals: Crl:CD(SD) Sprague Dawley rats (males and females). 10 rats/sex/group. Assigned reliability 1 by the Registrant.	3-(4-*tert*-butylphenyl)propionaldehyde EC 242-016-2 Purity: 99.0%. (Expiration date of the lot/batch: October 15, 2011). Vehicle: corn oil Doses: 0, 0.5, 1 and 5 mg/kg bw/day. Male rats were dosed once daily (oral gavage) beginning 14 days before cohabitation with treated females, during cohabitation and continuing through the day prior to scheduled euthanasia on Days 43 through 46. Female rats were dosed once daily (oral gavage) beginning 14 days before cohabitation with treated males and continuing through LD 12 (rats that delivered a litter) or GD 24 (rats that did not deliver a litter).	***Parental animals*** **Mortality and clinical observations** No clinical signs were observed. One female at 1 mg/kg bw/day found dead on GD 22. One female at 0.5 mg/kg bw/day found dead on LD 8. **Body weight** No effects on body weight were observed. **Reproductive performance** No effects on mating and fertility were observed. Viability index was 81.8%, 96.3%, 97.9% and 91.0% at 0, 0.5, 1 and 5 mg/kg bw/day, respectively. ***F1 generation*** No clinical signs were observed. ↓ pup body weight at 5 mg/kg bw/day, LD 9 (-11%; p≤0.05) and LD 12 (-12%; p≤0.01). **Thyroid hormone analysis** *Females* ↓ mean serum T4 at 0.5 mg/kg bw/day (-18%; not significant), at 1 mg/kg bw/day (-26%; p≤0.01), at 5 mg/kg bw/day (-26%; p≤0.01) on day 12. NOAEL: >5 mg/kg bw/day (male reproduction) NOAEL: 1 mg/kg bw/day (pups weight)	Study report, 2019. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Full study report was available to DS Additional data available in Confidential Annex Annex I Section 3.10.1.1
14 days dose range finding study for the OECD Guideline 422 study (described above) No guideline No information	3-(4-*tert*-butylphenyl)propionaldehyde EC 242-016-2 Purity: no information Vehicle: corn oil Doses: 0, 5, 25, 50	**Mortality and clinical observations** *Males* Two mortalities at 50 mg/kg bw/day (day 1 and 14). *Females* Clinical signs at 25 and 50 mg/kg bw/day included suspected dehydration, hunched	Dose-range finding study described in Study report 2019, the Robust study summary in Registration dossier, ECHA's

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
on GLP compliance Test animals: Crl:CD(SD) Sprague Dawley rats (males and females). 20 rats/sex, 5 rats/sex/group.	mg/kg bw/day. The substance was administered orally by gavage 3 times (approximately 6 hours apart) daily for 14 consecutive days	posture and thin appearance. **Body weight and Food consumption** *Females* ↓ body weight loss at 25 (-15.4 g vs. +13.3 g) and 50 mg/kg bw/day (-33.0 vs. +13.3 g) at day 1 to 15. ↓ mean body weight at 25 mg/kg bw/day (-9% on day 7 and -4% on day 15) and at 50 mg/kg bw/day (-18% on day 3 to -5% on day 15). ↓ food consumption at 25 mg/kg bw/day (-16% to -6%) and at 50 mg/kg bw/day (-64% to -17%). **Reproductive organs** *Males* ↓ testicular size at 50 mg/kg bw/day (1/20). Changes in absolute and relative organ weights in testes at 50 mg/kg bw/day (no details provided) Changes in absolute and relative liver weight at 5, 25 and 50 mg/kg bw/day (no details provided). Changes in testes and liver, correlated with hypertrophy or atrophy/degeneration (no details provided). Vacuolation and degeneration of seminiferous tubular epithelium, Sertoli cell vacuolation in testes at 5, 25 and 50 mg/kg bw/day. Cribriform change, cellular debris, and hypospermia in epididymides at 25 and 50 mg/kg bw/day. *Females* Changes in absolute and relative ovaries weight at 25 and 50 mg/kg bw/day (no details provided). Changes in absolute and relative uterus weight at 50 mg/kg bw/day (no details provided). Changes in uterus correlated with hypertrophy or atrophy/degeneration. **Sperm effects** ↓ sperm motility at 5 mg/kg bw/day (77% vs. 84% in controls) ↓ little to no sperm at 25 and 50 mg/kg bw/day	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**
		(-66% and -81%%, respectively). ↑ headless and detached sperm at 25 and 50 mg/kg bw/day. LOAEL: 5 mg/kg bw/day (male reproduction)	
Toxicity screening test No test guideline GLP compliant Test animals: Male Crl:CD® (SD)IGS BR rats 6 rats per dose group. Positive control group of six males received Lilial (lysmeral) at 250 mg/kg bw/day. Reliability 2 according to Registrant	3-(4-*tert*-butylphenyl)propionaldehyde EC 242-016-2 Purity 98.4% Vehicle: corn oil. Substance was administered orally (gavage) at doses 0, 25, 100 or 250 mg/kg bw/day once daily for 5 consecutive days	**Mortality and Clinical Observations** Three males at 250 mg/kg bw/day and 1 male at 100 mg/kg bw/day were killed 5 to 12 hours after first dose due to poor clinical conditions. Remaining animals at 250 mg/kg bw/day were killed day 2 prior to dosing. At 250 or 100 mg/kg bw/day (only after first dose) signs of underactivity, reduced body temperature, irregular breathing, piloerection, loose faeces and partially closed eyelids. **Body weight** ↓ body weight loss at 25 (-10 g after first dose), and at 100 mg/kg bw/day (-14 g after first dose, and at days 3-5) and at 250 mg/kg bw/day (-15 to -30 g in 3 males killed on day 2). **Organ weights** ↑ absolute epididymal weight at 100 mg/kg bw/day (no details provided, no information on statistical significance) ↓ testes weight at 100 mg/kg bw/day (no details provided, no information on statistical significance) Enlarged epididymides (3/6), kidney depressions (2/6), thickened forestomach (2/6), pale livers (5/6) at 100 mg/kg bw/day. Pale livers also observed at 25 mg/kg bw/day (6/6). Seminiferous tubular degeneration/atrophy, Sertoli cell vacuolation, multinucleate giant cell and luminal sloughing of spermatogenic cells in the testes at 100 mg/kg bw/day. Reduced numbers of spermatozoa, sloughed germ cells in lumen and inflammation in the epididymides at 100 mg/kg bw/day. **Urine analysis** Analysis of urine in males at 100 or 25 mg/kg bw/day demonstrated presence of metabolite 4-*tert*-butylbenzoic acid (TBBA). NOAEL: 25 mg/kg bw/day (male	Study report, 2009. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 2 Annex I Section 3.10.1.2

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		reproduction)	
4-*tert*-butyltoluene EC 202-675-9			
OECD TG 421 Reproduction / Developmental Toxicity Screening Test GLP compliant Sprague Dawley rats, males/females 12 males and 12 females per group. Assigned reliability 1 by the Registrant. Full study report was not available to DS. Differences in weight of parental animals were estimated from graphs and are associated with uncertainties.	4-*tert*-butyltoluene EC 202-675-9 Purity 96.94% Vehicle: Corn oil Substance was administered orally (gavage) at doses 0, 1.5, 5, 15, 50 mg/kg bw/day once daily. The administration period for males was total 50 to 52 days including 14 days before mating and subsequent 36 to 38 days (necropsy of males was separately conducted in 3 days since the observation of sperm requires 3 days). The administration period for females was total 41 to 45 days including 14 days before mating, mating period (14 days at the longest), gestational period, and first 3 days in lactation period. The starting day of administration was set as day 1.	***Parental animals*** **Mortality and Clinical observations** *Males* One death at 50 mg/kg bw/day (with transient salivation, decreased locomotor activity, soiled fur, reddish urine, and hypothermia). At 1.5 mg/kg bw/day transient salivation and at 50 mg/kg bw/day soiled fur in one animal. *Females* One death at 15 mg/kg bw/day and 6 deaths at 50 mg/kg bw/day (no detailed information provided). At 15 mg/kg bw/day: hypothermia, decreased locomotor activity, and transient salivation. At 50 mg/kg bw/day: hypothermia, prone position, decreased locomotor activity, piloerection, soiled fur, bradypnea, and transient salivation. Clinical signs at 15 mg/kg bw/day (transient salivation) and at 50 mg/kg bw/day (transient salivation, hypothermia, decreased locomotor activity, staggering gait, lacrimation, diarrhea, and muscle relaxation) **Body weight** *Males* ↓ body weight at 15 mg/kg bw/day (estimated from graph, -13%, day 49; p≤0.01) and 50 mg/kg bw/day (estimated from graph, -6%, day 4 and -19%, day 49; p≤0.01). *Females* ↓ body weight GD 7 at 5 mg/kg bw/day (-8%; p≤0.05 estimated from graph) and GD 14 (-10%; p≤0.05 estimated from graph) and at 15 mg/kg bw/day, GD 7 to 21, estimated from graph, -9% at GD 21; p≤0.01. ↓ body weight at 5 mg/kg bw/day at LD 4 (estimated from graph -13%, p≤0.01, correlated with significantly decreased food consumption). ↓ body weight at 15 mg/kg bw/day LD 4 (-11% estimated from graph, only one animal).	Study report, 2007a, Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 3 Annex I 3.10.1.3 Additional data available in Confidential Annex

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		↓ body weight at 50 mg/kg bw/day (from day 4 to 15, before mating (estimated from graph, -8%; p≤0.05). ↓ body weight, day of necropsy at 5 (-13%; p≤0.01), 15 (-18%; p≤0.01), and 50 mg/kg bw/day (-29%; p≤0.01). **Sperm effects** ↓ sperm motility, ratio, path velocity, straight line velocity, curvilinear velocity, viability, survivability, and sperm count at 15 and 50 mg/kg bw/day. **Reproductive organs** ↓ absolute weight epididymis at 15 mg/kg bw/day (-12%; p≤0.01). ↓ absolute weight testis (tendency) at 15 mg/kg (-8%). ↓ absolute and relative weights of testis (-82%; p≤0.01 and -44%) and epididymis (-34%; p≤0.01 and -20%) at 50 mg/kg bw/day. Atrophy of testes and epididymides at 15 (1/12) and 50 mg/kg bw/day (11/11). Testis atrophy of seminiferous tubules (4/12 animals), hyperplasia of Leydig Cells (2/12 animals), at 15 mg/kg bw/day. Atrophy of seminiferous tubules and hyperplasia of Leydig cells (11/11) at 50 mg/kg bw/day. ↓ sperm count at 15 mg/kg bw/day (4/12 animals) and at 50 mg/kg bw/day (11/11 animals). **Reproductive performance** One pair did not achieve copulation at 50 mg/kg bw/day. ↓ fertility index at 15 (33.3%) and 50 mg/kg bw/day (0%). ↓ gestation index at 15 mg/kg bw/day (66.7%). All newborn pups of one dam at 15 mg/kg bw/day died by day 1 of the lactation period. ↓ number of pups born (-26%; p≤0.05) and number of live pups (-58%; p≤0.01) at LD 0 at 15 mg/kg bw/day. ↓ delivery index (82.7% vs. 94.1% in controls), birth index (49.3% vs. 93.6% in controls), and live birth index (63% vs. 99.5%	

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		in controls) at 15 mg/kg bw/day. ↑ number of stillbirths at 15 mg/kg bw/day (4.7 vs. 0.1 in controls; not statistically significant). NOAEL: 5 mg/kg bw/day (male reproduction) ***F1 generation*** Offspring of one dam died by LD 1 at 15 mg/kg bw/day. ↓ number of live offspring at LD 4 (4.5 vs. 14.3 in controls, tendency) and viability index LD 4 (45% vs. 98.8% in controls) at 15 mg/kg bw/day. ↓ body weights of pups LD 0 (-11% and -8% in males and females, respectively) and LD 4 (-16% and -15% in males and females, respectively) at 5 mg/kg bw/day. ↓ body weights of pups at LD 0 (-32% in males and females) and LD 4 (-13% and -10% in males and females, respectively) at 15 mg/kg (based on pups from 1 dam). NOAEL: 1.5 mg/kg bw/day (pups weight)	
Testicular toxicity screening test No test guideline Not GLP compliant Test animals: male albino SPF rats 7 males per group Assigned reliability 2 by the Registrant.	4-*tert*-butyltoluene EC 202-675-9 No information on purity Vehicle: rape oil The substance was administered orally (gavage) at doses 0 and 200 mg/kg bw once daily for 5 consecutive days.	**Mortality and Clinical observations** No deaths, and no clinical signs were observed. **Body weight** Slight body weight loss was apparent in treated males up to 3 days following treatment (no information on statistical significance). **Reproductive organs** ↓ testes weight at 200 mg/kg bw/day. Effects in seminiferous tubules of all treated rats included lesions in epithelium with degeneration of spermatocytes and spermatids, reduction of spermatozoa and appearance of giant cells.	Study report 1982a. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 4 Annex I Section 3.10.1.4
Testicular toxicity screening test No test guideline Not GLP compliant Test animals:	4-*tert*-butyltoluene EC 202-675-9 No information on purity Vehicle: rape oil The substance was	**Mortality and clinical observations** No mortality. Clinical signs at 50 and 100 mg/kg bw/day included loss of hair, shaggy fur, hunched posture, lethargy and diarrhea. **Body weight**	Study report 1982b Robust study summary in Registration dossier, ECHA's dissemination

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
male albino SPF rats 8 males per dose group; 4 males in the vehicle control group Assigned reliability 2 by the Registrant.	administered orally (gavage) at doses 0, 12.5, 25, 50, 100 mg/kg bw/day once daily for 5 consecutive days.	↓ body weight loss at 100 mg/kg bw/day (-13% on day 6 compared to day 1, -24% compared to controls). **Organ weight and pathology** ↓ testes weight at 100 mg/kg bw/day (-23%). Delineation of hepatic lobules in liver of majority of rats at 50 (6/8) and 100 mg/kg bw/day (6/8). Pale livers (3/8) and pale kidneys (3/8) at 100 mg/kg bw/day. Severe cell-deformations in germinal epithelium at 50 and 100 mg/kg bw/day. Degenerated spermatids and spermatocytes, reduced spermatozoa, giant cells were observed sporadically. NOAEL 25 mg/kg bw/day (male reproduction)	site, 2022. Study 5 Annex I 3.10.1.5
Testicular toxicity screening test No test guideline Not GLP compliant Test animals: male Himalayan guinea pigs 5 males per group Assigned reliability 2 by the Registrant.	4-*tert*-butyltoluene EC 202-675-9 No information on purity Vehicle: rape oil The substance was administered orally (gavage) at doses 0 and 100 mg/kg bw once daily for 5 consecutive days.	**Mortality and Clinical observations** No deaths, no signs of toxicity were noted. **Body weight** No effects on body weights. **Male reproductive organs** Slight damage of germinal epithelium in testes both at 0 (2/5 animals) and 100 mg/kg bw/day (1/5 animals). Moderate damage of germinal epithelium at 100 mg/kg bw/day (1/5 treated animals).	Study report 1984a. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 6 Annex I 3.10.1.6
Testicular toxicity screening test No test guideline Not GLP compliant. Test animals: male Beagle dogs 1 control male and 2 dosed males Assigned reliability 2 by	4-*tert*-butyltoluene EC 202-675-9 No information on purity No vehicle The substance administered orally (capsule) at doses 0 and 100 mg/kg bw once daily for 5 consecutive days.	**Mortality and clinical observations** No deaths, no clinical signs were observed. **Body weight** No effects on body weight. **Male reproduction organs** A few multinucleated giant cells in seminiferous tubules of the control dog. Small quantity of seminiferous tubules with nearly total depopulation of germinal epithelium in both testes of treated dog 1. The concerned seminiferous tubules (ca. 20 in testis 1 and 10 in testis 2) showed early stages of spermatogenesis and Sertoli cells.	Study report,1984b. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 7 Annex I 3.10.1.7

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
the Registrant.		No changes were found in testes of second treated dog and no changes in epididymides of any dogs.	
Testicular toxicity screening test No test guideline Not GLP compliant Test animals: male albino mice 6 males per group Assigned reliability 2 by the Registrant.	4-*tert*-butyltoluene EC 202-675-9 No information on purity Vehicle: rape oil The substance was administered orally (gavage) at doses 0 and 100 mg/kg bw/day once daily for 5 consecutive days	**Mortality and clinical observations** No mortality or clinical signs were observed. **Body weight** No effects on body weight. **Organ weights** ↑ absolute testes weights of the dosed animals were increased at 100 mg/kg bw/day (+17%). Relative testis weight was decreased (-12%). No information on statistical significance. Slight damage of germinal epithelium in testes at 0 (1/6) and 100 mg/kg bw/day (3/6).	Study report 1984c. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 8 Annex I 3.10.1.8
Repeated Dose 28-Day Oral Toxicity Study in Rodents OECD TG 407 GLP compliant Test animals: Crj:CD(SD)IGS, SPF rats 12 males and 12 females per group Assigned reliability 1 by the Registrant.	4-*tert*-butyltoluene EC 202-675-9 Purity: 95.93% Vehicle: corn oil Substance was administered orally (gavage) at doses : 1.5, 5, 15, 50 mg/kg bw/day daily for 28 days Post-exposure recovery period of 14 days in satellite groups.	**Mortality and clinical observations** No mortality. Transient salivation in males and females at 15 and 50 mg/kg bw/day. **Body weight and food/water consumption** *Males* ↓ food and water consumption at 15 and 50 mg/kg bw/day. No details provided. *Females* ↓ food and water consumption at 50 mg/kg bw/day. No details provided. **Organ weights** *Males* ↓ absolute weights of testis and epididymis and relative weight of testis at 50 mg/kg bw/day. No details provided. Atrophy of the seminiferous tubules and hyperplasia of Leydig cells at 50 mg/kg bw/day (6/12 animals). ↓ sperm count at 50 mg/kg bw/day (6/12 animals) *Females* ↓ body weight at 50 mg/kg bw/day (day of necropsy). No details provided. ↓ absolute weight of ovary at 50 mg/kg bw/day. No details provided.	Study report 2007b. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 10 Annex I 3.10.1.10

