CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

Cumene

EC Number: **202-704-5**

CAS Number: **98-82-8**

Index Number: **601-024-00-X**

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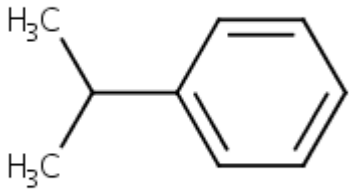
CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	**Cumene**
Other names (usual name, trade name, abbreviation)	(1-methylethyl)benzene, isopropylbenzene, propan-2-ylbenzene
ISO common name (if available and appropriate)	Not applicable
EC number (if available and appropriate)	202-704-5
EC name (if available and appropriate)	Cumene
CAS number (if available)	98-82-8
Other identity code (if available)	-
Molecular formula	C_9H_{12}
Structural formula	
SMILES notation (if available)	CC(C)C1=CC=CC=C1
Molecular weight or molecular weight range	120.194 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 80 wt %

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Cumene	≥ 80 wt % (mono-constituent)	Flam. Liq. 3 (H226), Asp. Tox. 1 (H304), STOT SE 3 (H335), Aquatic Chronic 2 (H411)	Registrants report the harmonised classification. In addition a self-classification is reported: Flam. Liq. 3 (H226), Asp. Tox. 1 (H304), STOT SE 3 (H335), Aquatic Chronic 3 (H413) 42 additional notifications (2152 notifiers, 14/3/2018) are available, see below this table.

The following C&L inventory information for self classification **for physicochemical and human health endpoints** is available for the general entry of cumene (CAS no: 98-82-8 on 14/3/2018)

Classification	Number of notifiers
Not classified	1
Flam Liq. 3 - H226	2149
Asp Tox. 1 – H304	2149
STOT SE 3 - H335, H370	2136
Acute Tox 4 – H332, 302	697
Skin Irrit. 2 – H315	4
Eye Irrit. 2 – H319	5
STOT RE 1 – H372	1

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
No data available				

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

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
No relevant additives					

Table 5: Test substances (non-confidential information) (this table is optional)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
No data available				

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	601-024-00-X	cumene	202-704-5	98-82-8	Flam. Liq. 3 Asp. Tox. 1 STOT SE 3 Aquatic Chronic 2	H226 H304 H335 H411	GHS02 GHS07 GHS08 GHS09 Dgr	H226 H304 H335 H411	-	-	Note C
Dossier submitters proposal	601-024-00-X	cumene	202-704-5	98-82-8	**Add** Carc. 2	**Add** H351		**Add** H351			
Resulting Annex VI entry if agreed by RAC and COM	601-024-00-X	cumene	202-704-5	98-82-8	Flam. Liq. 3 Carc. 2 Asp. Tox. 1 STOT SE 3 Aquatic Chronic 2	H226 H351 H304 H335 H411	GHS02 GHS07 GHS08 GHS09 Dgr	H226 H351 H304 H335 H411			Note C

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public 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	data conclusive but not sufficient for classification	Yes
Carcinogenicity	harmonised classification proposed	Yes
Reproductive toxicity	data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	hazard class not assessed in this dossier	No
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	hazard class not assessed in this dossier	No
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Current (harmonised) classification on cumene includes Hazard statements H226, H304, H335 and H411. These Hazard statements correspond to earlier classifications under the Directive on Dangerous Substances (67/548/EEC; 25th ATP) with risk phrase R10 (flammable) corresponding to H226, R65 (Harmful: may cause lung damage if swallowed) to H304, R37 (Irritating to respiratory system) to H335 and R51/53 (Toxic to aquatic organisms/May cause long-term adverse effects in the aquatic environment) to H411.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level, because this dossier only addresses hazard classes which shall normally subject to harmonized classification and labelling (Article 36 (1) of the CLP Regulation).

5 IDENTIFIED USES

Cumene is mainly used as an intermediate (approximately 95 %) for the production of phenol and acetone. In addition, the substance is a minor constituent of gasolines and solvents. Cumene is also used in the synthesis of alpha-methylstyrene, acetophenone and detergents, the manufacture of di-isopropylbenzene and as a catalyst for acrylic polyester-type resins. It can also be found as an isomer in the general C9 aromatic hydrocarbon content of solvents, especially in those used in the printing industry (ECB, 2001).

6 DATA SOURCES

This assessment is based on original study reports for each of the toxicological endpoints discussed (see specific Section for reference) and on most recent reviews and assessments, i.e., ACGIH (2017), DFG (2016), IARC (2013), NTP (2013), SCOEL (2015). In addition, relevant earlier assessments were considered for comparison (ECB, 2001; US EPA, 1997; WHO, 1999). (ECB, 2001; US EPA, 1997; WHO, 1999). The Klimisch criteria were used in each case for the reliability assessment. The database has substantially changed since publication of the NTP technical report on the toxicological and carcinogenesis studies of cumene (NTP, 2009), as evident from some more recent key studies (e.g., Chen *et al*., 2011; NTP, 2012)

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Colourless liquid with strong aromatic odour	(ECB, 2001)	
Melting/freezing point	-96 °C at 1013 h Pa	(ECB, 2001)	
Boiling point	152.7 °C at 1013 hPa	(ECB, 2001)	
Relative density	0.86 at 20 °C/4°C	(ECB, 2001)	
Vapour pressure	4.96 hPa at 20°C	(ECB, 2001)	Extrapolation; based on eight experimental data which fit well a linear regression (correlation coefficient = 0.999)
Surface tension	27.5 nN/m at 20 °C	(ECB, 2001)	Estimated
Water solubility	50 mg/L at 25 °C	(ECB, 2001)	Practically insoluble in water, soluble in ethanol and organic solvents
Partition coefficient n-octanol/water	3.55 at 23 °C	(ECB, 2001)	Measured, OECD 107

Property	Value	Reference	Comment (e.g. measured or estimated)
Flash point	31 °C (closed cup) 39 °C (closed cup)	(ECB, 2001)	
Flammability	0.9% in volume (LEL) 6.5% in volume (UEL)	(ECB, 2001)	Measured
Explosive properties	Explosive under influence of a flame	(ECB, 2001)	
Self-ignition temperature	424 °C at 1010 hPa	(ECB, 2001)	
Oxidising properties	None	(ECB, 2001)	
Granulometry	Not applicable		
Stability in organic solvents and identity of relevant degradation products	No data		
Dissociation constant	No data		
Viscosity	0.73 x 10^{-6} m²/s	(ECB, 2001)	

8 EVALUATION OF PHYSICAL HAZARDS

Not performed for this substance.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 9: Summary table of toxicokinetic studies

ADME endpoint	Species, Test conditions	Results	Reference
Absorption	**Humans, laboratory animals** (summary statement)	Cumene is readily absorbed following inhalation exposure in humans and after inhalation, oral or dermal exposure in laboratory animals	(NTP, 2013)
	Human data, inhalation	Mean* 50% (45% - 64%) retention in the respiratory tract, declining with exposure duration (*not specified: arithmetic or geometric)	(Senczuk und Litewka, 1976); (Brugnone *et al*., 1989)
	Human data, inhalation, chemical workers (no direct occupational exposure to cumene)	Blood concentrations of cumene ≈ 40 times higher than in alveolar air, supporting the blood/air partition coefficient of 37 for cumene	(Brugnone *et al*., 1989; NTP, 2013)
	Animal data, F344 rats; ♀, ♂, different routes of administration (oral and inhalation)	Readily absorbed from stomach and from lungs, after inhalation exposure cumene could be detected in blood within 5 min. In gavage studies in rats, maximum blood levels were reached within 4 h after a dose of 33 mg/kg or 8 to 16 h after a dose of 1350 mg/kg was applied.	(Research Triangle Institute, 1989) and (NTP, 2013)
	Rats, rabbits, dermal	Percutaneous absorption observed	(NTP, 2013)
	Animal studies, percutaneous flux with ethylbenzene	Ability of alkylbenzenes to penetrate the skin is significant	(DFG, 2016)
Distribution			

ADME endpoint	Species, Test conditions	Results	Reference
	Animal data, F344 rats; ♀, ♂, inhalation	Generally concentrations in the tissues are low since >90% were excreted Adipose tissues were observed to have slightly elevated concentrations at all doses, followed by liver and kidney. Inhalation studies in rats have reported half-lives of cumene disappearance from blood as 3.9 to 6.6 hours. There was no evidence of cumene accumulation in tissues following high or repeated oral doses in rats or mice.	(Research Triangle Institute, 1989) and (NTP, 2013)
	Animal data, F344 rats; ♀, ♂, gavage	Generally concentrations in the tissues are low as >90% were excreted. After exposure to a single dose of 33 mg/kg bw elevated levels of cumene were found in liver, kidney and adipose tissue. However, concentration was very low (<0.5% of total radioactivity). The findings after a multiple dosing of 33 mg/kg bw were similar to that after a single exposure. There is no indication that cumene or its radioactive metabolites accumulate in any tissue.	(Research Triangle Institute, 1989)
	Rats, mice; gavage (radiolabelled ^{14}C)	Single Exposure: a) Tissue retention Less than 3% tissue retention after 24 hrs. for rats; less than 1% for mice, (all tissues excluding stomach and intestines) b) Tissue concentration (mice) Similar for ♀ and ♂ mice, low dose (10 mg/kg); higher in ♀ mice at high dose (1000 mg/kg) <u>c) Tissue concentrations, (relative, rats vs. mice)</u> single exposure, higher in rats than in ♀ and ♂ mice, particularly in the kidneys d) Relevant tissues (rats vs. mice) Highest tissue concentrations in liver, kidney, lung (mice), or in adipose tissue, liver, kidney (rat) Repeated Exposure (only ♂rats, ♀ mice tested) After seven consecutive daily doses in mice: highest tissue concentrations in lungs of mice and in the kidney of rats Time-dependent ^{14}C accumulation in respective tissues ; Higher tissue concentrations in rat kidney and mouse lung studies correlate with higher incidence of tumours in these studies (see Section 10.7)	(NTP, 2013), (Chen *et al.*, 2011)

<table>
<tr><th>ADME endpoint</th><th>Species, Test conditions</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>Repeat dosing accumulation in liver, kidney, lung, as well as in blood, brain, heart, muscle, and spleen (only in mice)</td><td></td></tr>
<tr><td></td><td>Rats, inhalation, up to 150 days</td><td>Distribution to endocrine organs, central nervous system, bone marrow, spleen, liver</td><td>(WHO, 1999)</td></tr>
<tr><td>Metabolism</td><td>Human data, volunteers exposed to cumene vapour for 8 hrs.</td><td>2-phenyl 2-propanol [M14] or conjugates (35% of absorbed dose in urine, 48 hrs. after exposure)</td><td>(Senczuk and Litewka, 1976)</td></tr>
<tr><td></td><td>Animal data, ♂- rat, ♂-, ♀- mice, gavage, 14C radiolabelled (ring)</td><td>Cumene metabolites, urine, oral exposure; ranges after single exposure to 1.4-140 mg/kg (rats) or 10-1000 mg/kg (mice)
<table>
<tr><th rowspan="2">Substance [Id-Nr.]</th><th colspan="3">% of radiolabelled peaks</th></tr>
<tr><th>♂ rat</th><th colspan="2">mouse</th></tr>
<tr><th></th><th></th><th>♂</th><th>♀</th></tr>
<tr><td>[M1] unknown</td><td>N.D.</td><td>N.D.-trace</td><td>1.8-3.0</td></tr>
<tr><td>[M2] 2-(2-hydroxy-2-propyl) phenylsulfate</td><td>trace</td><td>N.D.-trace</td><td>N.D.-4.4</td></tr>
<tr><td>[M3] 4-(2-hydroxy-2-propyl) phenylsulfate</td><td>7-11.4</td><td>N.D.</td><td>N.D.-trace</td></tr>
<tr><td>[M4] unknown</td><td>5.2-5.6</td><td>N.D.</td><td>N.D.-trace</td></tr>
<tr><td>[M5] 2-hydroxy-2-phenylpropylsulfate</td><td>2.2-2.6</td><td>3-8.4</td><td>5.8-19.1</td></tr>
<tr><td>[M6] 2-phenyl-1,2-propandiol-2-glucuronide</td><td>N.D.-1.6</td><td>2.9-4-4</td><td>2.5-4.2</td></tr>
<tr><td>[M7] 2-phenyl-1,2-propandiol-1-glucuronide</td><td>17.8-20.1</td><td>8.6-16.9</td><td>6.1-16.5</td></tr>
<tr><td>[M8] 2-hydroxy-2-phenylproprionic acid</td><td>12.1-16.4</td><td>12.8-15.7</td><td>11.4-20.4</td></tr>
<tr><td>[M9] 2-phenyl-2-propanol glucuronide</td><td>38.1-48.4[a]</td><td>33.5-42.8</td><td>29.8-36.8</td></tr>
<tr><td>[M10] 2-phenylpropionyl glucuronide</td><td>b</td><td>N.D.</td><td>N.D.</td></tr>
<tr><td>[M11] 2-phenylpropionyl glycine</td><td>N.D.</td><td>5.1-11</td><td>2.8-3.7</td></tr>
<tr><td>[M12] S-(2-hydroxy-2-phenylpropyl)-N-acetylcysteine</td><td>4-4.9[c]</td><td>trace</td><td>trace</td></tr>
<tr><td>[M13] 2-phenyl-1-propanol glucuronide</td><td>4-4.9[c]</td><td>1.6-5.8[c]</td><td>1.5-2.3[c]</td></tr>
<tr><td>[M14] 2-phenyl-2-propanol</td><td>Trace-1.8</td><td>N.D.-1.5</td><td>N.D.</td></tr>
<tr><td>[M15] 2-phenyl-1-propanol</td><td>N.D.</td><td>N.D.-1.6</td><td>N.D.</td></tr>
<tr><td>[M16] 2-phenylpropionic acid</td><td>Trace-2.1</td><td>N.D.-trace</td><td>N.D.-trace</td></tr>
<tr><td colspan="4">N.D.=not detected; a= total M9 plus M10;</td></tr>
</table>
</td><td>(Chen et al., 2011; NTP, 2013)</td></tr>
</table>

ADME endpoint	Species, Test conditions	Results	Reference
		b=M10 reported as minor metabolite that co-eluted with M9; c= total of M12 plus M13	
	Rats, (F344); ♀, ♂, inhalation and oral	The major metabolite of urinary excretion (50% and more) was 2-phenyl-2-propanol and its glucuronide and/or sulphate conjugates	(Research Triangle Institute, 1989)
	Rats (F344) single i.v.	Very similar across all routes of application; >50% 2-phenyl-2-propanol and glucuronides or sulfate conjugates; unknown metabolite: possibly "a carboxylic acid metabolite of cumene"	(US EPA, 1997)
	Rats (F344) single inhalation		
	Rats (F344) single gavage		
	Rats (F344) repeated gavage		
	Rats, mice; oral exposure	More efficient metabolism suggested in mice compared to rats	(NTP, 2013)
	Rats, mice: radiolabelled Cumene, expired air analysis	In expired air: Cumene more than 95% of radiolabelled VOC, α-methylstyrene: 3-4% (mice), trace (rats)	(Chen *et al*., 2011)
	In vitro, rabbits, liver and lung	Highest metabolic rate for cumene in lung and liver, compared to other aromatic (and other) solvents	(Sato und Nakajima, 1987)
	In vitro, rat, mice liver and lung microsomes	α-Methylstyrene, 2-phenyl-2-propanol [M14],] 2-phenyl-1-propanol [M15]; mouse lung microsomes metabolised more cumene than microsomes from mouse liver, rat lung, or rat liver	(Chen *et al*., 2011)
Excretion	**Human data**, controlled exposure study, 10 individuals, inhalation exposure to 3 concentrations: 240, 480, 720 mg/m³, each for 8 hrs.)	Biphasic excretion of 2-phenyl 2-propanol [M14], initial excretion half-life 2 hrs, subsequent (post-exposure) half-life 10 hrs., approached zero after 48 hrs in urine	(Senczuk und Litewka, 1976)
	Human data, samples collected from healthy volunteers (from urban population and an U.S. Air force educational institution)	Some cumene eliminated in expired air (no details provided)	(NTP, 2013)
	Rats, (F344); ♀, ♂, inhalation	Urine was the major route of elimination (76.2 to 93.2%). Excretion was rapid with the majority of cumene being excreted within 24 h (78.6 to 84.6%) and after 72 h nearly complete excretion was observed (96.0 to 98.9%).	(Research Triangle Institute, 1989)
	Rats, (F344); ♀, ♂, oral	Urine was the predominant route of excretion. At 33 mg/kg bw after 8 h about 40% were excreted via urine. This value increased up to 90% after 72 h. A comparable course of excretion was also observed after a multiple exposure of 33 mg/kg bw. After an exposure to a high concentration of 1350 mg/kg bw excretion via urine was delayed. After 72 h excretion via urine was about 75%. Excretion via volatile breath was the predominant excretion pathway during the first hours after exposure accounting for 6 to 7% after 8 h compared to 3 to 4% excreted via urine at this time.	(Research Triangle Institute, 1989)
	Animal data, rats, inhalation	Half-lives, disappearance from blood, 3.9-6.6 hrs.	(NTP, 2013)
	Rats, gavage	Half-lives, disappearance from blood, 9-16 hrs.	(NTP, 2013)

