Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

2,4,6-tri-tert-butylphenol

EC Number: 211-989-5
CAS Number: 732-26-3; (1333-60-4); (11100-56-4); (19879-87-9); (50356-20-2); (53320-88-0)

CLH-O-0000006909-58-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

8 October 2020

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

2,4,6-tri-tert-butylphenol

EC Number: **211-989-5**

CAS Number: **732-26-3**

Index Number: **/**

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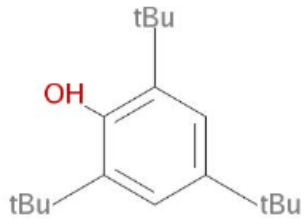
Version number: 2 **Date: 14 February 2019**

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)	2,4,6-tri-tert-butylphenol
Other names (usual name, trade name, abbreviation)	2,4,6-Tri-tert-butylphenol 2,4,6-tritert-butylphenol 2,4,6-tritertiary-butylphenol
ISO common name (if available and appropriate)	
EC number (if available and appropriate)	211-989-5
EC name (if available and appropriate)	2,4,6-tri-tert-butylphenol
CAS number (if available)	732-26-3
Other identity code (if available)	
Molecular formula	C18H30O
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	262.44 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)	≥99 - ≤100%(W/W)

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)**
2,4,6-tri-tert-butylphenol*1 **EC n° 211-989-5** **CAS n° 732-26-3**	**≥99 - ≤100%(W/W)*2**		

**1 The mono-constituent substance 2,4,6-tri-tert-butylphenol is not manufactured/imported in the EU as such, but is a component of the multi-constituent substances "reaction mass of 2,6-di-tert-butylphenol" and 2,4,6-tri-tert-butylphenol" and "reaction mass of 2-tert-butylphenol and 2,6-di-tert-butylphenol and 2,4,6-tri-tert-butylphenol". REACH registration of the multi-constituent substances is covered by the registration of the mono-constituent 2,4,6-tri-tert-butylphenol.*

**2 The concentration range given here corresponds to the mono-constituent test substance used in the studies.*

Impurities are not specified in the confidential IUCLID.

All studies were performed with the mono-constituent substance 2,4,6-tri-tert-butylphenol. Almost all of them were performed with a purity of 99.8%.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 3:

	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	None				/	/					
Dossier submitters proposal		2,4,6-tri-tert-butylphenol	211-989-5	732-26-3	Acute Tox. 4 Skin Sens. 1B Repr. 2 STOT RE 1	H302 H317 H361d H372 (liver)	 GHS07 GHS08	H302 H317 H361d H372		ATE (oral) : 500 mg/kg bw	
Resulting Annex VI entry if agreed by RAC and COM		2,4,6-tri-tert-butylphenol	211-989-5	732-26-3	Acute Tox. 4 Skin Sens. 1B Repr. 2 STOT RE 1	H302 H317 H361d H372 (liver)	 GHS07 GHS08	H302 H317 H361d H372		ATE (oral) : 500 mg/kg bw	

Table 4: 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	Acute Tox.4, H302	Yes
Acute toxicity via dermal route	data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Hazard class not assessed in this dossier	No
Skin corrosion/irritation	data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	data conclusive but not sufficient for classification	Yes
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Skin Sens. 1B, H317	Yes
Germ cell mutagenicity	data conclusive but not sufficient for classification	Yes
Carcinogenicity	data lacking	Yes
Reproductive toxicity	Repr. 2, H361d	Yes
Specific target organ toxicity-single exposure	data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	STOT RE 1, H372 (liver)	Yes
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

2,4,6-tri-tert-butylphenol (2,4,6-TTBP) is a chemical substance which is registered under REACH (1907/2006/EC). The mono-constituent substance 2,4,6-tri-tert-butylphenol is not manufactured/imported in the EU as such but is a component of the multi-constituent substances "reaction mass of 2,6-di-tert-butylphenol" and 2,4,6-tri-tert-butylphenol" and "reaction mass of 2-tert-butylphenol and 2,6-di-tert-butylphenol and 2,4,6-tri-tert-butylphenol". REACH registration of the multi-constituent substances is covered by the registration of the mono-constituent 2,4,6-tri-tert-butylphenol.

The substance is currently not listed in annex VI of CLP and classification and labelling was not previously discussed by the TC C&L The substance is classified in the public registration dossier as :

-2,4,6-tritertiary-butylphenol : data lacking for each property, not classified

-2,4,6-tri-tert-butylphenol (idem joint entries in C&L inventory)

Acute Tox.4 , H302
Skin Sens. 1B, H317
STOT RE 1, H372 (Liver)
Aquatic chronic 2, H411

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[A.] There is no requirement for justification that action is needed at Community level.

Based on the available data, the substance is considered toxic to reproduction.

[B.] Justification that action is needed at Community level is required.

- Differences in self-classification between different notifiers in the C&L inventory and/or between different registration dossiers.

Joint entry :

Acute Tox.4, H302

Skin Sens. 1B, H317

STOT RE 1, H372(Liver)

Aquatic chronic 2, H411

Other self-classifications :

Eye Irrit.2, H319

Skin Irrit.2, H315

STOT SE 3, H335 (Lung)

STOT RE 2, H373 (Liver)

Aquatic Acute 1, H400

Aquatic Chronic 1, H410

Aquatic Chronic 4, H413

Not classified

- Furthermore, harmonized classification is relevant for other legislation or processes

The substance is under substance evaluation (REACH). It was concluded that the substance fulfils

the PBT-criteria. In order to identify the substance as a SVHC, the T should be confirmed via a CLH.

5 IDENTIFIED USES

This substance is used by professional workers, in formulation or re-packaging and at industrial sites use of intermediates) in fuels.

The substance is used by professionals in indoor close systems (e.g., cooling liquids in refrigerators, oil-based electric heaters) and outdoor close systems(e.g. hydraulic liquids in automotive suspension, lubricants in motor oil and break fluids).

6 DATA SOURCES

REACH registration dossier (last modification 14 Nov 2018, consultation by the DS on 3 December 2018)

7 PHYSICOCHEMICAL PROPERTIES

Table 5: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Slightly yellow solid particulate powder	Anonymous 1, 2015	Rel.1 Purity : ≥99 - ≤100%(W/W)
Melting/freezing point	131 °C (at 1012 ± 1 hPa)	Anonymous 1, 2015	OECD TG 102 GLP Rel.1 Purity : ≥99 - ≤100%(W/W)
Boiling point	278 °C (at 1012 ± 1 hPa)	Anonymous 1, 2015	OECD TG 103 GLP Rel.1 Purity : ≥99 - ≤100%(W/W)
Relative density	Density : 0.977 g/cm³ at 20 °C Relative density : 0.977	Anonymous 1, 2015	OECD TG 109 GLP Rel.1 Purity : ≥99 - ≤100%(W/W)
Vapour pressure	3.5×10^{-2} Pa (2.6×10^{-4} mm Hg) at 20 °C 7.3×10^{-2} Pa (5.5×10^{-4} mm Hg) at 25 °C	Anonymous 1, 2015	OECD TG 104 GLP Rel.1 Purity : ≥99 - ≤100%(W/W)
Surface tension	Study scientifically not necessary		
Water solubility	0.063 mg/L (at 20°C)	Anonymous 1, 2015	OECD TG 105

Property	Value	Reference	Comment (e.g. measured or estimated)
			GLP Rel.1 Purity : ≥99 - ≤100%(W/W)
Partition coefficient n-octanol/water	Log Kow=7.1	Anonymous 1, 2015	OECD TG 117 GLP Rel.1 Purity : ≥99 - ≤100%(W/W)
Flash point	Study technically not feasible		
Flammability	Not highly flammable	Anonymous 1, 2015	EU method A.10 GLP Rel.1 Purity : ≥99 - ≤100%(W/W)
Explosive properties	No explosive properties	Anonymous 2, 2015	According to Guidance IR&CSA chapter R7a : structural examination and an oxygen balance calculation Non GLP Rel.2 Purity : ≥99 - ≤100%(W/W)
Self-ignition temperature	Study scientifically not necessary		
Oxidising properties	No oxidising properties	Anonymous 3, 2015	According to Guidance IR&CSA chapter R7a : structural examination Non GLP Rel.2 Purity : ≥99 - ≤100%(W/W)
Granulometry	Study technically not feasible		
Stability in organic solvents and identity of relevant degradation products	Study technically not feasible		
Dissociation constant	pKa=12.62		According to Guidance IR&CSA chapter R6 : PALLAS prediction Rel.2 Non GLP Purity : ≥99 - ≤100%(W/W)
Viscosity	Study technically not feasible		

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this CLH dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not evaluated in this CLH dossier.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Table 6: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Results	Reference
Acute oral toxicity study Gavage OECD TG 401 GLP	Rats (SD) 5/sex/dose	2,4,6-TTBP Purity : unknown Vehicle : arachis oil	200 and 2000 mg/kg bw Single exposure Post exposure period : 14d	200 mg/kg bw : no effect observed 2000 mg/kg bw : 2 females were found dead 1d after treatment and 3 females and 1 male were killed in extremis 1 or 4d after exposure. Animals exhibited clinical signs (ataxia, hunched posture, lethargy, decreased respiratory rate and laboured respiration, ptosis and loss of lighting reflex) and at necropsy, haemorrhagic lungs, dark or pale liver, haemorrhagic or pale gastric mucosa were noted LD50 : > 200 and < 2000 mg/kg bw	Anonymous 4, 1992

No human data and no other studies available

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

In an acute oral toxicity study (Anonymous 4, 1992), following OECD TG 401, groups of 5 male and 5 female rats (SD) were given once, by gavage, 2,4,6-TTBP at a concentration of 200 or 2000 mg/kg bw.