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		NOAEL: 15 mg/kg bw/day (male reproduction)	
4-*tert*-butylbenzaldehyde EC 213-367-9			
Testicular toxicity screening test No test guideline GLP compliance not specified Test animals: male SPF albino rats Control group 4 males, test groups per dose: 8 males Assigned reliability 2 by the Registrant.	4-*tert*-butylbenzaldehyde EC 213-367-9 No information on purity Vehicle: rape oil The substance was administered orally (gavage) at doses of 6.5, 12.5, 25 and 50 mg/kg bw once daily for 5 consecutive days.	**Mortality and Clinical observations** No mortality. Three rats at 12.5 mg/kg bw/day showed slight aggressiveness on days 3 and 4. Slight hair loss at 50 mg/kg bw/day (one animal). **Body weight** ↓ body weight at 50 mg/kg bw/day (-4% on day 6 compared with day 1, -13% compared to controls on day 6). **Organ weights** ↓ testes weights at 50 mg/kg bw/day (-14%) Disorganisation of the epithelial structure, degeneration of cells, and reduction of spermatozoa. Moderate to severe injuries in seminiferous epithelia at 50 mg/kg bw/day (8/8). NOAEL: 12.5 mg/kg bw/day (male reproduction)	Study report 1981. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 11 Annex I 3.10.1.11
Testicular toxicity screening test No test guideline GLP compliance not specified Test animals: Male SPF albino mice 6 animals per dose group Assigned reliability 2 by the Registrant.	4-*tert*-butylbenzaldehyde EC 213-367-9 No information on purity Vehicle: rape oil Substance was administered orally (gavage) to male mice for 5 consecutive days at doses 0 and 100 mg/kg bw/day	**Mortality and clinical signs** No mortality or clinical effects were observed. **Body weight** No effects on body weight. **Organ weights** No effect on testes weight. **Male reproductive organs** Slight damage of germinal epithelium in testes at 0 (1/6) and 100 mg/kg bw/day (4/6).	Study report 1984d. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 12 Annex I 3.10.1.12
Testicular toxicity screening test No test guideline GLP compliance not specified Test animals:	4-*tert*-butylbenzaldehyde EC 213-367-9 No information on purity Vehicle: rape oil Substance was	**Mortality and clinical observations** No mortality or clinical effects were observed. **Body weights** No effect on body weight was seen. **Male reproductive organs**	Study report1984e. Robust study summary in Registration dossier, ECHA's dissemination site, 2022.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Male Himalayan guinea pigs 5 animals per dose group Assigned reliability 2 by the Registrant.	administered orally (gavage) to guinea pigs for 5 consecutive days at doses 0 and 100 mg/kg bw/day.	Slight damage of germinal epithelium at 0 (2/5) and at 100 mg/kg bw/day (1/5). Lumen of the seminiferous tubules at 100 mg/kg bw/day was more detritus than in controls.	Study 13 annex I 3.10.1.13
Testicular toxicity screening test No test guideline GLP compliance not specified Test animals: male Beagle dogs 2 treated males and one control male. Assigned reliability 2 by the Registrant.	4-*tert*-butylbenzaldehyde EC 213-367-9 No information on purity Substance was administered orally one daily for 5 consecutive days via gelatine capsule Doses: 0 and 100 mg/kg bw/day	**Mortality and clinical observations** No mortality or clinical signs were observed. **Body weight** ↓ body weight at 100 mg/kg bw /day (-5% and -10%, from day 1 to day 6, respectively for both dogs). No information provided on the control dog. **Male reproductive organs** Seminiferous tubules with nearly total depopulation of germinal epithelium (both testes of one dog) with only early stages of spermatogenesis and Sertoli cells preserved. Occurrence of multinucleated giant cells in testes of the other treated dog.	Study report 1984f. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 14 - Annex I 3.10.1.14
Testicular toxicity screening test No test guideline GLP compliance not specified Test animals: male SPF albino rats. 7 male animals per dose group Assigned reliability 2 by the Registrant.	4-*tert*-butylbenzaldehyde EC 213-367-9 No information on purity Vehicle: rape oil Substance was administered orally via gavage once daily for 5 consecutive days. Doses: 0 and 100 mg/kg bw/day	**Mortality and clinical observations** No mortality or clinical signs were observed. **Body weight** ↓ body weight (-8% on day 2, with subsequent weight gain at the end of treatment; -8% compared to control). **Organ weights** ↓ testes weight at 100 mg/kg bw/day (-15%). **Male reproductive organs** Injuries of the seminiferous epithelium at 100 mg/kg bw/day. Minimal to moderate degeneration of spermatids and spermatocytes (5/7). One treated animal showed a Minimal reduction of spermatozoa (1/7), minimal to moderate appearance of multinucleate giant cells (7/7).	TSCATS, NTIS/OTS0505405, New Doc. I.D. 88-8100336, 1982. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 15 Annex I 3.10.1.15
Methyl 4-*tert*-butylbenzoate EC 247-768-5			
No studies are available	methyl 4-*tert*-butylbenzoate EC 247-768-5		

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral) EC 201-289-8 [10]			
One-generation range finding study (non-guideline, non-GLP) Rat (Wistar) Oral: via diet	2-(4-*tert*-butylbenzyl)propionaldehyde Concentrations of 0, 400, 800, 1700, 3400 ppm in the diet. Concentrations of 0, 14, 28, 62.6, 116.8 mg/kg bw/day (doses males). Concentrations of 0, 10-15, 18.3-29.4, 62.7, 123.2 mg/kg bw/day (doses / dose range females). Purity: 30.7% (a.i. encapsulated).	**Fertility/reprod. performance - Main effects** Testicular toxicity / spermatotoxic effects Effects on reproductive parameters **General systemic toxicity - Main effects** ↓ body weights /FC changes in liver associated parameters (clinical chemistry, ↑ liver weights) ↑ relative kidney weights **Developmental toxicity - Main effects (coinciding with maternal toxicity):** ↓ pup body weights	BASF SE 2006C Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde.
One-generation range finding study (non-guideline, GLP) Rat (Wistar) Oral: via diet	2-(4-*tert*-butylbenzyl)propionaldehyde Concentrations of 0, 230, 750, 2300 ppm in the diet. Concentrations of 0, 2.3-2.8, 7.4-9.1, 25.1-27.5 mg/kg bw/day (dose range males). Concentrations of 0, 3.3-3.7, 10.6-11.9, 21-34.7 mg/kg bw/day (dose range females). Purity: 17.7% (a.i. encapsulated)	**Fertility/reprod. performance - Main effects** Testicular toxicity / spermatotoxic effects Effects on reprod. parameters **General systemic toxicity - Main effects** ↓ body weights /FC Changes in liver associated parameters (clinical chemistry, ↑ liver weights, macroscopic changes), Hematological changes **Developmental toxicity - Main effects (coinciding with maternal toxicity)** ↓ pup body weights and early pup survival	BASF SE 2017B Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde.

[10] The majority of studies included are copied information from Table 18, CLH report 2-(4-tert-butylbenzyl)propionaldehyde (ECHA,2017a). Study details can be found in the original report.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Modified extended one generation reproduction toxicity study (OECD Guideline 443) GLP Rat (Wistar) Oral: via diet	2-(4-*tert*-butylbenzyl)propionaldehyde Concentrations of 0, 75, 230, 750 ppm in the diet. Concentrations of 0, 1, 3, 10 mg/kg bw/day (nominal dose). Concentrations of 0, 1.4, 4.5, 15.1 mg/kg bw/d (overall mean dose) Purity: 17.7% (a.i. encapsulated)	**General systemic toxicity - Main effects** ↓ body weights/FC, Hematological changes Changes in liver associated parameters (clinical chemistry, ↑ liver weights, histopathology) **Developmental toxicity - Main effects (coinciding with maternal toxicity)** ↓ Pup body weights. NOAEL (general systemic toxicity): 3 (4.5) mg/kg bw/d NOAEL (developmental toxicity): 3 (4.5) mg/kg bw/d NOAEL (developmental neurotoxicity): 10 (15.1) mg/kg bw/d NOAEL (developmental immunitoxicity): 10 (15.1) mg/kg bw/d NOAEL (fertility/reprod. performance): 10 (15.1) mg/kg bw/d	BASF SE (2017) Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde.
Nonguideline GLP n=5 male rats/dose group	2-(4-*tert*-butylbenzyl)propionaldehyde Dermal at 250, 500, 1000, 2000 mg/kg bw/day; daily for 6 hours, 5 days. Purity: 99.1%	**Testicular toxicity** Marked disorganization of epithelial structure in tubuli seminiferi; ↓ germ cell nr ↑ degenerating germ cell nr. inclusive giant cells (5/5); slight -moderate immature/ degenerating germ cells in epididymides (5/5); spermatocele (1/5) **Additional systemic toxicity** ↓ body weights (slight) LOAEL: 2000 mg/kg bw/day NOAEL: 1000 mg/kg bw/day	Givaudan 1991A Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde.
Nonguideline non-GLP 5 male rats/ time point investigated	2-(4-*tert*-butylbenzyl)propionaldehyde p.o. at 50 mg/kg bw/day for 1, 2, 3, 4, 14 days Purity: 99.1%	**Testicular toxicity** slight to severe testicular atrophy (2/5 at day 1; 5/5 at later time points). **Spermatotoxicity** ↓ sperm motility ↓ testes spermatid count; ↓ cauda epididymal sperm count; affected	BASF SE 2006A Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		sperm morphology. **Additional systemic toxicity** ↓ body weights (day 14)	
Nonguideline GLP n=8 male rats/dose group	2-(4-*tert*-butylbenzyl)propionaldehyde p.o. at 25, 50, 100, 200, 400 mg/kg bw/day, daily for 5 days	**Testicular toxicity** degeneration and loss of seminiferous epithelium. **Additional systemic toxicity** clinical signs; initial body weight loss; macroscopic liver changes; ↓ kidney/ testes weights (at doses above LOAEL). NOAEL: 25 mg/kg bw/day LOAEL: 50 mg/kg bw/day	Givaudan 1986B Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde.
Nonguideline GLP n=5 male rats/dose group	2-(4-*tert*-butylbenzyl)propionaldehyde p.o. at 25, 50, 100 mg/kg bw/day; daily for 5 days Purity: 99.1%	**Testicular toxicity** minimal and moderate to marked atrophy of testes **Additional systemic toxicity** initial body weight loss NOAEL: 25 mg/kg bw/day LOAEL: 50 mg/kg bw/day	Givaudan 1991A Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde.
Nonguideline n=8 male rats/dose group	2-(4-*tert*-butylbenzyl)propionaldehyde p.o. at 50, 100, 200, 400 mg/kg bw/day; daily for 5 days	**Testicular toxicity** testicular tubule epithelial degeneration Additional systemic toxicity ↓ body weights ↓ testis/ kidney weights (at doses above LOAEL). NOAEL: 50 mg/kg bw/day LOAEL: 100 mg/kg bw/day	Newberne 1990A Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde.
OECD TG 408 GLP n= 14 rats / sex /dose group	2-(4-*tert*-butylbenzyl)propionaldehyde p.o. at 2, 5, 25, 50 mg/kg bw/day. 5 days/week (90 days) Purity: 97.8%	**Testicular toxicity** Details provided in CLH report, table 16. **Additional systemic toxicity** clinical signs, changes in liver associated parameters (clinical chemistry, ↑ liver weights, histopathology) NOAEL: 25 mg/kg bw/day LOAEL: 50 mg/kg bw/day	Givaudan 1986A Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde.
Nonguideline	2-(4-*tert*-	Info given refers to male animals	BASF SE

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
non-GLP n=10 rats / sex and dose group	butylbenzyl)propionaldehyde Feed; 400, 800, 1700, 3400 ppm; daily Purity: 30.7% (a.i. Encapsulated)	**Testicular toxicity** ↓ testis/epididymis weights; moderate diffuse testes degeneration (8/10); moderate to severe focal testes degeneration (2/10); aspermia of epididymides (10/10). **Spermatotoxicity** 6 mio. testicular spermatid heads (vs. 121 mio. in ctrl.); 2 mio. epididymal sperm heads (vs. 591 mio. in ctrl.); 0% motile sperm; 84.5% morphologically normal sperm. **Effects on reprod. parameters** see CLH report table 20. **Additional systemic toxicity** ↓ body weights; changes in liver associated parameters (clinical chemistry, ↑ liver weights) ↑ relative kidney weights and ↓ seminal vesicle/prostate weights (at doses above LOAEL); minimal to slight hyperplasia of Leydig cells (9/10 males; at doses above LOAEL). NOAEL: 28.0 mg/kg bw/day (800 ppm) LOAEL: 62.6 mg/kg bw/day (1700 ppm)	2006C Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde.
Nonguideline GLP n=10 rats / sex and dose group	2-(4-*tert*-butylbenzyl)propionaldehyde Feed; 230, 750, 2300 ppm; daily, 10 weeks Purity: 17.7% (a.i. encapsulated)	Info given refers to male animals **Testicular toxicity** ↓ testis/epididymis weights; minimal to moderate tubular degeneration in testis in 3/10 (vs. 1/10 in ctrl.)); minimal to moderate ductal atrophy in epididymis (8 /10); slight to moderate oligospermia (6/10); slight to moderate cellular debris (2/10); not observed in placebo control. **Spermatotoxicity** ↓ mean fraction of motile sperm (25% vs 85% in ctrl.); ↑ mean fraction of abnormal sperm (72.3% vs 6.2% in ctrl.) ↓ mean sperm head count (469 vs 674 mio/g in ctrl.) in cauda epididymis.	BASF SE 2017B Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		Effects on reprod. Parameters see CLH report Table 21 **Additional systemic toxicity** ↓ body weights; changes in liver associated parameters (clinical chemistry, ↑ liver weights, macroscopic changes) hematological changes. NOAEL: 7.4-9.1 mg/kg bw/day (750 ppm) LOAEL: 25.1-27.5 mg/kg bw/day (2300 ppm)	
OECD TG 443 GLP n=10-40 rats / sex and dose group	2-(4-*tert*-butylbenzyl)propionaldehyde Feed; 75, 230, 750 ppm; daily up to 25 weeks Purity: 17.7% (a.i. encapsulat ed)	Info given refers to male animals. NOAEL: 10.2-15.3 mg/kg bw/day (750 ppm)	BASF SE 2017 Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde.
Nonguideline GLP n=4 male dogs / dose group	2-(4-*tert*-butylbenzyl)propionaldehyde Gelatine capsule at 40, 200, 1000/500 mg/kg bw/day; daily, 14 days Purity: 99.1%	**Testicular toxicity** (1/4) ↓ size testis/epididymis ↓ weight testis; massive diffuse degeneration of seminiferous tubules; slight hyperplasia of Leydig cells; aspermia and epithelial vacuolation in epididymides; not observed in low/high dose animals. **Additional systemic toxicity** clinical signs; ↓ body weights; changes in liver associated parameters (clinical chemistry, ↑ liver weights, histopathology).	BASF SE 2008A Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde.
Nonguideline GLP n=10 male dogs /dose group	2-(4-*tert*-butylbenzyl)propionaldehyde Gelatine capsule at 200 mg/kg bw/day, daily, 14 days Purity: 99.1%	**Testicular toxicity** ↓ weight testis/prostate (slight) ↓ testicular length or width of >= 3 mm (6/10); slight to severe degeneration of seminiferous tubules (9/10); minimal to moderate multi focal prostate atrophy (3/10); not observed in ctrls. **Spermatotoxic effects**	BASF SE 2008B Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		↓ % progressively motile spermatozoa (8/10); ↑ % spermatozoa with damaged plasma membrane (3/10); ↑ % morphological altered spermatozoa (9/10). **Additional systemic toxicity** body weight loss; clinical signs, changes in liver associated parameters (clinical chemistry, ↑ liver weights, histopathology); hematological changes.	
Non-guideline n=2 male dogs / dose group	2-(4-*tert*-butylbenzyl)propionaldehyde Gelatine capsule, 47 – 564 mg/kg bw/day, 9 weeks Purity: 95%	**Testicular toxicity** (2/2): Mild atrophy of seminiferous tissues (necrosis of germ cells, multinucleated giant cells in lumen of tubules) **Additional systemic toxicity** clinical signs, body weigh loss; clinical chemistry; liver histopathology.	Givaudan 1990A Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde.
Similar to OECD TG 409 GLP n=3 dogs/sex and dose group	2-(4-*tert*-butylbenzyl)propionaldehyde Gelatine capsule at 4.4, 22.3, 44.6 mg/kg bw/day; daily, 90 days Purity: 97.6%	NOAEL: 44.6 mg/kg bw/day LOAEL: > 44.6 mg/kg bw/day	Givaudan 1990B Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde.
Non-guideline non-GLP n=5 male mice/time point investigated	2-(4-*tert*-butylbenzyl)propionaldehyde p.o. 50 mg/kg bw/day; daily for 1, 2, 3, 4, 14 days Purity:99.1%	NOAEL: 50 mg/kg bw/day	BASF SE 2006B Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde.
Non-guideline non-GLP n=5 male mice/dose group	2-(4-*tert*-butylbenzyl)propionaldehyde p.o. 100 mg/kg bw/day; daily, 5 days	NOAEL: 100 mg/kg bw/day	Givaudan 1983 Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde.
Non-guideline non-GLP	2-(4-*tert*-butylbenzyl)propionaldehyde	NOAEL: 100 mg/kg bw/day	Givaudan 1983 Reviewed in CLH report for

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
n=5 male guinea pigs / dose group	p.o. 100 mg/kg bw/day; daily, 5 days		2-(4-*tert*-butylbenzyl)propionaldehyde.
Non-guideline non-GLP n=2 male rhesus monkey / dose group	2-(4-*tert*-butylbenzyl)propionaldehyde feed; 100 mg/kg bw/day; daily, 5 days	NOAEL: 100 mg/kg bw/day	Givaudan 1984G Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde.
Non-guideline non-GLP n=5 male rabbits/dose group	2-(4-*tert*-butylbenzyl)propionaldehyde p.o. 30, 100, 300 mg/kg bw/day; daily, 15 days Purity: 99.1%	NOAEL: 300 mg/kg bw/day	BASF SE 2008C Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde.
52 days repeated dose toxicity study* Non-guideline Non GLP 20 animal per group male/female Fuellinsdorft albino rats	2-(4-*tert*-butylbenzyl)propionaldehyde p.o. 0 and 50 mg/kg bw/day; daily, 24 days or 52 days Purity: 97.4%	↓ absolute mean weight of testes at 50 mg/kg bw/day (-25% after 24 days and -40% after 52 days). ↓ absolute mean weight of seminal vesicles at 50 mg/kg bw/day (-12% after 24 days and -9% after 52 days). ↓ absolute mean weight of the prostate (-12% after 52 days).	*not reviewed in CLH report Study report, 1987 Study summary in Registration dossier, ECHA's dissemination site, 2022.
4-*tert*-butylbenzoic acid (TBBA) EC [11]			
Wistar rats 10 males/group	4-*tert*-butylbenzoic acid Concentrations of 0, 20, 100, 500 ppm in feed, 70 days before matings. Doses were 1.6 (20 ppm), 7.9 (100 ppm) and 41 (500 ppm) mg/kg	Reversible reduction in body weight at 41 mg/kg bw/day. Body weight -14% compared to controls after 70 days of exposure. No pregnant females at 41 mg/kg bw/day. At second mating trial 70 days after the end of the treatment period the recovery group males were all fertile. Mean testes weight (-12%) after recovery at	Hoechst, 1987 Reviewed in CLH report for 4-*tert*-butylbenzoic acid.