ADME endpoint	Species, Test conditions	Results	Reference
	Rats, mice, oral	No evidence of accumulation	(NTP, 2013)
	Rats, mice, all routes of administration	Excreted in urine (70-90%), in feces (1-5.3%), expired air (radiolabelled VOC) (<1% - 22%); At higher doses: higher excretion via expired air ♀ mice showed higher excretion than ♂ mice via expired air	(NTP, 2013)
	Rabbits, oral	90% recovered as metabolites in urine within 24hrs.	(WHO, 1999)
	Rats, gavage	Practically no radioactivity eliminated in form of $^{14}CO_2$	(Chen *et al*., 2011; DFG, 2016)
	Rats, mice; oral administration	Minor difference between single or repeated exposure pattern in excretion	(NTP, 2013)
	Rats; i.v. administration	Enterohepatic circulation of cumene glucuronide metabolites implied Elimination half-life 8.6 hrs. for ♂, 7.3 hrs. for ♀-rats	(Chen *et al*., 2011)

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

Cumene is readily absorbed following inhalation exposure in humans and after inhalation, oral or dermal exposure in experimental animals. From rodent studies it can be concluded that cumene is widely distributed in the body, extensively metabolised and rapidly excreted, primarily in the urine (NTP, 2013; Research Triangle Institute, 1989). Most findings on metabolism of cumene are based on studies by Chen *et al*. (2011), as shown in **Figure 9-1**, with some details on quantitative comparative data for mice vs. rats documented in Table 9.

It can be concluded that oxidation to metabolite 2-phenyl-2-propanol [M14] is a key step, both in humans and in experimental animals, and that excretion in rats and in mice primarily includes metabolites 2-phenyl-1,2-propandiol 1-glucuronide [M7], 2-hydroxy-2-phenylproprionic acid [M8] and 2-phenyl-2-propanol glucuronide [M9]. Other metabolic pathways are quantitatively less relevant, but include reactive metabolites:

- One pathway includes side-chain oxidation of [M14] to α-methyl styrene (AMS). AMS induced carcinogenic effects in experimental animals (NTP, 2007). AMS probably is further oxidised to AMS-oxide, a substance, which has been demonstrated to be mutagenic in *S. typh*. (see Section 10.6).
- Another metabolic pathway is ring oxidation, with two metabolites, 2-(2-hydroxy-2-propyl) phenylsulfate [M2] and 4-(2-hydroxy-2-propyl) phenylsulfate [M3], found *in vivo* after cumene exposure in the urine of mice and rats. However, the aggregated amount of those two metabolites [M2, M3] is limited with 7-11.4% in rats and up to 4.4% in mice (% of total ^{14}C recovered). Because of the postulated intermediates (arene oxides, catechol and quinonemethide) this metabolic pathway is regarded as potentially relevant for adverse health effect: For example, for another alkylated benzene substance, styrene, ring hydroxylation is associated with mouse specific metabolism in lung Clara cells, leading to cytotoxicity, Clara cell destruction and lung tumours in male mice (Cruzan *et al*., 2012).

Chen *et al*. (2011) demonstrated accumulation of ^{14}C radioactivity in the female mouse lung after repeated oral cumene exposure, in contrast to male rats, where no accumulation in the lung was observed. However, the authors did not link the recorded radioactivity to a certain metabolic pathway, did not identify specific metabolites or specific responsible enzymes (within CYP subfamily, critical for metabolism in the respiratory tract) and thus do not allow further insight into the toxicokinetics in the respiratory tract of mice or rats (further discussion in Section10.7.1). In the study by Research Triangle Institute (1989) rats were

exposed via inhalation or gavage against radiolabelled cumene. The authors did not show an accumulation of ^{14}C in the lung. Adipose tissues were observed to have slightly elevated concentrations at all doses, followed by liver and kidney.

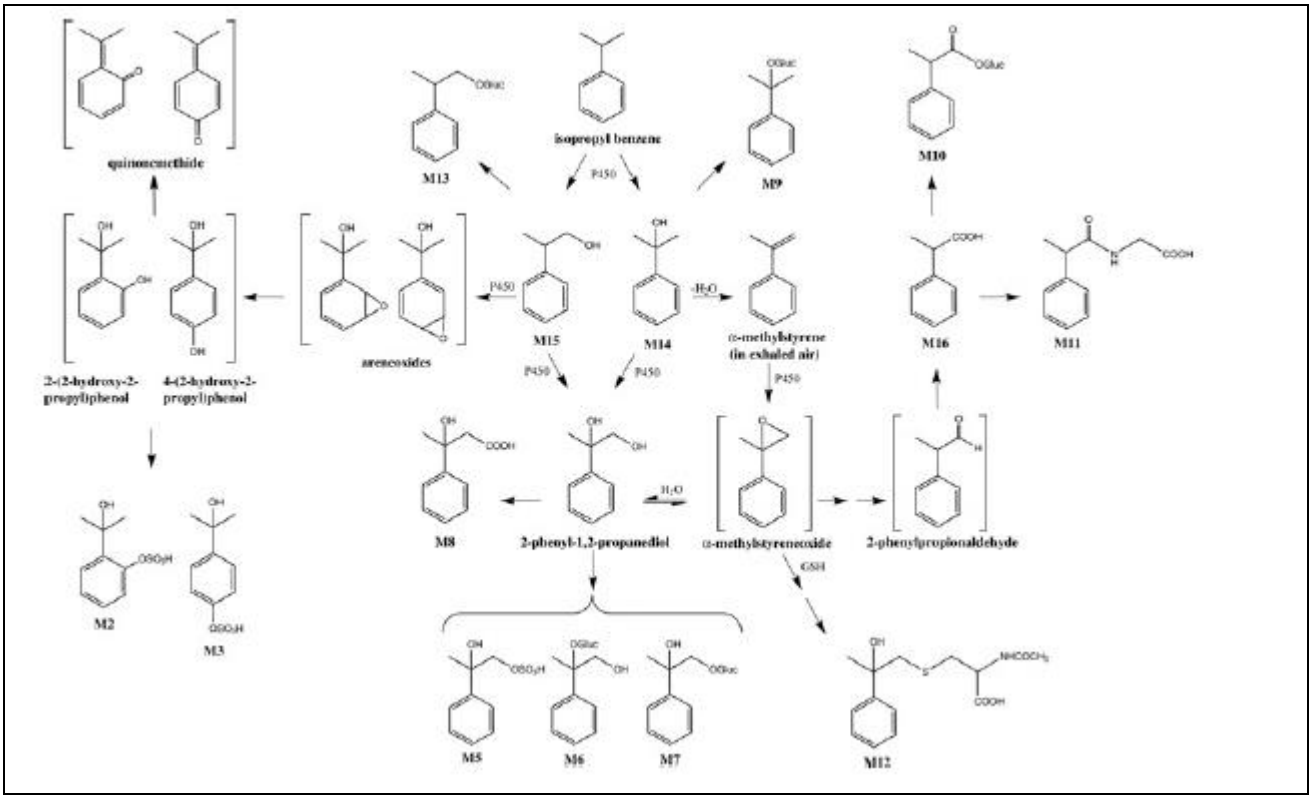


Figure 9-1: Detected and postulated metabolites of cumene adopted from DFG (2016), as modified from Chen *et al.* (2011) (mainly based on data from rodents, but assumed to be applicable to humans)

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity

Evaluation not performed for this substance

10.2 Skin corrosion/irritation

Evaluation not performed for this substance

10.3 Serious eye damage/eye irritation

Evaluation not performed for this substance

10.4 Respiratory sensitisation

Evaluation not performed for this substance

10.5 Skin sensitisation

Evaluation not performed for this substance

10.6 Germ cell mutagenicity

Table 10: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Bacterial gene mutation (Ames test)				
S. typhimurium (TA 98, TA 100), ± S9-mix, preincubation (Tests 1-4), *E.coli* WP2 ± S9-mix, preincubation (Tests 5,6) No explicit mentioning of OECD-TG or GLP, equivalent reliability, but only three strains tested Reliability according to disseminated database: study not reported Reliability according to authors of this evaluation: 3	Cumene Purity: >99%; no impurities > 0.1% observed Vehicle DMSO	<u>All tests:</u> Measures taken to avoid influence from volatility (sealed tubes), results reported by mean ± SEM, triplicate test <u>Test 1 (TA 100)</u> a) without activation: 0 - 250 µg/plate (≥125 µg/plate (slightly toxic or toxic) + positive control (sodium azide), b) with activation (10% phenobarbital/benzoflavone-induced rat liver S9-mix): 0 - 500 µg/plate (≥250 µg/plate (slightly toxic) + positive control (benzo(a)pyrene) <u>Test 2 (TA 100)</u> a) without activation: 0 - 250 µg/plate (≥100 µg/plate	<u>All tests:</u> without and with activation (S9-mix): → negative	(NTP, 2012)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		(slightly toxic) + positive control (sodium azide) b) 10% phenobarbital/benzoflavone-induced rat liver S9-mix 0 - 250 µg/plate(250 µg/plate (slightly toxic) + positive control (benzo(a)pyrene) <u>Test 3 (TA 98)</u> a) without activation: 0 - 500 µg/plate (≥250 µg/plate (slightly toxic) + positive control (2-nitrofluorene), b) with activation (10% phenobarbital/benzoflavone-induced rat liver S9-mix–mix): 0 - 500 µg/plate + positive control (2-aminoanthracene) <u>Test 4 (TA 98)</u> a) without activation: 0 - 250 µg/plate (≥100 µg/plate (slightly toxic or toxic, resp.) + positive control (2-nitrofluorene) b) 10% phenobarbital/benzoflavone-induced rat liver S9-mix: 0 - 500 µg/plate (≥250 µg/plate (slightly toxic) + positive control (2-aminoanthracene) <u>Test 5 (*E.coli* WP2)</u> a) without activation: 0 - 500 µg/plate (≥100 µg/plate (slightly toxic or toxic, resp.)) + positive control (4-nitroquinoline-N-oxide) b) with activation (10% phenobarbital/benzoflavone-induced rat liver S9-mix): 0 - 500 µg/plate (500 µg/plate (slightly toxic)) + positive control (2-aminoanthracene) <u>Test 6 (*E.coli* WP2)</u> a) without activation: 0 - 500 µg/plate (≥125 µg/plate (slightly toxic or toxic, resp.)) + positive control (4-nitroquinoline-N-oxide) b) with activation (10%		

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		phenobarbital/benzoflavone-induced rat liver S9-mix): 0- 500 µg/plate (500 µg/plate (toxic)) + positive control (2-aminoanthracene)		
S. typhimurium (TA 97, TA 98, TA 100, TA 1535) ± S9-mix, preincubation NTP conducts its studies in compliance with FDA Good Laboratory Practice Regulations. Limited remaining uncertainties because of a) potential volatility losses, b) highest concentrations not always reaching toxicity level, but TA 102 or E. coli strains not tested Reliability according to disseminated database: 1 Reliability according to authors of this evaluation: 3	Cumene Purity: >99.9%; no impurities > 0.1% observed Vehicle: no data	<u>All tests:</u> Each trial: triplicate plates plus concurrent positive and negative controls, 5 doses of cumene. All trials repeated. Negative trials with S9-mix repeated with higher S9-mix concentrations (10%; 30%). In some, but not in all tests, the highest concentration (i.e., 166 or 333 µg/plate) was slightly toxic. For TA97 (-S9-mix) already 100 µg/plate was slightly toxic. a) without activation: 0-333 µg/plate or 0-166 µg/plate positive controls: sodium azide (TA100; TA1535); 9-aminoacridine (TA 97), 4-nitro-o-phenylenediamine (TA98) b) with activation (from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9-mix): positive controls (all strains): 2-aminoanthracene	<u>All tests:</u> without and with activation (S9-mix): → negative	(NTP, 2009) Study also reported in (ECHA Dissemination, 2018, genetic toxicity, in vitro, key #001)
S. typhimurium (TA 98, TA 100, TA 1535, TA1537) ± S9-mix, preincubation Certified compliance with GLP, but TA 102 or E. coli strains not tested Reliability according to disseminated database: 1 Reliability according to authors of this evaluation: 3	Cumene; Purity: no data Vehicle: Pluronic F127, prepared at 50% (w/w) in ethanol	<u>All tests:</u> 7 dose levels of cumene (minimum 4 non-toxic dose levels) along with untreated, vehicle and positive control, ± 10% S9-mix, plus additional positive control in F127; plus single maximally water soluble dose of cumene tested on all four tester strains ± S9-mix. Prior range finding study to determine toxic potency. Dose range: 33, 67, 100, 333, 667, 1000, 2000 µg/plate All experimental results confirmed in repeat experiment. a) without activation: positive control: TA98, 5.0 µg 2-nitrofluorene TA100, TA1535: 2.5 µg sodium azide	<u>All tests:</u> without and with activation (S9-mix): → negative	(Lawlor und Wagner, 1987) Study also reported in (ECHA Dissemination, 2018, genetic toxicity, in vitro, #005,supporting)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		TA1537, 75 µg 9-aminoacrdine b) with activation (Aroclor induced rat liver microsomes S9-mix): positive control: TA98, TA100, TA1535, TA1537: 2.0 µg aminoanthracene		
S. typhimurium (TA 98, TA 100, TA 1535, TA1537, TA1538) ± S9-mix Reliability according to disseminated database: 4 Reliability according to authors of this evaluation: 4	Cumene, no details provided	Tested up to 5000 µg/plate, no details provided	<u>All tests:</u> without and with activation (S9-mix): → negative	(Huels, 1987; unpublished, cited from ECHA Dissemination, 2018, genetic toxicity, in vitro, #008, other)
S. typhimurium (TA 98, TA 100, TA 1535, TA1537) ± S9-mix, Reliability according to disseminated database: 4 Compliance with Monsanto Standard Operation Procedures. According to test authors: "minor GLP violations did not impact study results" Potential Influence of volatility may not be excluded Reliability according to authors of this evaluation: 2	Cumene Purity: 99.5% Vehicle: ethanol	<u>All tests:</u> Up to 0.2 µl/plate and 20 µl/spot (spot test) ± S9-mix; 0.2 µg/plate were toxic to all four test strains ± S9-mix; triplicate testing, solvent controls, non-solvent controls, positive controls a) without activation: Positive controls TA98, TA100: 4-nitroquinoline-N-oxide TA1535: $NaNO_2$ TA1537: 9-aminoacridine b) with activation (S9-mix from livers of Aroclor 1254-induced male Sprague-Dawley rats and male CD-1 mice): Positive controls TA98: 2-acetylaminofluorene TA100: benzo(a)pyrene TA1535, TA1537: 2-aminoanthracene	<u>All tests:</u> without and with activation (S9-mix): → negative	(Monsanto Co, 1985) Study also reported in: (ECHA Dissemination, 2018, genetic toxicity, in vitro, #010, other)
S. typhimurium (TA 98, TA 100, TA 1535,	Cumene Purity: no	3.6- 3606.0 µg/plate (0.03 - 30 µmol/plate) ± S9-mix (4 doses);	<u>All tests:</u> without and with	(Florin *et al.*, 1980)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
TA1537) ± S9, spot test and plate incorporation TA 102 or E. coli strains not tested Reliability according to disseminated database: 4 Reliability according to authors of this evaluation: 3	data Vehicle: no data	toxic dose ≥ 3 µmoles/plate; No details on substance specific test outcome provided (qualitative result documentation) a) without activation: positive control all testers: N-methyl-N'-nitro-N-nitrosoguanidin b) with activation (S9-mix: from Aroclor 1254 or Methylcholanthrene induced rats) positive control all testers: 2-aminoanthracene	activation (S9-mix): → negative	Study also reported in (ECHA Dissemination, 2018, genetic toxicity: in vitro, #011 other))
S. typhimurium (TA 98, TA 100, TA 1535, TA1537, TA1538) ± S9, plate incorporation Reliability according to disseminated database: study not reported Reliability according to authors of this evaluation: 4	Cumene, reagents of the highest available purity, but generally purity was not determined and not specified	Study included without details in report on 300 chemicals tested, identified in drinking water. Max. dose 5 mg/plate or a (lower) dose giving a toxic response (not reported for cumene). No explicit details on S9-mixmetabolic activation provided. Positive and negative (solvent) controls were included.	<u>All tests:</u> without and with activation (S9-mix): → negative Cumene was also tested negative in *S. typhimurium* desiccator testing (no strain and no details provided)	(Simmon *et al*., 1977)
S. typhimurium (TA 100) ± S9-mix, Spot test Reliability according to disseminated database: 3 Reliability according to authors of this evaluation: 4	Cumene Purity: no data	Study included without details in report on almost 300 chemicals tested, identified in tap water. Approximately one third of those have been spot-tested in TA100 ±S9-mix. No details provided.	Compounds tested and found to be mutagenic in *S. typhimurium* TA100 include isopropylbenzene (cumene) → positive	(Tardiff *et al*., 1978) Study also reported in (ECHA Dissemination, 2018, genetic toxicity, in vitro, #007, other)
Yeast *S. cerevisiae*				
Yeast *S. cerevisiae* D3 assay, suspension Reliability according to disseminated database: study not reported Reliability according to authors of this evaluation: 4	Cumene, reagents of the highest available purity, but generally purity was not determined and not specified	Study included without details in report on 300 chemicals tested, identified in drinking water, only "some of which" were tested in *Saccharomyces cerevisiae* D3. Positive and negative (solvent) controls were included. Cytotoxicity not reported.	negative	(Simmon *et al*., 1977)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Mammalian Cells				
CHO/HGPRT mutation assay ± S9-mix Reliability according to disseminated database: 1 Certified to be conducted in compliance with GLP, but stability of test or control substances have not been determined. Reliability according to authors of this evaluation: 2	Cumene Purity: 99.7% Vehicle: Pluronic polyol F127 (1:1 in ethanol)	Test for ability to induce forward mutations at the HGPRT-locus of Chinese Hamster Ovary Cells (CHO-cells). <u>All tests:</u> Untreated control, solvent control (F127), three doses of positive controls 9 concentrations in dose range: 8-225 µg/ml a) without activation: positive control: ethyl methanesulfonate (0.2 µl/ml) (toxic > 125 µg/ml) b) with activation (S9-mix from livers of Aroclor 1254-induced rats): positive control: benzo(a)pyrene (4 µg/ml) (toxic > 125 µg/ml)	<u>All tests:</u> without and with activation (S9-mix): → negative	(Yang, 1987) Study also reported in (ECHA Dissemination, 2018, genetic toxicity: in vitro, #004, key))
CHO/HGPRT mutation assay ± S9-mix Reliability according to disseminated database: 4 Reliability according to the authors of this evaluation: 2	Cumene, Purity: no data Vehicle: Pluronic F127 (1:1 in ethanol)	Test for ability to induce forward mutations at the HGPRT-locus of Chinese Hamster Ovary Cells (CHO-cells). <u>All tests:</u> Untreated control, solvent control (F127), positive controls <u>Test 1</u> (November, 1984) 8, 16, 32, 64, 128, 150, 175 µg/ml ±S9-mix; a) without activation: cytotoxicity (colony counts) ≥ 128 µg/ml Positive control: Ethylmethanesolfonate b) with activation (S9-mix from livers of Aroclor 1254-induced rats): cytotoxicity (colony counts) ≥ 128 µg/ml; cell count reduction already at ≥ 16 µg/ml Positive control: Benzo(a)pyrene <u>Test 2</u> (February, 1985)	Test 1, negative ± S9-mix, but potential positive outlier at 175 µg/ml (+S9-mix). Confirmation of effect as outlier by test 2 (negative in test 2) → negative	(Gulf Oil Corporation, 1985a) Study also reported in (ECHA Dissemination, 2018, genetic toxicity, in vitro, #009, other))