No adverse effects were observed at the lowest dose level.

However, at the high dose, 2 females were found dead 1d after exposure and 3 females and 1 male were killed in extremis 1 or 4 d after treatment. Animals exhibited clinical signs such as ataxia, hunched posture, lethargy, decreased respiratory rate, laboured respiration, ptosis and loss of righting reflex. Surviving animals recovered 3 or 10d after exposure.

At necropsy, animals, which died or which were killed, showed haemorrhagic lungs, dark or pale liver, patchy pallor of the liver or red-coloured possible necrosis of the liver and haemorrhagic or pale gastric mucosa.

10.1.2 Comparison with the CLP criteria

Oral acute toxicity criteria	Results of the available study
Category 4 : LD50 between 300 and 2000 mg/kg bw	LD50 between 200 and 2000 mg/kg bw (200 mg/kg bw : no mortality ; 2000 mg/kg bw : 2 females found dead and 3 females and 1 male killed)

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the available results (LD50 between 200 and 2000 mg/kg bw), a classification as **Acute Tox. Cat. 4 H302 (Harmful if swallowed)** is warranted. Moreover, based on the table 3.1.2 of the CLP Regulation, an ATE of 500 mg/kg bw is warranted.

10.2 Acute toxicity - dermal route

Table 7: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
Acute dermal toxicity study Occlusive Area covered : approx. 10% of the tot. body surface OECD TG 402 GLP	Rat (Wistar) 5/sex/group	2,4,6-TTBP Purity : 99.88% Vehicle : corn oil	2000 mg/kg bw Duration of exposure : 24h Post exposure period : 2w	No mortality, nor bw and macroscopic changes were observed 1 female exhibited a general erythema 2d after exposure LD_{50} > 2000 mg/kg bw	Anonymous 5, 2015

No human data and no other studies available

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

In a dermal toxicity study (Anonymous 5, 2015), following the OECD TG 402, groups of 5 male and 5 female Wistar rats were exposed during 24h to 2,4,6-TTBP at a concentration of 2000 mg/kg bw.

No mortality was observed. Moreover, no body weight or macroscopic changes were noted. Only 1 female exhibited a general erythema 2d after exposure.

The LD50 was higher than 2000 mg/kg bw.

10.2.2 Comparison with the CLP criteria

Dermal acute toxicity criteria	Results of the available study
Category 4 : LD50 between 1000 and 2000 mg/kg bw	LD50 > 2000 mg/kg bw

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Based on the results of the dermal acute toxicity study (LD50 > 2000 mg/kg bw), no classification is warranted.

10.3 Acute toxicity - inhalation route

Not evaluated in this CLH dossier

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Oral Route

The dossier submitter (DS) proposed classification of 2,4,6-tri-tert-butylphenol as Acute Tox. 4 with hazard statement H302: Harmful if swallowed - based on results of an acute toxicity study performed in GLP conditions according to OECD TG 401. Five female and 5 male rats were given the substance by gavage in two doses, 200 and 2000 mg/kg bw. No toxicity was observed in animals receiving a dose of 200 mg/kg bw, while all five females and one male administered a dose of 2000 mg/kg bw were found dead or were killed in extremis at 1 or 4 days after exposure. LD_{50} was found to be in a range between 200 and 2000 mg/kg bw.

Dermal Route

In the acute dermal toxicity study in female and male rats, carried out according to OECD TG 402 in GLP conditions, no mortality was observed after occlusive application on skin of 2,4,6-tri-tert-butylphenol at a dose of 2000 mg/kg bw on approx. 10% of the total body surface, therefore the DS proposed no classification for acute dermal toxicity.

Inhalation Route

Not evaluated by the DS.

Comments received during the general consultation

One MSCA supported classification of 2,4,6-tri-tert-butylphenol as Acute Tox. 4, H302: Harmful if swallowed.

Assessment and comparison with the classification criteria

Comparison with the criteria

Oral route

Taking into account that in a reliable acute oral toxicity study no symptoms were observed in 10 rats administered by gavage 2,4,6-tri-tert-butylphenol a dose of 200 mg/kg bw, while 5 out of 5 females and 1 out of 5 males were killed by a dose of 2000 mg/kg bw, RAC is of the opinion that oral LD_{50} for female rats of 2,4,6-tri-tert-

butylphenol is in a range of 300 – 2000 mg/kg bw, thus meeting the classification criteria for Category 4 of acute oral toxicity.

Since the exact value of oral LD_{50} is not defined, a converted acute toxicity point estimate for Category 4 equal to 500 mg/kg bw according to Table 3.1.2 of Regulation (EC) No 1272/2008 (CLP Regulation) should be used as an oral ATE in the formulas for the classification of mixtures.
Therefore, **classification as Acute Tox. 4, H302: Harmful if swallowed, with an ATE of 500 mg/kg bw**, is warranted.

Dermal route

Taking into account the dermal LD_{50} value in male and female rats, which is above the threshold value for classification (2000 mg/kg bw), 2,4,6-tri-tert-butylphenol **does not warrant classification for acute dermal toxicity** according to the CLP criteria.

10.4 Skin corrosion/irritation

Table 8: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
Skin irritation study Semi-occlusive OECD TG 404 GLP	Rabbit (NZW) 3 males	2,4,6-TTBP Purity : unknown Vehicle : distilled water	Dose : 0.5g Duration of exposure : 4h	Mean erythema score (mean of the 24, 48 and 72h examination) : 0.22/4 (fully reversible within 72h) Mean edema score (mean of the 24, 48 and 72h examination) : 0/4	Anonymous 6, 1992

No human data and no other studies available

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

In a skin irritation study (Anonymous 6, 1992), following OECD TG 404, 3 male rabbits were exposed to 0.5g of 2,4,6-TTBP in distilled water during 4 hours. Rabbits were observed 1, 24, 48 and 72h after patch removal.

One rabbit exhibited a slight erythema 1, 24 and 48h after patch removal. The 2 others exhibited a slight erythema only 1h after patch removal. One rabbit showed a slight edema, only at the first observation time point (see table 9).

Table 9 : Erythema and edema score

	animals	Observation time (h)				Mean of the 24, 48 and 72h examinations
		1	24	48	72	
erythema	1	1	1	1	0	0.22/4

	2	1	0	0	0	
	3	1	0	0	0	
Oedema	1	1	0	0	0	0/4
	2	0	0	0	0	
	3	0	0	0	0	

10.4.2 Comparison with the CLP criteria

CLP criteria Skin Irrit. Cat. 2	Results of the available study
• Mean value of ≥ 2.3 - ≤ 4.0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from grading at 24, 48 and 72 hours after patch removal or if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or • Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or • In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above	Mean erythema score (mean of the 24, 48 and 72h examinations) : 0.22/4 (fully reversible within 72h Mean oedema score (mean of the 24, 48 and 72h examinations) : 0/4

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Based on the available results, a classification as Skin irritation is not warranted.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

No human data are available. In the skin irritation study in rabbits carried out according to OECD TG 404 and in GLP conditions (Anonymous 6, 1992), mild erythema (score 1) was observed after 24h and 48h only in one out of three tested rabbits. The erythema was reversible 72 hours after treatment. No oedema was observed in any of three exposed rabbits. Based on these data the DS is of the opinion that a classification for Skin Irritation for 2,4,6-tri-tert-butylphenol is not warranted.

Comments received during general consultation

One MSCA supported no classification of 2,4,6-tri-tert-butylphenol as a skin irritant.

Assessment and comparison with the classification criteria

In the dermal irritation study (Anonymous 6, 1992) adult New Zealand white rabbits (3 males) were exposed to 0.5 g of 2,4,6-tri-tert-butylphenol, applied to the intact shaved flank under a semi-occlusive dressing for 4 hours. Skin reactions were scored at 1, 24, 48 and 72 hours after removal of the dressings. No clinical signs were observed in the animals during the study and no mortality occurred. Mild erythema (score 1) was observed only in one animal and it was no longer observable 72 hours after exposure. The primary irritation index (calculated by totalling the mean cumulative scores at 24, 48 and 72 hours for each animal and then dividing by the number of animals) was 0.67 for erythema and 0.0 for oedema. Taking these results into account RAC is of the opinion that 2,4,6-tri-tert-butylphenol **does not fulfil the criteria for classification for skin corrosion/irritation**.