[11] The data included are copied information from CLH report on 4-*tert*-butylbenzoic acid (ECHA, 2010).

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
	bw/day. Each male was mated to two non-exposed females (first mating trial). Males that had not been fertile during the first trial were kept for another 70 days without dietary exposure acid and then again mated to females (second mating trial). The latter were designated as recovery group.	41 mg/kg bw/day. Lesions in the germinative epithelium at 41 mg/kg bw/day. NOAEL: 1.6 mg/kg bw/day LOAEL: 7.9 mg/kg bw/day	
Carworth Farm rats 10 Animals/sex	4-*tert*-butylbenzoic acid Diets containing 0, 100, 316, 1000, 3160, or 10 000 ppm (0, 6, 21, and 75 mg/kg bw/d for males, 0, 8, 27, 89 mg/kg bw/d for females with no calculation on the top two doses). Exposure period of 90 days.	Premature deaths or animals to be killed in extremis at 3160 and 10000 ppm. ↓ terminal body weights at 316 and 1000 ppm ($p<0.01$) compared to controls. Absolute and relative weight impairment of liver, kidney. ↓ absolute and relative testes weights at 316 (-23%) and 1000 ppm (-65%) ($p<0.05$). Renal tubular and papillary necrosis at 100, 316 and 1000, ppm. Histopathological investigations revealed Testes atrophy caused by destruction of the epithelium of the seminiferous tubules. Atrophy of the testis was found already at 100 ppm. LOAEL: 6 mg/kg bw/day (male reproductive organs)	Hunter et al., 1965 Reviewed in CLH report for 4-*tert*-butylbenzoic acid.
Subchronic dermal toxicity study Fischer 344 rats in groups of 20 animals/sex	4-*tert*-butylbenzoic acid Topical application (once a day /five days a week) on skin clipped free of hair for 7 weeks (7 animals/sex/group) or 13 weeks (13 animals/sex/group) . Resulting daily	↓ body weights and body weight gain in males and females at 140 mg/kg bw and in females at 70 mg/kg bw. Absolute and relative organ weight impairment of liver and kidney at 17.5 mg/kg bw (both 7 and 13 weeks). ↓ absolute and relative testes weights at 70 and 140 mg/kg bw. Sperm head count and LDH-X enzyme activities were reduced at 70 and 140 mg/kg bw.	Cagen et al., 1989 Reviewed in CLH report for 4-*tert*-butylbenzoic acid.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
	exposures were 0 (deionized water), 17.5 (11.7), 35 (21.6) 70 (41.3) and 140 (82.6) mg/kg bw/day.	Lesions in liver, kidneys and testes. Testicular changes at 70 and 140 mg/kg bw/day. ↓ number of spermatogenic cell types and absence of late spermatids.	
Dermal study Groups of 8 male Carworth Farm E strain rats	4-*tert*-butylbenzoic acid Concentrations of 0, 7.5, 15, 30 and 60 mg/kg bw/d topically on shaved skin for 28 days.	↓ growth rates at 30 and 60 mg/kg bw/d resulting in significantly ↓ final body weight at these dose groups. ↓ relative and absolute testes weights at 60 mg/kg bw/day. Histopathology revealed degeneration of germinal epithelium. NOAEL: 30 mg/kg bw/day (male reproductive organs) LOAEL: 60 mg/kg bw/day	Shell, 1975 Reviewed in CLH report for 4-*tert*-butylbenzoic acid.
Inhalation toxicity study in Fischer 344 rats	4-*tert*-butylbenzoic acid Concentrations of 495, 668, 958, or 1802 mg/m3 for 4 hours (6 animals/group), (as dust in air) Control groups may have been inappropriate, as these were not dust exposed but exposed to air only.	Dose-related testicular effects at all doses. ↓ mean testis weights ($p<0.05$) at all doses. ↓ testicular sperm count -85%, -84%, -91% and -99% at 495, 668, 958, or 1802 mg /m3. Histopathological analysis revealed absence of late spermatids in the seminiferous tubules of the lower exposed group. All stages differentiating spermatids were absent at 1802 mg dust/ m3). Tubules containing Sertoli cells only and tubules with multinucleated giant cells were prevalent.	Shell, 1982a Reviewed in CLH report for 4-*tert*-butylbenzoic acid.
Inhalation toxicity study in Fischer 344 rats	4-*tert*-butylbenzoic acid Concentrations of 12.5, 106, or 525 mg/m3 6 hours/day for 4 consecutive days, followed by 3 days rest and 3 days of exposure (8 animals/group). Control groups may have been inappropriate, as these were not dust exposed but exposed to air only	Death of 2 out of 8 males at 106 mg in air/m3 and of 7 out of 8 males at 525 mg in air/m3. Lower testis weights were reported for survivors from mid and high exposure dose groups. ↓ testicular sperm counts -21%, -61% and -96% at 12.5, 106, and 525 mg/m3. Absence of late spermatids, presence of multinucleated giant cells, and reduction in spermatogenic cell types were observed in testes from survivors at 106 mg/m3.	Shell, 1982b Reviewed in CLH report for 4-*tert*-butylbenzoic acid.

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**
4-*tert*-butylbenzoic acid (TBBA) EC 202-696-3				
Human observational studies Not GLP	Occupational exposure to 4-*tert*-butylbenzoic acid (TBBA)	The possible testicular effects resulting from occupational exposure to TBBA were studied in 90 male volunteers employed at the Martinez, California, facility of the Shell Chemical Company. The comparison data used were obtained from an external reference group of 103 male volunteers not exposed to any known testicular toxin. Exposure indices were based on the calendar years of employment in a given job category. Outcome variables included sperm count, history of fathering children, and gonadotropin levels.	Analysis of the sperm count data of the 51 individuals of the study group (the number of subjects was considered too small to evaluate sperm-count results by job category) yielded a median sperm count of 72 million sperm/ml semen, while that of the control group was 78 million sperm/ml. 8 individuals in the study group (15.7 %) had sperm counts of less than 20 million sperm/ml (e.g. in the sub-fertile range), compared to 7 subjects in the control group. The authors calculated that this difference was not significant and concluded that PTBBA, at the exposures experienced at that plant, had no clinically detectable effect on testicular function of the workers. Also, there were no indications that PTBBA caused infertility in men who took part in this study. No adverse effects on liver and kidney function or on blood composition were observed. The levels of the hormones studied were in the normal range in the semen providing and the other participants. Of the group of non-exposed men (then numbering 335), 25 (7.5%) had sperm counts less than 20 million/ml. It is reported, that depending on the process used for statistical analysis, the slight difference between the study subjects and the non-exposed group might or might not have been significant. Closer analysis of the urological-clinical data for the men with oligospermia in the study group of the plant revealed that a multitude of other potential factors, such as orchitis after mumps, testicular hernias and sclerosis of the penis could have been responsible for the reduced sperm density. The urological-clinical data for the control group could not be evaluated to further improve the statistical analysis. The small size of the study group together with the manifold urological findings make the biological significance of the difference from the control group questionable.	Study report 2009 Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Reviewed in European Union Risk Assessment Report, 4-tert-butylbenzoic acid, CAS No: 98-73-7, EINECS No: 202-696-3, 4.1.2.9. Toxicity for reproduction, p. 68-75, Final Approved Version, July 2009 Original article: Whorton et al. 1981.

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**
4-*tert*-butylbenzoic acid (TBBA) EC 202-696-3				
Ex vivo study using a 3D cell culture with primary seminiferous tubules from male juvenile Sprague Dawley rats (Bio-Alter®). No guideline Not GLP compliant *meta*-3-*tert*-butylbenzoic acid (*m*-TBBA, CAS 7498-54-6) and the positive control methoxyacetic acid were additionally included in the study. Assigned reliability 1 by the Registrant	4-*tert*-butylbenzoic acid (TBBA, EC 202-696-3)	Cytotoxicity, blood-testis barrier functionality via trans-epithelial electrical resistance (TEER) measurements and cell numbers of different somatic and germ cell populations were quantified. The content of TBBA conjugated with CoA and the metabolome was assessed in cell culture lysates.	The effect of TBBA on the blood-testis barrier was low. A transient and slight decrease versus control was observed. ↑ number of somatic cells (dose dependently) at day 7. ↑ number of spermatogonia at day 7 (50µM) and at day 14 (3 tested concentrations). TBBA affected the meiotic process of germ cells, starting at the stage of middle to late pachytene spermatocytes; ↓ number of middle to late pachytene spermatocytes, ↓ number of secondary spermatocytes, ↓ number of round spermatids. In samples dosed with 10 and 50 µM of TBBA, the corresponding CoA-conjugate *p-tert*-butyl-benzoyl-CoA was detectable both at 8 and 15 days, while trace amounts were detected in the samples dosed with 2 µM.	Study report, 2019a. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 16 Annex I 3.10.1.16
Ex vivo study using a 3D cell culture with primary seminiferous tubules from juvenile Sprague Dawley rats (Bio-Alter®).	4-*tert*-butylbenzoic acid (TBBA, EC 202-696-3)	Cytotoxicity and cell numbers of different somatic and germ cell populations were quantified.	↑ number of somatic cells (dose-dependently) at day 8. ↑ number of spermatogonia at day 8 (10µM and 50µM). At day 8 and 15, TBBA affected the meiotic process of germ cells, starting at the stage of middle to late pachytene spermatocytes; ↓ number of middle to late pachytene spermatocytes, ↓ number of secondary spermatocytes,	Study report, 2020 Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 17 Annex I 3.10.1.17

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No guideline Not GLP compliant *meta*-3-*tert*-butylbenzoic acid (*m*-TBBA, CAS 7498-54-6) and the positive control methoxyacetic acid were additionally included in the study. Assigned reliability 1 by the Registrant			↓ number of round spermatids.	
Ex vivo study using a 3D cell culture with primary seminiferous tubules from a transgender male who underwent castration. *meta*-3-*tert*-butylbenzoic acid (*m*-TBBA, CAS 7498-54-6) and the positive control methoxyacetic acid were additionally included in the study. Assigned reliability 2 by the Registrant	4-*tert*-butylbenzoic acid (TBBA, EC 202-696-3)	Cytotoxicity and cell numbers of different somatic and germ cell populations were quantified. The content of TBBA conjugated with CoA was assessed in cell culture lysates.	↓ numbers of somatic cells at day 14 (not dose-dependently). ↑ total number of germ cells and the number of spermatogonia at day 14 and day 21 at the highest tested concentration (50µM). No clear dose-related effect was observed on pachytene spermatocytes, secondary spermatocytes and round spermatids. In culture samples dosed with 2 and 10 µM of TBBA, the corresponding CoA-conjugate (ptBBA-CoA) was below the limit of quantification in all samples at both time points. Trace amounts around the detection limit were detected in two out of six samples dosed with 50 µM *p*-TBBA after 22 days of treatment, while *p*-TBBA-CoA was not detectable in the remaining 4 samples at this time point and not in any sample of the tissues dosed for 15 days.	Study report, 2019b. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 18 Annex I 3.10.1.18

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
2-(4-*tert*-butylbenzyl)propionaldehyde EC 201-289-8				
In vitro test using estrogen responsive MCF7 human breast cancer cell line and human recombinant ER alpha and ER beta. Non guideline Not GLP compliant Assigned reliability 3 by the Registrant	2-(4-*tert*-butylbenzyl) propionaldehyde EC 201-289-8 ≥95% purity		**Ligand Binding to Human ER** At 3 000 000- fold molar excess, lysmeral gave 27% inhibition of [3H]estradiol binding to ERα, and approx 15% inhibition (estimated from graph) of [3H]estradiol binding to Erβ. **Competitive Binding Assay to ER of MCF7 Cytosol** The maximal inhibition of [3H]estradiol binding at 3 000 000-fold molar excess of lysmeral was 47%. **Assay of stably transfected ERE-CAT reporter gene in MCF7 cells** Lysmeral induced CAT gene expression, although in no case of the same magnitude as with 17β-oestradiol. **Cell Proliferation Experiments** Lysmeral increased growth of MCF7 cells (after 7 days in a dose-dependent manner). Cell density reached near confluence after 14 days with 10^-8M 17β-estradiol and after 35 days with 10^-4M lysmeral. Stimulatory action of 10^-10M 17β-oestradiol on MCF7 cell growth was slightly inhibited by 10^-4M but not by 10^-5M lysmeral. **RT-PCR analysis** Following 7 days of estrogen deprivation, a 24 h exposure to lysmeral increased expression of the estrogen-regulated gene pS2 mRNA, although in no case to the same extent as with exposure to 17β-estradiol.	Charles and Darbre, 2009. Study summary in Registration dossier, ECHA's dissemination site, 2022. Study 19 Annex I 3.10.1.19

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

3-(4-*tert*-butylphenyl)propionaldehyde

OECD TG 422 study in rats (Study report, 2019)

In an OECD TG 422 (GLP compliant) study from 2019, male and female Sprague Dawley rats (10 per sex/group) were exposed to 3-(4-*tert*-butylphenyl)propionaldehyde at 0, 0.5, 1 and 5 mg/kg bw/day. Male rats were dosed once daily (oral gavage) beginning 14 days before cohabitation with treated females, during cohabitation and continuing through the day prior to scheduled euthanasia on days 43 through 46. F1 generation pups were not directly exposed to the test or control substance.

Of note, this study was conducted in 2019 but according to the study report the expiration date of the lot/batch of the substance used in the study was in October 2011.

Parental animals

There were no effects on reproductive function or reproductive performance and no effects were observed on oestrous cycle. Sperm measures were not examined.

There were no effects on mating and fertility. The days in cohabitation (2.8 to 3.5 days), mating index (90% or 100%), and fertility index (90% or 100%) at 0.5, 1, and 5 mg/kg bw/day were similar to controls. Pregnancy occurred in 9 (90%), 10 (100%), 9 (90%), and 10 (100%) of the 10 mated females at 0, 0.5, 1, and 5 mg/kg bw/day, respectively. Of these pregnant females, 8 to 10 females across the groups delivered their litters and one dam at 1 mg/kg bw/day was found dead on GD 22 (not considered test substance-related by study authors). There were no effects on any natural delivery or litter parameter at any dose. The mean number of implantation sites per delivered litter, dams with stillborn pups, dams with no liveborn pups, gestation index (number of rats with live offspring/number of pregnant rats), mean number of dams with all pups dying (days 0 to 3 postpartum and days 4 to 12 postpartum), mean number of pups delivered (liveborn and stillborn), pups found dead or presumed cannibalized, percent male pups per number of pups sexed per litter, surviving pups per litter, lactation index, and live litter size were similar among the four dose groups.

There was a statistically significant decrease in the number of pups found dead between days 1 and 3 postpartum at 0.5 and 1 mg/kg bw/day resulting from an increase in pup mortality in the control group during this same period. Consequently, the viability index at 0.5 and 1 mg/kg bw/day was higher than the control group.

General toxicity (parental animals)

Non adverse clinical signs or mortality were observed among male rats. Mean body weights, mean body weight gains, and mean food consumption values were similar across all groups in the P generation males. There were no effects on any neurobehavioral parameter (functional observation battery or motor activity) at any dose. There were no macroscopic or microscopic observations or alterations in organ weights at any dose. In the P generation males, mean serum T4 concentrations were 104%, 87%, and 90% of controls at 0.5, 1, and 5 mg/kg bw/day, respectively, on day 43/44 (not statistically significant). This effect was not associated with any macroscopic or microscopic observations or alteration in thyroid weight. The study authors considered this change as not related to substance.

There was no mortality in the P generation females at any dose, no effects on body weight and weight change, food consumption, clinical parameters.

F1 generation

There were no clinical signs observed in the F1 generation pups at any dose and no mortality observed. There were no effects on mean body weights in the F1 generation pups at 0.5 and 1 mg/kg bw/day. However, the mean pup body weight was statistically significantly reduced at 5 mg/kg bw/day compared to control values on days 9 and 12 postpartum (-12% to -11%) (the reduced pup weights were within the range observed historically at the testing facility).