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		because of a potential positive outlier at 175 µg/ml +S9-mix Test 1 a repeat test (Test 2) was performed 150,175 µg/ml +S9-mix Positive control: Benzo(a)pyrene		
Chromosomal aberrations (CHO cells) ± S9-mix Reliability according to disseminated database: 1 Compliance with GLP stated by authors. Reliability according to authors of this evaluation: 2	Cumene Purity: 99.7%, Vehicle: Pluronic F127 (1:1 in ethanol)	Test for chromosomal aberrations in Chinese hamster ovary cells: negative control: untreated cells, plus vehicle control (F127) a) without activation: Dosing 0, 19-200 µg/ml (7 doses), Toxic at 200 µg/mL, high dose positive control: triethylenemelamine b) with activation (S9-mix from livers of Aroclor induced rats): Dosing 0, 24-225 µg/ml (6doses), Toxic at 225 µg/mL (+S9-mix), high dose	Negative –S9-mix, Increased vs. vehicle control at 156 µg/ml + S9-mix (low F127 in control), but no statistically significant increase compared to untreated control and within historical control range, regarded as negative by authors → inconclusive	(Putman, 1987a) Study also reported in (ECHA Dissemination, 2018, genetic toxicity: in vitro, #003, key)
UDS –S9-mix Reliability according to disseminated database: no documented study Authors state GLP compliance Reliability according to authors of this evaluation: 2	Cumene Purity: no data Vehicle: pluronic F68 Polyol	Tested for unscheduled DNA synthesis using primary rat hepatocytes. Negative control: vehicle (F68), untreated control, positive control: 2-acetylaminofluorene, dosing (triplicate test) : 8 – 128 µg/ml (5 doses), 128 µg/ml toxic	An increase in grain counts was obtained at 16 and 32 µg/ml. Although this increase in grain counts was not regarded to be clearly positive by authors, there was a significant increase ($p<0.01$) in percentage of cells in repair at those dose levels. → positive, retested by Curren (1987)	(Gulf Oil Corporation, 1984)
UDS – S9-mix Reliability according to disseminated database: 1 Authors state GLP compliance, but note that, e.g., purity and stability	Cumene Purity: 99.7% Vehicle: Pluronic Polyol F127 (1:1; ethanol)	Tested for unscheduled DNA synthesis using primary rat hepatocytes. Primary hepatocytes, rat F344, without metabolic activation, vehicle control, negative control, positive control, test specific confounding factors not reported	No significant increase in unscheduled DNA synthesis as measured by mean number of net grain counts (i.e., an increase of at least 5 counts over control)	(Curren, 1987) Study also reported: (ECHA Dissemination, 2018, genetic toxicity, in vitro,

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
of test and control substance have not been determined Reliability according to authors of this evaluation: 2		13 doses: 1-128 µg/mL; doses > 24 µg/mL toxic; fully evaluated at 6 dose levels (1-24 µg/ml). This study was performed as a retest because of increased cell repair observed in Gulf Oil Cooperation (1984)	→ negative	key #002)
(Assumed or confirmed) METABOLITES				
α – methyl styrene				
Salmonella typhimurium TA97, TA98, TA100, TA 1535 ± (rat or hamster) S9-mix, *in vitro* TA 102 or E. coli strains not tested Reliability according to disseminated database: study not reported Reliability according to authors of this evaluation: 3	α - Methyl styrene Purity: 99.5% Vehicle: no data	<u>All tests:</u> Each trial consisted of triplicate plates including concurrent positive and negative controls, plus 5 doses α - Methyl styrene. High dose limited by toxicity. All trial repeated at the same or higher S9-mix fraction Dosing: 1-3333 µg/plate a) without activation positive control: TA100, TA1535: sodium azide TA97: 9-aminoacridine TA98: 4-nitro-o-phenylenediamine slight toxicity at 333 µg/plate b) with activation (S9-mix from Aroclor 1254-induced male Sprgue-Dawley rat or Syrian hamster liver): slight toxicity at 1000 µg/plate (S9-mix) positive control: all strains 2-aminoanthracene	<u>All tests:</u> without and with activation (S9-mix): → negative	(NTP, 2007)
Chromosomal aberrations, CHO cells, no metabolic activation Reliability according to disseminated database: 1 Reliability according to authors of this evaluation: 2	α - Methyl styrene Purity: 99.5% Vehicle: DMSO	<u>All tests:</u> Negative control: vehicle (DMSO) 2 trials <u>Trial 1:</u> Dosing: 100-200 µg/ml (3 doses) positive control: Mitomycin C not toxic up to highest dose	<u>In both trials:</u> → negative	(NTP, 2007) Study also reported in: (ECHA Dissemination, 2018, genetic toxicity: in vitro, #007, supporting)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		Trial 2: Dosing: 37.7-251.3 µg/ml (4 doses) positive control: Mitomycin C toxic at highest dose: 251.3 µg/ml		
SCE, *in vitro*, tested only –S9-mix Reliability according to disseminated database: 2 Reliability according to authors of this evaluation: 3	α - Methyl styrene Purity: > 97% Vehicle: acetone	Test for induction of sister-chromatid exchanges by test substance in cultured human lymphocytes Control cultures treated with vehicle (acetone) Dosing: 5 doses (0.1-4 mM), cell cycle delay (measure for toxicity) increased at 4 mM. Limited documentation (only graphical presentation of results, no individual results for test substance and controls reported)	Weakly positive – S9-mix at > 1 mM (less than doubling of SCEs compared to corresponding controls)	(Norppa und Vainio, 1983) Study also reported in: (ECHA Dissemination, 2018, genetic toxicity, in vitro, #010, supporting)
SCE, *in vitro*, ± S9-mix Reliability according to disseminated database: 2 According to disseminated database, study design comparable to OECD guideline 479 Reliability according to authors of this evaluation: 2	α - methyl styrene Purity: 99.5% Vehicle: DMSO	All tests: Negative control: vehicle (DMSO) a) without activation: Dosing: 5-166.7 µg/ml (4 doses) positive control: Mitomycin C 166.7 µg/ml toxic b) two trials with activation (S-9 mix from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) Dosing Trial 1: 5-166.7 µg/ml (4 doses) Dosing Trial 2: 50-149.9 µg/ml (3 doses) positive control: cyclophosphamide 166.7 µg/ml toxic	→ Negative without S9-mix Trial 1: → at 50 µg/ml +S9 relative change in SCEs/chromosome: 28.42% , trend: $p \leq 0.001$ → positive Trial 2: Dose related increase of SCE at 50, 124.4, or 149.9 µg/ml +S9 (relative change in SCEs/chromosome: 39.59, 49.16, 82.77 %, respectively) trend: $p \leq 0.001$ → positive	(NTP, 2007) Study also reported in: (ECHA Dissemination, 2018, genetic toxicity, in vitro, #003, key)
α - methyl styrene oxide				
S. Typh. TA100, preincubation	α - methyl styrene	Dosing: 7 doses (0.01-10 µmoles/ preincubation tube) plus DMSO	Dose related increase in number of	(Rosman *et al.*,

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Reliability according to disseminated database: substance not registered under REACH Reliability according to authors of this evaluation: 3	oxide Purity: no data Vehicle: DMSO	(negative control), highest dose (10 µmole) toxic, triplicate plates. Positive control: glycidol (no results on positive control reported), but specific potency data for other derivatives of α - methyl styrene oxide	revertants → positive	1986)

Table 11: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Micronuclei				
F344 ♂rats, i.p. No explicit mentioning of OECD-TG. NTP conducts its studies in compliance with FDA Good Laboratory Practice Regulations. Reliability according to disseminated database: 1 Reliability according to authors of this evaluation: 2	Cumene Purity: >99.9%; no impurities > 0.1% observed Vehicle: corn oil	Endpoint: micronucleated polychromatic erythrocytes, in bone marrow. 2 Trials: n=5 animals/dose/trial Negative controls: corn oil (vehicle) Positive controls: injected 25 mg/kg (3x, intervals 24h) cyclophosphamide <u>Trial 1:</u> Dosing: 6 groups: 78.13 - 2500 mg/kg three times (intervals 24h); 2500 mg/kg high mortality <u>Trial 2:</u> 0, 312-2500 mg/kg three times; 2500 mg/kg elevated mortality	<u>Trial 1:</u> Pairwise comparison. Highest statistically evaluated dose (1250 mg/kg) significantly elevated number of micronucleated PCE (P=0.0001), trend (P<0.001; highest dose, 2500 mg/kg, excluded from statistical analysis) → positive <u>Trial 2:</u> Pairwise comparison. Micronuclei elevated at all four tested doses (312, 625, 1250 mg/kg, 2500 mg/kg) (P= 0.0052, P= 0.0194, P=0.0033; P=0.0192), but not significant (criterion: P≤0.006), nonsignificant trend (P=0.085) → questionably positive → combined: positive	(NTP, 2009) Study also reported in (ECHA Dissemination, 2018, genetic toxicity, in vivo, #002, key)
F344/ DuCrl, ♂rats, gavage No explicit mentioning of OECD-TG or GLP, but equivalent reliability ensured.	Cumene Purity: >99%; no impurities > 0.1% observed Vehicle: Corn oil	6 animals/dose group, vehicle control (corn oil), 1x/day, 4 consecutive days, gavage, 0, 200, 400, 800 mg/kg/d; positive control: ethyl methanesulfonate (200 mg/kg/d) % of circulating reticulocytes significantly reduced at top dose (30%),	No significant increases in micronucleated erythrocytes (NCE) or reticulocytes (PCE) were observed → negative	(NTP, 2012)

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Reliability according to disseminated database: study not reported Reliability according to authors of this evaluation: 1		supports dose selection		
$B6C3F_1$ mice, ♀,♂; gavage No explicit mentioning of OECD-TG or GLP, but equivalent reliability ensured. Reliability according to disseminated database: study not reported Reliability according to authors of this evaluation: 1	Cumene Purity: >99%; no impurities > 0.1% observed Vehicle: Corn oil	6 animals/dose group, vehicle control (corn oil), 1x/day, 4 consecutive days, gavage, 0, 312,625,1250, mg/kg/d (♂) or 0,250,500,1000 mg/kg/d (♀) ; positive control: ethyl methanesulfonate (150 mg/kg/d; n=5)	No significant increases in micronucleated erythrocytes (NCE) or reticulocytes (PCE) were observed → negative	(NTP, 2012)
$B6C3F_1$ mice, ♀,♂; inhalation No explicit mentioning of OECD-TG. NTP conducts its studies in compliance with FDA Good Laboratory Practice Regulations. Reliability according to disseminated database: 1 Reliability according to authors of this	Cumene Purity: >99.9 %; no impurities > 0.1% observed	Endpoint: micronucleated cells in normochromatic erythrocytes after 3 month inhalation exposure Concentrations: ♂: 0, 62.5, 125, 250, 500, 1000 ppm (306-4900 mg/m³) ♀: 0, 62.5, 125, 250, 500 ppm (306-2450 mg/m³) n=9 or 10 animals/dose group; exposure 6 hrs. per day, 5 days/week, 14 weeks	Peripheral Blood Erythrocytes of Mice following inhalation treatment for 3 months: no significant difference from concurrent air control group, no significant trend → negative	(NTP, 2009) Study also reported in (ECHA Dissemination, 2018, genetic toxicity: in vivo, #001,key)