10.5 Serious eye damage/eye irritation

Table 10 : Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results - Observations and time point of onset - Mean scores/animal - Reversibility**	**Reference**
Eye irritation study OECD TG 405 GLP	Rabbit (NZW) 2 males and 1 female	2,4,6-TTBP Purity : unknown Vehicle : unchanged	0.1 ml (approx. 62mg) Single exposure The left eye remained untreated and was used as control washing : no	Mean score of the 24, 48 and 72h examinations : Cornea opacity score : 0/4 Iris score : 0/2 Conjunctivae score (redness) : 0.22/3 Chemosis score : 0/4	Anonymous 7, 1992

No human data and no other studies available

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

In an eye irritation study (Anonymous 7, 1992), following OECD TG 405, 2 males and 1 female rabbit (NZW) were exposed to 0.1ml of 2,4,6-TTBP. The animals were observed at 1, 24, 48 and 72h after exposure. The left eye remained untreated and was considered as control.

No corneal opacity was noted. Slight iridial inflammation was observed in 2 animals 1h after exposure. Moreover, all animals exhibited a minimal conjunctival irritation 1h after treatment and in 2 animals this effects was also observed 24h after treatment. The overall irritation score was of 8.7, 2.0, 0.0 and 0.0 respectively after 1, 24, 48 and 72h of exposure.

Table 11 : Eye irritation scores

Rabbit	Male 1				Male 2				Female 1				Mean score (24, 48 and 72h)
Observation time (hours)	1	24	48	72	1	24	48	72	1	24	48	72	

Cornea	Degree of opacity	0	0	0	0	0	0	0	0	0	0	0	0	0/4
	Area of opacity	0	0	0	0	0	0	0	0	0	0	0	0	
Iris		1	0	0	0	1	0	0	0	0	0	0	0	0/2
Conjunctivae	Redness	1	1	0	0	1	0	0	0	1	1	0	0	0.22/3
	Chemosis	1	0	0	0	1	0	0	0	0	0	0	0	0/4
	Discharge	1	1	0	0	1	0	0	0	1	0	0	0	
Mean score for ocular irritation		11	4	0	0	11	0	0	0	4	2	0	0	

10.5.2 Comparison with the CLP criteria

CLP criteria Eye Irrit. Cat. 2	Results of the available study
Irritating to eyes if, when applied to the eye of an animal, a substance produces : • At least in 2 of 3 tested animals, a positive response of : o Corneal opacity ≥ 1 and/or o Iritis ≥ 1 and/or o Conjunctival redness ≥2 and/or o Conjunctival oedema (chemosis) ≥2 • Calculated as the mean score following grading at 24, 48 and 72 hours after instillation of the test material, and which fully reverses within an observation period of 21days.	Mean score of the 24, 48 and 72h examinations : Mean corneal opacity score : 0/4 Mean iris score : 0/2 Mean conjunctival redness score : 0.22/3 Mean chemosis score : 0/4

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Based on the available results, a classification as Eye irritation is not warranted.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

No human data are available. In the only available animal eye irritation study conducted under GLP and following the OECD TG 405 (Anonymous 7, 1992), 3 adult New Zealand white rabbits (2 males and 1 female) were administered 0.1 ml of 2,4,6-tri-tert-butylphenol to the conjunctival sac. No clinical signs and no lethality were observed in the animals during the study.

The degree of eye irritation/corrosion was evaluated by scoring lesions of conjunctiva, cornea, and iris, at 1, 24, 48 and 72 hours after instillation. The mean scores at the 24, 48 and 72 h examinations per animal were: for corneal opacity – 0/0/0, for iris response

– 0/0/0, for conjunctival redness – 0.33/0/0.33 and for chemosis – 0/0/0. All eye lesions fully reversed within 72 hours after installation. Based on these data the DS is of the opinion that a classification for Eye Irritation for 2,4,6-tri-tert-butylphenol is not warranted.

Comments received during general consultation

One MSCA supported no classification of 2,4,6-tri-tert-butylphenol as an eye irritant.

Assessment and comparison with the classification criteria

Noting that in the reliable eye irritation study (Anonymous 7, 1992) all scores of ocular lesions in three New Zealand white rabbits after conjunctival installation of 2,4,6-tri-tert-butylphenol were well below the threshold of cornea, iris and conjunctival effects defined as criteria for classification as Eye Irrit. 2 of the CLP Regulation and, further noting the full reversibility of the observed eye responses within a 72-hour period after instillation, RAC considers that this substance **does not warrant classification as an eye irritant**.

10.6 Respiratory sensitisation

Not evaluated in this CLH dossier

10.7 Skin sensitisation

Table 12: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels	Results	Reference
LLNA OECD TG 429 GLP	Mouse (CBA/J) 5 females/group	2,4,6-TTBP Purity : 99.88% Vehicle : N,N-dimethyl formamide	0, 10, 25 and 50 % w/w	No mortality and no clinical signs observed No body weight changes The auricular lymph nodes of 2 animals exposed to 25% and of all animals exposed to 50% appeared larger in size SI : 1.7, 3.3 and 4.6 respectively at 10, 25 and 50% EC3 value of 22.2%	Anonymous 8, 2015

No human data and no other studies available

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

In a local lymph node assay (Anonymous 8, 2015), following OECD TG 429, groups of 5 female mice were treated with 2,4,6-TTBP at a concentration of 0, 10, 25 or 50% on 3 consecutive days, by open application

on the ears. 3d after the last exposure, all animals received by injection ^{3}H-methyl thymidine and after 5h, animals were killed.

No mortality, clinical signs or body weight changes were observed during the study. Furthermore, no irritation of the ears was noted.

The auricular lymph nodes appeared larger in size in 2 animals exposed to 25% and in all animals exposed to 50% of 2,4,6-TTBP. SI value was of 1.7, 3.3 and 4.6 respectively at 10, 25 and 50%. Based on these values, the EC3 was of 22.2%.

10.7.2 Comparison with the CLP criteria

Criteria for Skin Sens. 1A	Criteria for Skin Sens. 1B	Results of the study available
"Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered." EC3 value ≤ 2	"Substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans. Severity of reaction may also be considered." EC3 value > 2%	SI or 1.7, 3.3 and 4.6 respectively at 10, 25 and 50% EC3 of 22.2%

10.7.3 Conclusion on classification and labelling for skin sensitisation

Based on the results of the study, a classification as **Skin Sens. Cat. 1B, H317 (may cause an allergic skin reaction)** is warranted**.**

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Skin sensitisation potential was assessed in a mouse Local Lymph Node Assay (LLNA) study conducted under GLP and following the OECD TG 429 (Anonymous 8, 2015) using four groups of five female CBA/J mice. 2,4,6-tri-tert-butylphenol was applied topically on the skin of the ears of animals as 0, 10, 25 and 50% w/w solution in N, N-dimethylformamide for 3 consecutive days. Three days after the last exposure all animals were administered ^{3}H-methyl thymidine by injection and 5 hours later the animals were killed to remove the ear lymph nodes. The Stimulation Index (SI) defined as the ratio of the mean proliferation of lymphocytes in lymph nodes in mice treated with 10, 25 and 50% solutions to that in the concurrent vehicle control group, amounted to 1.7, 3.3, and 4.6. The estimated concentration needed to produce a stimulation index of 3 (EC3) was calculated to be 22.2%. No human data or other animal studies are available. Based on the results of this LLNA study the DS has proposed to classify 2,4,6-tri-tert-butylphenol as Skin Sens. 1B, H317: May cause an allergic skin reaction.

Comments received during general consultation

One MSCA supported classification of 2,4,6-tri-tert-butylphenol as Skin Sens. 1B.

Assessment and comparison with the classification criteria

In the reliable mice LLNA study (Anonymous 8, 2015) the estimated concentration of 2,4,6-tri-tert-butylphenol needed to produce a stimulation index of 3 (EC3) was 22.2%, which is within the limit for classification in sub-category 1B (a substance is to be classified as 1B if the EC3 value is higher than 2%). It can be excluded that the substance can meet the criterion to be classified in sub-category 1A, induction of SI of ≥ 3 in concentrations below 2%, because in the LLNA study it has been demonstrated that even at a concentration of 10% the SI was only 1.7. Since classification to sub-category 1A can be excluded, while criteria for classification to lower sub-category are met RAC is of the opinion that 2,4,6-tri-tert-butylphenol **warrants classification as Skin Sens. 1B, H317**: May cause an allergic skin reaction.