There were no effects observed on food consumption and compound intake. In the F1 generation male pups, mean serum T4 concentrations were -2%, -18%, and -22% of controls at 0.5, 1, and 5 mg/kg bw/day, respectively, on day 12 postpartum (not statistically significant). In the F1 generation female pups, mean serum T4 concentrations were -18%, -26%, and -26% of controls at 0.5, 1, and 5 mg/kg bw/day, respectively, on day 12 postpartum (statistically significant). There were no microscopic changes in the thyroid or parathyroid glands of the single F1 generation pup/sex/litter that was microscopically examined from 5 mg/kg bw/day. There were no differences in mean anogenital distance in the F1 generation males or females at any dose on day 1 postpartum. There were no differences on nipple retention in the F1 generation male pups in any dose group. No male pups had nipples present on PND 12.

The doses used in this study were considerably lower compared to doses used in other studies with similar substances. For example, the highest dose selected in the OECD TG 422 study from 2019 (5 mg/kg bw/day) was the lowest dose used in the dose range finding study (described below).

Conclusions

In the screening study from 2019 no effects on reproductive function or reproductive performance were seen in parental animals and no general toxicity was reported in any dose group. Sperm measures were not examined.

In the F1 generation no effects were observed except a statistically significantly reduced mean pup body weight at 5 mg/kg bw/day on days 9 and 12 postpartum (-11 and -12%).

The doses used in the study were relatively low compared to other toxicity studies conducted on this substance, with the highest dose being 5 mg/kg bw/day.

Dose range finding study for OECD TG 422 study in rats (Study report, 2019)

In the 14-day dose range finding study conducted on 3-(4-*tert*-butylphenyl)propionaldehyde, male and female Sprague Dawley rats (5 rats/sex/group) were dosed with 0, 5, 25 and 50 mg/kg bw/day orally by gavage three times (approximately 6 hours apart) daily.

At necropsy examination, macroscopic findings demonstrated decreased testicular size which was observed in one male at 50 mg/kg bw/day. In addition, differences in mean absolute and relative organ weights were observed in the testes (50 mg/kg bw/day) and liver (at 5, 25 and 50 mg/kg bw/day) in the males and the ovaries (at 25 and 50 mg/kg bw/day) and uterus (50 mg/kg bw/day) in females. Each of the changes in the testes, liver, and uterus had a histologic correlate of hypertrophy or atrophy/degeneration.

In males, microscopic findings were observed in the testes at 5, 25 and 50 mg/kg bw/day (vacuolation and degeneration of seminiferous tubular epithelium, Sertoli cell vacuolation) with secondary effects in the epididymides at 25 and 50 mg/kg bw/day (cribriform change, cellular debris, and hypospermia). The vacuolation noted in the seminiferous tubule was characterized by fine microvesicular vacuolation within the cytoplasm of seminiferous tubule epithelium and uniformly affected all stages of spermatogenesis (spermatogonia, spermatocyte, and spermatid). In females, microscopic findings were observed in the uterus at 50 mg/kg bw/day (uterine atrophy). Microscopic findings were also observed in the liver of both males and females (see also Annex I section 3.10.1.1).

Effects in sperm motility were observed at all doses, including reductions in sperm motility at 5 mg/kg bw/day (77% vs. 84% in controls) and little to no sperm at 25 and 50 mg/kg bw/day (18% and 3%, respectively, vs. 84% in controls). All sperm samples that were analyzed at 25 and 50 mg/kg bw/day contained headless and detached sperm, with the exception of one sperm sample at 25 mg/kg bw/day. The infrequent increased spermatid head retention by the Sertoli cells, degeneration of maturing spermatids, round spermatids, and/or elongating spermatids, exfoliation/degeneration of germ cells, increased cellular debris, and moderate to marked hypospermia that was observed microscopically in the seminiferous tubules or epididymides may have contributed to the overall decrease in sperm motility.

General toxicity

There were no mortalities in females at any dose or among males at 5 and 25 mg/kg bw/day. There were two mortalities at 50 mg/kg bw/day in males. One male was found dead on day 14, with no clinical signs prior to death or macroscopic findings during necropsy examination. The other male was euthanized on day 1 due to adverse clinical condition. All other animals survived to scheduled euthanasia on day 15.

Clinical signs were limited to females in the 25 and 50 mg/kg bw/day dose groups and included suspected dehydration and a low incidence of hunched posture and thin appearance.

In females, mean body weight losses of -15.4 g and -33.0 g were observed at 25 and 50 mg/kg bw/day, respectively, compared to a mean body weight gain of +13.3 g in controls (day 1 to 15). In addition, lower mean body weights were observed in females on day 7 and 15 at 25 mg/kg bw/day (-9% and -4%) and on day 3 and 15 at 50 mg/kg bw/day (-18% and -5%). There were no effects on mean body weights or mean body weight gain in males at any dose.

Lower mean food consumption was observed in females at 25 mg/kg bw/day (up to -16%) and at 50 mg/kg bw/day (up to -64%). There were no effects on mean absolute food consumption in males at any dose.

Conclusions

In males, decreased testicular size and statistically significant difference in testes weight, which correlated with histological findings were seen at the highest dose 50 mg/kg bw/day. However, excessive toxicity in this dose group was reported and these findings should therefore not be considered for classification. Microscopic findings in testes/semimiferous tubule and effects on sperm motility were demonstrated at all doses. There was clear evidence of spermatotoxicity at mid- and high doses.

In females, reduced uterus weight and uterine atrophy was seen at the highest dose in presence of adverse general toxicity (reduced body weight).

Toxicity screening test in rats (Study report, 2009)

In a toxicity screening test, (GLP compliant), male Crl:CD® (SD)IGS BR rats (6 per group) were exposed to 3-(4-*tert*-butylphenyl)propionaldehyde at doses 0, 25, 100 and 250 mg/kg bw/day once daily for 5 consecutive days. Males treated at 250 mg/kg bw/day were all killed on day 2 of the study due to welfare reasons.

Findings considered related to treatment were seen in testes and epididymides. Five of 6 animals treated with 100 mg/kg bw/day showed treatment-related effects in the testes and epididymides, and 3/6 animals had enlarged epididymides. More details on other organs including liver, kidney and stomach in Annex I section 3.10.1.2.

Seminiferous tubular degeneration/atrophy, Sertoli cell vacuolation, multinucleate giant cell and luminal sloughing of spermatogenic cells in the testes and, reduced numbers of spermatozoa, sloughed germ cells in lumen and inflammation in the epididymides were seen at 100 mg/kg bw/day.

Analysis of urine from males treated with 100 or 25 mg/kg bw/day demonstrated presence of the metabolite, TBBA, mean concentrations were 275 and 35.8 µg/ml, respectively.

General toxicity

Two males treated at 250 mg/kg bw/day were killed for welfare reasons after administration of the first dose due to poor clinical condition. Another male treated at 250 mg/kg bw/day and one animal at 100 mg/kg bw/day were killed for welfare reasons approximately 10 and 12 hours after administration of the first dose, respectively, due to poor clinical condition. Since half of the animals at the high dose had been killed, the remaining animals were killed on the morning of day 2 of study prior to dosing. Signs of underactivity, reduced body temperature, irregular breathing, piloerection, loose faeces and partially closed eyelids were recorded after dosing at 250 or 100 mg/kg bw/day, but all of the signs at 100 mg/kg bw/day with the exception of underactivity were only recorded after administration of the first dose. More details in Annex I section 3.10.1.2.

All of the three males treated at 250 mg/kg bw/day and killed on day 2 of study showed body weight loss of 15-30 g. Treatment at 100 mg/kg bw/day was associated with mean body weight loss of 14 g following administration of the first dose. This was followed by mean body weight stasis during days 2-3 of study and mean body weight loss during days 3-4 and 4-5 of study.

Conclusion

All six animals at 250 mg/kg bw/day and 1 animal at 100 mg/kg bw/day were killed for welfare reasons due to marked general toxicity. Remaining five males exposed to 100 mg/kg bw/day demonstrated effects in testes (reduced weight) and epididymides, and 3 of 6 animals had enlarged epididymides observed in absence of marked general toxicity.

In addition, in the same dose group, seminiferous tubular degeneration and atrophy were seen, along with Sertoli cell vacuolation, multinucleate giant cell and luminal sloughing of spermatogenic cells in the testes and, reduced numbers of spermatozoa, sloughed germ cells in lumen and inflammation in the epididymides.

4-*tert*-butyltoluene

OECD TG 421 study in rat (Study report, 2007a)

In an OECD TG 421 (GLP compliant) study from 2007, male and female Sprague Dawley rats (12 per sex and group) were dosed at 0, 1.5, 5, 15, 50 mg/kg bw/day. The administration period for males was 50- 52 days including 14 days before mating and subsequent 36 to 38 days. The administration period for females was total 41 to 45 days including 14 days before mating, mating period, gestational period, and first 3 days in lactation period.

Males

There were statistically significant decreases in sperm motility ratio, path velocity, straight line velocity, curvilinear velocity, sperm viability, sperm survivability, sperm count, and sperm count per one gram of the left cauda epididymis at 15 mg/kg bw/day compared to control group. There were statistically significant increases in beat cross frequency and ratios of morphological abnormality of sperms (ratios of abnormality in the head, tail and the total of those) in the same group. No statistically significant effects compared to control were seen at 1.5 and 5 mg/kg bw/day.

There were significant decreases in sperm motility ratio, sperm count, sperm count per one gram of the cauda epididymis at 50 mg/kg bw/day compared with the control group. Among the animals with low numbers of motile sperms, there was only one animal that could be used for measurements of path velocity, straight line velocity, curvilinear velocity, beat cross frequency, sperm viability, and sperm survivability. However, there were decreases in all the parameters. The authors could conduct the observation of sperm morphology on only 5 animals at 50 mg/kg bw/day. However, there were significant increases in the ratios of morphological abnormality of sperms (ratios of abnormality in the head, tail and the total of those).

There were statistically significant decreases in absolute weight of the epididymis (-12%) and a decreasing tendency of absolute weight of the testis (-8%) at 15 mg/kg bw/day compared to control group. There were significant decreases in absolute and relative weights of the testis (- 54% and -44%, respectively) and epididymis (-34% and -20%, respectively) at 50 mg/kg bw/day compared with the control group.

There was atrophy of the testes, epididymides, seminal vesicles, and prostate in the dead animals at 50 mg/kg bw/day. There was atrophy of the seminiferous tubules in 4 animals, hyperplasia of Leydig Cells in 2 animals, and remaining spermatids at step 19 in the seminiferous tubules of groups 3 and 4 in one animal at 15 mg/kg bw/day. There was atrophy of the seminiferous tubules and hyperplasia of Leydig cells in all 11 animals at 50 mg/kg bw/day. In the testis, there was no abnormality at 0, 1.5 and 5 mg/kg bw/day.

In epididymis, there was no abnormality at 0, 1.5 and 5 mg/kg bw/day. There was a decrease in sperm count in 4 animals at 15 mg/kg bw/day. There was a decrease in sperm count in all 11 males at 50 mg/kg bw/day.

Females

No changes attributable to administration of the test substance were noted in the numbers of estrous cases. There were statistically significant decreases in body weight at the day of necropsy at 5 (-13%), 15 (-18%), and 50 (-29%) mg/kg bw/day compared with the control group. There were no significant differences in absolute and relative weights of the ovary in any of the treated groups compared to controls.

Reproductive performance

There was no significant difference in frequency of estrus during the administration period (14 days) before mating between each group and the control group. There was no significant difference in days required for copulation between each group and the control group. One pair of animals did not achieve copulation at 50 mg/kg bw/day. There was no significant difference in copulation index between each group and the control group. There were 8 non-pregnant females at 15 mg/kg bw/day. There was no pregnant female at 50 mg/kg bw/day. There were significant decreases in fertility index at 15 and 50 mg/kg bw/day compared with the control group.

There was no significant difference in gestational period at 1.5, 5, and 15 mg/kg bw/day compared with the control group. There was no abnormality of delivery status at 0, 1.5 and 5 mg/kg bw/day. No newborn offspring were obtained with one dam at 15 mg/kg bw/day since the litters were all dead. There were no significant differences in number of pregnant corpora lutea, number of implantations, and implantation index at 1.5, 5, and 15 mg/kg bw/day compared with the control group. The gestation index was 100% at 1.5 and 5 mg/kg bw/day. The gestation index at 15 mg/kg bw/day was 66.7% since one dam did not deliver live offspring.

In the observation of lactation status, there was no abnormality at 0, 1.5, and 5 mg/kg bw/day. All newborn pups of one dam at 15 mg/kg bw/day died by day 1 of the lactation period.

There were statistically significant decreases in number of offspring born (-26%) and number of live pups born (-58%) and an increase in number of stillbirths at 15 mg/kg bw/day (4.7 vs. 0.1 in controls), compared with the control group. There was decreasing tendency of delivery index (94.1%, 93.8%, 98.1% and 82.7%), birth index (93.6%, 91.2%, 94.3% and 49.3%), and live birth index (99.5%, 97.3%, 96.2% and 63.0%) at 0, 1.5, 5. and 15 mg/kg bw/day, respectively.

F1 generation

The newborn offspring of one dam died by LD 1 at 15 mg/kg bw/day, and there were decreasing tendencies of number of live offspring and viability index at LD 4.

There was no abnormality in the observation of external abnormality of newborn offspring in any group or in the observation of clinical signs on the newborn offspring.

There were significant decreases in body weights of males and females at LD 0 (-11% and -8%, respectively) and LD 4 (-16% and -15%, respectively) at 5 mg/kg bw/day compared with the control group. There were decreasing tendencies of body weights of males and females at LD 0 and LD 4 at 15 mg/kg bw/day compared with the control group.

General toxicity (parental animals)

Males

There was one death at 50 mg/kg bw/day, the animal showed transient salivation, a decrease in locomotor activity, soiled fur, reddish urine, and hypothermia. In the observation of clinical signs on the live animals, no abnormality was observed in the control group. There were significant decreases in body weight from day 18 to day 49 of the administration period at 15 mg/kg bw/day compared with the control group (-13% at day 49, estimated from graph). There were significant decreases in body weight day 4 to 49 of the administration period at 50 mg/kg bw/day compared with the control group (estimated from graph -6% at day 4 and -19% at day 49). At the time of mating (Day 15) the mean body weight of males at 50 mg/kg bw/day was statistically significantly lower (approximately -5%, estimated from graph).

There were no significant differences in food consumption at any day of measurement at 1.5, 5, and 15 mg/kg bw/day compared with the control group. There was a significant decrease in food

consumption at day 48 of the administration period at 50 mg/kg bw/day compared with the control group.

There were statistically significant decreases in body weight at the day of necropsy at 15 and 50 mg/kg bw/day compared with the control group.

Females

There was one death at 15 mg/kg bw/day and 6 deaths occurred at 50 mg/kg bw/day.

There were statistically significant decreases in body weight from day 4 to 15 of the administration period at 50 mg/kg bw/day compared with the control group (estimated from graph, -8%).

There were statistically significant decreases in body weight at days 7 and 14 of the gestational period at 5 mg/kg bw/day compared with the control group (estimated from graph, -8% GD 7 and -10% GD 14). There were significant decreases in body weight from day 7 to day 21 of the gestational period at 15 mg/kg bw/day compared with the control group (roughly estimated from graph, -9%, GD 21).

There was a significant decrease in body weight at day 4 of the lactation period at 5 mg/kg bw/day compared with the control group (estimated from graph, -13%). There was a decreasing tendency of body weight in one animal at 15 mg/kg bw/day at day 4 of the lactation period (i.e no -dose response).

There were some effects on food consumption at 5 mg/kg bw/day and in one animal at 15 mg/kg bw/day during lactation period.

Full study report was not available to DS. Differences in weight of parental animals were estimated from graphs and are associated with uncertainties.

Conclusions

In the Reproduction/Developmental Toxicity Screening Test from 2007, effects on sperm motility and viability and morphology were seen in males at 15 and 50 mg/kg bw/day in presence of general toxicity. The weights of epididymides and testis were reduced in these males, dose-dependently, and up to -54% in the highest dose group. Atrophy of the seminiferous tubules and hyperplasia of Leydig cells were seen.

Six females at 50 mg/kg bw/day died and effects in this dose group were therefore not considered further for classification purposes. There were 8 non-pregnant females at 15 mg/kg bw/day.

There were statistically significant decreases in number of offspring born and number of live pups born and an increase in number of stillbirths at 15 mg/kg bw/day in presence of significant maternal toxicity (decreased body weight >10%).

There were significant decreases in body weights of male and female pups in the beginning of lactation period at 5 mg/kg bw/day, up to -16% (and decreasing tendencies at 15 mg/kg bw/day).

OECD TG 407 study in rats (Study report, 2007b)

In an OECD TG 407 study from 2007, male and female Sprague-Dawley rats (12 per sex/group) were exposed to 4-*tert*-butyltoluene by oral gavage at 1.5, 5, 15, 50 mg/kg bw/day, daily for 28 days. Post-exposure recovery period of 14 days in satellite groups.

In males, there were significant decreases in absolute weights of the testis (-65%) and epididymis (-23%) and relative weight of the testis (-61%) at 50 mg/kg bw/day, which appeared to last through the recovery period. In females, there was a significant decrease in absolute weight of the ovary at 50 mg/kg bw/day (-28%).

At termination of the administration period, testes of male rats had atrophy of the seminiferous tubules and hyperplasia of Leydig cells in 6 of 12 animals at 50 mg/kg bw/day and in epididymis, there was a decrease in sperm count in the lumen of the ductus epididymis in 6 or 12 animals (statistically significant effects compared to controls).