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
evaluation: 2				
Swiss Mice, ♀,♂; gavage No explicit mentioning of OECD-TG, GLP compliance is given (including certificate) Reliability according to disseminated database: 2 Reliability according to authors of this evaluation: 3	Cumene; purity: 2.5 g cumene in 50 mL paraffin oil (5% w/v) (according to Disseminated database)	Test for micronucleated polychromatic erythrocytes in bone marrow Dosing: 0.25, 0.5, 1 g/kg body weight, gavage, BR Swiss mice, 2 days (highest dose some only 1 single day), at ≥ 1.25 g/kg ♀mortality. N= 10/dose/sex. Negative control: paraffin oil (20 ml/kg), Positive control: cyclophosphamide (N=4)	No effect on micronucleated polychromatic erythrocytes in bone marrow under conditions of this test → negative	(Gulf Oil Corporation, 1985b) Study also reported in (ECHA Dissemination, 2018genetic toxicity: in vivo, #003, supporting)
Comet Assays				
♂, F344/N, gavage No explicit mentioning of OECD-TG or GLP, but equivalent reliability ensured. Reliability according to disseminated database: study not reported Reliability according to authors of this evaluation: 1	Cumene Purity: >99%; no impurities > 0.1% observed Vehicle: corn oil	DNA-damage analysed in blood, lung, kidney, liver 6 animals/dose group, vehicle control (corn oil), 1x/day, 4 consecutive days, gavage, 0, 200, 400, 800 mg/kg/d; positive control: ethyl methanesulfonate (200 mg/kg/d)	Liver positive for % tail DNA (p=0.004 at highest dose: 800 mg/kg/d; p=0.002 for trend), all other sites negative (blood, lung, kidney) → weakly positive for liver, male rats	(NTP, 2012)
♀,♂; B6C3F$_1$ mice, gavage No explicit mentioning of OECD-TG or GLP, but equivalent	Cumene Purity: >99%; no impurities > 0.1% observed Vehicle: corn	Blood, lung, kidney, liver 6 animals/dose group, vehicle control (corn oil), 1x/day, 4 consecutive days, gavage, 0, 312, 625, 1250, mg/kg/d (♂) or 0, 250, 500, 1000 mg/kg/d (♀) ; positive	♀: lung positive for % tail DNA (p=0.016 at highest dose: 1000 mg/kg/d; p=0.008 for trend), all other sites negative (blood, lung, kidney) ♂: negative all sites	(NTP, 2012)

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
reliability ensured. Reliability according to disseminated database: study not documented Reliability according to authors of this evaluation: 1	oil	control: ethyl methanesulfonate (150 mg/kg/d; n=6)	→ weakly positive for lung, female mice	
FLARE: Fragment Length Analysis with Repair Enzyme Reliability according to disseminated database: study not reported Reliability according to authors of this evaluation: 4	Cumene Purity: no data	80 SD rats were assigned to 4 dose groups, exposed to cumene vapour for 90 days, 6h/d, to 0, 8.05, 80.13 or 800.85 ppm (40-3920 mg/m³). In hepatocytes and lymphocytes olive tail moment and tail length were measured and rOGG1 mRNA expression from hepatocytes was scored for cells from different exposure durations (1d, 14d, 28d, 90d). OGG1 is a DNA damage repair gene. The other assays indicate DNA damage similar to the Comet assay.	Significant changes olive tail moment and tail length, indicating DNA damage in hepatocytes and lymphocytes from cumene exposure. OGG1 gene expression to repair oxidative DNA damage in liver was inhibited after significant increase at the first day of exposure. The results demonstrate oxidative DNA damage, but a dose-response or duration-effect relationship cannot be established from this study.	(Kim *et al.*, 2008)
Mutations in Tumours				
Biochemical analysis (including mutation analysis) Reliability according to disseminated database: study not reported Reliability according to authors of this evaluation: 1	Cumene Purity: >99.9%; no impurities > 0.1% observed (from NTP, 2009)	Study includes 52 cumene induced lung tumours examined for K-*ras* mutations, p53 mutations, p*53* protein expression and "loss of heterozygosity" (LOH). (52= 6 adenoma, 46 carcinoma) and compared to control (concurrent: n=7 tumours; historical: n=117 tumours). 45 tumours in exposed ♂ examined, 9 tumours in ♀.	**<u>K-*ras* or p*53* mutation in tumours observed:</u>** 50% in adenoma (3/6), 52% in carcinoma (24/46) **<u>K-*ras*:</u>** Mutations in cumene induced lung tumours -dose response (treatment ppm; no. of tumours with K-*ras* mutations %) Control (historical): 0 ppm:28% Control (concurrent): 0 ppm: 14% Exposed 125 ppm: 25% Exposed 250 ppm: 77% Exposed 500 ppm: 94% Exposed 1000 ppm: 100%	(Hong *et al.*, 2008)

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			Exposed (total): 87% K-*ras* mutations more prevalent in ♂ (91%; n=41/45) vs. ♀ (57%; n=4/7) (few tumours from ♀ analysed!) <u>K-ras mutation spectra:</u> <u>predominant K-ras mut. in exposed:</u> -codon 12, G→T transversion (36%), G→T transversion in hist. control (18%) -codon 61, A→G transitions (29%), A→G transitions in hist. control (6%) <u>predominant K-ras mut. in control:</u> Codon 12, G→A transitions (42%) **<u>p*53*:</u>** Control (historical): no data provided Control (concurrent): 0 ppm: 0% Exposed (total): 52% p*53* mutations more prevalent in ♂ (58%; n=26/45) vs. ♀ (14%; n=1/7) (few tumours from ♀ analysed!) p53 mutations were correlated with increased p53 protein expression and protein expression was exposure related: p53 <u>protein expression</u> changed in tumours: control: 1/7 (14%) 125 ppm: 1/4 (25%) 250 ppm: 6/13 (46%) 500 ppm: 8/18 (44%) 1000 ppm: 14/17 (82%) Exposed (total): 29/52 (56%) **LOH analysis:** LOH on chromosome 6 near K-*ras* gene was observed: 12% in carcinomas (cumene exposed) 0% in adenoma (cumene exposed) 0% in spontaneous carcinoma LOH of the C3H/He allele was observed on chromosome 4 near p*16* gene (allele loss of p16 detected in human cancer):	

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			13% in carcinomas (cumene exposed) 17% in adenoma (cumene exposed) 0% in spontaneous carcinoma	
(Assumed or confirmed) metabolites				
α - methyl styrene				
Micronuclei, *in vivo*, ♂,♀ in mice Reliability according to disseminated database: 2 Reliability according to authors of this evaluation: 2	α - methyl styrene Purity: 99.5%	3-month inhalation exposure of ♂ and ♀mice (♂,♀: 0, 75, 150, 300, 600, 1000 ppm; i.e., 0, 360-4800 mg/m³, n=10/sex/group). Peripheral blood samples were scanned for the frequency of micronuclei in normochromatic erythrocytes (NCE) and in polychromatic erythrocytes (PCE)	<u>Peripheral Blood:</u> ♀: Trend ($p \leq 0.001$) and highest concentration (1000 ppm: p=0.0006) positive for increase of micronucleated cells (NCE); no increase in micronucleated PCE seen at the 1000 ppm dose. No dose-response in the percent PCE ♂ negative response, no dose related changes → weakly positive, significance uncertain	(NTP, 2007) Study also reported in (ECHA Dissemination, 2018, genetic toxicity, in vivo, key)
Micronuclei, *in vivo*, ♂, in mice Reliability according to disseminated database: study not reported Reliability according to authors of this evaluation: 2	α - methyl styrene Purity: 99%	*I*CR-mice, (n= 6/dose, orally) dosage: 0, 500, 1000, 2000 mg/kg, single exposure. Bone marrow cells were scanned for the frequency of micronuclei in polychromatic erythrocytes (PCE). Positive control: mitomycin C. No inhibition of proliferation within the dose range of this test. No further data on cytotoxicity provided.	→ negative	(Rim *et al.*, 2012)

For α - methyl styrene only the most relevant studies on genotoxicity are provided in Table 10 or Table 11, respectively. A more complete overview is found, e.g., in NTP (2007).

Table 12: Summary table of human data relevant for germ cell mutagenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No human data on cumene are available, which are relevant for germ cell mutagenicity assessment				

10.6.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

No human data were available for the assessment of genotoxicity of cumene. Results of *in vitro*- or *in vivo*-testing are summarised in Table 10 or Table 11, respectively.

Cumene was not mutagenic in the *Salmonella typh.* mutagenicity assay in a variety of strains[1], in *E. coli* or in yeast with or without metabolic activation. A single study with a positive result in *S. typh*. TA100 (Tardiff *et al*., 1978) is not regarded as reliable because of insufficient reporting. Cumene was tested in mammalian cells for genotoxicity (CHO/HGPRT assay; chromosomal aberrations in CHO cells and UDS test) with negative results; however, some early inadequate tests with equivocal or positive findings had to be repeated and, then, confirmed the overall negative outcome (NTP, 2013).

The metabolite, α-methylstyrene, was negative in *Salmonella typh.,* and did not induce chromosomal aberrations *in vitro*. However, positive effects in sister chromatid exchange *in vitro* were observed (Norppa und Vainio, 1983; NTP, 2007). The putative metabolite, α-methylstyrene oxide, yielded positive results in a reverse mutation test in *S. typh.* (Rosman *et al*., 1986). No data were available on genotoxicity of other metabolites of cumene.

In vivo, intraperitoneal injection induced small, but significant increases in micronuclei in the bone marrow of male F344 rats in two trials (NTP, 2009). However, the substance was found to be negative in a gavage test for micronuclei with male F344/DuCrl rats in a more recent assessment (NTP, 2012). Further tests on micronuclei with mice ($B6C3F_1$, Swiss) with gavage or inhalation exposure provided negative results. Comet assays in male rats and female or male mice gave largely negative results in blood, lung, kidney or liver cells. However, the response was weakly positive for male rats in the liver (trend, highest dose) and for female mice in the lung (trend, highest dose) (NTP, 2012). To clarify the DNA damage from reactive oxygen species, Kim *et al*.(2008) performed a "Fragment Length Analysis with Repair Enzyme" (FLARE) test in combination with a Comet assay after subchronic inhalation exposure in hepatocytes and lymphocytes of SD rats. The authors found some indications for oxidative DNA damage from cumene exposure; however, there was no clear duration-response relationship observed and the study is qualified as being insufficient in reporting of methods and results (NTP, 2013).

Analysis of mutations in the cumene-induced lung tumours in mice from the NTP carcinogenicity study (Table 11) found significant increases of K-*ras* und P*53*- mutations and different types of mutations from cumene exposed mice compared to mutations in spontaneous tumours in the control group. In addition, loss of heterozygosity (LOH) was detected in cumene induced tumours, with no such changes in spontaneous tumours. The authors discuss a (primary or secondary) genotoxic and/or an epigenetic mode of action for the observed changes (Hong *et al*., 2008; NTP, 2013; Wakamatsu *et al*., 2008). G→T transversions, as observed predominantly in cumene induced lung tumours, are associated with active oxygen species and are consistent with 8-OH-G adducts produced during oxidative damage to DNA. G→T transversions in K-ras codon 12 is the most common mutation detected in human adenocarcinoma (Hong *et al*., 2008).

The metabolite, α-methylstyrene, was positive *in vivo* in female mice in normochromic erythrocytes for micronuclei induction (trend, highest dose tested), but neither an increase of micronucleated polychromatic erythrocytes nor genotoxicity were observed in male mice (NTP, 2007). Another recent test on micronuclei formation in male mice bone marrow cells was negative (Rim *et al*., 2012). There are no *in vivo* data available for the postulated metabolite α-methylstyrene oxide or for other metabolites of cumene.

In conclusion, there are no data on germ cell mutagenicity from cumene or metabolites. For somatic cells, the vast majority of available tests gave negative results and there are only few indications for a genotoxic potential:

- Some DNA damage in male liver or female mice may not be excluded, as evidenced by recent Comet assay analysis from NTP (2012). It is speculated that this DNA damage may be a secondary genotoxic effect, e.g., due to oxidative damage in target organs,
- The postulated metabolite α-methylstyrene oxide may be mutagenic; however, the quantitative relevance of this substance for cumene metabolism has not been assessed and the finding is not confirmed by direct observations with cumene,

[1] Tests for reverse mutations in bacteria mostly lack strains for detecting cross-linking activity (TA102, E. coli WP2 strains) and in consequence were rated Reliability 3. But cross-linking activity is not assumed to be critical for cumene and negative results obtained in these tests (e.g. NTP, 2012) are considered meaningful for the assessment of mutagenic effects of cumene in bacteria.

- Changed profiles and increases of K-*ras* and p*53* mutations in cumene induced lung tumours may either point to mutagenicity of cumene or secondary genotoxicity (e.g., from reactive oxygen species, resulting in genetic instability and/or impairment of repair mechanisms) or epigenetic changes (e.g., from altered histone deacetylase, as discussed in Section 10.7.1).

Conclusions with respect to classification for germ cell mutagenicity are shown in Section 10.6.3.

10.6.2 Comparison with the CLP criteria

For potential classification on germ cell mutagenicity, criteria from CLP Regulation (EC, 2017) were applied:

a) Comparison with Category 1 criteria

- *The classification in Category 1A is based on positive evidence from human epidemiological studies (EC, 2017)*

There are no epidemiological data to support classification of cumene in Category 1A.

- *The classification in Category 1B is based on positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals (EC, 2017)*

There exist no *in vivo* heritable germ cell mutagenicity tests in mammals for cumene.

- *Classification in Category 1B can also be based on "positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells" (EC, 2017).*

This criterion is rejected as, for cumene, no primary *in vivo* somatic cell mutagenicity has been demonstrated and, by a weight of evidence approach, there only exists a concern for secondary genotoxicity and epigenetic interactions with DNA. There is not sufficient evidence that cumene interacts with the genetic material of germ cells. For metabolites, there are no studies to indicate that α-methyl styrene, a confirmed metabolite of cumene, interacts with the genetic material of germ cells. There is insufficient evidence to qualify α-methyl styrene as an *in vivo* mutagen in somatic cells. The postulated metabolite α-methyl styrene oxide, which is assumed to be genotoxic in somatic cells (Rosman *et al*., 1986), has not been shown to interact with the genetic material of germ cells.

Therefore, there is no evidence that the substance has the potential to cause germ cell mutations. Classification in Category 1 is not justified.

b) Comparison with Category 2 criteria

- *Classification in category 2 is based on somatic cell mutagenicity tests in vivo, in mammals (ECHA, 2017)*

For cumene primary somatic cell mutagenicity has not been demonstrated *in vivo* or *in vitro*. However, there is some evidence for potential DNA damages from cumene exposure shown by the Comet assay only in specific target tissues in high concentrations from inhalation exposure (NTP, 2012) and from intraperitoneal application in male rat (NTP, 2009). These genotoxic events are not regarded as a mutagenic effect. Mutations have been observed in cumene-induced tumours, but those are regarded as induced via epigenetic or secondary genotoxic in mode of action (Wakamatsu *et al*., 2008). One of the discussed "mode of action" proposes that epigenetic events from cumene exposure lead to amplifications of pre-existing spontaneous mutations. Potential genotoxic "modes of action" refer to genotoxicity secondary to oxidative stress (Kim *et al*., 2008; NTP, 2009; Wakamatsu *et al*., 2008). This interaction is not believed to lead to germ cell mutagenicity without an effect threshold.

- *Classification in category 2 is also based on other in vivo somatic cell genotoxicity, which are supported by positive results from in vitro mutagenicity assays (ECHA, 2017)*

The application of this criterion is rejected, as there are no sufficient indications of *in vitro* mutagenicity for cumene. Mutagenicity, as has been shown for the postulated metabolite α-methyl styrene oxide (Rosman *et al*., 1986), has not been evidenced to be relevant for exposures to cumene.

- *This hazard class is primarily concerned with substances that may cause mutations in the germ cells of humans that can be transmitted to the progeny. However, the results from mutagenicity or genotoxicity tests in vitro and in mammalian somatic and germ cells in vivo are also considered in classifying substances and mixtures within this hazard class" (EC, 2017).* ECHA (2017) further comments:

  Thus, classification as a germ cell mutagen (Category 1A, 1B, and 2) classifies for the hazard heritable genetic damage as well as providing an indication that the substance could be carcinogenic.

10.6.3 Conclusion on classification and labelling for germ cell mutagenicity

There is no evidence that cumene is a germ cell mutagen.