10.8 Germ cell mutagenicity

Table 13 : 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 reverse mutation assay AMES test OECD TG 471 GLP	2,4,6-TTBP Purity : 99.88% Vehicle : DMSO	*S. Typh* TA1535, TA1537, TA98 and TA100 + *E. Coli* WP2uvrA With and without S9-mix First experiment : dose levels : 1.7, 5.4, 17, 52, 164, 512, 1600 and 5000 µg/plate (for strains TA100 and WP2uvrA) and 17, 52, 164, 512 and 1600 µg/plate (for strains TA1535, TA1537 and TA98) Second experiment : dose levels : up to the dose level of 1600 µg/plate	Genotoxicity : negative for all bacterial strains (no increase in the number of revertants) Cytotoxicity : only in tester strains *S. Typh* TA1535 and TA1537 without S9-mix	Anonymous 9, 2015
In vitro mammalian cell gene mutation OECD TG 476 GLP	2,4,6-TTBP Purity : 99.88% Vehicle : DMSO	Mouse lymphoma L5178Y cells Dose levels : First experiment : 3h of treatment : 0.1 to 45 µg/ml (without S9-mix) and 0.1 to 100 µg/ml (with S9-mix) Second experiment : 3h of treatment : 0.01 to 25 µg/ml (without S9-mix) and 0.01 to 60 µg/ml (with S9-mix) Third experiment : 24h of treatment : 0.1 to 30 µg/ml (without S9-mix)	Genotoxicity : negative Cytotoxicity : yes In absence or presence of metabolic activation, 2,4,6-TTBP did not induce a sign. increase in the mutation frequency in the two experiment	Anonymous 10, 2015
In vitro mammalian	2,4,6-TTBP	Chinese hamster ovary (CHO)	Genotoxicity : negative	Anonymous 11, 1998

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
chromosome aberration test Japabese guideline (for screening mutagenicity) GLP	Purity : unknown Vehicle : DMSO	With and without S9-mix Dose levels : without S9-mix : 0.015, 0.022, 0.024 and 0.026 mg/ml for 6h treatment ; 0.0098, 0.013, 0.017 and 0.022 mg/ml for 24h treatment ; 0.010, 0.020, 0.025 and 0.030 mg/ml for 48h treatment With S9-mix : 0.026, 0.035, 0.047, 0.062, 0.083, 0.11 and 0.15 mg/ml for 6h treatment	Cytotoxicity : yes	

No *in vivo* data and no human data available

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

A bacterial reverse mutation assay, following OECD TG 471, was performed in 2 steps (Anonymous 9, 2015).
First, a direct plate assay was done and 2,4,6-TTBP was tested in the tester strains *S. Typh.* TA100 and *E. Coli* WP2uvrA with concentrations of 1.7, 5.4, 17, 52, 164, 512, 1600 and 5000 µg/plate in the absence and presence of rat liver S9-mix. Based on the results, the doses of 17, 52, 164, 512 and 1600 µ/plate were used for the assay with the tester strains *S. Typh* TA1535, TA1537 and TA98 in the absence and presence of rat liver S9-mix.
No significant increase in the number of revertants was noted (see table 14). And no reduction in the bacterial background lawn was observed.

Table 14 : Mean number of revertant colonies/3 replicate plates

Dose level (µg/plate)	S. Typh				E. Coli
	TA98	TA100	TA1535	TA1537	WP2uvrA
Without S9-mix					
Positive control	13040±64	776±46	760±64	339±27	1326±78
Negative control	17±6	103±19	12±10	5±4	28±3
1.7	NT	113±18	NT	NT	26±11
5.4	NT	108±17	NT	NT	27±6
17	19±2	114±27	6±1	6±2	27±8
52	10±4	92±8	13±2	7±2	29±13
164	14±11	96±8	11±1	11±3	30±1
512	15±4	104±24	12±4	10±2	30±8
1600	7±1	85±4	6±2	4±2	24±2
5000	NT	99±10	NT	NT	23±5
With S9-mix					
Positive control	908±359	1389±88	275±64	324±28	218±13
Negative control	24±2	108±10	10±5	8±3	33±3
1.7	NT	106±17	NT	NT	36±6
5.4	NT	108±17	NT	NT	52±27
17	25±2	116±24	8±4	11±1	37±5
52	18±2	97±6	11±6	6±4	34±7
164	24±5	98±13	8±4	10±2	35±2

512	9±2	105±11	10±4	4±2	35±6
1600	13±2	89±13	8±2	6±2	31±2
5000	NT	111±9	NT	NT	32±7

In a second experiment, a pre-incubation assay, was performed based on the result of the first test. 2,4,6-TTBP was tested up to the dose level of 1600 µg/plate in the tester strains S. Typh TA1535, TA1537, TA98, TA100 and E. Coli WP2uvrA.
No dose relationship changes in number of revertants was observed. (see table 15)

Table 15 : Mean number of revertant colonies/3 replicate

Dose level (µg/plate)	S. Typh				E. Coli
	TA98	TA100	TA1535	TA1537	WP2uvrA
Without S9-mix					
Positive control	1964±166	699±73	72±24	62±4	153±32
Negative control	10±3	86±12	9±2	4±4	24±7
17	11±6	91±16	6±6	5±5	21±2
52	11±3	79±16	5±3	5±5	26±7
164	12±6	90±2	9±3	4±1	30±10
512	11±4	89±8	17±4	7±4	23±11
1600	8±1	81±3	4±1	2±2	28±4
With S9-mix					
Positive control	627±35	875±128	775±73	147±17	341±19
Negative control	15±4	62±4	7±4	6±1	51±4
17	17±6	73±3	4±1	6±2	50±10
52	12±3	64±4	11±3	7±3	47±6
164	11±4	68±5	8±3	8±6	61±7
512	19±8	65±6	6±2	3±2	44±5
1600	15±7	86±25	8±2	5±0	58±8

An *in vitro* mammalian cell gene mutation test (Anonymous 10, 2015) was performed according to OECD TG 476. 2,4,6-TTBP was tested with a 3-hour treatment period in the presence of S9-mix (concentration range of 0.1 to 100 µg/ml) and in the absence of S9-mix (concentration range of 0.1 to 45 µg/ml). Additionally, a second test with a 3-hour treatment period was performed in the absence of S9-mix (concentration range of 0.01 to 25 µg/ml) and presence of S9-mix (concentration range of 0.01 to 60 µg/ml). A third experiment with a 24-hour treatment period was done in the absence of S9-mix (concentration range of 0.1 to 30 µg/ml).

Table 16 : First experiment : cytotoxicity and mutagenic response after 3h treatment

Dose level (µg/ml)	RSG (%)	CE D2 (%)	RS D2 (%)	RTG (%)	Total mutation frequency (per 10^6 survivors)
Without S9-mix					
Negative control 1	100	50	100	100	159
Negative control 2	100	65	100	100	126
0.1	116	57	98	114	99
1	103	111	193	199	49
5	101	50	86	87	128
10	127	70	122	155	100
20	47	55	95	44	120
25	21	102	177	37	123

35	7	81	141	10	108
45	12	57	98	11	114
Positive control	68	39	67	46	671
With S9-mix					
Negative control 1	100	56	100	100	135
Negative control 2	100	60	100	100	83
0.1	104	57	98	101	133
1	91	61	106	96	105
10	101	68	118	118	77
20	82	67	116	95	130
50	67	84	145	97	106
70	22	49	84	18	106
80	14	47	81	11	189
100	11	72	125	13	124
Positive control	40	23	40	16	1676

Table 17 : Second experiment : cytotoxicity and mutagenic response after 3h treatment

Dose level (µg/ml)	RSG (%)	CE D2 (%)	RS D2 (%)	RTG (%)	Total mutation frequency (per 10^6 survivors)
Without S9-mix					
Negative control 1	100	120	100	100	66
Negative control 2	100	110	100	100	86
0.01	94	101	88	83	87
0.1	108	120	104	113	56
0.5	112	110	96	107	75
1	96	129	112	107	76
5	113	111	97	110	82
10	105	101	88	92	92
20	60	127	111	66	75
25	26	111	97	25	89
Positive control	87	59	52	45	994
With S9-mix					
Negative control 1	100	91	100	100	103
Negative control 2	100	75	100	100	127
0.01	108	94	113	122	85
0.1	95	89	107	102	101
1	105	81	98	103	82
5	90	102	123	111	75
10	93	111	134	125	58
35	47	118	142	67	74
50	23	98	118	28	90
60	11	93	112	13	102
Positive control	52	42	51	27	1752

Table 18 : Third experiment : cytotoxicity and mutagenic response after 24h treatment

Dose level (µg/ml)	RSG (%)	CE D2 (%)	RS D2 (%)	RTG (%)	Total mutation frequency (per 10^6 survivors)
Without S9-mix					

Negative control 1	100	90	100	100	92
Negative control 2	100	98	100	100	92
0.1	93	94	100	93	101
1	102	104	110	112	109
5	95	120	127	121	101
10	83	108	115	95	73
15	80	63	67	54	48
20	51	137	145	73	69
25	33	108	115	38	95
30	20	107	114	23	106
Positive control	84	81	87	72	798

In the absence and presence of S9-mix, 2,4,6-TTBP did not induce a significant increase in the mutation frequency in the 3 experiments.