General toxicity
There was no death or morbidity in males and females in any group. Transient salivation was found in males and females at 15 and 50 mg/kg bw/day. There was no statistically significant difference in body weight in males or females at any dose groups compared with the controls, except in high dose females at the day of necropsy (-13%).

In males, there was a significant increase in water consumption at day 17 of the administration period at 15 mg/kg bw/day, and at 50 mg/kg bw/day from day 3 to day 24. In females, there was a significant increase in water consumption at day 10 at 50 mg/kg bw/day compared with the control group.

In males, at the end of administration period there was a statistically significant increase in relative weight of the liver at 15 (+20%) and 50 mg/kg bw/day (+55%). There was a significant increases in absolute weight of the liver at 50 mg/kg bw/day (+39%) and periportal hepatocyte hypertrophy was observed in 4 of 12 animals at 50 mg/kg bw/day. Further, there was a significant decrease in absolute weight of the heart at 50 mg/kg bw/day (-13%).

In females, there were significant increases in absolute (+37%) and relative (+48%) weights of the liver at 15 mg/kg bw/day. There were significant increases in absolute weight of the liver (+60%) and relative weights of the liver (+83%), kidney (+20%), and adrenal gland (+15%) at 50 mg/kg bw/day. Further, there was a significant decrease in absolute weight of the thymus at 50 mg/kg bw/day (-26%).

Haematology
In males, there were statistically significant decreases in APTT and fibrinogen at 5, 15, and 50 mg/kg bw/day (-16%, -16% -17% and -10%, -16%, -24%, respectively), and at 15 and 50 mg/kg bw/day, there were statistically significant decreases in MCH (-3% and -4%, respectively). After recovery, there were statistically significant decreases in erythrocyte count (-5% and -6%, respectively), hemoglobin concentration (-6% and -7%, respectively), and hematocrit value (-6% and -6%, respectively) at 15 and 50 mg/kg bw/day.

In females, at 5 mg/kg bw/day, there was a statistically significant decrease in platelet (-17%). At 15 mg/kg bw/day, there was a statistically significant increase in fibrinogen (-26%) and decreases in MCHC and platelet (-2% and -16%). At 50 mg/kg bw/day, there was statistically significant decreases in fibrinogen, MCH and MCHC (-27%, -5% -3%, respectively), and a significant increase in PT (+9%). After recovery, there were significant decreases in hemoglobin (-6%) and hematocrit (-6%) at 50 mg/kg bw/day.

Clinical chemistry
In males, at termination of administration period, there were statistically significant decreases in total protein (-6%) and triglyceride (-51%) and statistically significant increases in AST (+30%), ALT (+38%), blood urea nitrogen (+39%), and inorganic phosphorus (+9%) at 5 mg/kg bw/day. At 15 mg/kg bw/day, there were significant decreases in total protein (-12%), albumin (-7%), and triglyceride (-73%) and significant increases in AST (+27%), A/G (+15%), total bilirubin (+27%), blood urea nitrogen (+31%), and inorganic phosphorus (+9%).

At 50 mg/kg bw/day, there were statistically significant decreases in total protein (-13%), albumin (-9%), total cholesterol (-34%), triglyceride (-74%), and Na (-2%), and significant increases in AST (+43%), A/G (+14%), total bilirubin (+64%), blood urea nitrogen (+152%), creatinine (+27%), and inorganic phosphorus (+14%).

In females, there were stasistically significant decreases in total protein (-9%), albumin (-11%), total cholesterol (-31%), triglyceride (-67%), and Ca (-8%), and a significant increase in gamma-GTP (+184%) at 15 mg/kg bw/day. At 50 mg/kg bw/day, there were significant decreases in total protein (-9%), albumin (-12%), triglyceride (-61%), K (-10%), and Ca (-5%), and a decreasing tendency of total cholesterol (-28%), and significant increases in gamma-GTP (+274%) and total bilirubin (+54%).

Urinanalysis
In males, before termination of administration period, there was a statistically significant increase in urine volume at 15 and 50 mg/kg bw/day. There was a significant decrease in urine specific gravity and decreasing tendencies in pH and protein at 50 mg/kg bw/day. Before termination of recovery period there was a statistically significant increase in urine volume and a significant decrease in urine specific gravity at 50 mg/kg bw/day. In females, there was a statistically significant increase in urine volume and a decreasing tendency in pH at 50 mg/kg bw/day.

Additional data in Annex I section 3.10.1.10.

Conclusions

Significant decreases in weights of the testis and epididymis were reported at 50 mg/kg bw/day in male rats and significant decreased ovary weights in females. At the same dose level, atrophy of the seminiferous tubules, hyperplasia of Leydig cells and statistically significantly decreased sperm count were observed in the absence of severe general toxicity.

Testicular toxicity screening test in rats (Study report, 1982a)

In a testicular toxicity screening test, male albino SPF rats (7 per group) were dosed with 4-*tert*-butyltoluene at 0 and 200 mg/kg bw/day once daily for 5 consecutive days.

There was a treatment-related decrease in the testes weight of treated rats when compared with controls. The seminiferous tubules of all dosed rats were changed. Lesions seen in the epithelium comprised degeneration of spermatocytes and spermatids, reduction of spermatozoa as well as appearance of giant cells. Sertoli cells and interstitial cells of Leydig were unaffected.

General toxicity

No deaths occurred and all animals appeared normal. Slight body weight loss was apparent in treated males up to 3 days following treatment. A tendency to return to normal was noted at the end of treatment.

Conclusions

Decreased testes weight was seen in rats treated short-term at 200 mg/kg bw/day. Analysis demonstrated lesions in epithelium of the seminiferous tubules and included degeneration of spermatocytes and spermatids, reduction of spermatozoa as well as appearance of giant cells. No marked systemic toxicity was observed.

Testicular toxicity screening test in rats (Study report, 1982b)

In a testicular toxicity screening test, male albino SPF rats (8 males per group, 4 males in control group) were dosed with 4-*tert*-butyltoluene at 0, 12.5, 25, 50 and 100 mg/kg bw/day once daily for 5 consecutive days.

The mean testes weight of rats at 100 mg/kg bw/day was approximately 23% lower than those of control rats. The seminiferous epithelium at 0, 12.5 and 25 mg/kg bw/day did not exhibit any histological changes. At 50 and 100 mg/kg bw/day the germinal epithelium showed severe cell-deformations. Spermatids and spermatocytes were mainly degenerated. Spermatozoa were reduced. Giant cells were observed sporadically.

General toxicity

Clinical signs of toxicity were observed in rats at 50 and 100 mg/kg bw/day and comprised loss of hair, shaggy fur, hunched posture, lethargy, and diarrhea. No deaths occurred. A marked progressive

loss of body weight was seen in the majority of rats at 100 mg/kg bw/day throughout the study (average body weight -24% of controls).

Figures from testes evaluation in table 11 below demonstrate an increased proportion of injured testis tissue with increasing dose.

Table 11: Histological assessment of seminiferous tubules per testis (adapted from registration dossier).

Concentration (mg/kg bw)	Grading mean %*			
	0	1	2	3
Control	85.6	14.4	0	0
12.5	86.1	13.9	0	0
25.0	84.7	15.3	0	0
50.0	10.3	40.4	29.1	20.2
100.0	0	1.3	12.4	86.3

*examination/grading of 100 randomly selected, cross-sectioned tubules in a respective testis section.

Conclusions

Decreased testes weight was seen in rats treated at 100 mg/kg bw/day. Lesions in the seminiferous tubules included degeneration of spermatocytes and spermatids, reduction of spermatozoa as well as appearance of giant cells. The reported effects were observed in presence of marked general toxicity.

Testicular toxicity screening test in guinea pigs (Study report, 1984a)

In a testicular toxicity screening test, male Himalayan guinea pigs (5 males per group) were dosed with 4-*tert*-butyltoluene at 0 and 100 mg/kg bw/day once daily for 5 consecutive days.

Mean testes weights were similar in both dosed and control animals. A slight damage of germinal epithelium was seen in testes of 2/5 control animals and in 1/5 treated animals. Furthermore, 1/5 treated animals exhibited a moderate damage of germinal epithelium. There were no other testicular or epididymal changes.

General toxicity

No deaths were observed, and no signs of toxicity were noted. Body weight gains were similar in treated and control animals.

Figures from testes evaluation in table 12 below demonstrate a very slight increased proportion of injured testis tissue at 100 mg/kg bw/day.

Table 12: Histological assessment of seminiferous tubules per testis (adapted from registration dossier).

Concentration (mg/kg bw/day)	Grading mean %*			
	0	1	2	3
Control	97.5	2.3	0	0.2
100	91.0	4.5	1.8	2.7

*examination/grading of 100 randomly selected, cross-sectioned tubules in a respective testis section.

Conclusions

Slight or moderate damage of germinal epithelium in testes were seen in two guinea pigs of 5 at 100 mg/kg bw/day. No other signs of toxicity were observed.

Testicular toxicity screening test in beagle dogs (Study report, 1984b)

In a testicular toxicity screening test, 3 male beagle dogs were dosed with 4-*tert*-butyltoluene at 0 or 100 mg/kg bw/day once daily for 5 consecutive days.

A few multinucleated giant cells were seen in the lumen of seminiferous tubules of the control dog (dog no. 1).

There was a small quantity of seminiferous tubules with nearly total depopulation of germinal epithelium in both testes of dog no. 2 (treated). The concerned seminiferous tubules (approx. 20 in testis 1 and 10 in testis 2) showed early stages of spermatogenesis and Sertoli cells. No changes were found in testes of dog no. 3 (treated) and in epididymides of all dogs. See Annex I section 3.10.1.7

General toxicity

No clinical symptoms were noted. None of the dogs died. Body weight was not affected.

Table 13: Grading of histological findings (adapted from registration dossier).

Organ	Finding	Dog 1 (control)	Dog 2 (treated)	Dog 3 (treated)
Testes	Occurrence of multinucleated giant cells in the lumen of seminiferous tubules (disseminated at random throughout the testis section)	none to minimal	none	none
Testes	Occurrence of seminiferous tubules with severe depopulation of germinal epithelium (disseminated at random throughout the testis section)	none	none to minimal	none
Epididymides		No change in any dog		

Conclusions

In one of the treated dogs the germinal epithelium of the seminiferous tubule was affected. No effects were observed in the other treated dog.

Testicular toxicity screening test (Study report, 1984c)

In a testicular toxicity screening test, male albino mice (6 males per group) were dosed with 4-*tert*-butyltoluene at 0 and 100 mg/kg bw/day once daily for 5 consecutive days.

Mean testes weights of the dosed animals were different when compared with controls (+17% absolute weight and -12% relative weight, compared to controls). A slight damage of germinal epithelium was seen in testes of 1/6 control animals and in 3/6 treated animals.

General toxicity

No treatment-related deaths were observed. No signs of toxicity were noted. Body weight gains were similar in treated and control animals (Annex I section 3.10.1.8).

Figures from testes evaluation in table 14 below demonstrate a very slight increased proportion of injured testis tissue at 100 mg/kg bw/day.

Table 14: Histological assessment of seminiferous tubules per testis (adapted from registration dossier).

Concentration (mg/kg bw/day)	Grading mean %*			
	0	1	2	3
Control	95.75	4.08	0	0.17
100	94.83	4.25	0.25	0.67

*examination/grading of 100 randomly selected, cross-sectioned tubules in a respective testis section.

Conclusions

Testes weights of treated mice were affected. Slight damage of the germinal epithelium of testes was seen in 50% of treated animals. Effects were observed in absence of general toxicity.

4-*tert*-butylbenzaldehyde

Testicular toxicity screening test in rats (Study report, 1981)

In a testicular toxicity screening test from 1981 male SPF albino rats (8 males per group, 4 males in control group) were dosed orally by gavage to 0, 6.5, 12.5, 25 and 50 mg/kg bw/day daily for 5 consecutive days.

Testes weights of rats treated with 50 mg/kg bw/day were significantly lower than these recorded for the controls. The changes of testes caused by the treatment were limited to the seminiferous epithelium. Interstitial cells and Sertoli cells were unaffected. Disorganisation of the epithelial structure, degeneration of cells, and reduction of the spermatozoa were observed. A testis of a control rat showed about 80 % convoluted tubules with a normal epithelium (grade 0) and about 20 % convoluted tubules with a normal epithelium but with degenerated cells or detritus in the lumina (grade 1). This ratio occurred also at 6.5 and 12.5 mg/kg bw/day. An alteration of this ratio was seen at 25 and 50 mg/kg bw/day. Moderate to severe injuries were discovered in the seminiferous epithelia of all rats treated at 50 mg/kg bw/day.

Table 15: Histological assessment of seminiferous tubules per testis (adapted from registration dossier).

Concentration (mg/kg bw)	Grading mean %*			
	0	1	2	3
Control	78.1	21.9	0	0
6.5	82.5	17.5	0	0

12.5	83.0	17.0	0	0
25.0	53.6	34.6	5.7	6.1
50.0	1.5	27.6	43.8	27.1

*examination/grading of 100 randomly selected, cross-sectioned tubules in a respective testis section.

General toxicity
All animals survived the experimental period. Three rats treated at 12.5 mg/kg bw/day showed slight aggressiveness on test days 3 and 4. From days 3 to 6, a slight loss of hair was seen in one animal at 50 mg/kg bw/day. Rats treated at 25 mg/kg bw/day initially showed a slight weight loss and returned to normal at the end of the treatment. The animals in the highest dose group demonstrated weight loss throughout the study (-13% on day 6 compared to controls).

Conclusions

Testes weights of rats treated at 50 mg/kg bw/day were significantly reduced. The changes of testes caused by treatment were limited to the seminiferous epithelium and included disorganisation of the epithelial structure, degeneration of cells, and reduction of the spermatozoa. Males in the highest dose group demonstrated weight loss throughout the study.

Testicular toxicity screening test in mice (Study report, 1984d)

In a testicular toxicity screening test from 1984 male SPF albino mice (6 animals per group) were dosed orally by gavage to 0 and 100 mg/kg bw/day daily for 5 consecutive days. The testes weights of the treated animals showed no effect when compared to controls. A slight damage of germinal epithelium was seen in testes of 1 control and 4 treated animals. Other testicular changes and changes of epididymides were not observed.

Table 16: Histological assessment of seminiferous tubules per testis (adapted from registration dossier).

Concentration (mg/kg bw/day)	Grading mean %±SD*			
	0	1	2	3
0	95.75±1.29	4.08±1.00	0	0.17±0.58
100	94.42±2.39	4.08±1.73	0.33±0.65	1.17±2.12

*examination/grading of 100 randomly selected, cross-sectioned tubules in a respective testis section.

Conclusions

In mice, no effects on testis weight were seen, however slight damage of the germinal epithelium was seen in the testes of 4 of 6 treated animals.

Testicular toxicity screening test (Study report, 1984e)

In a testicular toxicity screening test from 1984, male Himalayan guinea pigs (5 males per group) were dosed orally by gavage at 0 and 100 mg/kg bw/day daily for 5 consecutive days. A slight damage of germinal epithelium was seen in 2 control animals and in 1 treated animal, however the lumen of the seminiferous tubules of the treated animals showed more detritus than those of the control animals.

Other testicular changes and changes of epididymides were not observed. No significant difference in testes weights was observed between treated and control group.

General toxicity

No death occurred. There were no effects on body weights.

Table 17: Histological assessment of seminiferous tubules per testis (adapted from registration dossier).

Concentration (mg/kg bw/day)	Grading mean %±SD*			
	0	1	2	3
Control	97.5±1.18	2.3±1.06	0	0.2±0.42
100	89.7±2.41	10.1±2.51	0.1±0.32	0.1±0.32

*examination/grading of 100 randomly selected, cross-sectioned tubules in a respective testis section.

Conclusions

In guinea pigs, no effects on testis weight were seen, however slight damage of the germinal epithelium was seen in the testes of 1 of 5 treated animals. The lumen of the seminiferous tubules of the treated animals showed more detritus than those of the control animals

Testicular toxicity screening test in dogs (Study report, 1984f)

In a testicular toxicity screening test from 1984, 3 male Beagle dogs were dosed orally by capsule at 0 or 100 mg/kg bw/day daily for 5 consecutive days.
There were 60 cross-sectioned seminiferous tubules with nearly total depopulation of germinal epithelium in both testes of one treated dog. In these seminiferous tubules, early stages of spermatogenesis and Sertoli cells were preserved only. With the exception of the occurrence of multinucleated giant cells (a background finding seen also in the control animal) no abnormalities were discovered in the testes of the other treated dog. No changes were seen in epididymides.

General toxicity

Both treated dosed dogs showed a slight weight loss (dog 1; -5% and dog 2; -10%, from day 1 to 6). There was no information about the control dog.

Conclusions

In one of the treated dogs the germinal epithelium of the seminiferous tubule was affected. No effects were seen in the other treated dog. Both treated dogs showed a slight weight loss.

TSCATS study 1982

In a screening study from 1982, male SPF albino rats (7 males per group) were dosed with 4-*tert*-butylbenzaldehyde at 0 and 100 mg/kg bw/day for 5 consecutive days.

Testes weights of the treated animals were lower than those of the controls. One of the 7 tested animals had an agenesia of the testis. The treated animals showed injuries in the seminiferous epithelium. Five treated animals showed minimal to moderate degeneration of spermatids and spermatocytes. One treated animal showed a minimal reduction of spermatozoa and all treated animals showed minimal to moderate appearance of multinucleate giant cells. Sertoli cells and Leydig cells were unaffected.

General toxicity

No mortality occurred throughout the study and all animals appeared normal. During the first two days an initial body weight loss was observed, but these rats showed subsequent weight gain at the end of the treatment period.

Conclusions

The testes weights of treated rats were reduced. The treated animals showed injuries in the seminiferous epithelium and minimal to moderate degeneration of spermatids and spermatocytes.