Accordingly, a "weight of evidence" approach is taken, as Guidance on the application of CLP criteria specifically requests: "If there is also negative or equivocal data, a weight of evidence approach using expert judgement has to be applied" (ECHA, 2017).

Basically,

- Cumene might have caused some DNA damage in male liver of rats or lungs of female mice, as evidenced by recent Comet assay analysis from NTP (2012). It is speculated that this DNA damage may be a secondary genotoxic effect due to oxidative damage or genomic instability in the target organ (Hong *et al*., 2008),
- There are some indications of genotoxicity for the metabolite α-methylstyrene due to increased sister chromatid exchanges in vitro, but those data are not sufficient to classify α-methylstyrene as a genotoxic substance (EC, 2017),
- the postulated metabolite α-methylstyrene oxide may be mutagenic,
- some further metabolites from ring-oxidation of cumene are assumed to be reactive,
- changed profiles and increases of K-*ras*, p*53* mutations in cumene induced lung tumours may be either point to mutagenicity of cumene or secondary genotoxicity (e.g., from reactive oxygen species, resulting in genetic instability and/or impairment of repair mechanisms).

In conclusion, the evidence for a primarily genotoxic mode of action for cumene carcinogenicity is unlikely.

Therefore no classification as a germ cell mutagen is warranted for cumene.

10.7 Carcinogenicity

Table 13: Summary table of animal studies on carcinogenicity (overall rates according to NTP, additional information on historical control data is available in Annex I)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
2-year carcinogenicity study according to OECD 451 in Mice, B6C3F$_1$ (♂) 50 males per concentration group GLP: according to FDA Good Laboratory Practice Regulations (21 CFR, Part 58) Reliability according to disseminated database: 2 Reliability according to authors of this evaluation: 1	Cumene Purity: 99.9 % Inhalation exposure: 0, 250, 500, and 1000 ppm (0, 1225, 2450, 4900 mg/m³) 6 h/d plus T_{90} (12 min), 5 d/w, 105 w	**<u>Lung</u>** Alveolar epithelium, bronchiole, metaplasia[a]: 5/50, 43/50**, 42/50**, 39/50** Bronchiole, hyperplasia: 0/50, 11/50**, 17/50**, 18/50** Alveolar/bronchiolar adenoma, multiple: 1/50, 12/50**, 15/50**, 20/50** Alveolar/bronchiolar adenoma (includes multiple)[b]: 13/50 P<0.001[c], 31/50***, 31/50***, 29/50*** Alveolar/bronchiolar carcinoma, multiple: 0/50, 8/50**, 20/50**, 17/50** Alveolar/bronchiolar carcinoma (includes multiple [b]): 9/50 P<0.001[c], 19/50*, 32/50***, 33/50*** Alveolar/bronchiolar adenoma or carcinoma[b,d]: 19/50 P<0.001[c], 38/50***, 42/50***, 43/50*** **<u>Liver</u>** Eosinophilic foci[a]: 6/50, 5/50, 16/50**, 14/50* Hepatocellular adenoma, multiple: 17/50, 20/50, 22/50, 26/50 Hepatocellular adenoma (includes multiple): 34/50, 33/50, 37/50, 35/50 Hepatocellular carcinoma, multiple: 3/50, 1/50, 4/50, 7/50 Hepatocellular carcinoma (includes multiple): 13/50, 18/50, 21/50, 17/50 Hepatocellular adenoma or carcinoma[b,e]: 40/50 P=0.250[c], 42/50, 43/50, 41/50 **<u>Hemangiosarcoma</u>** Hemangiosarcoma, spleen[b,f]: 0/50 P=0.002[c], 0/50, 0/49, 4/50* Hemangiosarcoma, all organs[g,h]: 0/50 P=0.015[c], 1/50, 2/50, 4/50* **<u>Thyroid gland</u>** Follicular cell, hyperplasia[a]: 7/50, 7/50, 7/49, 11/50 Follicular cell, adenoma[b,i]: 0/50 P=0.010[c], 0/50, 0/49, 3/50[j]	(NTP, 2009) Study also reported in (ECHA Dissemination, 2018, carcinogenicity, #002, key)
2-year	Cumene	**<u>Lung</u>**	(NTP, 2009)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
carcinogenicity study according to OECD 451 in Mice, B6C3F$_1$ (♀) 50 females per concentration group GLP: according to FDA Good Laboratory Practice Regulations (21 CFR, Part 58) Reliability according to disseminated database: 2 Reliability according to authors of this evaluation: 1	Purity: 99.9 % Inhalation exposure: 0, 125, 250, and 500 ppm (0, 1225, 2450, 4900 mg/m³) 6 h/d plus T_{90} (12 min), 5 d/w, 105 w	Alveolar epithelium, bronchiole, metaplasia[a]: 0/50, 42/50**, 49/50**, 47/50** Bronchiole, hyperplasia: 0/50, 17/50**, 10/50**, 14/50** Alveolar/bronchiolar adenoma, multiple: 0/50, 13/50**, 20/50**, 30/50** Alveolar/bronchiolar adenoma (includes multiple)[b]: 1/50 P<0.001[c], 26/50***, 36/50***, 38/50*** Alveolar/bronchiolar carcinoma, multiple: 0/50, 6/50*, 7/50**, 19/50** Alveolar/bronchiolar carcinoma (includes multiple)[b]: 3/50 P<0.001[c], 16/50***, 20/50***, 34/50*** Alveolar/bronchiolar adenoma or carcinoma[b,k]: 4/50 P<0.001[c], 31/50***, 42/50***, 46/50*** **Liver** Eosinophilic focus[a]: 8/50, 11/50, 7/50, 14/50 Hepatocellular adenoma, multiple: 9/50, 13/50, 9/50, 10/50 Hepatocellular adenoma (includes multiple)[b]: 18/50 P=0.040[c], 23/50, 27/50[l], 29/50* Hepatocellular carcinoma, multiple: 2/50, 1/50, 2/50, 0/50 Hepatocellular carcinoma (includes multiple): 10/50, 7/50, 6/50, 12/50 Hepatocellular adenoma or carcinoma[b,m]: 25/50 P=0.024[c], 26/50, 29/50[l], 36/50*	Study also reported in (ECHA Dissemination, 2018, carcinogenicity, #002, key)
2-year carcinogenicity study according to OECD 451 in Rat, F344/N (♂) 50 males per concentration group GLP: according to FDA Good Laboratory Practice Regulations (21 CFR, Part 58) Reliability	Cumene Purity: 99.9 % Inhalation exposure: 0, 250, 500, and 1000 ppm (0, 1225, 2450, 4900 mg/m³) 6 h/d plus T_{90}	**Nose** Olfactory epithelium, hyperplasia, basal cell[a]: 0/50, 19/50**, 27/49**, 26/50** Respiratory epithelium, hyperplasia: 0/50, 15/50**, 16/49**, 23/50** Goblet cell, hyperplasia: 3/50, 11/50*, 7/49, 5/50 Glands, respiratory epithelium, adenoma: 0/50, 0/50, 1/49, 0/50 Respiratory epithelium, adenoma, multiple: 0/50, 1/50, 2/49, 6/50* Respiratory epithelium, adenoma (includes multiple and all sites)[b,n]: 0/50 P=0.004[c], 7/50**, 18/49***, 10/50*** **Kidney** Renal tubule, hyperplasia[a]: 0/50, 3/50, 8/50**, 6/50* Papilla, mineralisation: 5/50, 35/50**, 44/50**, 41/50** Pelvis, transitional epithelium, hyperplasia: 3/50, 5/50, 14/50**,	(NTP, 2009) Study also reported in (ECHA Dissemination, 2018, carcinogenicity, #001, key)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
according to disseminated database: 2 Reliability according to authors of this evaluation: 1	(12 min), 5 d/w, 105 w	15/50** Nephropathy: 47/50, 47/50, 47/50, 50/50 Renal tubule, adenoma[b]: 1/50 P=0.219[c], 4/50, 5/50, 4/50 Renal tubule, carcinoma, bilateral: 0/50, 0/50, 1/50, 0/50 Renal tubule, carcinoma (includes bilateral)[b]: 1/50 P=0.180[c], 1/50, 3/50, 3/50 Renal tubule, adenoma or carcinoma[b,o]: 2/50 P=0.087[c], 5/50, 8/50*, 7/50 Renal tubule, lipoma: 1/50, 0/50, 0/50, 1/50 **Testis** Interstitial cell, hyperplasia[a]: 12/50, 18/50, 19/50, 9/50 Bilateral interstitial cell, hyperplasia: 0/50, 0/50, 0/50, 1/50 Interstitial cell, adenoma: 18/50, 14/50, 13/50, 9/50 Bilateral interstitial cell, adenoma: 18/50, 24/50, 27/50, 37/50 Interstitial cell, adenoma (includes bilateral)[b,p]: 36/50 P=0.006[c], 38/50, 40/50, 46/50**	
2-year carcinogenicity study according to OECD 451 in Rat, F344/N (♀) 50 females per concentration group GLP: according to FDA Good Laboratory Practice Regulations (21 CFR, Part 58) Reliability according to disseminated database: 2 Reliability according to authors of this	Cumene Purity: 99.9 % Inhalation exposure: 0, 250, 500, and 1000 ppm (0, 1225, 2450, 4900 mg/m³) 6 h/d plus T_{90} (12 min), 5 d/w, 105 w	**Nose** Olfactory epithelium, hyperplasia, basal cell[a]: 0/50, 14/48**, 25/50**, 31/50** Respiratory epithelium, hyperplasia: 0/50, 0/48, 4/50, 6/50* Respiratory epithelium, adenoma[q]: 0/50 P=0.320[c], 5/48*, 4/50, 3/50 **Kidney** Nephropathy[a]: 38/50, 37/50, 41/50, 44/50	(NTP, 2009) Study also reported in (ECHA Dissemination, 2018, carcinogenicity, #001, key)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
evaluation: 1			

Footnotes:

Significant difference from chamber control group determined by Poly-3 test: * P ≤ 0.05, ** P ≤ 0.01,*** P ≤ 0.001.

[a] Overall rate, number of animals with lesion per number of animals examined microscopically
[b] Overall rate, number of animals with neoplasms per number of animals with lung/liver/tissue/thyroid gland/nose/kidney/testis examined microscopically (only with regard to the organ under investigation)
[c] For chamber control incidence, P value is given that is associated with the trend test determined by Poly-3 test (accounts for differential mortality in animals that do not reach terminal sacrifice).
[d] Historical incidence for inhalation studies: 146/449 (32.5% ± 5.9%), range 26%-44%
[e] Historical incidence for inhalation studies: 264/449 (58.8% ± 9.6%), range 50%-80%
[f] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 6/444 (1.4% ± 1.5%), range 0%-4%
[g] Overall rate, number of animals with neoplasm per number of animals necropsied
[h] Historical incidence for inhalation studies: 21/450 (4.7% ± 3.7%), range 0%-12%
[i] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 5/441 (1.1% ± 2.0%), range 0%-6%
[k] Historical incidence for inhalation studies: 34/449 (7.6% ± 4.0%), range 2%-14%
[l] One animal with adenoma also had hepatoblastoma
[m] Historical incidence for inhalation studies: 145/447 (32.4% ± 8.8%), range 22%-50%
[n] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 1/447 (0.2% ± 0.7%), range 0%-2%
[o] Historical incidence for inhalation studies: 6/449 (1.3% ± 1.4%), range 0%-4%
[p] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 345/449 (76.8% ± 5.9%), range 66%-84%
[q] Historical incidence for inhalation studies: 0/496

Table 14: Summary table of human data on carcinogenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No human data on cumene are available, which are relevant for carcinogenicity classification assessment				

Table 15: Summary table of other studies relevant for carcinogenicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Transformation assay; BALB/3T3 cells; without activation Certified GLP compliance, but stability of test and control substances	Cumene Purity: 99.7% Vehicle: F127	3 day exposure of BALB/3T3 cells to 0, 50-200 µg/ml (4 doses) with survival of 102%, 87%, 19% and 4%, respectively. Positive control: 3-methylcholanthrene Retest based on Gulf Oil	No increase of Type III (or Type II) foci in cumene treated cells compared to vehicle treated cells. → negative	(Putman, 1987b) Study is also reported in (ECHA Dissemination, 2018, genetic toxicity, in vitro, #006,

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
have not been tested Reliability according to disseminated database: 1 Reliability according to authors of this evaluation:3		Corporation (1984)		supporting)
Transformation assay; BALB/3T3 cells; without activation Compliance with GLP stated by authors Reliability according to disseminated database: study not reported Reliability according to authors of this evaluation:3	Cumene Purity: not disclosed, but may be requested from the sponsor of the study Vehicle: Pluronic F68 Polyol	Mouse embryo cells (BALB/3T3), dosing: (untreated, vehicle, 5-90 µg/ml, 4 doses, positive control), highest dose (90 µg/ml) extremely toxic (eliminated from study). Colony forming efficiency reduced at 60 µg/ml Positive control: 3-methylcholanthrene	Transformation (type III foci) observed at 60 µg/ml. → positive Re-tested by Putman (1987b)	(Gulf Oil Corporation, 1984)
Mechanistic study: Gene expression analysis Reliability according to disseminated database: study not reported Reliability according to authors of this evaluation:1	Cumene Purity: >99.9%; no impurities > 0.1% observed (from NTP, 2009)	8/23 tissue from cumene induced lung tumours in mice (→NTP, 2009, Table 13) plus 4 normal lung tissues (untreated mice) were selected for gene expression analysis. Lung tumour tissues were chosen based on the absence of necrosis and inflammatory cell infiltration. In a microarray analysis gene expression changes were separated into 3 groups: control lung tissue, tumours with K-*ras* mutations, tumours without K-*ras* mutations. Specific analysis focused on gene expression linked to	281 Genes different between normal lung and tumours without K-*ras*; 627 genes differed between normal lung and tumours with K-*ras* mutation. K-*ras* 66 genes were differently expressed between tumours with K-*ras* and tumours without K-*ras* or normal lung tissue. <u>Gene expression profile of cumene-induced lung tumours linked the MAPK signalling pathway:</u> Many of the significantly altered genes in cumene-induced lung tumours were associated with the MAPK signalling pathway. The majority of genes associated with MAPK pathway were significantly altered only in tumours with K-*ras* mutations (genes known to promote MAPK activation, genes activated by MAPK signalling, genes involved in the inactivation of MAPK	(Wakamatsu *et al*., 2008)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		MAP kinase (MAPK) signalling pathway and to potential histone modification due to histone deacetylases (HDACs) activity changes, which may influence DNA unfolding and transcription.	pathway were downregulated) Some genes linked to tumour suppression, invasion and metastasis were only significantly altered in cumene induced tumours with K-ras mutations. Gene expression profile of cumene-induced lung tumours linked to histone modification: Genes associated with the HDAC complex were significantly altered in tumours; K-ras mutation status of the tumours appeared to correlate with upregulated genes. The HDAC complex has been shown to play a role in human cancer. Both, genetic and epigenetic factors may contribute to cumene-induced lung cancer. Epigenetic alterations in gene expression are likely to be involved in cumene-induced lung neoplasms.	

10.7.1 Short summary and overall relevance of the provided information on carcinogenicity

There are no human data available for assessment of the carcinogenic hazard of cumene.

From the data presented in Table 13 cumene is carcinogenic in experimental animals. The effect is more pronounced in mice after chronic inhalation exposure, with

- significantly increased lung alveolar/bronchial adenoma or carcinoma (combined) in male and female mice,
- significantly increased liver adenoma or carcinoma (combined) in female mice,
- slight increase of renal tubular cell adenoma or carcinoma (combined) in male rats,

  significantly increased nose adenoma in the respiratory epithelium in male rats
- various further tumour sites with increased tumour incidence at the highest exposure concentration, which, however, are not in focus for a more detailed analysis (rat: adenoma in testes; male mice: haemangiosarcoma in spleen and in all organs (combined), follicular-cell adenoma of the thyroid gland).

Each observed tumour type will be discussed separately, taking into account available information on the Mode of Action (MoA). However, even if each tumour site is discussed *per se*, the final conclusion (see Section 10.7.3) also needs to consider that cumene appears to be a carcinogen in experimental mice as a clear evidence for a carcinogenic effect is observed for lung tumours in mice.