An *in vitro* mammalian chromosome aberration test (Anonymous 11, 1998), was done following a Japanese guideline (MHW, notification n°24 of the Pharmaceutical Affairs Bureau, Japanese Ministry of Health and Welfare, 1989). 2,4,6-TTBP was tested in the absence of S9-mix with a 6-hour treatment (at a concentration range of 0.015 to 0.026 mg/ml), with a 24-hour treatment (at a concentration range of 0.0098 to 0.022 mg/ml) and with a 48-hour treatment (at a concentration range of 0.010 to 0.030 mg/ml). Furthermore, 2,4,6-TTBP was tested in the presence of S9-mix with a 6-hour treatment at a concentration range of 0.026 to 0.15 mg/ml.

Table 19 : Chromosome aberration

Treatment time (h)	Met. act.	Dose level (mg/ml)	Mitotic index	Nb. of cells scored	Mean aberrations per cell	Cells with aberrations (%)	
						Numerical	Structural
6	-	Neg. control	8.0	200	0.010	2.5	1.0
		Neg. control	8.3	200	0.015	1.0	1.5
		0.015	9.7	200	0.025	3.0	2.0
		0.022	7.0	200	0.010	2.0	1.0
		0.024	6.4	200	0.025	2.5	2.5
		0.026	4.6	200	0.090	4.0	3.5
		Pos. control	9.1	200	0.335	3.5	24.5**
24	-	Neg. control	4.0	200	0.025	1.5	2.5
		Neg. control	3.6	200	0.115	1.0	5.5
		0.0098	6.2	200	0.035	2.0	3.5
		0.013	4.2	200	0.050	3.5	4.5
		0.017	5.5	200	0.030	0.0	3.0
		0.022	4.0	200	0.050	2.5	4.5
		Pos. control	5.5	200	0.630	3.5	39.0**
48	-	Neg. control	5.0	200	0.015	4.5	1.5
		Neg. control	4.7	200	0.005	3.0	0.5
		0.010	5.1	200	0.005	3.5	0.5
		0.020	4.5	200	0.015	4.5	1.5
		0.025	5.1	200	0.005	3.0	0.5
		0.030	0.8	200	0.010	5.5	1.0
		Pos. control	5.2	200	0.940	8.0	37.5**
6	+	Neg. control	7.6	200	0.045	2.5	4.5
		Neg. control	8.0	200	0.045	4.0	4.0
		0.026	10.1	200	0.050	6.0	3.5
		0.035	8.1	200	0.065	2.5	5.5

		0.047	7.7	200	0.025	3.5	2.5
		0.062	5.5	200	0.045	5.5	4.5
		Pos. control	7.2	200	3.030	3.0	74.0**

** : $p<0.01$

2,4,6-TTBP did not induce structural and numerical chromosome aberrations.

10.8.2 Comparison with the CLP criteria

Criteria for germ cell mutagens Category 1	Criteria for germ cell mutagens Category 2	Results of the available studies
"Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans. Category 1A : the classification in Category 1A is based on positive evidence from human epidemiological studies. Category 1B : the classification in Category 1B is based on : - Positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or - 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 the supportive 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; or - Positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of	"substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans The classification in Category 2 is based on : - Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from (somatic cell mutagenicity tests in vivo in mammals or other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays."	3 *in vitro* studies were available and all were negative Furthermore, no *in vivo* studies were available.

transmission to progeny."		

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Based on the results, a classification as germ cell mutagenicity is not warranted.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Three *in vitro* studies were provided for assessment of germ cell mutagenicity of 2,4,6-tri-tert-butylphenol.

In the bacterial reverse mutation assay (Ames test OECD TG 471, GLP; Anonymous 9, 2015) in the absence and presence of rat liver S9-mix 2,4,6-tri-tert-butylphenol did not induced increase in number of revertants in bacterial strains *S. typhimurium* TA100 and *E. Coli* WP2uvrA in the first series of first experiments in concentrations 1.7, 5.4, 17, 52, 164, 512, 1600 and 5000 µg/plate and also no increase of revertants was observed in a second series of first experiments in the tester strains *S. Typhimurium* TA1535, TA1537 and TA98 in concentrations 17, 52, 164, 512 and 1600 µ/plate. In the second experiment with and without S9-mix, 2,4,6-tri-tert-butylphenol did not induced an increase in the number of revertants in concentrations of 17, 52, 164, 512 and 1600 µg/plate in *S. Typhimurium* TA98, TA100, TA1535, TA1537 and *E. Coli* WP2uvrA. Reduction in the number of revertants due to cytotoxicity was noted at concentration 1600 µg/plate in *S. Typhimurium* TA98 and TA1535 without S9-mix. A considerable increase of revertants was noted in both experiments in positive control groups confirming validity of the test system, although the name of the substance used in positive control was not provided.

In the *in vitro* mammalian cell gene mutation (OECD TG 476, GLP, Anonymous 10, 2015) 2,4,6-tri-tert-butylphenol did not increase a gene mutation frequency in mouse lymphoma L5178Y cells in three experiments. First: 3h exposure at concentrations of 0.1, 1, 5, 10, 20, 25, 35 and 45 µg/ml without S9 mix and of 0.1, 1, 10, 20, 50 70, 80 and 100 µg/ml with S9 mix; Second: at several concentrations in a range of 0.01- 25 µg/ml without S9 mix and in several concentrations in a range of 0.01 - 60 µg/ml with S9 mix; Third: at concentrations in a range of 0.1 - 30 µg/ml without S9 mix. The large increase of number of mutant cells per 10^6 of cloneable cells was noted in all three experiments in positive controls confirming validity of the test system, although name of substance used in positive control was not provided.

In the *in vitro* mammalian chromosome aberration test (notified Japanese guideline for screening mutagenicity, GLP, Anonymous 11, 2015), 4,6-tri-tert-butylphenol tested in the absence of S9-mix: with a 6-hour treatment (at a concentration range of 0.015 to 0.026 mg/ml); with a 24-hour treatment (at a concentration range of 0.0098 to 0.022 mg/ml), and with a 48-hour treatment (at a concentration range of 0.010 to 0.030 mg/ml) and in the presence of S9-mix with a 6-hour treatment at a concentration range of 0.026 to 0.15 mg/ml did not increase the frequency of numerical and structural chromosomal aberrations in Chinese hamster ovary cells. The over 10-fold increase in the number of structural chromosomal aberrations was noted in all series of the study in positive control wells confirming validity of the test system, although the name of the substance used in positive

control was not provided.

Based on the results of these studies the DS concluded that a classification of 4,6-tri-tert-butylphenol for germ cell mutagenicity is not warranted.

Comments received during general consultation

One MSCA supported no classification of 2,4,6-tri-tert-butylphenol to a hazard class of germ cell mutagenicity.

Assessment and comparison with the classification criteria

Taking into account that 2,4,6-tri-tert-butylphenol in the relevant and reliable *in vitro* studies did not induce gene mutations in bacterial and mammalian cells or numerical and structural aberrations in mammalian cells, RAC is of the opinion that this substance **does not require classification for germ cell mutagenicity**.

10.9 Carcinogenicity

Table 20 : Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Chronic toxicity study Feed Rat / Wistar 40/sex/group No guideline followed GLP : not specified	2,4,6-TTBP Purity : 97% Vehicle : unknown 24m Dose level : 0, 30, 100, 300 and 1000 ppm (equivalent to approx. 0, 2.51, 8.35, 25.05 and 83.5 mg/kg bw/d)	No mortality and no clinical signs were observed. Significant lower bw in females at the highest dose (after 12m of exposure and thereafter) Significant changes were observed at the hematology and clinical chemistry examination. Necropsy : Only liver, kidneys and adrenals were analysed Liver : significant increase of relative liver weight + swelling, focal necrosis and vacuolisation of hepatocytes Kidneys : significant increase of relative kidney weights Adrenals : significant increase of adrenal weights No neoplastic lesions observed (no more information available)	Matsumoto K. *et al.*, 1991

No human data and other studies available

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

In a chronic toxicity study (Matsumoto K. *et al.*, 1991), groups of 40 male and 40 female Wistar rats were given 2,4,6-TTBP at a concentration of 0, 30, 100, 300 or 1000 ppm during 24 months. The dose levels were equivalent approximately to 0, 2.51, 8.35, 25.05 and 83.5 mg/kg bw/d. After 6, 12, 18 and 24 months of

exposure, hematology, clinical chemistry, organ weight (only liver, kidneys and adrenals) and histopathology examinations (only liver, kidneys and adrenals) were performed in some animals per groups.