2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral)

The substance 2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral) already has a harmonised classification as Repr.1B (H360Fd). No change to the current harmonised classification is proposed by the DS. An adapted summary from RAC's opinion on the previous proposal can be found below.

Adapted from RAC Opinion: Assessment of fertility (ECHA, 2019)

Several repeated dose toxicity studies in rats and other species as well as four reproductive toxicity studies in rats of lysmeral were available. Lysmeral elicited adverse effects on male reproductive organs in rats and in dogs.

In the two one-generation range finding studies in rats via the oral route, male fertility was markedly affected at doses starting from 25 mg/kg bw/d. Findings are summarised in the table below.

Effects on testes included reduced organ weights and degeneration. Spermatotoxic effects included reduced sperm counts and increased numbers of abnormal sperms resulting in markedly reduced fertility indices.

Doses eliciting adverse testicular effects and spermatotoxicity also lead to hepatotoxicity represented by increased organ weights and changes in clinical chemistry. Similar effect patterns were observed in the repeated dose toxicity studies in rats and dogs, presented as supporting evidence. LOAELs for male fertility were 50 mg/kg bw/d in rats and 200 mg/kg bw/d in dogs.

After dermal application, testicular effects were observed in rats at doses of 2000 mg/kg bw/d (above the limit dose). No effects on fertility were seen in other species up to oral doses of 100 mg/kg bw/d in rhesus monkeys, mice and Guinea pigs, and 300 mg/kg bw/d in rabbits.

Based on a number of in vivo and in vitro toxicokinetic studies with Lysmeral there is evidence that species differences exist, but these differences are considered as quantitative rather than qualitative. The proposed MoA includes the formation of stable TBBA-CoA conjugates from the main metabolite TBBA, the amount of which was shown to be species dependent. High levels of stable TBBA-CoA have been measured in rat hepatocytes after incubation with Lysmeral, while levels in human hepatocytes were around 5 times lower. TBBA-CoA formation also occurs in rat testicular tissue, although to a much lesser extent than in hepatocytes. Dermal penetration studies in rats and humans showed that Lysmeral is absorbed via the dermal route in both species, although the amount absorbed may differ.

The classification criteria for reproductive toxicity state that "the classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate."

RAC considers the testicular toxicity and spermatotoxicity shown in two species (rats and dogs) relevant for humans despite a quantitatively different metabolism of the compound in different species.

Effects were consistently observed in several repeated dose toxicity studies in rats and dogs and in the two one-generation range finding studies in rats. RAC considers that the doses used in other species (mice, Guinea pigs, rhesus monkeys) may have been too low, and exposure periods (5 days) too short, to induce testicular effects in these species. RAC hence considers data from these species not sufficient to deem them "non-responders". In addition, doses in the EOGRTS were chosen to certainly produce offspring and may therefore also have been too low to induce similar effects as seen in other studies. RAC considers the proposed MoA, although plausible, not sufficient to preclude relevance for humans. It is not clear how relevant mechanistic findings from in vitro tests in hepatocytes are for the effects seen on testes tissue. For example, severe atrophy were seen already after only 24 hours after exposure. Although TBBA-CoA-conjugates were also formed in rat testes tissue ex vivo, concentrations were approximately 100-fold lower than in hepatocytes. Therefore, a direct effect of Lysmeral on this tissue cannot be ruled out. Even though some quantitative differences have been shown between rats and humans, no mechanistic data is available for dogs, the second species in which testicular effects were observed after exposure to Lysmeral. As supporting evidence, the metabolite considered to be responsible for the compound's testicular and sperm toxicity – TBBA – is classified as Repr. 1B H360F.

Table: *Summary of findings in male rats in two one-generation range finding studies*

Method, Duration of study, Route of exposure, Guideline, GLP status	**Species, Strain, Sex, No/group**	**Test substance, Vehicle, Dose levels Duration of exposure**	**NOAELs, LOAELs**
One-generation range finding Oral, diet 12 weeks Non-TG, non-GLP (BASF SE, 2006c)	Rat (Wistar) 10 males and 10 females per group	30.7% Lysmeral in sunflower oil, microencapsulated in gelatin Nominal doses*: 0, 400, 800, 1700, 3400 ppm For dams adjusted to 0, 200, 400, 850, 1700 ppm during gestation and lactation Actual intake*: Males: 0, 14, 28, 62.6, 116.8 mg/kg bw/d Exposure: from 6 weeks prior mating to PND21 *doses and intake refer to pure substance	LOAEL (general toxicity, males): 28 mg/kg bw/d NOAEL (male fertility): 28 mg/kg bw/d
Results: **Mating indices:** 100, 100, 100, 80, 50%	**0 mg/kg bw/d:** Fertility index: 100% **14 mg/kg bw/d:** Fertility index: 100% **≥ 28 mg/kg bw/d:** ↑ rel. liver weights, 10-20% Fertility index: 100% **≥ 62.6 mg/kg bw/d:** ↑ ALAT, 20-45%; ↑ ALP, 30-55%; ↑ GLDH, 4-5-fold; ↓ rel. testes weights, 30-45%; ↓ rel. cauda epididymis weights, 30-40%; Diffuse testes degeneration (8/10) moderate to severe focal testes degeneration (2/10), aspermia of the epididymis (10/10); ↓ testicular spermatid heads (6 mio vs 121 mio in controls); ↓ epididymal sperm heads (2 mio vs 591 mio in controls); 0% motile sperm Fertility index: 10% **116.8 mg/kg bw/d:** ↓ food consumption, 15%; ↑ rel. kidney weights, 15%; ↑ gamma-GT, 100%; ↓ seminal vesicle weights, 10%; ↓ prostate weights, 20%; Minimal to slight hyperplasia of Leydig cells (9/10) Fertility index: 0%		
Method, Duration of study, Route of exposure, Guideline, GLP status	**Species, Strain, Sex, No/ group**	**Test substance Vehicle, Dose levels Duration of exposure**	**NOAELs, LOAELs**
One-generation range finding	Rat (Wistar)	17.7% Lysmeral in sunflower oil	LOAEL (general toxicity, males):

Oral, diet 8 weeks Non-TG, GLP (BASF SE, 2017b)	10 males and 10 females per group	microencapsulated in alginate; Nominal doses*: 0, 230, 750, 2300 ppm For dams adjusted to 0, 115, 375, 1150 ppm during lactation Actual intake*: males, pre-mating: 0, 2.8, 9.1, 27.5 mg/kg bw/d males, post-mating: 0, 2.3, 7.4, 25.1 mg/kg bw/d Exposure: from 2 weeks prior mating to PND21 *doses and intake refer to pure substance	27.5/25.1 mg/kg bw/d NOAEL (male fertility): 9.1/7.4 mg/kg bw/d
Results: **Mating indices:** 100, 100, 100, 90%	**0 mg/kg bw/d:** Fertility index: 100% **2.8/2.3 mg/kg bw/d:** ↑ rel. liver weights, < 10% Fertility index: 90% **9.1/7.4 mg/kg bw/d:** ↓ food consumption within week 1 of treatment, 7%; ↓ total protein; ↑ rel. liver weights, < 10% Fertility index: 100% **27.5/25.1 mg/kg bw/d:** ↓ bwg, 45-84%; ↓ food consumption within 1st week of treatment, 9%; ↓ total protein, albumin, globulin, cholesterol, triglycerides, sodium, calcium; ↑ ALAT, 26%; ↑ rel. liver weights, 14-30%, ↑ incidences of discolouration; ↓ rel. cauda epididymis weights, 19%; ↓ epididymis weights, 16%; ↓ seminal vesicle weights, 19%; Minimal to moderate tubular degeneration (3/10 vs 1/10 in controls); Minimal to moderate ductal atrophy in epididymis (8 /10); Slight to moderate oligospermia (6/10); Slight to moderate cellular debris (2/10); ↓ epididymal sperm heads (469 mio vs 674 mio in controls); Testicular spermatid head counts not affected (115 mio vs 124 mio in controls); 25% motile sperm; 72% abnormal sperm; Fertility index: 40%		

4-*tert*-butylbenzoic acid (TBBA)

The substance 4-*tert*-butylbenzoic acid (TBBA) already has a harmonised classification as Repr.1B (H360F). No change to the current classification regarding fertility is proposed by the DS. An adapted summary on reproductive toxicity from the previous CLH report is provided below (ECHA, 2010).

Summary and discussion on fertility adapted from CLH report for TBBA (ECHA, 2010)

With regard to male fertility, several studies with rats with different routes of application (oral, inhalation, dermal) are available revealing a toxic potential of 4-*tert*-butylbenzoic acid with induction of testicular lesions, spermatotoxic effects and (reversible at test dose of 41 mg/kg) infertility already at relatively low dosages/concentrations. Consistently and independent from route of application testes toxicity was characterised by lower absolute and relative organ weights, testes atrophy from

seminiferous tubular degeneration, destruction of the germinative epithelium resulting in disturbance of spermatogenesis and in particular in loss of late spermatids.

Concern on possible spermatotoxic effects of 4-*tert*-butylbenzoic acid also in humans might be given but remains uncertain. A study on occupationally exposed workers provided some indication for slightly higher numbers of individuals with low sperm count (less than 20 million sperm/ml) in exposed participants compared to non-exposed participants. However the findings could be biased by other factors and uncertainty remains due to the low numbers of participants.

Hazard assessment for 4-*tert*-butylbenzoic acid with respect to female fertility is not possible, since there are no data available.

Table 5: NOAEL/C and LOAEL/C values from different administration routes for fertility risk characterisation

Route of application (duration)	NOAEL/C	LOAEL/C	Reference
Oral (70 days)	1.6 mg/kg bw/d	7.9 mg/kg bw/d	Hoechst, 1987
Oral (90 days)	-	6 mg/kg bw/d	Hunter et al., 1965
Dermal (7 and 13 weeks)	35 mg/kg bw/d	70 mg/kg bw/d	Cagen et al., 1989
Dermal (28 days)	30 mg/kg bw/d	60 mg/kg bw/d	Shell, 1975
Inhalation (4 days (3 days rest) 3 days)	-	12.5 mg/m^3	Shell, 1982b

In some studies testes toxicity occurs at same doses where body weight gain was also significantly affected (Hoechst, 1987, Shell, 1975). However, there are other studies reporting that testes toxicity was evident at doses/concentration without any sign of general toxicity (Hunter et al., 1965, Cagen et al., 1989, Shell, 1982b). Due to the fact that testes toxicity was observed in some studies without significant general toxicity it could not be interpreted as secondary effect. In summary, there is a clear-cut toxic potential specifically adverse to male gonads and resulting in impaired male fertility in rats for 4-*tert*-butylbenzoic acid in several studies and consistently across various routes of administration the substance.

RAC's Opinion (ECHA 2011)[12]

Based on the available comparison of reproductive toxicity data with classification criteria RAC supports the actual proposal of the dossier submitter (CLP Repr. 1B H360F).

10.10.3 Comparison with the CLP criteria

The criteria for classification in Repr. 1B for adverse effects on sexual function and fertility are considered fulfilled for 3-(4-*tert*-butylphenyl)propionaldehyde, 4-*tert*-butyltoluene, 4-*tert*-butylbenzylaldehyde and methyl 4-*tert*-butylbenzoate based on a weight of evidence approach and read-across within this group. The available data for substances within the category, including 2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral) and 4-*tert*-butylbenzoic acid (TBBA), which already have harmonised classification as Repr.1B (H360F), demonstrate a clear toxic effect on male reproductive organs, including testicular toxicity and toxicity to the sperm.

Similar type of reproductive toxicity is seen across different species (rats, mice, guinea pigs, dogs), although most studies are conducted in rats and the degree of toxicity appears to differ between species. The DS agrees with the previous assessment made by RAC on the substance lysmeral (ECHA, 2019), that the data to support a species-specific mechanism of action in rats, as proposed by some registrants

[12] Copy from RAC's opinion 4-tert-Butylbenzoic acid, 2011.

are not sufficient to preclude relevance for humans, and therefore considers the effects as relevant to humans.

Effects on the male reproductive system (testicular toxicity and spermatotoxicity) are reported for five of six substances in the category. Infertility was clearly demonstrated in rats dosed at 15 and 50 mg/kg bw/day in the OECD TG study 421 on 4-*tert*-butyltoluene (Study report 2007), which can be correlated with the testicular toxicity and sperm effects demonstrated in the same study. Although general toxicity was evident in the highest dose group, especially among female rats, body weights were only slightly affected among males at the time of mating.

Another test guideline study (OECD TG study 422) demonstrated a negative outcome on fertility, however, the doses used were relatively low (up to 5 mg/kg bw/day). Studies available on fertility for 2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral) and 4-*tert*-butylbenzoic acid (TBBA), which already have harmonised classification as Repr.1B (H360F), report similar adverse findings on the male reproductive system and further support the conclusion on reproductive toxicity for this category of substances.

Four of the substances included in the proposal (lysmeral, 3-(4-*tert*-butylphenyl)propionaldehyde, 4-*tert*-butyltoluene and, 4-*tert*-butylbenzylaldehyde) have demonstrated experimentally *in vivo* to form *tert*-butylbenzoic acid (TBBA), which is considered responsible for the toxicity seen in male rats. No toxicokinetics or experimental studies on reproductive toxicity is available for the substance methyl 4-*tert*-butylbenzoate.

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 substances and 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 is considered as clear evidence and not some evidence.

10.10.4 Adverse effects on development

Table 18: 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**
3-(4-*tert*-butylphenyl)propionaldehyde EC 242-016-2			
OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test) No deviations GLP compliant Test animals: Crl:CD(SD) Sprague Dawley rats (males and females). 10 rats/sex/group. Assigned reliability 1 by the	3-(4-*tert*-butylphenyl)propionaldehyde EC 242-016-2 Purity: 99.0%. Vehicle: corn oil Expiration date of the lot/batch: October 15, 2011. Doses: 0, 0.5, 1 and 5 mg/kg bw/day.	***F1 generation*** **Clinical observations** No clinical signs, no mortality in F1 generation. **Body weight** ↓ mean pup body weight at 5 mg/kg bw/day days 9 and 12 postpartum (-11% and -12%, p≤0.05 and p≤0.0, respectively). ↓ mean serum T4 concentrations in females at 1 and 5 mg/kg bw/day (both -26%, day 12 postpartum, p≤0.01).	Study report, 2019. Robust study summary in Registration dossier, ECHA's dissemination site, 2022.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Registrant.	Male rats were dosed once daily (oral gavage) beginning 14 days before cohabitation with treated females, during cohabitation and continuing through the day prior to scheduled euthanasia on Days 43 through 46. Female rats were dosed once daily (oral gavage) beginning 14 days before cohabitation with treated males and continuing through LD 12 (rats that delivered a litter) or GD 24 (rats that did not deliver a litter).	NOAEL : 1 mg/kg bw/day (pups weight) **Parental animals** No clinical signs or effects on body weights in parental animals.	Full study report was available to DS Additional data available in Confidential Annex Study 1- Annex I Section 3.10.1.1
4-*tert*-butyltoluene EC 202-675-9			
OECD TG 421 Reproduction / Developmental Toxicity Screening Test GLP compliant Sprague Dawley rats, males/females 12 males and 12 females per group. Assigned reliability 1 by the Registrant. Full study report was not available to DS. Differences in weight of parental animals were estimated from graphs and are associated with uncertainties.	4-*tert*-butyltoluene EC 202-675-9 Purity 96.94% Vehicle: Corn oil Substance was administered orally (gavage) at doses 0, 1.5, 5, 15, 50 mg/kg bw/day once daily The administration period for males was total 50 to 52 days including 14 days before mating and subsequent 36 to 38 days (necropsy of males was separately conducted in 3 days since the observation of sperm requires 3 days). The administration period for females	**F1 generation** No pups produced at 50 mg/kg bw/day. All newborn pups of one dam at 15 mg/kg bw/day died by LD 1. ↓ number of pups born (-26%; p≤0.05) and number of live pups (-58%; p≤0.01) at day 0 at 15 mg/kg bw/day. ↓ delivery index (82.7% vs. 94.1% in controls), birth index (49.3% vs. 93.6% in controls), and live birth index (63% vs. 99.5% in controls), viability index (45% vs. 98.8% in controls) at 15 mg/kg bw/day. ↑ number of stillbirths at 15 mg/kg bw/day (4.7 vs. 0.1 in controls; not statistically significant, but slightly increasing trend with dose). ↓ number of live offspring (4.5 vs. 14.3 in controls, tendency) and viability index at LD 4 (45% vs. 98.8% in controls, tendency). ↓ body weights of pups at LD 0 (-11% and -8% in males and females, respectively) and LD 4 (-16% and -15% in males and females, respectively) at 5 mg/kg bw/day. ↓ body weights of pups at LD 0 (-32% in males	Study report, 2007. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 3–- Annex I 3.10.1.3 Additional data available in Confidential Annex.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	was total 41 to 45 days including 14 days before mating, mating period (14 days at the longest), gestational period, and first 3 days in lactation period. The starting day of administration was set as the day 1.	and females) and LD 4 (-13% and -10% in males and females, respectively) at 15 mg/kg (based on pups from 3 dams and 1 dam, respectively). NOAEL: 1.5 mg/kg bw/day (pups weight) **Maternal animals:** ↓ body weight GD 7 at 5 mg/kg bw/day (-8%; p≤0.05 estimated from graph) and GD 14 (-10%; p≤0.05 estimated from graph) and at 15 mg/kg bw/day, GD 7 to 21, estimated from graph, -9% at GD 21; p≤0.01. ↓ body weight at 5 mg/kg bw/day at LD 4 (estimated from graph -13%, p≤0.01, correlated with significantly decreased food consumption). ↓ body weight at 15 mg/kg bw/day LD 4 (-11% estimated from graph, only one animal). ↓ body weight, day of necropsy at 5 (-13%; p≤0.01), 15 (-18%; p≤0.01), and 50 mg/kg bw/day (-29%; p≤0.01).	
Prenatal developmental toxicity test Not according to test guideline. Test animals: Female Wistar rats. No. of animals per sex per dose: no data GLP not specified. Exposure via inhalation, but no details on type of inhalation. Assigned reliability 4 by the Registrant.	4-*tert*-butyltoluene EC Number: 202-675-9 Duration of exposure: days 7 through 20 of gestation Frequency of treatment:6 hours/day Duration of test: until 22 months after delivery Concentrations: ca. 0.12 mg/l (20 ppm)	**Mortality or clinical observations** No decreased viability of offspring. Lowered pup body weight until day 10 and delayed ontogeny of reflexes were observed. Increased latencies and swim length were observed during learning period in treated female offspring at 3 months of age. Indications of memory impairments 3 weeks later (not statistically significant). At 22 months, increased latencies and swim length were observed (indicating memory impairments) The substance did not induce maternal toxicity.	Hass et al 1996. Long-lasting learning and memory impairments induced by prenatal exposure to 4-tert-butyltoluene in rats Teratology 53: 22A, abstract no. F15. Summarised in Registration dossier
2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral) EC 201-289-8 [13]			