10.7.1.1 Lung tumours in B6C3F$_1$ mice

In the study by NTP (2009) lung tumours were statistically increased in mice, as evidenced by alveolar/bronchiolar adenoma or carcinoma in males (19/50, 38/50***, 42/50***, 43/50*** (*** $p \leq 0.001$; P for trend: $P<0.001$)) and in females (4/50, 31/50***, 42/50***, 46/50***, P for trend: $P<0.001$), for further

explanation on the data, see Table 13). Even if some of the background dose response data are not strictly monotonously increasing with dose (e.g., male bronchiole metaplasia (0/50; 42/50; 49/50; 47/50)), this does not invalidate this overall clear evidence for an exposure related causal effect in both sexes. The question of human relevance is separately discussed below.

a) Genotoxicity as MoA

As discussed in Section 10.6.3, a direct interaction of cumene or metabolites with DNA (primary genotoxicity) is not a likely MoA for lung tumours. However, in a Comet assay significant increases in DNA damage in lungs of female mice were observed *in vivo* (only weak, but significant increase at the highest dose tested and significant trend) (NTP, 2012).

In addition, K-*ras* mutations and p*53* mutations were evaluated in spontaneously occurring and cumene-induced tumours in mice (see Table 11 for details). The data show differences in the incidence of K-*ras* mutations between cumene-induced (87%) and spontaneous lung tumours (14%) and historical controls (28%). The type of K-ras mutations differed between tumours from exposed and unexposed animals (e.g., predominant K-*ras* mutations in lung tumours from cumene-exposed mice were codon 12 G→T transversions (36% vs. 18% in historical controls); in contrast, codon 12 G→A transitions (42%) was the most common mutation in spontaneous lung tumours). Mutations in the p*53* tumour suppressor gene were not observed in spontaneous lung tumours of the concurrent controls, but were evident in 52% of the cumene-induced lung tumours (no historical control data provided). Furthermore, a loss of heterozygosity (LOH) occurred in cumene-induced mouse lung tumours, but not in spontaneous tumours in control mice (Hong *et al*., 2008). The K-*ras* and p*53* mutations showed a dose-dependent increase (total of all exposed groups) and similar mutation rates were reported for adenomas and carcinomas. However, as such mutations were more prevalent in exposed males than females, this observation would not be in accordance with the elevated female sensitivity compared to males for cumene-induced lung tumours.

As a further possible MoA, leading to secondary genotoxicity, induction of reactive oxygen species (ROS) by cumene is discussed. ROS-dependent changes including oxidative damage could explain the positive results in the Comet assay (Hong *et al*., 2008). However, no specific studies with cumene providing evidence for this potential MoA (e.g., analyses of oxo-deoxyguanosine adducts) were identified.

The observed increase in K-*ras* and p*53* mutations may also be caused by an epigenetic MoA: cumene is discussed to cause growth advantage for preneoplastic or neoplastic cells carrying these (possibly spontaneous) mutations and "these molecular changes may be an effect rather than a cause" of the multistage carcinogenic process (NTP, 2013).

In conclusion, genotoxicity can currently not be excluded as contributing to MoA for lung cancer in mice from cumene exposure, but the relevance within this process is currently unknown.

b) Increased K-*ras* mutations or p*53* mutations and their relevance to humans

The observed increase of K-*ras* mutations in tumours from cumene-exposed mice may or may not be due to a genotoxic event, but this increased incidence is possibly involved in the MoA of cumene carcinogenesis in mice.

An analysis compared the gene expression patterns in cumene-induced tumours with K-*ras* mutations with those in spontaneous occurring tumours without K-*ras* mutations (Wakamatsu *et al*., 2008). The former were associated with increased expression of genes

- involved in the mitogen activated protein kinase (MAPK) signalling pathway,
- linked to invasion and metastasis,
- linked to inhibition of apoptosis,
- linked to increased angiogenesis,
- linked to increased metastatic potential.

According to the authors of this analysis, the difference in gene expression suggests that cumene-induced carcinomas with K-*ras* mutations have a higher degree of malignancy than tumours without K-*ras* mutations.

There is no indication that these K-*ras* mutations are a species-specific factor in tumorigenesis in mice. In contrast, K-*ras* and p*53* mutations have also been found in human lung cancer (Hoenerhoff *et al*., 2009). Activation of the K-*ras* proto-oncogene and inactivation of the p*53* tumour suppressor gene were also frequently observed in human pulmonary adenocarcinoma (NTP, 2013). From that, NTP concludes that "many of the genes with altered expression in the mouse tumor model represent major genes that may play a role in lung and other cancers in humans".

c) Lung tumours in mice from exposure to alkylbenzenes

A publication by Cruzan *et al*. (2009) compared tumour incidences in rodents for several alkylbenzenes and other aromatic compounds (such as styrene, ethylbenzene, cumene, α-methylstyrene, coumarin, naphthalene) and found an obvious discrepancy in tumour incidence in the different rodent species: generally, the incidence of bronchiolo-alveolar adenomas or carcinomas in lungs of mice was significantly increased for most of those substances, whereas no such lung tumours were observed in rats. With focus on more specific studies on styrene, the authors hypothesised that the observed carcinogenicity is linked to a hydroxylation of the aromatic ring (not the side-chain epoxide), for which a specific CYP enzyme (CYP 2F2) is responsible, leading to a reactive metabolite. Cytotoxicity mediated by reactive metabolites formed from CYP2F2 metabolism precedes hyperplasia and finally (at a late stage) tumours. CYP2F2 is expressed in the Clara cells (club-cells) of mice, and is expressed to a much lesser extent in rats and humans. In addition, Clara cells in the lower respiratory tract of mice differ significantly in quantity, function and distribution from humans. Based on those observations, a workshop by "Toxicology Excellence for Risk Assessment" (TERA) was organised in 2013 to discuss the relevance of the respective mouse lung tumours to humans (TERA, 2013). The workshop participants largely confirmed the view of Cruzan *et al*. and concluded that this issue was similar in relevance as the one recently discussed for the peroxisome proliferator-activated receptor alpha (PPARα) - MOA and the interspecies extrapolation of the respective mouse liver cancer. PPARα is – in general – not regarded as quantitatively relevant for humans. The workshop participants stated:

"Therefore, while this mode of action is theoretically possible in humans if sufficient concentrations of active metabolites are produced, this is highly unlikely to occur given the cross-compound evidence of the central role of mouse-specific CYP2F2 in mediating cytotoxicity. Thus, the hypothesized MOA developed from this cross-compound analysis suggests these chemicals are not expected to cause lung tumors in humans"(TERA, 2013).

Based on the observations by Cruzan *et al*. (2009) mainly on styrene and further evidence provided by later studies (Cruzan *et al*., 2012), some recent assessors assume such an analogy of cumene to styrene (e.g., AGS, 2014; DFG, 2016; SCOEL, 2015) and conclude, e.g.: "*For the induction of observed lung … tumours species-specific mechanisms appear to be decisive*" (SCOEL, 2015).

However, the panel of the TERA workshop also proposed that the following evaluations and criteria would be necessary to demonstrate this MOA (as validated for styrene) for other compounds:

- Evaluate the ring oxidation potential of the chemical's structure, looking for demonstration of ring-oxidized metabolites, including *in vitro* CYP2F2 metabolism studies
- Look at the genetic activity profiles (GAPs), to determine if mutation is an early and influential key event in the mode of action
- Look for evidence of acute cytotoxicity in mice and rats (*in vivo*)
- If the cytotoxicity response is specific to mice (and not rats), then use CYP2F2 knockout mouse to demonstrate that the response is dependent upon CYP2F2 metabolism
- Lastly, test in the humanized TG mouse to confirm humans will not metabolize sufficient compound via CYP2F1 to produce lung tumors in a "susceptible" system (TERA, 2013).

Apparently none of those criteria fits to the current toxicological state of knowledge on cumene: neither the respective validations have been performed (TG mouse test; CYP2F2 knockout mouse test; GAP analysis, *in vitro* CYP2F2 studies) nor the data generated for cumene support the suggested MOA. *Inter alia*, the postulated significant participation of CYP2F2 in cumene metabolism in the mice lung has not been demonstrated.

Therefore, current conclusions that the mouse model would not be appropriate for cumene from analogy to other alkylbenzenes are, at least, premature. In fact, several indications suggest a MoA for cumene different from styrene:

- Metabolites for cumene from ring-hydroxylation were found only in small quantities in ^{14}C analysis by Chen *et al*. (2011) (see metabolites [M2], [M3] in Table 9) and there is no obvious quantitative difference indicating that mice were more prone to this metabolic pathway compared to the rat.
- Cruzan *et al*. (2009) postulated that the CYP2F2 pathway leads to cytotoxicity as an essential step for subsequent hyperplasia and tumours. However, in the long-term NTP studies on cumene there was no observed cytotoxicity in the lower respiratory tract in mice preceding hyperplasia (NTP, 2009; 2013).
- There has been Clara cell loss in bronchioles with styrene exposure. However, this loss has not been observed for (ethylbenzene and) cumene (US EPA, 2014).
- The observed K-*ras* mutations in tumours from cumene exposure may be part of an alternative MoA (see above), which has not been observed or discussed for styrene.

Due to these findings, the postulated analogy to styrene is questionable. In an even more recent workshop by U.S. EPA on the relevance of mice tumours for humans, the similarity of tumours from exposure to alkylbenzenes in mice was further discussed and it was concluded: "*Although structurally related chemicals may cause lung tumors in the B6C3F1 mouse, the mechanism may not be similar"*(Pandiri, 2015; US EPA, 2014).

In conclusion, there are indications from other alkylbenzenes that lung tumours observed in the mouse model would not be relevant for humans because of a (largely) species specific MoA. However, there is insufficient evidence that this MoA seen with other alkylbenzenes is applicable to cumene. Therefore, significant concerns remain that the observed adenoma and adenocarcinoma in the lung in $B6C3F_1$ mice from exposure to cumene may in fact be meaningful for humans.

10.7.1.2 Liver tumours in female $B6C3F_1$ mice

In the study by NTP (2009) liver tumours were statistically increased in female mice, as evidenced by hepatocellular adenoma or carcinoma in exposed animals (25/50, 26/50, 29/50, 36/50* (* $p<0.05$; P for trend =0.024), further explanation on the data, see Table 13). Even if some of the background dose response data are not increasing with dose (e.g., hepatocellular carcinoma only (10/50; 7/50; 6/50; 12/50)), this does not invalidate this overall clear evidence for an exposure related causal effect in female mice. The question of human relevance is separately discussed below.

a) Genotoxicity as MoA

As discussed in Section 10.6.3, a direct interaction of cumene or metabolites with DNA (primary genotoxicity) is probably not a critical MoA for liver tumours of $B6C3F_1$ mice. Also the Comet assay did not show significant DNA damage in the liver of mice, but only in rat (NTP, 2012). There is only limited evidence that the metabolite α-methylstyrene (AMS; not marked as one of the [M1]-[M16]-metabolites by Chen *et al*.(2011)), may be genotoxic (Norppa und Vainio, 1983; NTP, 2007; Rim *et al*., 2012). AMS oxide, a postulated metabolite from cumene and AMS, is genotoxic *in vitro* (see Section 10.6.1 for details). With small amounts of AMS detected in exhaled air from cumene exposure (see Section 9.1 for details), a (primary or secondary) genotoxic MoA for hepatocellular adenoma and carcinoma of cumene in mice cannot be fully excluded; however, indications of a genotoxic MoA are limited.

b) Relevance of liver tumours in B6C3F$_1$ mice for humans

Hepatocellular adenoma and carcinoma are frequently observed in B6C3F$_1$ mice and were observed in female mice after long term inhalation exposure to cumene. This strain of mice is associated with a high background incidence of liver tumours (NTP, 2009, Table 24). With low evidence of a genotoxic MoA, the relevance of increased liver tumours in B6C3F$_1$ mice for human exposure has been questioned. Felter *et al*. (2018) reported the results of a workshop on "human relevance of rodent liver tumors". Workshop discussions focused on two nuclear receptor-mediated MoAs ("Constitutive Androstane Receptor" (CAR) and "Peroxysome Proliferator Activated Receptor-alpha" (PPARα)) and on cytotoxicity. Most, but not all, participants considered the CAR and the PPARα MoAs as not relevant to humans based on quantitative and qualitative differences. In contrast, cytotoxicity was considered as clearly relevant to humans, but associated with a threshold MoA.

There are no data to link cumene to either a CAR- or PPARα-like MoA, with a general deficit to analyse CYP-specifications and critical metabolites for cumene in the species of interest. However, many heterogeneous substances activate the CAR and lead to tumour promotion in mice, as does the model substance phenobarbital (Elcombe *et al*., 2014). Examples are pyrene (Zhang *et al*., 2015), ethyl isobutyl ketone (Hughes *et al*., 2016), or tetrahydrofuran (Choi *et al*., 2017). Sweeney *et al*. (2015) assume this MoA also for ethylbenzene, which is similar to cumene in structure, but details are not available.

There are no obvious indications of cytotoxicity preceding neoplastic effects in the chronic inhalation study with cumene (NTP, 2009). In the 14-weeks studies there was some minimal chronic focal liver inflammation in female mice exposed to the lowest concentration (62.5 ppm) of the test regimen, but without a relation between dose and response, and some increase in relative liver weights at elevated exposures (125 ppm) (DFG, 2016). However, DFG (2016) suggests "chronic organ damage" as a non-genotoxic MoA of cumene: "*In analogy to ethylbenzene, the isopropyl benzene-induced neoplasms in the liver of female mice and the eosinophilic foci of male animals could therefore be the result of increased cell proliferation following chronic organ damage*". If this consideration was confirmed by better data, it would support categorising cumene as a threshold carcinogen.

In summary, relevance for humans of the observed liver tumours in mice from cumene exposure may be low or not existing. However, the MoA is still largely unknown and has been insufficiently examined. Therefore, these data support the conclusion that cumene's induction of liver tumours in female mice is uncertain with respect to the relevance for humans.

10.7.1.3 Renal tumours in F344/N male rats

In the study by NTP (2009) a suggestive increase of renal tubular cell adenoma or carcinoma (combined) in male rats due to exposure to cumene was observed.

(i) The incidence of renal tubular hyperplasia was increased significantly in all exposed groups (hyperplasia is possibly linked to the MoA of cancer),

(ii) The incidence of renal tubular adenoma was (insignificantly) higher than control in all exposure groups,

(iii) Renal tubular carcinoma were (insignificantly) increased above control in the two higher exposure groups,

(iv) Renal tubular cell adenoma or carcinoma (combined) were elevated in all exposure groups, close to significance (but not significant for trend (P=0,087)), and significantly increased in the mid exposure group (8/50* vs. 2/50 in control),

(v) For one of the metabolites of cumene (alpha-methylstyrene) there was also some evidence of carcinogenic activity in male F344/N rats based on increased incidences of renal tubule adenomas and carcinomas (combined) (NTP, 2007).

The question of human relevance is separately discussed below.

a) Genotoxicity as MoA

As discussed in Section 10.6.3, a direct interaction of cumene or metabolites with DNA (primary genotoxicity) is not a plausible MoA for renal tumours. The results of a Comet assay did not show a significant increase in DNA damage in the kidneys of male rats *in vivo* after exposure to cumene (NTP, 2012). There is only limited evidence that the metabolite α-methylstyrene (AMS; not marked as one of the [M1]-[M16]-metabolites by Chen *et al*. (2011)) may be genotoxic (Norppa und Vainio, 1983; NTP, 2007; Rim *et al*., 2012). AMS oxide, a postulated metabolite of cumene and AMS, is genotoxic *in vitro* (see Section 10.6.1 for details). With small amounts of AMS detected in exhaled air after cumene exposure (see Section 9.1 for details), a (primary or secondary) genotoxic MoA for renal tumours of cumene in the rat is unlikely, however cannot be fully excluded.

b) Relevance of renal tumours in male rats for humans

Cumene leads to renal tumours in male rats after inhalation exposure. One of the metabolites, confirmed in rodents, also is associated with renal tubular adenoma and carcinoma (combined) in male but not in female rats (NTP, 2007). However, α-methylstyrene apparently is only a minor metabolite of cumene (see Section 9.1).