A significant higher body weight was observed in females exposed to 1000 ppm, after 12 months of exposure and thereafter.

At necropsy, the liver was modified. The relative liver weight was significantly higher at 300 and 1000 ppm in males and in all female treated groups (except at 30 ppm after 12 months of exposure). Additionally, swelling, focal necrosis and vacuolisation of hepatocytes were noted after 6 months of exposure and thereafter (no more information available). Moreover, the relative kidney and adrenal weights were modified in a few groups.

No neoplastic lesions were observed (no more information available).

Table 21 : Relative organ weights

		Males					Females				
Dose level (in ppm)		0	30	100	300	1000	0	30	100	300	1000
After 6m of expo	liver w (g%)	2.4	2.29	2.39	2.56*	3.14**	2.42	2.72**	2.90**	3.36**	5.02**
	kidney w (g%)	0.49	0.50	0.48	0.48	0.52	0.63	0.61	0.59	0.63	0.65
	adrenal w (mg%)	11	12	12	11	14*	25	26	25	24	26
After 12m of expo	liver w (g%)	2.30	2.39	2.32	2.76**	3.40**	2.19	2.19	2.53**	3.02**	5.39**
	kidney w (g%)	0.49	0.51	0.50	0.51	0.56**	0.55	0.52*	0.56	0.57	0.76**
	adrenal w (mg%)	11	11	11	12	13*	18	17	17	18	22**
After 18m of expo	liver w (g%)	2.47	2.57	2.63	2.85**	3.86**	2.09	2.44**	2.61**	3.22**	5.26**
	kidney w (g%)	0.53	0.54	0.51	0.52	0.58	0.56	0.57	0.57	0.65	0.78**
	adrenal w (mg%)	11	12	11	12*	15	17	18	16	19	20
After 24m of expo	liver w (g%)	2.76	2.72	3.46*	3.62**	5.58**	2.44	2.80**	3.40**	4.65**	6.61**
	kidney w (g%)	0.61	0.59	0.86	0.65	0.89*	0.59	0.65	0.73**	0.89**	1.12**
	adrenal w (mg%)	16	14	21	15	18	19	17	20	22	26*

* : $p<0.05$; ** : $p<0.01$

10.9.2 Comparison with the CLP criteria

Criteria for carcinogen Category 1	Criteria for carcinogen Category 2	Results of the available studies
"known or presumed human carcinogens A substance is classified in category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as : Category 1A : known to have carcinogenic potential for humans, classification is largely based on human evidence, or Category 1B : presumed to have carcinogenic potential for humans, classification is largely based on animal evidence."	"Suspected human carcinogens 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."	Only one study with minimal descriptions of method and results was available. Moreover, macroscopic and microscopic examinations were only performed on liver, kidneys and adrenals. Based on the limitations of the available study, a classification is not warranted due to data lacking.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Based on the limitations of the available study, a classification as carcinogen is not warranted (data lacking).

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The results of chronic toxicity study (OECD TG 452, GLP not specified, Matsumoto *et al.*, 1991) in which Wistar rats (40/sex/dose) were given for 24 months 2,4,6-tri-tert-butylphenol in feed at concentrations of 0, 30, 100, 300 and 1000 ppm (approx. 0, 2.51, 8.35, 25.05 and 83.5 mg/kg bw/d) were provided for evaluation of carcinogenicity. No neoplastic lesions were observed, and the DS concluded that no classification is warranted due to lack of appropriate data.

Comments received during general consultation

One MSCA supported no classification of 2,4,6-tri-tert-butylphenol for carcinogenicity noting limitations in the submitted data (i.e. histopathological examinations were only done for three organs: liver, kidney and adrenals).

Assessment and comparison with the classification criteria

Taking into account the serious limitations of the submitted chronic toxicity study, in particular, restriction of histopathological examinations of the internal organs only to liver, kidney and adrenals, RAC is of the opinion that 2,4,6-tri-tert-butylphenol **does not warrant classification for carcinogenicity due to inconclusive data**.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 22: 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
Combined 28d repeated dose toxicity study with the reproduction/developmental toxicity screening test OECD TG 407, 421 and 422 Rat (Wistar) 10/sex/dose GLP	2,4,6-TTBP Purity : 99.88% Vehicle : corn oil Dose levels : 0, 3, 10 and 30 mg/kg bw/d Gavage Duration of exposure : Males : 29D (beginning 2w prior mating) Females : 2w prior mating and until lactation D4	No mortality and no clinical signs observed. Significant bw changes (see table 23). Fertility index : unaffected Mean nb of corpora lutea, implantations and duration of gestation : no dose related effects Higher percentage of postnatal loss (0.0, 0.0, 6.6 and 12.8 % respectively at 0, 3, 10 and 30 mg/kg bw/d) (Significant increase of the number of dead pups (0, 0, 8** and 12** respectively at 0, 3, 10 and 30 mg/kg bw/d) Liver weight significantly modified in females at 10 mg/kg bw/d and in both sexes at 30 mg/kg bw/d. Additionally, hypertrophy of hepatocytes was noted.	Anonymous 12, 2015
Range finding study of the combined 28d repeated dose toxicity study with the reproduction/developmental toxicity screening test Rat (Wistar) 3 females/group No guideline followed GLP	2,4,6-TTBP Purity : 99.88% Vehicle : corn oil Dose levels : 50, 100 and 250 mg/kg bw/d Duration of exposure : 10d	Mortality : 1 animal found dead on d9 and 2 animals sacrificed in extremis on d10 Clinical signs : ≥50 mg/kg bw/d : hunched posture ≥100 mg/kg bw/d : lethargy 250 mg/kg bw/d : uncoordinated movements, abnormal gait, labored respiration, Slight reduced bw at the 2 highest dose levels Macroscopic examination : enlarged liver was observed at the 2 highest dose. + at 250 mg/kg bw/d, hardened liver (2 animals), irregular surface of the forestomach (1 animal), black brown foci on the adrenal glands (1animal), reddish foci on the mesenteric lymph nodes (2 animals) Organ weight : liver weight was higher at 50 and 100 mg/kg bw/d (not determined for the highest dose because all animals sacrificed/found dead before scheduled necropsy)) (no more information available)	Anonymous 12, 2015

** : $p<0.01$

No human data and other studies available

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

In a combined 28d repeated dose toxicity study with the reproduction/developmental toxicity screening test (Anonymous 12, 2015), following OECD TG 407, 421 and 422, groups of 10 male and 10 female Wistar rats were given, by gavage, 2,4,6-TTBP at a concentration of 0, 3, 10 or 30 mg/kg bw/d. Exposure of males began 2 weeks before mating and continued for a total exposure of 29 days. Females were exposed 2 weeks prior mating until lactation d4.

All animals survived during the exposure period. Excessive salivation was observed immediately after exposure in males exposed to 3, 10 and 30 mg/kg bw/d and occasionally in a single female exposed to 3 mg/kg bw/d. One female of the highest dose level exhibited piloerection (only observed 2d during treatment).

Examination of body weight revealed slight increases in females (see table 23). Some slight changes in haematology and clinical biochemistry parameters were also observed (see tables 24 and 25).

Table 23 : bw data (in g) (10 animals examined per group)

		Males				Females			
Dose level (in mg/kg bw/d)		0	3	10	30	0	3	10	30
Premating	D1	317	315	317	314	215	216	215	220
	D8	340	341	347	345	226	230	2280	232
Mating	D1	359	357	363	362	227	233	235*	235*
	D15	370	382	391*	384	251 (n=1)	288 (n=1)	/	/
Gestation	D0					231 (n=9)	234 (n=9)	238	240*
	D14					278 (n=9)	288	287	284
	D20					345 (n=9)	358	353	347
Lactation	D1					267 (n=9)	275	276	277
	D4					270	280	271	273

* : $p<0.05$

Table 24 : Significant haematology changes (5 animals examined per group)

	Males				Females			
Dose level (in mg/kg bw/d)	0	3	10	30	0	3	10	30
Neutrophils (% WBC)	15.2	17.9	16.0	19.4	31.9	31.3	28.7	20.2*
Lymphocytes (% WBC)	81.9	79.1	81.7	78.1	65.5	66.5	68.4	77.1*
RBC (10^{12}/L)	8.39	8.51	8.37	8.54	6.8	6.64	7.22	7.41*
Reticulocytes (% RBC)	2.2	2.2	2.1	2.2	6.8	5.4	4.0	3.9*
Haemoglobin (mmol/L)	9.5	9.4	9.6	9.5	8.1	7.8	8.1	7.9
Haematocrit (L/L)	0.447	0.447	0.444	0.444	0.386	0.370	0.384	0.379
MCV (fL)	53.3	52.6	53.0	52.1	56.8	55.7	53.2*	51.1**
MCH (fmol)	1.13	1.11	1.14	1.11	1.19	1.18	1.12**	1.07**
PT (s)	16.4	16.1	15.5	15.9	15.8	15.4	15.5	14.3**