[13] Studies reporting on developmental toxicity in CLH report of 2-(4-tert-butylbenzyl)propionaldehyde, 2017.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
One-generation range finding study (non-guideline, non-GLP) rat (Wistar)	2-(4-*tert*-butylbenzyl)propionaldehyde oral: via diet 0, 400, 800, 1700, 3400 ppm in the diet 0, 14, 28, 62.6, 116.8 mg/kg bw/d (doses Lysmeral males) 0, 10-15, 18.3-29.4, 62.7, 123.2 mg/kg bw/d (doses / dose range Lysmeral females) purity: 30.7% (a.i. encapsulated)	**Fertility/reprod. performance - Main effects** **General systemic toxicity - Main effects** ↓ Body weights /FC Changes in liver associated parameters (clinical chemistry, ↑ liver weights) ↑ Rel. kidney weights **Developmental toxicity - Main effects (coinciding with maternal toxicity):** ↓ pup body weights	BASF SE 2006C Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde
One-generation range finding study (non-guideline, GLP) rat (Wistar)	2-(4-*tert*-butylbenzyl)propionaldehyde oral: via diet 0, 230, 750, 2300 ppm in the diet 0, 2.3-2.8, 7.4-9.1, 25.1-27.5 mg/kg bw/d (dose range Lysmeral males) 0, 3.3-3.7, 10.6-11.9, 21-34.7 mg/kg bw/d (dose range Lysmeral females) purity: 17.7% (a.i. encapsulated)	**Fertility/reprod. performance - Main effects** **General systemic toxicity - Main effects** ↓ Body weights /FC Changes in liver associated parameters (clinical chemistry, ↑ liver weights, macroscopic changes), Hematological changes **Developmental toxicity - Main effects (coinciding with maternal toxicity)** ↓ Pup body weights and early pup survival	BASF SE 2017B Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde
Modified extended one generation reproduction toxicity study (OECD Guideline 443, GLP) rat (Wistar)	2-(4-*tert*-butylbenzyl)propionaldehyde oral: via diet 0, 75, 230, 750 ppm in the diet 0, 1, 3, 10 mg/kg bw/d (nominal dose Lysmeral) 0, 1.4, 4.5, 15.1 mg/kg bw/d (overall mean dose Lysmeral)	**General systemic toxicity - Main effects** ↓ Body weights/FC, Hematological changes Changes in liver associated parameters (clinical chemistry, ↑ liver weights, histopathology) **Developmental toxicity - Main effects (coinciding with maternal toxicity)** ↓ Pup body weights. NOAEL (general systemic toxicity): 3 (4.5) mg/kg bw/d NOAEL (developmental toxicity):3 (4.5)	BASF SE (2017) Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	purity: 17.7% (a.i. encapsulated)	mg/kg bw/d NOAEL (developmental neurotoxicity): 10 (15.1) mg/kg bw/d NOAEL (developmental immunitoxicity): 10 (15.1) mg/kg bw/d NOAEL (fertility/reprod. performance): 10 (15.1) mg/kg bw/d	
Prenatal Developmental Toxicity Study (OECD Guideline 414, GLP) rat (Wistar)	2-(4-*tert*-butylbenzyl)propionaldehyde oral: gavage 0, 5, 15, 45 mg/kg bw/d (nominal dose) 0, 4.1, 12.7, 40.7 mg/kg bw/d (actually ingested) purity: 98.1%	**General maternal toxicity - Main effects** Clinical signs, ↓ body weights (incl. transient body weight loss), changes in liver associated parameters (clinical chemistry, ↑ liver weights). **Prenatal developmental toxicity - Main effects (coinciding with maternal toxicity)** ↑ total resorptions/postimplantation loss, ↓ mean gravid uterus weights, ↓ in fetal body weights and associated ↑ in skeletal variations. NOAEL (maternal toxicity): 5 (4.1) mg/kg bw/d NOAEL (prenatal developmental toxicity): 5 (4.1) mg/kg bw/d	BASF SE (2004) Reviewed in CLH report for 2-(4-*tert*-butylbenzyl)propionaldehyde

Table 19: 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 20: 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

3-(4-*tert*-butylphenyl)propionaldehyde

OECD TG 422 study in rats (Study report, 2019)

In an OECD TG 422 (GLP compliant) study from 2019, male and female Crl:CD(SD) Sprague Dawley rats (10 per sex/group) were exposed to 3-(4-*tert*-butylphenyl)propionaldehyde at 0, 0.5, 1 and 5 mg/kg bw/day. Male rats were dosed once daily (oral gavage) beginning 14 days before cohabitation with treated females, during cohabitation and continuing through the day prior to scheduled euthanasia on days 43 through 46. F1 generation pups were not directly exposed to the test or control substance.

Of note, this study was conducted in 2019, and according to the study report the expiration date of the lot/batch of the substance used in the study was in October 2011.

General toxicity maternal animals

There was no mortality in the P generation females at any dose, no effects on body weight and weight change, food consumption, clinical parameters. There were no effects on reproductive function or reproductive performance.

F1 generation

There were no clinical signs observed in the F1 generation pups at any dose and no mortality observed. There were no effects on mean body weights in the F1 generation pups at 0.5 and 1 mg/kg bw/day. The mean pup body weight was statistically significantly reduced at 5 mg/kg bw/day compared to controls on days 9 and 12 postpartum (-12% and -11%). (The reduced pup weights were within the range observed historically at the testing facility).

There were no effects observed on food consumption.

In male pups, mean serum T4 concentrations were -2%, -18%, and -22% at 0.5, 1, and 5 mg/kg bw/day, respectively, on day 12 postpartum (not statistically significant). In the F1 generation female pups, mean serum T4 concentrations were -18%, -26% (significant) and -26% (significant) at 0.5, 1, and 5 mg/kg bw/day, respectively, on day 12 postpartum. There were no microscopic changes in the thyroid or parathyroid glands of the single F1 generation pup/sex/litter that was microscopically examined at 5 mg/kg bw/day.

There were no differences in mean anogenital distance in the F1 generation males or females at any dose on Day 1 postpartum. There were no differences on nipple retention in the F1 generation male pups in any dose group. No male pups had nipples present on PND 12.

The doses used in this study were considerably lower compared to doses used in other studies with this substance.

Conclusions

In the F1 generation a statistically significantly reduced mean pup body weight at 5 mg/kg bw/day, up to -12%, was observed on days 9 and 12 postpartum. There were no signs of marked general toxicity in parental animals.

4-*tert*-butyltoluene

OECD TG 421 study in rats (Study report, 2007)

In an OECD TG 421 (GLP compliant) study from 2007, male and female Sprague Dawley rats (12 per group and dose) were dosed by gavage at 0, 1.5, 5, 15, 50 mg/kg bw/day once daily. The administration period for males was total 50 to 52 days including 14 days before mating and subsequent 36 to 38 days (necropsy of males was separately conducted in 3 days since the observation of sperm requires 3 days). The administration period for females was total 41 to 45 days including 14 days before mating, mating period (14 days at the longest), gestational period, and first 3 days in lactation period. The starting day of administration was set as day 1.

Maternal animals

There was one death among females at 15 mg/kg bw/day and 6 deaths (50%) occurred at 50 mg/kg bw/day.

There were statistically significant decreases in body weight from day 4 to 15 of the administration period at 50 mg/kg bw/day compared with the control group (estimated from graph, -8%). There were statistically significant decreases in body weight at GD 7 and GD 14 at 5 mg/kg bw/day compared with the control group (estimated from graph, -8% and -10%, respectively, $p \leq 0.01$). There were statistically significant decreases in body weight on GD 7 to 21 at 15 mg/kg bw/day compared with the control group (estimated from graph, -9% at GD 21).

There was a statistically significant decrease in body weight at LD 4 at 5 mg/kg bw/day compared with the control group (estimated from graph, -13%). There was a decreasing tendency of body weight in one animal at 15 mg/kg bw/day at LD4 (i.e no dose response).

There were statistically significant decreases in body weight at the day of necropsy at 5 (-13%), 15 (-18%), and 50 (-29%) mg/kg bw/day compared with the control group. There were statistically significant effects on food consumption at 5 mg/kg bw/day and in one animal at 15 mg/kg bw/day at the beginning of the lactation period (estimated from graph, -30%).

The full study report was not available to the DS. Differences in weight of parental animals were estimated from graphs and are associated with uncertainties.

Reproductive performance

There was no significant difference in frequency of estrus during the administration period (14 days) before mating between each group and the control group. There was no significant difference in days required for copulation between each group and the control group. One pair of animals did not achieve copulation at 50 mg/kg bw/day. There was no significant difference in copulation index between each group and the control group.

There were 8 non-pregnant females (of 12) at 15 mg/kg bw/day. There was no pregnant female at 50 mg/kg bw/day. There were significant decreases in fertility index at 15 and 50 mg/kg bw/day compared with the control group.

There was no significant difference in gestational period at 1.5, 5, and 15 mg/kg bw/day compared with the control group. There was no abnormality of delivery status at 0, 1.5 and 5 mg/kg bw/day. No newborn offspring were obtained with one dam at 15 mg/kg bw/day since the litters were all dead. There were no significant differences in number of pregnant corpora lutea, number of implantations, and implantation index at 1.5, 5, and 15 mg/kg bw/day compared with the control group. The gestation index was 100% at 1.5 and 5 mg/kg bw/day. The gestation index at 15 mg/kg bw/day was 66.7% since one dam did not deliver live offspring.

In the observation of lactation status, there was no abnormality at 0, 1.5, and 5 mg/kg bw/day.

There were statistically significant decreases in number of offspring born (-26%) and number of live pups born (-58%) and an increase in number of stillbirths at 15 mg/kg bw/day (4.7 vs. 0.1 in controls), compared with the control group. There was decreasing tendency of delivery index (94.1%, 93.8%, 98.1% and 82.7%), birth index (93.6%, 91.2%, 94.3% and 49.3%), and live birth index (99.5%, 97.3%, 96.2% and 63.0%) at 0, 1.5, 5. and 15 mg/kg bw/day, respectively. There was an increase in number of stillbirths at 15 mg/kg bw/day (4.7 vs. 0.1 in controls).

F1 generation

The newborn offspring of one dam died by LD 1 at 15 mg/kg bw/day, and there were decreasing tendencies of number of live offspring and viability index at LD 4.

There was no abnormality in the observation of external abnormality of newborn offspring in any group or in the observation of clinical signs on the newborn offspring.

There were statistically significant decreases in body weights of male and female pups at LD 0 (-11% and -8%, respectively) and LD 4 (-16% and -15%, respectively) at 5 mg/kg bw/day. The body weights of male and female pups were reduced compared to controls at LD 0 (-32% and -32%, respectively), and LD 4 (-13% and -10%, respectively, only 1 dam) at 15 mg/kg bw/day compared with the control group. These number as based on litter from 3 (LD 0) and 1 (LD 4) dams and no statistical analysis is available.

Conclusions

In the Reproduction/Developmental Toxicity Screening Test OECD TG 421 on 4-*tert*-butyltoluene there were statistically significant decreases in number of offspring born and number of live pups born and an increase in number of stillbirths at 15 mg/kg bw/day. All pups of one female at 15 mg/kg bw/day were all dead and the litter of another dam died at LD 1.

There were significant decreases in body weights of pups at the beginning of the lactation period at 5 mg/kg bw/day (up to -16%). Body weights of pups at 15 mg/kg bw/day at LD 0 were even lower (-32%), numbers were based on litters from 2 dams only.

In maternal animals at 50 mg/kg bw/day there was excessive toxicity, since 50% of the females died. No offspring was produced at 50 mg/kg bw/day, possible at least partly due to testicular and spermatotoxic effects in paternal animals. At 15 mg/kg bw/day one female (of 12; 8.3%) died and only 3 females became pregnant.

Maternal animals at 5 mg/kg bw/day demonstrated less general toxicity compared to dams at higher doses, and included reduced body weight during gestation, approximately -10% (estimated from graph) and no other toxicity reported. Average body weight at necropsy at this dose was -13%.

Non-guideline prenatal developmental toxixty study in rats (Hass et al. 1996)

Long-lasting learning and memory were studied following prenatal exposure (days 7 to 20 of gestation) to 4-*tert*-butyltoluene in Wistar rats by inhalation. Pups were followed until 22 months after delivery. There was no maternal toxicity (no details available).

Prenatal exposure did not affect the viability of the offspring. There was lowered pup body weight until day 10 and delayed ontogeny of reflexes.

At the age of 3 months, increased latencies and swim length were observed in the learning period of treated female offspring. Three weeks later, indications of memory impairments were noted (not statistically significant). No substance-related effects were observed at 17 months. At the age of 22 months, increases in latencies and swim length indicating memory impairments were observed in the first 3 trials and in the trials following a 4-days break in testing

According to the authors, the impairment in exposed female offspring was not considered to be related to poorer swimming capability since swim lengths were increased in proportion to the increased latencies; swim speed was similar to control. The results indicated that substance-related neurobehavioral impairments could interact with the consequences of aging.

Conclusions
In this non-guideline study with limited information, results indicate effects on neurobehavior of offspring exposed *in utero*. In addition, results demonstrate lower pups body weight until day 10 postpartum and delayed ontogeny of reflexes.

2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral)

The substance 2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral) already has a harmonised classification as Repr.1B (H360Fd). No change in the current classification is proposed by the DS. An adapted summary from RAC's opinion on the previous proposal can be found below.

Adapted from RAC Opinion: Assessment of developmental toxicity (ECHA, 2019)

Effects on pre- and postnatal development after exposure to Lysmeral in doses up to 45 mg/kg bw/d were shown in rats. Findings from the two one-generation range finding studies in rats are summarised in the table below.

Table: *Summary of the findings in dams and foetues/pups in two one-generation range finding studies*

Method, Duration of study, Route of exposure, Guideline, GLP status	**Species, Strain, Sex, No/group**	**Test substance, Vehicle, Dose levels Duration of exposure**	**NOAELs, LOAELs**
One-generation range finding Oral, diet 12 weeks Non-TG, non-GLP (BASF SE, 2006c)	Rat (Wistar) 10 males and 10 females per group	30.7% Lysmeral in sunflower oil, microencapsulated in gelatin Nominal doses*: 0, 400, 800, 1700, 3400 ppm For dams adjusted to 0, 200, 400, 850, 1700 ppm during gestation and lactation Actual intake*: Females: 0, 10-15, 18.3-29.4, 62.7, 123.2 mg/kg bw/d Exposure: from 6 weeks prior mating to PND21 *doses and intake refer to pure substance	LOAEL (general toxicity, females): 18.3-29.4 mg/kg bw/d NOAEL (female fertility): 18.3-29.4 mg/kg bw/d
Results: **Mating indices:** 100, 100, 100, 80, 50%	**0 mg/kg bw/d:** Mean implantation sites: 9.9; Mean post implantation loss: 5.1±9.27%; Mean pups delivered: 9.4±3.95; Number of litters: 10 Fertility index: 100% **≥ 10-15 mg/kg bw/d:** ↓ ChE, 50-60%; ↑ gamma-GT, 2-8-fold; Mean implantation sites: 8.5; Mean post implantation loss: **16.2±30.3%**; Mean pups delivered: 8.7±1.41; Number of litters: 9 Fertility index: 100% **18.3-29.4 mg/kg bw/d:** ↓ bwg 10-30% before/during mating; ~10% during gestation/lactation; ↓ food consumption, 20% during lactation; ↑ GLDH, 5-75%; Mean implantation sites: 8.8; Mean post implantation loss: **11.1±10.16%**; Mean pups delivered: 7.9±2.23; Number of litters: 10 Fertility index: 100% **62.7 mg/kg bw/d**:** Mean implantation sites: 1; Mean post implantation loss: 100%; Mean pups delivered: 0; Number of litters: 0		