A major MoA of renal tumours in male rats is α_{2u}-globulin accumulation, observed as hyaline droplets, in proximal tubule. This may lead to epithelial degeneration and necrosis, granular casts, cell proliferation, chronic progressive nephropathy (more often in older rats), atypical hyperplasia within the proximal tubules, and progression to tumours (Capen *et al*., 1999; Swenberg und Lehman-McKeeman, 1999).

The International Agency for Research on Cancer (IARC) developed a list of criteria, which must be met, for identifying agents where this is the sole MoA of renal tumours. Those criteria are:

- Lack of genotoxic activity,
- Male rat specificity for nephropathy and renal tumorigenicity,
- Indication of the characteristic sequence of histopathological changes, of which protein droplet accumulation is obligatory,
- Identification of the protein accumulating in the tubule cells as α_{2u}- globulin,
- Reversible binding of the chemical or metabolite to α_{2u}- globulin,
- Induction of sustained increased cell proliferation in the renal cortex,
- Similarities in dose-response relationship of the tumour outcome with the histopathological endpoints (protein droplets, α_{2u}- globulin accumulation, cell proliferation) (Capen *et al*., 1999).

The NTP (2009) concluded that the lesions observed in male rats were characteristic of α_{2u}-globulin accumulation. However, not all of the IARC-criteria were met: male specificity of nephropathy (some nephrotoxicity was also observed in females), and evidence of sustained cell proliferation were not provided; reversible binding to α_{2u}- globulin was not assessed and genotoxicity as a possible MoA is not completely ruled out. IARC (2013) points out the "*one of the mutagenic metabolites of cumene, α-methylstyrene oxide, could play a role in the initiation of such tumours*" and concludes "*the data do not support a mechanism that involves α_{2u}-globulin- associated nephropathy in the development of these kidney tumours*." Note that IARC regards α-methylstyrene oxide as a confirmed metabolite of cumene, which is, however, only a proposed metabolite according to Chen *et al*. (2011). Therefore, at least the quantitative relevance of this metabolic pathway is uncertain. NTP (2016) concludes: "*Overall, the data provide evidence that cumene causes kidney tumors largely via α2u-globulin nephropathy; however, it cannot be ruled out that other mechanisms, such as genotoxicity, also contribute to kidney tumor formation. Although it is likely that genotoxicity plays a role in cumene-induced carcinogenicity at some tissue sites, the strongest evidence for genotoxicity was found for lung and liver tumors, and the extent to which genotoxicity contributes to the formation of kidney tumors is unknown. Thus, the relevance of the kidney tumors in male rats to human cancer is uncertain.*" (NTP, 2016).

Based on relevant indications of a species specific effect, we agree with this NTP conclusion ("human relevance is uncertain").

10.7.1.4 Nasal tumours in male F344/N rats

In the experimental study by NTP (2009) nasal tumours were statistically increased in male rats, as evidenced by adenoma of the respiratory epithelium (including multiple and all sites (0/50, 7/50**, 18/49***, 10/50*** (*** p≤0.001; P for trend: P<0.001)) and in females ((4/50, 31/50***, 42/50***, 46/50***, P for trend: P=0.004),for further explanation of the data, see Table 13). Even if response data are not monotonously increasing with dose, this does not invalidate this overall clear evidence for an exposure related causal effect in male rats. The relevance of these findings is further supported by an (insignificant) increase of respiratory epithelium adenoma in female rats (0/50; 5/48*; 4/50; 3/50; P at low dose exposure <0.05; for details see Table 13). A potential progression to malignant tumours is separately discussed below.

Increases in the incidence of benign nasal tumours (adenoma of the respiratory epithelium) were observed in rats of both sexes (NTP, 2009). NTP (2016) assumes that this kind of tumours cannot progress to malignancy. They cited a publication by Brown (1991) as evidence. However, from analysis of the original study by Brown (1991), no such definite statement was found. In NTP (2009) a different conclusion was reported: "Progression of nasal respiratory epithelial adenomas to malignancy has been described in the literature".

DFG (2016) believes that the CYP enzymes probably responsible for transforming cumene to reactive metabolites in the nasal cavity are much less expressed in humans compared to other mammalian species. Therefore no relevance for human cancer risk is assumed, "*however, according to current data* [the relevance of nasal tumours for humans] *cannot be excluded*". AGS (2014) uses the dose-response data for nasal tumours in rats to calculate cancer risks, to support the occupational exposure limit (OEL) derived from non-cancer effects quantitatively and derived a very low associated excess risk at air concentrations of 10 ppm (occupational exposure scenario) implicitly acknowledging a possible relevance of this cancer endpoint for human exposure.

Overall, the observed effect in male rats is regarded relevant for human exposure, but progression to malignancy is uncertain. A significant contribution of cytotoxicity (secondary to cytotoxicity) to hyperplasia and subsequent occurrence of tumours (i.e., threshold-type mode of action) is possible.

10.7.1.5 Other tumour sites

In male mice, haemangiosarcoma of the spleen and follicular-cell adenoma in the thyroid gland may have been treatment related based on marginal increases over historical control values. However, haemangiosarcoma occur in multiple tissue types and are not specific to or rare in the spleen (NTP, 2013). In addition, the incidence in all organs was within the historical control ranges for inhalation studies and for all routes. Follicular-cell adenoma were only insignificantly increased at the highest exposure group; only the trend was significant (P=0.01). The unadjusted overall tumour rate for thyroid adenoma (6%) at the high exposure concentration was within the historical control range (0%–6%) for inhalation studies and for all routes.

Further, the incidence of interstitial-cell adenoma of the testes of rats was significantly increased at the highest exposure level with a positive trend and exceeded the historical control from inhalation studies. However, this type of adenoma does not progress to malignancy (NTP, 2013).

Table 16: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
mice, $B6C3F_1$	a) lung alveolar/bronchiolar adenoma or carcinoma (background incidence: 19/50, males; 4/50 females)	yes, see Table 13	yes, see Table 13	Yes (first incidence high dose group; males: 420 days, compared to 628 days in control; females: 513 days, compared to 533 days)	both sexes	not assumed to be due to excessive toxicity	inhalation	possibly relevant; discussed in detail in Section 10.7.1.1
	b) liver high background incidence of adenoma or carcinoma in this strain (40/50 males, 25/50 females)		yes, see Table 13	Yes (males), no (females) (first incidence high dose group; males: 391 days, compared to 551 days in control; females: 662 days, compared to 607 days)	significant only in females			relevance uncertain; discussed in detail in Section 10.7.1.2
	c) hemangiosarcoma in males (background incidence: 0/50; incidence of treated animal within historical control ranges)		tumour is malignant	No tumours in control group	only observed in male mice			relevance uncertain; see Section 10.7.1.5
	d) thyroid gland follicular cell adenoma in males (background incidence: 0/50 (only hyperplasia in control); unadjusted overall tumour rate within historical control)		only (non-malignant) adenoma observed	No tumours in control group	only observed in male mice			inadequate evidence; see Section 10.7.1.5
rat, F344/N	a) nose adenoma respiratory epithelium (no elevated background incidence: 0/50 males, 0/50 females)	yes, see Table 13	no, see Table 13, but cannot be excluded (see Section 10.7.1.4)	No tumours in control group	both sexes (but not clearly significant in females)	not assumed to be due to excessive toxicity	inhalation	uncertain, but low evidence; discussed in detail in Section 10.7.1.4

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
	b) kidney (renal tubule adenoma or carcinoma in control males only: 2/50)		yes, see Table 13	Yes (first incidence high dose group; males: 618 days, compared to 729 days in control)	males only			uncertain, but low evidence; discussed in detail in Section 10.7.1.3
	c) testes (adenoma; high background incidence, males: 36/50)		only (non-malignant) adenoma observed, does not progress to malignancy	Inconclusive (first incidence high dose group; males: 541 days, compared to 558 days in control)	males only			inadequate evidence; discussed in detail in Section 10.7.1.5

10.7.2 Comparison with the CLP criteria

IARC (2013) classified cumene "possibly carcinogenic to humans (Group 2B)", based on "sufficient evidence in animals" and "no data available in humans", according to the IARC criteria.

For potential classification on carcinogenicity, criteria of the CLP Regulation (EC, 2017) were applied.

- *Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence (EC, 2017)*

As indicated, there are no relevant data on exposure to cumene for classification into Category 1A.

- *Evidence for Category 1B is derived from animal experiments for which there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen) (EC, 2017)*

Based on the results provided by NTP (2009) with studies in mice and rats, overall there is sufficient evidence in animals for carcinogenicity. This conclusion is in agreement with IARC (2013). However, the criterion above is closely linked to the relevance for humans (see remark in brackets: "presumed human carcinogen"), as further discussed by EC:

- *Substances which have induced benign and malignant tumours in well performed experimental studies on animals are considered also to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans. (EC, 2017)*

As evidenced in detail in Section 10.7.1, the relevance for humans of observed tumours in animal studies has been seriously questioned in the case of cumene. In further specifications, EC requests:

- *Additional considerations (as part of the weight of evidence approach) …a number of other factors need to be considered that influence the overall likelihood that the substance poses a carcinogenic hazard to humans….Some important factors which may be taken into consideration, when assessing the overall level of concern are…mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity. (EC, 2017)*

These considerations are described in detail in Section 10.7.1 for each tumour site and animal species discussed as potentially relevant for carcinogenicity classification. It is concluded that there are serious doubts that the respective modes of action for carcinogenic effects in experimental animals are relevant for humans in case of cumene, but that relevant concerns remain.

- *Category 2: suspected human carcinogen. The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations." (EC, 2017)*

When assessing the overall level of concern for classification of carcinogenicity for cumene, according to criteria in Section 3.6.2.2.6 (EC, 2017), we conclude that

(a) tumour type and background incidence;

Observed tumor types (lung alveolar/bronchiolar adenoma or carcinoma as evidenced from mice; hepatocellular adenoma or carcinoma as evidenced from female mice; renal tubular adenoma or carcinoma found (insignificantly) increased in male rats, respiratory adenoma as evidenced from male rats) are generally assumed to be relevant for classification. However, for some of those observed tumours in rodent studies the mode of action may possibly not be relevant for humans (see criterion k, below) or progression to malignancy has been questioned (see criterion c, below). Background incidence for hepatocellular tumours in $B6C3F_1$–mice is high, adding to uncertainties on the relevance for classification based on respective observations in this mouse strain, which is very sensitive to respective hepatocellular tumours. For hepatocellular tumours in $B6C3F_1$-mice quantitative species extrapolation is not possible, if the mode of action is not clearly genotoxic. The qualitative relevance is uncertain.

(b) multi-site responses;

Based on overall evidence, cumene is regarded to show multi-site tumour responses in the rodent studies (lung in mice, liver in female mice, renal tumours in male rats, nasal epithelium in rats) and, furthermore, but with less evidence: testes in rats, thyroid gland and hemangiosarcoma in spleen of mice. However, this does not imply that those tumours are all induced by one overall relevant mode of action. Furthermore, it does not imply that all of those tumours in rodents are relevant to humans.

(c) progression of lesions to malignancy;

Some of the observed tumours in rodent studies may potentially progress to malignancy as assumed by default (lung tumours in mice, hepatocellular tumours in mice, kidney tumours in rats, follicular cell adenoma in the thyroid gland), for other tumours this progression to malignancy is uncertain (this type of nasal adenoma in rats) or not expected (interstitial cell adenoma in testes of rats).

(d) reduced tumour latency;

This criterion was not addressed and is not regarded influencing classification for the tumours observed in the rodent experimental studies on cumene.

(e) whether responses are in single or both sexes;

Observed tumours were not significantly increased in both sexes for all tumour sites apart from the lung, which was affected similarly and significantly in male and female mice. Specifically, hepatocellular tumours have only been increased significantly in female mice (and only insignificantly in male mice); kidney tumours have only insignificantly been increased in male rats, with only insignificant increase of nonmalignant nephropathic effects in female rats; and nasal adenoma were significantly increased only in male rats with significant (but not neoplastic) hyperplasia respiratory epithelium and only insignificant increase of adenoma in female rats. From these observations, cumene is not regarded to be tumourigenic to only one sex. For some tumour locations a sex- and species-specific mode of action is discussed (e.g., kidney tumours in male rats), however, without firm conclusions.

(f) whether responses are in a single species or several species;

Tumourigenic responses have been observed in more than one species (i.e., rats and mice). However, the tumour sites usually differed between the two species and some of the observed tumours may be species-specific.

(g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity;

Some other aromatic hydrocarbons are associated with similar tumours, but the evidence for their carcinogenicity does not help to eliminate uncertainties on the mode of action and human relevance of the tumours observed in the NTP studies on cumene. Specifically, for relevance of tumours in mice to humans comparisons with other aromatic hydrocarbons were discussed intensively (TERA, 2013; US EPA, 2014), as also addressed in Section 10.7.1.1 of this report.

(h) routes of exposure;

This criterion was not addressed and is not regarded influencing classification for the tumours observed in the rodent experimental studies on cumene. Only inhalation studies on carcinogenicity were available. However, *in vivo* genotoxicity studies also included data from oral (gavage) exposure.

(i) comparison of absorption, distribution, metabolism and excretion between test animals and humans;

Even though absorption, distribution, metabolism and excretion appear to be similar between animal species and humans, some differences in quantitative metabolism have been reported (see Section 9, this report). Specifically, higher tissue concentrations in rat kidney and mouse lung studies correlate with higher incidence of tumours in these studies. There are relevant uncertainties on the metabolism of cumene in the mouse lung and whether this metabolism is species specific.

(j) the possibility of a confounding effect of excessive toxicity at test doses;

There is no reason to assume relevant influences of confounders in the outcome of the critical studies on cumene.

(k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity.

These aspects are discussed in detail in Section 10.7.1, of this report.

From the definition of Category 2 in Regulation 1272/2008 (EC, 2017), and from the overall summary discussion based on Regulation criteria a-k, Section 3.6.2.2.6 (above) , and from the discussion described in detail in Section 10.7.1, for each tumour site and animal species for cumene, the substance is regarded a "suspected human carcinogen". The relevance of the observed tumours in experimental animals is uncertain (less than sufficient evidence), which would be needed for classification in Category 1B.

10.7.3 Conclusion on classification and labelling for carcinogenicity

Cumene is a multi-site carcinogen in rodent inhalation studies. No data exist for other exposure pathways. There are, however, several indications of limited or no relevance of the observed tumours for human exposure. Various MoAs have to be considered and a definite conclusion on a species-specific MoA, not relevant for human exposure, is not possible. Specifically,

- for lung tumours in mice, transformation of cumene in the lower respiratory tract by CYP2F enzymes with ring oxidation in mice Clara cells would be a possible species specific mode of action, but some observations (like the increase incidence of K-ras mutations in tumours of exposed mice and the lack of cytotoxic effects in the animals) point to alternative MoAs or are not in compliance with the postulated mode of action. Moreover, involvement of specific enzymes in the metabolism of cumene in the lungs have not been shown and no knock out-model experiments are available to validate the hypothesised species specific MoA;
- for liver tumours in mice, there are indications for species specific MoA via nuclear receptors (CAR, PPRAα), but no evidence is available for the involvement of these receptors. Furthermore, a cytotoxic MoA is also discussed. This would also be relevant for human exposure, but would implicate an effect threshold for tumorigenicity. However, as no clear cytotoxic effects were observed in mice, this MoA also cannot be confirmed;
- for renal tumours in male rats, a MoA has been suggested which involves α_{2u}-globulin and hyaline droplets accumulation to finally result in species and sex specific tumours. Even though this MoA is supported by the observed effects, full compliance with the IARC criteria for this kind of mechanism is not provided. Thus, human relevance cannot be excluded;
- also, for further tumour sites in experimental animals (i.e., hemangiosarcoma of the spleen, follicular cell adenoma in the thyroid gland and testis adenoma) there also remain some uncertainties with regard to causality by cumene exposure and/or relevance to humans;
- for nasal tumours in rats, relevance to humans can be assumed, however progress to malignancy has been questioned and is regarded uncertain;
- the majority of *in vitro* and *in vivo* studies on genotoxicity do not indicate a genotoxic MoA for any of the cancer sites. However, a) increase of specific K-*ras* and p*53*-mutations in mouse lung tumours induced by cumene exposure, b) minor DNA damage, as indicated by Comet assays *in vivo*, c) potential genotoxicity of the presumed metabolite α-methylstyrene oxide, as evidenced from *in vitro* testing, and d) some further reactive metabolites formed by ring-hydroxylation of cumene (i.e, quinone methide or catechol via isopropylphenol formation) may be involved in the mode of action. The relevance of primary genotoxicity mediated by α-methylstyrene oxide may be questioned, because of the low concentrations of this presumed metabolite. However, available data are insufficient for further conclusions. Some of the observed genetic alterations could result from secondary genotoxicity or from epigenetic mechanisms.