* : $p<0.05$; ** : $p<0.01$

Table 25 : Clinical biochemistry data (5 animals examined per group)

	Males				Females			
Dose level (in mg/kg bw/d)	0	3	10	30	0	3	10	30
ASAT (U/L)	81.0	76.3	77.0	82.3	71.5	77.8	87.6**	74.8
Tot. protein (g/L)	58.6	58.9	57.6	59.0	63.1	62.5	64.3	70.3**

Albumin (g/L)	31.6	31.7	30.6	30.8	31.7	31.5	32.8	36.8**
Tot. bilirubin (µmol/L)	2.4	2.1	2.0**	1.7**	2.3	2.1	1.9**	1.5**
Glucose (mmol/L)	8.41	7.70	9.24	9.17	5.79	6.10	6.81	7.39*
Cholesterol (mmol/L)	1.62	1.94	1.99	2.23	1.52	2.17	2.82**	4.57**
Potassium (mmol/L)	3.87	4.16	4.16	4.20*	3.46	3.63	3.72	3.98**
Calcium (mmol/L)	2.50	2.51	2.53	2.49	2.62	2.65	2.61	2.80*

* : p<0.05 ; ** : p<0.01

Following a premating period of minimum 14 days, one female was cohabited with one male of the same treated dose level. Detection of mating was confirmed by evidence of sperm in the vaginal lavage or by the appearance of an intravaginal copulatory plug. This day was designated as D0 post coitum.

The fertility index was unaffected in all treated group (90, 100, 100 and 100% respectively at 0, 3, 10 and 30 mg/kg bw/d). No dose related changes were observed concerning corpora lutea, implantation and duration of gestation (see table 26). No information about the oestrus cycle, resorptions, the pre and post implantation loss were available.

Table 26 : Reproductive data

Dose level (in mg/kg bw/d)	0	3	10	30
Number of females/litter examined	10	10	10	10
Mean precoital time (d)	3.6	3.0	3.3	3.3
Mean nb of corpora lutea	12.9	13.8	15.2	13.7
Mean nb of implantations	12.7	13.3	12.9	11.4
Mean duration of gestation (d)	21.2	21.0	21.0	21.4
Number of litter examined	9	10	10	10
Nb. of dead pups at first litter check	0	0	1	0
Nb. of living pups at first litter check (mean)	101 (11.2)	125 (12.5)	121 (12.1)	94 (9.4)
% of postnatal loss (nb. of dead pups)	0.0	0.0	6.6 (8**)	12.8 (12**)

* : p<0.05 ; ** : p<0.01

At the end of the study, parents were necropsied. Macroscopic examination revealed an enlargement of the liver in 3 males and in 1 female exposed to 30 mg/kg bw/d. The liver weight was significantly increased at the mid dose in females and at the high dose in both sexes (see table 27). The microscopic liver evaluation showed slight to moderate hepatocellular hypertrophy at the mid and high dose levels. Additionally, hepatocellular necrosis was noted in 1 male and 1 female exposed to 30 mg/kg bw/d. Mucosal hypertrophy in caecum was present in males at the mid and high dose levels. Moreover, a lower hematopoiesis was observed in females at the 2 highest dose levels. (see table 28)

Table 27 : Organ weight data

		Males				Females			
Dose level (in mg/kg bw/d)		0	3	10	30	0	3	10	30
FBW (g)		351 (n=10)	363 (n=10)	371* (n=10)	367 (n=10)	235 (n=5)	249 (n=5)	246 (n=5)	246 (n=5)
Brain (5/sex/group)	Abs (g)	1.97	2.01	2.08*	1.99	1.91	1.87	1.89	1.93
Epididymides (10/sex/group)	Abs (g)	1.091	1.134	1.156	1.122				
	Rela (%)	0.310	0.314	0.311	0.307				
Liver (5/sex/group)	Abs (g)	8.07	8.68	9.24	11.38**	7.09	7.98	8.95**	12.08**
	Rela (%)	2.25	2.40	2.52	3.13**	3.01	3.20	3.64**	4.91**
Prostate (5/sex/group)	Abs (g)	0.542	0.570	0.663	0.623				
	Rela (%)	0.151	0.158	0.181	0.171				
Sem. ves. (5/sex/group)	Abs (g)	1.485	1.472	1.420	1.644				

	Rela (%)	0.413	0.410	0.388	0.450				
Spleen (5/sex/group)	Abs (g)	0.531	0.563	0.667*	0.586	0.587	0.567	0.518	0.511
	Rela (%)	0.148	0.156	0.182*	0.160	0.251	0.227	0.211	0.208
Testes/ovaries (10 males/group and 5 females/group)	Abs (g)	3.31	3.55	3.66	3.41	0.125	0.140	0.190	0.135
Thyroid (5/sex/group)	Abs (g)	0.016	0.018	0.013	0.017	0.012	0.014	0.013	0.017
Uterus (5/sex/group)	Abs (g)					0.721	0.687	0.658	0.655
	Rela (%)					0.307	0.276	0.268	0.268

* : p<0.05 ; ** : p<0.01

Table 28 : Microscopic data

			Males				Females			
Dose level (in mg/kg bw/d)			0	3	10	30	0	3	10	30
Cecum	Nb. examined		5	5	5	5	5	/	/	5
	Hypertrophy, mucosa	Tot. affected	0	0	1	3	0			0
		Grade 1	0	0	1	1	0			0
		Grade 2	0	0	0	2	0			0
Liver	Nb. examined		5	5	5	5	5	5	5	5
	Hypertrophy hepatocellular	Tot. affected	0	0	2	5	0	0	5	5
		Grade 1	0	0	2	1	0	0	0	0
		Grade 2	0	0	0	4	0	0	5	0
		Grade 3	0	0	0	0	0	0	0	5
	Necrosis hepatocellular	Grade 1	0	0	0	1	0	0	0	1
Spleen	Nb. examined		5	/	1	5	5	5	5	5
	Hematopoiesis	Tot. affected	2	/	1	2	5	5	5	5
		Grade 1	2	/	1 (n=1)	2	0	0	3	2
		Grade 2	0	/	0	0	1	2	1	3
		Grade 3	0	/	0	0	4	3	1	0

Grade 1 : minimal/very few/very small ; Grade 2 : slight/few/small ; Grade 3 : moderate/moderate number/moderate size

Before the performance of the combined 28d repeated dose toxicity study with the reproduction/developmental toxicity screening test, a range finding study (Anonymous 12, 2015) was done. Groups of 3 females rats were exposed to 2,4,6-TTBP at a concentration of 50, 100 or 250 mg/kg bw/d during 10 days.

At the highest dose, 1 animal was found dead on d9 and the remaining 2 animals were sacrificed in extremis on day 10. Hunched posture was observed in all groups. At the 2 highest dose, lethargy, piloerection and uncoordinated movements were noted and at the highest dose, animals exhibited abnormal gait, labored respiration, ventro-lateral recumbency and deep respiration.

At necropsy, terminal body weight, kidney and liver weight were determined. A higher liver weight was noted at 50 and 100 mg/kg bw/d. The liver weight of animals exposed to 250 mg/kg bw/d was not determined as all animals were sacrified/found dead before scheduled necropsy. Moreover, enlarged liver with yellowish foci was noted in all animals exposed at the 2 highest dose levels. At 250 mg/kg bw, hardened liver (2 animals), irregular surface of the forestomach (1 animal), black brown foci on the adrenal glands (1animal), reddish foci on the mesenteric lymph nodes (2 animals) were observed. Microscopic examination was not performed.

10.10.3 Comparison with the CLP criteria

Criteria for reproductive toxicant Category 1	Criteria for reproductive toxicant Category 2
"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 on development in humans 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). Category 1A : the classification of a substance in category 1A is largely based on evidence from humans Category 1B : the classification of a substance in category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in category 2 may be more appropriate."	"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, or on development, and where the evidence is not sufficiently convincing to place the substance in category1. If deficiencies in the study make the quality of evidence less convincing, category 2 could be more appropriate classification."

Since no human studies are available for effects on fertility, classification in Repr. 1A is not appropriate

Moreover, in the combined repeated dose toxicity with reproductive/developmental toxicity screening test (anonymous 12, 2015), reproductive parameters (fertility index, corpora lutea, number of implantations and duration of gestation) were unaffected. And, at necropsy, weight of reproductive organs was not significantly modified. As no effects on fertility were observed, a classification in Repr. 1B or 2 is not appropriate. However, the tested doses are very low.