	Fertility index: 13% **123.3 mg/kg bw/d**:** Mean implantation sites: 0; Mean pups delivered: 0; Number of litters: 0 Fertility index: 0% ** general toxicity was not evaluated due to absence of offspring		
Method, Duration of study, Route of exposure, Guideline, GLP status	**Species, Strain, Sex, No/ group**	**Test substance Vehicle, Dose levels Duration of exposure**	**NOAELs, LOAELs**
One-generation range finding Oral, diet 8 weeks Non-TG, GLP (BASF SE, 2017b)	Rat (Wistar) 10 males and 10 females per group	17.7% Lysmeral in sunflower oil microencapsulated in alginate; Nominal doses*: 0, 230, 750, 2300 ppm For dams adjusted to 0, 115, 375, 1150 ppm during lactation Actual intake*: females, premating/gestation: 0, 3.3-3.6, 10.6-11.9, 30.6-34.7 mg/kg bw/d females, lactation: 0, 3.7, 10.7, 21.0 mg/kg bw/d Exposure: from 2 weeks prior mating to PND21 *doses and intake refer to pure substance	LOAEL (general toxicity, females): 10.6-11.9 mg/kg bw/d NOAEL (female fertility): 10.6-11.9 mg/kg bw/d
	0 mg/kg bw/d: Mean implantation sites: 11.5; Mean post implantation loss: 3.8±6.85%; Mean pups delivered: 11.1±1.91; Number of litters: 10 Fertility index: 100% **3.3-3.7 mg/kg bw/d:** Mean implantation sites: 11.8; Mean post implantation loss: 3.9±6.29%; Mean pups delivered: 11.3±1.66; Number of litters: 9 Fertility index: 90% **10.6-11.9 mg/kg bw/d:** ↓ bwg and body weights during premating and gestation; ↓ body weights, ~10% during lactation, recovery at end of lactation; ↓ food consumption, during week 1 and 2 of lactation; ↓ triglycerides, sodium, calcium; ↑ ASAT, 23-47%; ↑ gamma-GT, 9-24-fold; Mean implantation sites: 10.1; Mean post implantation loss: 3.7±7.77%; Mean pups delivered: 9.7±2.36; Number of litters: 10 Fertility index: 100% **21.0-34.7 mg/kg bw/d:** ↓ bwg 32-59% during premating and gestation; ↓ body weights, ~10% during lactation, recovery at end of lactation;		

	↓ total protein, albumin, globulin, cholesterol, triglycerides, sodium, calcium, creatinine, total bilirubin, chloride, inorganic phosphate; ↑ ASAT, 23-47%; ↑ gamma-GT, 9-24-fold; ↓ food consumption, 14% during 1st week, 44-48% during lactation; Mean implantation sites: 4.5; Mean post implantation loss: **16.7±23.57%**; Mean pups delivered: 4.0±3.16; Number of litters: 4 Fertility index: 44%

In the first one-generation range finding study, post-implantation loss was increased starting from the dose of 10-15 mg/kg bw/d. Starting at the same dose, pup weights were significantly reduced at birth and at weaning, down to 22% below controls. In maternal animals, this and higher doses were associated with decreased choline esterase levels (50-60% below controls) and two- to eight-fold increased gamma-GT levels compared to controls, but liver weights were not affected. At the lowest dose, mean post-implantation loss showed a high variation (16.2±30.3%) and was higher than at the next dose level of 18.3-29.4 mg/kg bw/d (11.1±10.16%). At this dose level, maternal toxicity additionally consisted of a decreased body weight gain (up to 30% before and during mating, and around 10% during gestation and lactation), a decreased food consumption (-20% during lactation), and increased GLDH levels (up to 75% above control levels). Starting from the dose level of 62.7 mg/kg bw/d, there was only one implantation site, and general toxicity was not assessed due to lack of offspring. Taking into account the developmental effects and the maternal toxicity seen, RAC does not consider it clear that the developmental effects seen at these doses, in particular at 10-15 mg/kg bw/d, are secondary non-specific consequences of the maternal toxicity observed at 10-15 and 18.3-29.4 mg/kg bw/d.

In the second one-generation range finding study, post-implantation loss was increased up to 4-fold compared to controls at the dose of 21.0-34.7 mg/kg bw/d, which was the highest dose tested. This dose level was associated with a decrease in maternal body weight gain of up to 59% during premating and gestation, and a decrease in food consumption of up to 48% during lactation. Accordingly, body weights were decreased (10-16% below controls) from gestation day 14 into lactation, but had recovered at the end of lactation. Clinical chemistry parameters were also altered (see table above), but not liver weights. In contrast to the first study, in this study post-implantation loss was not affected at a slightly lower dose of 10.6-11.9 mg/kg bw/d (3.7±7.77% vs. 3.8±6.85% in controls).

In this second one-generation range finding study, pup survival was decreased on postnatal days 0 to 4 in the high and mid dose groups (75% and 86%, respectively, compared to 99% and 95% in the low dose and control groups, respectively). Furthermore, and similar to the first range finding study, a significant decrease in pup birth weights (17% and 18% below controls in the mid and high dose groups, respectively) and pup weights at weaning (up to 21% and 32% below controls, respectively) were observed in these dose groups. Again, according to RAC it is not considered clear that the effects on pup survival and pup body weight development at 10.6-11.9 mg/kg bw/d are secondary, non-specific consequences of the maternal toxicity observed.

In the EOGRTS, the mean number of implantation sites in the F1 generation was statistically significantly reduced in the highest dose group (mean dose of 15.1 mg/kg bw/d administered to male and female rats throughout the whole study). The number of implantation sites were 10.5±2.13 per dam, compared to 12.3±1.82 in controls. Consequently, F1 high dose dams delivered statistically significantly less pups (10.1±2.19 vs. 12.0±2.06 in controls). In the F0 generation high dose group, these parameters were not affected. Mean post-implantation loss was slightly, but not statistically significantly, increased in F0 and F1 dams starting from the lowest dose level. However, these changes were not dose-dependent and showed high variations. RAC notes that in this study, dose levels were chosen with the aim to produce enough viable offspring for the additional cohorts, and they are considered too low to induce the same effect on post-implantation loss as was seen in the range finding studies where higher doses were used.

Maternal toxicity in F0 and F1 high dose dams consisted of increased ALAT (up to 30% above controls) and glutamate dehydrogenase levels (79% above controls), decreased choline esterase levels

(down to 45% below controls), and increased relative liver weights (up to 28% above controls) with associated histopathology. Mean maternal body weight change during gestation was slightly, but statistically significantly, decreased in both high dose F0 and F1 dams (12 and 11% below controls, respectively), and mean maternal food consumption in these dams was slightly decreased during lactation (5 and 12% below controls, respectively). Accordingly, body weights at gestation day 20 and lactation day 14 were somewhat lower (4-8%) than in controls. Body weights of high dose F1 and F2 pups were decreased to 16% below controls at birth and did not recover until weaning, when pup body weights were still decreased (10% below controls). Decreased pup weights were associated with decreased organ weights (brain, thymus, spleen). Pup survival was not affected. A statistically significantly reduced anogenital distance was observed in F2 offspring (2.97/1.49 mm in males and females vs 3.08/1.55 mm in controls, respectively), but not in the F1 offspring (3.01/1.47 mm in males and females vs. 3.08/1.48 mmm in controls, respectively).

RAC also consulted the full study report, and found no correlation between individual maternal weight loss and the respective pup weights. Therefore, RAC considers the effects on pup body weights not secondary to maternal toxicity, and thus relevant for classification.

In the prenatal developmental toxicity study, developmental effects were observed in the mid and high dose groups of nominal 15 and 45 mg/kg bw/d, respectively (12.7 and 40.7 mg/kg bw/d effective doses). These consisted mainly of skeletal variations (delayed ossification and supernumerary ribs), post-implantation loss and decreased foetal weights. For the skeletal variations, only the incidences in the high dose group were outside the extended historical control range until 2012. Mean foetal weights were statistically significantly reduced in the mid and high dose groups, but at the mid dose the reduction was only slight (8% below controls). Post-implantation loss was increased only in the high dose group with a high variation (15.1±20.25% vs. 4.4±7.35% in controls). Malformations were observed at the top dose in 3 out of 170 foetuses (1.8%), but without a consistent pattern and at an incidence within the historical control range (0 – 2.7%). Maternal toxicity in the mid and high dose groups consisted of increased relative liver weights (11 and 19% above controls, respectively) and increased ALAT and choline esterase levels. The level of maternal toxicity was more marked at the high dose, with also a decrease in maternal food consumption (by 18%) on gestation days 6 to 8, resulting in body weight loss on these days. Mean body weights in this group were also decreased on gestation days 13 to 20, leading to a 25% decreased body weight gain over the treatment period as compared to controls. The corrected mean body weight gain was 32% below controls. As only the incidences for skeletal variations in the high dose group were outside the HCD, and malformations were also observed in this group only, RAC considers these findings per se not enough to warrant classification.

The CLP criteria states that: “Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity.” (CLP Regulation, Annex I, 3.7.2.4.2)

Effects on post-implantation loss were seen consistently in several studies, albeit with a high variation. These effects were associated with doses also leading to clinical chemistry changes indicative of maternal liver toxicity; however, only in some cases these were accompanied by changes in liver weight and liver histopathology, or by markedly reduced maternal body weights or food consumption. Effects on pup body weights were also consistently observed; starting from a dose of 10-15 mg/kg bw/d, i.e. doses without marked maternal toxicity.

In the PNDT study, skeletal variations at an incidence outside the range of the extended historical control data were only observed at the high dose of 40.7 mg/kg bw/d, and are likely secondary to the marked maternal toxicity and decreased foetal weights at this dose. Malformations observed in this high dose group lacked a consistent pattern and occurred at a very low incidence inside the historical control range. Hence, RAC does not consider these effects as relevant for classification.

However, the effects on post-implantation loss and pup body weights are considered to warrant classification, as RAC considers these not unequivocally attributable to the maternal toxicity seen in the studies.

10.10.6 Comparison with the CLP criteria

The criteria for classification in Repr. 2 for adverse effects on development is considered fulfilled for 3-(4-*tert*-butylphenyl)propionaldehyde, 4-*tert*-butyltoluene and 4-*tert*-butylbenzylaldehyde, methyl 4-*tert*-butylbenzoate and *tert*-butylbenzoic acid (TBBA) based on a read-across approach within the category. A statistically significant effect on pup weights (up to 16% lower than controls) at low doses (5 mg/kg bw/day) was demonstrated in two test guideline studies (OECD TG 415; 4-*tert*-butyltoluene and OECD TG 422; 3-(4-*tert*-butylphenyl)propionaldehyde). The effect on pup weights at 5 mg/kg bw/day were not considered secondary to maternal toxicity as no marked toxicity was noted in maternal animals at this dose level. Data from the OECD TG 421 study on 4-*tert*-butyltoluene also indicate post-implantation loss, as there were statistically significant decreases in number of offspring born, number of live pups born and increase in number of stillbirths at 15 mg/kg bw/day. There is no indication that these effects observed were secondary to maternal toxicity. Additionally, and to further support the read across within the group, these results are in line with effects observed for 2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral) (i.e. post-implantation loss and pups weight), and which formed the basis for the previous harmonised classification of this substance as Repr. 2, H361d. Death of the developing organism and altered growth are listed among the major manifestation of developmental toxicity (ECHA, 2017b). There are no substance-specific data available on developmental toxicity for 4-*tert*-butylbenzylaldehyde, methyl 4-*tert*-butylbenzoate and TBBA.

The available data provide, in a weight of evidence approach and using a read-across approach some 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. 2, 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.1B is not appropriate as the evidence for adverse effects on development from existing experimental data is considered as some evidence and not clear evidence.

10.10.7 Adverse effects on or via lactation

Table 21: 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**
3-(4-*tert*-butylphenyl)propionaldehyde EC 242-016-2			
OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test) No deviations GLP compliant Test animals: Crl:CD(SD)	3-(4-*tert*-butylphenyl)propionaldehyde EC 242-016-2 Purity: 99.0%. Vehicle: corn oil Expiration date of the lot/batch: October 15, 2011. Doses: 0, 0.5, 1 and 5 mg/kg bw/day. Male rats were dosed once daily (oral gavage) beginning 14 days before cohabitation with treated females, during	***F1 generation*** **Clinical observations** No clinical signs, no mortality in F1 generation. **Body weight** ↓ mean pup body weight at 5 mg/kg bw/day days 9 and 12 postpartum (-11% and -12%, p≤0.05 and p≤0.0, respectively). ↓ mean serum T4 concentrations in females at 1 and 5 mg/kg bw/day (both -26%, day 12 postpartum, p≤0.01). NOAEL : 1 mg/kg bw/day (pups weight) **Parental animals**	Study report, 2019. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Full study report was available to DS

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Sprague Dawley rats (males and females). 10 rats/sex/group. Assigned reliability 1 by the Registrant.	cohabitation and continuing through the day prior to scheduled euthanasia on Days 43 through 46. Female rats were dosed once daily (oral gavage) beginning 14 days before cohabitation with treated males and continuing through LD 12 (rats that delivered a litter) or GD 24 (rats that did not deliver a litter).	No clinical signs or effects on body weights in parental animals.	Additional data available in Confidential Annex Study 1- Annex I Section 3.10.1.1
4-*tert*-butyltoluene EC 202-675-9			
OECD TG 421 Reproduction / Developmental Toxicity Screening Test GLP compliant Sprague Dawley rats, males/females 12 males and 12 females per group. Assigned reliability 1 by the Registrant. Full study report was not available to DS. Differences in weight of parental animals were estimated from graphs and are associated with uncertainties.	4-*tert*-butyltoluene EC 202-675-9 Purity 96.94% Vehicle: Corn oil Substance was administered orally (gavage) at doses 0, 1.5, 5, 15, 50 mg/kg bw/day once daily The administration period for males was total 50 to 52 days including 14 days before mating and subsequent 36 to 38 days (necropsy of males was separately conducted in 3 days since the observation of sperm requires 3 days). The administration period for females was total 41 to 45 days including 14 days before mating, mating period (14 days at the longest), gestational period, and first 3 days in lactation period. The starting day of administration was set as the day 1.	**F1 generation** No pups produced at 50 mg/kg bw/day. All newborn pups of one dam at 15 mg/kg bw/day died by LD 1. ↓ number of pups born (-26%; p≤0.05) and number of live pups (-58%; p≤0.01) at day 0 at 15 mg/kg bw/day. ↓ delivery index (82.7% vs. 94.1% in controls), birth index (49.3% vs. 93.6% in controls), and live birth index (63% vs. 99.5% in controls), viability index (45% vs. 98.8% in controls) at 15 mg/kg bw/day. ↑ number of stillbirths at 15 mg/kg bw/day (4.7 vs. 0.1 in controls; not statistically significant, but slightly increasing trend with dose). ↓ number of live offspring (4.5 vs. 14.3 in controls, tendency) and viability index at LD 4 (45% vs. 98.8% in controls, tendency). ↓ body weights of pups at LD 0 (-11% and -8% in males and females, respectively) and LD 4 (-16% and -15% in males and females, respectively) at 5 mg/kg bw/day. ↓ body weights of pups at LD 0 (-32% in males and females) and LD 4 (-13% and -10% in males and females, respectively) at 15 mg/kg (based on pups from 3 dams and 1 dam, respectively). NOAEL: 1.5 mg/kg bw/day (pups weight) **Maternal animals:** ↓ body weight GD 7 at 5 mg/kg bw/day (-8%; p≤0.05 estimated from graph) and GD 14 (-10%; p≤0.05 estimated from graph) and at 15 mg/kg bw/day, GD 7 to 21, estimated from graph, -9%	Study report, 2007. Robust study summary in Registration dossier, ECHA's dissemination site, 2022. Study 3 - Annex I 3.10.1.3 Additional data available in Confidential Annex.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		at GD 21; p≤0.01. ↓ body weight at 5 mg/kg bw/day at LD 4 (estimated from graph -13%, p≤0.01, correlated with significantly decreased food consumption). ↓ body weight at 15 mg/kg bw/day LD 4 (-11% estimated from graph, only one animal). ↓ body weight, day of necropsy at 5 (-13%; p≤0.01), 15 (-18%; p≤0.01), and 50 mg/kg bw/day (-29%; p≤0.01).	

Table 22: 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 23: 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

Two studies (according to OECD TG 422; 3-(4-*tert*-butylphenyl)propionaldehyde and OECD TG 421; 4-*tert*-butyltoluene) are available on pups during lactation, until lactation day 12 and lactation day 4, respectively.

Statistically significantly reduced weights were seen on LD 9-12 and on LD 4 in pups of treated females. There was also a tendency of reduced number of live offspring and viability index at LD 4 (4-*tert*-butyltoluene). The DS considers the available data not sufficient to determine whether the effects seen are caused by exposure via lactation. In addition, there are no studies available on the quantity, quality, or composition of the milk.

It was concluded in the RAC Opinion of 2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral) that no data concerning effects on or via lacatation were available and RAC considered classification for lactation effects not warranted for lysmeral (ECHA, 2019).

10.10.9 Comparison with the CLP criteria

Since data on effects on or via lactation are insufficient, comparison with the CLP criteria is inapplicable.

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.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Classification of this category of substances for adverse effects on sexual function and fertility and adverse effects on the development of the offspring is warranted: Repr. 1B H360Fd. 2-(4-*tert*-butylbenzyl)propionaldehyde (lysmeral) and 4-*tert*-butylbenzoic acid (TBBA) already have harmonised classification as Repr.1B (H360Fd and H360F, respectively). No change to the existing classification is proposed for lysmeral. Classification in Repr.2 (H361d) for adverse effects on the development of the offspring is proposed to be added to the existing harmonised classification of TBBA.

A specific concentration limit for adverse effects on sexual function and fertility is not proposed since the ED10 values for both 3-(4-*tert*-butylphenyl)propionaldehyde and 4-*tert*-bultyltoluene (effects on fertility and lack of pregnant females) 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). The same argument applies in the case of lysmeral and TBBA.

For effects on pups weight, the two calculated ED10 values were 4.1 mg/kg bw/day (3-(4-*tert*-butylphenyl)propionaldehyde) and 3.9 mg/kg bw/day (4-*tert*-butyltoluene), which both are at the level of justifying an SCL. However, since the specific concentration limit for developmental effects in category 2 would be similar to the generic concentration limit for effect on fertility category 1, an SCL is not proposed. The same argument applies for lysmeral.

10.10.11 Specific target organ toxicity-single exposure

Not evaluated in this CLH proposal.

10.10.12 Specific target organ toxicity-repeated exposure

Not evaluated in this CLH proposal.

10.10.13 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.

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15 ANNEXES

Annex I to the CLH report.

Confidential Annex to the CLH report.