Therefore, classification to

Carc. 2, 'H351: Suspected of causing cancer',

is warranted.

10.8 Reproductive toxicity

10.8.1 Adverse effects on sexual function and fertility

Table 17: 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
90d, repeated dose study Rat, F344/N ♂,♀, reproductive effects Similar to OECD 413. NTP conducts its studies in compliance with FDA Good Laboratory Practice Regulations Reliability according to disseminated database: study not reported Reliability according to authors of this evaluation: 1	Cumene, Purity: 99.9 % Inhalation exposure: 3 months exposure (n=10/sex and concentration) 0, 62.5, 125, 250, 500, and 1000 ppm (0, 306, 612, 1225, 2450, 4900 mg/m³) <u>Males:</u> Rats (0, 250, 500, and 1000 ppm) examined for -weight changes of cauda epididymis, epididymis and testes, -spermatid parameters (spermatid heads and spermatid counts), and -epididymal spermatozoal parameters (sperm motility, sperm concentration) Complete histopathology performed on 0 and 1000 ppm (core study) rats. Reproductive tissues examined: testis (with epididymis and seminal vesicle) <u>Females:</u> Rats (0, 250, 500, and 1000 ppm) examined for - Oestrus cycle length - Oestrus stages (% of cycle in dioestrus, prooestrus, oestrus, metoestrus) **Complete histopathology performed on 0, 500, 1000 ppm (core study) rats. Reproductive tissues examined: clitoral gland, mammary gland, and uterus.**	<u>Males:</u> No dose dependent significant differences between exposed and chamber control males in reproductive tissue evaluations. No histological changes in examined tissues from reproductive organs. For example: Testis weight (g): 1.41 ± 0.03, 1.46 ± 0.01, 1.43 ± 0.03, 1.45 ± 0.02 Spermatid count (10^6/cauda epididymis): 100.28 ± 5.52, 88.53 ± 4.55, 95.54 ± 3.36, 90.53 ± 2.32 Sperm motility (%): 85.45 ± 3.10, 81.28 ± 2.83, 84.10 ± 2.03, 87.62 ± 1.30 → negative <u>Females:</u> Exposed female groups differ significantly from the chamber control females in the relative length of time spent in the oestrous stages. Exposed females spent more time in oestrus and less time in proestrus than chamber control females (not dose dependent). No histological changes in examined tissues from reproductive organs. Estrous stages (% of cycle): Diestrus: 49.2, 41.7, 41.7, 44.2 Proestrus: 19.2, 14.2, 9.2, 11.7 Oestrus: 15.8, 25.8, 28.3, 25.0 Metestrus: 15.8, 18.3, 20.8, 19.2 → positive with questionable relevance Other toxicological endpoints assessed after three months of exposure: relative liver and kidney weights were significantly decreased in female rats at	(NTP, 2009)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		250 ppm and above. Relative kidney weight (g): 62.5 ppm: 3.322 ± 0.057 125 ppm: 3.439 ± 0.057* 250 ppm: 3.486 ± 0.044** 500 ppm: 3.612 ± 0.040** Relative liver weight (g): 62.5 ppm: 30.094 ± 0.634* 125 ppm: 31.289 ± 0.412** 250 ppm: 32.286 ± 0.386** 500 ppm: 36.958 ± 0.724** *:p≤0.05, **: p≤0.01 Markers of hepatocyte injury and hepatobiliary function were also altered at or above 250 ppm. No cross lesions and other persistent effects were observed.	
90d, repeated dose study Mice, B6C3F$_1$, ♂,♀, reproductive effects Similar to OECD 413. NTP conducts its studies in compliance with FDA Good Laboratory Practice Regulations Reliability according to disseminated database: study not reported Reliability according to	Cumene, Purity: 99.9 % Inhalation exposure: 3 months exposure (n=10/ sex and concentration) <u>Males:</u> 0, 62.5, 125, 250, 500, and 1000 ppm (0, 306, 612, 1225, 2450, 4900 mg/m³) Mice (0, 250, 500, and 1000 ppm) examined for -weight changes of cauda epididymis, epididymis and testes, -spermatid parameters (spermatid heads and spermatid counts), and -epididymal spermatozoal parameters (sperm motility, sperm concentration) Complete histopathology was performed on 0 and 1000 ppm (core study) mice. Reproductive tissues examined: testis (with epididymis and seminal vesicle) <u>Females:</u> 0, 62.5, 125, 250, 500, and 1000 ppm (0, 306, 612, 1225, 2450, 4900 mg/m³) Mice (0, 125, 250, and 500 ppm) examined	<u>Males:</u> At 1000 ppm significant reduction in cauda epididymis weight (p≤0.05) and in spermatid counts (p≤0.05), no other significant differences between exposed and exposed and chamber control mice. No histological changes in examined tissues from reproductive organs. For example: Cauda epididymis weight (g): 0.0196 ± 0.001, 0.019 ± 0.0007, 0.0173 ± 0.0006, 0.0171 ± 0.0006* (p≤0.05) Spermatid count (10^6/cauda epididymis): 18.05 ± 0.95, 17.62 ± 1.11, 17.53 ± 1.04, 14.70 ± 0.87* (p≤0.05) Sperm motility (%): 85.44 ± 1.96, 82.75 ± 2.41, 79.95 ± 2.13, 83.65 ± 2.43 → positive with questionable relevance Other toxicological endpoints assessed after 3 months of exposure: Final mean body weights and body weight gains of males exposed to ≥250 ppm generally less than of chamber controls. Significant increases in absolute liver weights in mice exposed to ≥500 ppm, significant increases in relative liver weights at ≥125 ppm. Minimal to mild liver necrosis significantly increased in mice at 1000	(NTP, 2009)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
authors of this evaluation: 1	for -Oestrus cycle length - Oestrus stages (% of cycle in dioestrus, prooestrus, oestrus, metoestrus) Complete histopathology was performed on 0 and 1000 ppm (core study) mice. Reproductive tissues examined: clitoral gland, mammary gland, and uterus.	ppm. Females: Exposed females do not differ significantly from the chamber control females in the relative time spent in the oestrous stages No histological changes in examined tissues from reproductive organs. → negative	
13w inhalations study, rats ♂, ♀ According to OECD 413 Reliability according to disseminated database: 1 Reliability according to authors of this evaluation: 3	Cumene, Purity: ≥99.94% ♂, ♀ - F344 rats, N=21 rats/sex/ group, exposure concentration: 0, 100, 500, 1200 ppm (0, 490 – 5880 mg/m³), 13 weeks, 6h/d, 5d/w. Males: Epididymides of 15 male animals per group were excised, and evaluated for sperm count and sperm morphology. Right testis of the 1200 ppm-group and control group was evaluated for stages of spermatogenesis. Females: Necroscopic examination of ovaries, uteri, cervix, vagina, oviducts and mammary tissue	Males: No cumene-related differences in the counts of testicular sperm heads or epididymal spermatozoa. Morphology and stages of spermatogenesis in the testes of 1200 ppm-group normal (1 rat exposed to 1200 ppm with diffuse testicular atrophy). For epididymal spermatozoa no abnormalities involving head portion of sperms. Only at 500 ppm-group increased frequency of sperm head abnormalities, but relatively infrequent and no dose response pattern observed. Sperm anomalies (%): 1.4, 1.6, 3.4, 2.3 Sperm head anomalies (%): 0.5, 0.5, 1.1, 0.7 Sperm tail anomalies (%): 0.5, 0.6, 1.5, 1.0 → negative Females: No cumene exposure related weight differences for ovaries compared to control were found. Lack of (adverse) findings for female rats. → negative	(CMA, 1989a; Cushman *et al*., 1995) Study also reported in (ECHA Dissemination, 2018, toxicity to reproduction)

Table 18: 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
No human data on adverse effects on sexual function and fertility from cumene exposure are available				

Table 19: 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
No other relevant studies on cumene are available, which are relevant for reproductive toxicity classification assessment				

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

There are no data on adverse effects on sexual function and fertility from human studies. No experimental studies on fertility following OECD guidelines are available. Therefore, observations on endpoints such as fertility impairment in either male or female experimental animals are not reported. However, subchronic repeated dose studies specifically addressed some indicative parameters, relevant for the assessment of adverse effects on sexual function and fertility. From those data there is only very limited indication of some adversity in reproductive parameters in experimental animals:

- In male B6C3F$_1$ mice at high concentrations (1000 ppm inhalation exposure) a significant reduction in cauda epididymis weight was observed and spermatid count was reduced (NTP, 2009). This concentration caused already significant reductions in body weight gain and was a hepatotoxic exposure concentration.
- In male F344 rats at high concentrations (1200 ppm inhalation exposure) one rat showed diffuse testicular atrophy. At 500 ppm increased frequency of sperm head abnormalities have been found, but not in a dose-related manner and relatively infrequent (Cushman *et al*., 1995). However, at (and below) 1000 ppm no adverse effects on testes or sperm parameters were seen in the study by NTP (2009).
- In female F344 rats the relative length of time spent in the oestrus stages were shifted compared to chamber control (NTP, 2009). This effect was not dose-related. Female rats in this study showed signs of hepatotoxicity and kidney weight changes at exposure concentrations leading to changes in oestrus cycle.

No other possible impairments of reproductive function were reported. This includes a study by Darmer *et al*. (1997) on developmental toxicity (see Table 20 for details) with some examinations on reproductive parameters of pregnant does.

10.8.3 Comparison with the CLP criteria

The available data were compared with the CLP criteria. In general, any effect of substances that has the potential to interfere with sexual function and fertility, is addressed. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems. For potential classification of cumene, classification criteria were analysed accordingly:

- *Category 1: Known or presumed human reproductive toxicant. Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).*

There are no data on adverse effects on sexual function and fertility associated with cumene exposure supporting category 1 classification.

- *Category 2: Suspected human reproductive toxicant. Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed 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 the other toxic effects.*

For cumene, only minor effects on male or female reproductive capacity were observed. These effects are not sufficient for classification to Category 2 (see also below).

- *No classification: If, in some reproductive toxicity studies in experimental animals the only effects recorded are considered to be of low or minimal toxicological significance, classification may not necessarily be the outcome.*

For cumene, the observed effects are of low or minimal toxicological significance and human relevance is questionable:

a) Changes in relative length of time spent in the oestrous stages, as indicated in a three month repeated dose study (NTP, 2009), were not dose-related and no other adverse effects on reproductive parameters were shown in female rats. Indication of liver and kidney toxicity occurred at equivalent concentrations. Another study with pregnant rats did not provide any indication on changes in gestational parameters from exposure to cumene (Darmer *et al*., 1997).

b) Testicular atrophy in a male rat at high inhalation exposures (1200 ppm) (Cushman *et al*., 1995) may not have been causally related to cumene exposure, because this effect occurred in just one animal and is not supported by the outcome of a second study with male rats with cumene inhalation exposure (NTP, 2009) and occurred at otherwise toxic exposure concentrations.

c) Similarly, sperm head abnormalities in one dose group of the above mentioned repeated dose study (Cushman *et al*., 1995) were not dose related and only minor in degree. Moreover this isolated effect was not confirmed in the other repeated dose study (NTP, 2009).

d) Another isolated effect in cauda epididymis weight and in spermatid counts occurred in mice at the highest exposure concentration (NTP, 2009). However, this effect was accompanied by significant other indications of toxicity in the male mice.

10.8.4 Adverse effects on development

Table 20: 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**
Developmental toxicity study, ♀, CD Sprague-Dawley rat Guideline	Cumene Purity: >99.9% 25 pregnant ♀ rats/concentration; mated with unexposed males 6h/d; GD 6-15	Maternal effects: increased relative liver weight, perioral wetness and incrustation at 1200 ppm, NOAEC 100 ppm, because of reduced food consumption at and above 500 ppm on GD 6-15 NOAEC: developmental effects ≥1200 ppm Gestational parameters not affected, no changes	(CMA, 1989b; Darmer *et al*., 1997) Study also reported in (ECHA Dissemination, 2018,

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
study OECD 414; compliant with GLP Reliability according to disseminated database: 1 Reliability according to authors of this evaluation: 2	0, 100, 500, 1200 ppm vapour (490-5880 mg/m³) , sacrificed at GD 21	in number of live litters and litter size. F1: sex ratio: not affected; no significantly increased frequencies in malformations or external variations.	developmental toxicity/ teratogenicity, #001, key)
Developmental toxicity study, ♀, New Zealand White rabbits Guideline study OECD 414 compliant with GLP Reliability according to disseminated database: 1 Reliability according to authors of this evaluation: 2	Cumene Purity: >99.9% 15 pregnant rabbits/concentration; mated with unexposed males 6h/d; GD 6-18 0, 500, 1200, 2300 ppm vapour (2450-11270 mg/m³), post exposure observation until GD29	Maternal effects: increased relative liver weight, perioral wetness at 2300 ppm, NOAEC <500 ppm, because of reduced food consumption at and above 500 ppm on GD 6-18 NOAEC: developmental effects ≥ 2300 ppm Gestational parameters not affected, no changes in number of live litters and litter size. Nonviable implants were found in one doe at 500 and 1200 ppm, respectively F1: sex ratio: not affected; no treatment related significantly increased frequencies in malformations or variations. An external variation (ecchymosis) was statistically significant only in the 500-ppm group (incidence of this variation 35.7% vs. 0% in control, but historical incidence 0-66.7% in unexposed rabbits). → negative	(CMA, 1989c; Darmer *et al.*, 1997) Study also reported in (ECHA Dissemination, 2018, developmental toxicity/ teratogenicity, #002, key)

Table 21: 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
No human data on adverse effects on development from cumene exposure are available				

Table 22: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No other relevant studies on cumene are available, which are relevant for reproductive toxicity classification assessment				

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

There is no available data on adverse effects on development from exposure to cumene in humans. There were no developmental effects observed in either New Zealand White Rabbits or CD Sprague Dawley rats in an OECD guideline study (OECD 414) as published by Darmer *et al*. (1997). This publication is consistent with an earlier internal study report (CMA, 1989b; c).

10.8.6 Comparison with the CLP criteria

Cumene is negative in developmental toxicity studies. CLP criteria for developmental toxicity do not apply to cumene.

10.8.7 Adverse effects on or via lactation

Table 23: 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
No relevant studies on cumene are available, which are relevant for "effects on or via lactation" assessment			

Table 24: 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
No human data on adverse effects on or via lactation are available				

Table 25: 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
No other relevant studies on cumene are available, which are relevant for "effects on or via lactation" assessment				

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

No data available.

10.8.9 Comparison with the CLP criteria

Not applicable, as no data are available for this endpoint.

10.8.10 Conclusion on classification and labelling for reproductive toxicity

As outlined in Section 10.8.3, a weight of evidence approach indicates that the minor observed effects on sexual function and fertility are not sufficient to meet the criteria for classification of cumene for this endpoint. However, it should be noted that adequate studies to examine fertility and other endpoints of reproductive toxicity have not been performed.

There is no evidence of adverse effects from cumene exposure on developmental toxicity (see Section 10.8.5). There are no data to assess adverse effects on or via lactation from cumene exposure. Overall the information is conclusive, but not sufficient for classification of cumene as a reproductive toxicant.

10.9 Specific target organ toxicity-single exposure

Evaluation not performed for this substance

10.10 Specific target organ toxicity-repeated exposure

Evaluation not performed for this substance

10.11 Aspiration hazard

Evaluation not performed for this substance

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Evaluation not performed for this substance

12 EVALUATION OF ADDITIONAL HAZARDS

Evaluation not performed for this substance

13 ADDITIONAL LABELLING

No additional labelling relevant for this substance.

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15 ANNEXES

[Please add ANNEX I to the CLH report and potential other annexes.]