10.10.4 Adverse effects on development

Table 29 : 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**
Combined 28d repeated dose toxicity study with the reproduction/developmental toxicity screening test	2,4,6-TTBP Purity : 99.88% Vehicle : corn oil	Dams : No mortality Sign. bw changes (however not at all observation	Anonymous 12, 2015)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD TG 407, 421 and 422 Rat (Wistar) 10/sex/dose GLP	Dose levels : 0, 3, 10 and 30 mg/kg bw/d Gavage Duration of exposure : males : 29D (beginning 2w prior mating) Females : 2w prior mating and until lactation D4	time) Higher percentage of postnatal loss (0.0, 0.0, 6.6 and 12.8 respectively at 0, 3, 10 and 30 mg/kg bw/d) (Sign. increase of the number of dead pups (0, 0, 8** and 12** respectively at 0, 3, 10 and 30 mg/kg bw/d) Liver weight sign. modified in females at 10 mg/kg bw/d and in both sexes at 30 mg/kg bw/d. Additionally, hypertrophy of hepatocytes were noted. <u>Pups</u> % of males/females of living pups at the first litter check unaffected Sign. lower viability index at the 2 highest dose levels (100, 100, 93.4** and 87.2** respectively at 0, 3, 10 and 30 mg/kg bw/d) Pups bw : sign. lower at 10 and 30 mg/kg bw/d	

* : p<0.05 ; ** : p<0.01

No human data and other studies available

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

<u>In a combined 28-day repeated dose toxicity study with the reproduction/developmental toxicity screening test (Anonymous 12, 2015)</u>, following OECD TG 407, 421 and 422, groups of 10 male and 10 female Wistar rats were given, by gavage, 2,4,6-TTBP at a concentration of 0, 3, 10 or 30 mg/kg bw/d. Exposure of males began 2 weeks before mating and continues for a total exposure of 29 days. Females were exposed 2 weeks prior mating and until lactation day 4.

Parental information was available in section 10.10.2.

Following a premating period of minimum 14 days, one female was cohabited with one male of the same treated dose level. The fertility index was unaffected in all treated group (90, 100, 100 and 100% respectively at 0, 3, 10 and 30 mg/kg bw/d). Moreover, the number of dead pups at the first litter was similar in all groups. However, the percentage of postnatal loss was higher at 10 and 30 mg/kg bw/d (significant increase of the number of dead pups) (see table 26). At the mid dose, 3 dams exhibited postnatal loss (1 each for 2 litters and 6 for another litter) while at the highest dose, 5 dams showed postnatal loss (1 each for 3 litter, 4 for one litter and 5 for another litter). Parturition and maternal care was unaffected by the treatment.

At the end of the study, animals were necropsied. (see results (organ weight, macroscopic and microscopic examinations) at the chapter 10.10.2)

The litters were examined to determine mortality, clinical signs, bw, sex, moreover all external abnormalities were recorded. The % of males/females of living pups at the first litter check was unaffected (50/50, 50/50, 50/50 and 55/45 respectively at 0, 3, 10 and 30 mg/kg bw/d). Significant lower viability index were observed at the 2 highest dose levels (100.0, 100.0, 93.4** and 87.2** respectively at 0, 3, 10 and 30 mg/kg bw/d). Furthermore, body weight of pups were significantly reduced at 10 and 30 mg/kg bw/d (see table 30). The observation of the maternal care did not exhibited modifications.

Table 30 : Body weight of pups (in g)

Dose level (in mg/kg bw/d)		0	3	10	30
D1	M	6.3	5.9	5.7**	5.7**
	F	6.1	5.7	5.4**	5.4*
	M+F	6.2	5.8	5.5**	5.6*
D4	M	9.6	8.8*	8.2**	7.7**
	F	9.4	8.3	7.7**	7.5**
	M+F	9.5	8.5	8.0**	7.6**

* : $p<0.05$; ** : $p<0.01$

Surviving pups showed incidental clinical symptoms (low incidence) such as pallor, absence of milk in the stomach, missing tail, dehydrated appearance. In control group, 2 pups exhibited blue spot snout and 2 pups showed blue spot head. In the lowest dose level, 3 pups exhibited blue spot back, 3 others showed scabs and 1 showed blue snout. At the mid dose level, 1 pup exhibited blue spot neck and 3 other showed pallor. Whereas, at the highest dose level, 1 pups exhibited pallor, 1 other showed absence of milk in stomach, 1 had missing tail, 2 other showed tail point and 2 had dehydrated appearance.

10.10.6 Comparison with the CLP criteria

Criteria for reproductive toxicant Category 1	Criteria for reproductive toxicant Category 2
"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 on development in humans 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). Category 1A : the classification of a substance in category 1A is largely based on evidence from humans Category 1B : the classification of a substance in category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in category 2 may be more appropriate."	"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, or on development, and where the evidence is not sufficiently convincing to place the substance in category1. If deficiencies in the study make the quality of evidence less convincing, category 2 could be more appropriate classification."

Since no human studies are available for effects on fertility, classification in Repr. 1A is not appropriate.

In the combined repeated dose toxicity with reproduction/developmental toxicity screening test (Anonymous 12, 2015), the mean number of living pups at the first litter check was only slightly reduced (11.2, 12.5, 12.1 and 9.4 respectively at 0, 3, 10 and 30 mg/kg bw/d). However, a treatment-related and significant increase of the percent of postnatal loss was observed (0.0, 0.0, 6.6** and 12.8** respectively at 0, 3, 10 and 30 mg/kg bw/d). At the mid dose, a total of 3 dams exhibited postnatal loss (1 dead or missing pup for 2 litter and 6 missing pups for another litter) and at the highest dose, a total of 5 dams showed postnatal loss (1 missing pup for 3 litters and 4 or 5 missing pups for 2 litters). The viability index was then significantly and treatment-related reduced (100.0, 100.0, 93.4** and 87.2** respectively at 0, 3, 10 and 30 mg/kg bw/d). Furthermore, the mean pup body weight was significantly reduced at D1 and D4 at the 2 highest dose levels. At d4, the mean pup body weight were approximately 16 and 20% lower than the control at the mid and high doses, respectively. These developmental effects were observed at very low dose.

The macroscopic examination performed in pups did not reveal treatment related changes.

During the study, dams exhibited severe liver toxicity. Hypertrophy hepatocellular was observed at the 2 highest doses and 1 dams exposed to 30 mg/kg bw/d exhibited hepatocellular necrosis. However, no treatment-related change was noted in the maternal care, furthermore, dams did not exhibited clinical signs and the maternal body weight was not modified. Despite the liver toxicity, dams were in good condition to take care of their progeny.

In the guidance on the application of the CLP criteria, 3.7.2.4.2 (annex I) it is mentioned that "Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case by case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant postnatal functional deficiencies."

In the combined study (Anonymous 12, 2015), a significant higher percent of postnatal loss and viability index, both treatment related justify a classification in category 2 as these effects are not considered to be a secondary non-specific consequence of the maternal toxic effects.

10.10.7 Adverse effects on or via lactation

No animal studies, no human data and no other studies available

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

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10.10.9 Comparison with the CLP criteria

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10.10.10 Conclusion on classification and labelling for reproductive toxicity

Based on the results, a classification as **Repr. 2 H361d (Suspected of damaging unborn child)** is warranted.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

One study was submitted for evaluation of reproductive toxicity of 2,4,6-tri-tert-butylphenol: the combined repeated dose toxicity study with reproduction/developmental toxicity screening test (GLP, OECD TG 422, Anonymous 12, 2015) in which Wistar rats (10/sex/dose) were exposed by gavage to 2,4,6-tri-tert-butylphenol dissolved in corn oil at doses 0, 3, 10 and 30 mg/kg bw/d: males - 2 weeks before start of mating till the end of mating; females - during 2 weeks before mating, during gestation and until day 4 of lactation. The doses were chosen based on results of the range finding study (Anonymous 12, 2015) demonstrating that 2,4,6-tri-tert-butylphenol at doses of 100 and 250 mg/kg bw/d for 10 days is causing severe toxicity which could interfere with reproductive functions due to systemic effects such as lethargy, uncoordinated movements and abnormal gait, labored respiration, ventrolateral recumbency, deep respiration, mortality.

Parental toxicity at doses 3, 10 and 30 mg/kg bw/d: no mortality and clinical symptoms throughout the study period, statistically significant, although minor, increases of body weight of males at a dose of 10 mg/kg bw/d, and in females at doses of 10 and 30 mg/kg bw/d, but they were not observed at the end of pregnancy and on 4th day of lactation despite continuation of exposure. The following effects were observed: an increase of bilirubin in blood of males and females in the 10 and 30 mg/kg bw/d groups, an increase of absolute and relative liver weight in females of 10 mg/kg bw/d group and in females and males at 30 mg/kg bw/d, slight to moderate hepatocellular hypertrophy in females at 10 and 30 mg/kg bw/d and slight liver hypertrophy in males at 30 mg/kg bw/d. In addition, in the 30 mg/kg bw/d group, hepatocellular necrosis of very small area (grade 1) was observed in 1/10 male and 1/10 female.

Fertility and sexual behaviour: number of oestrous cycles until mating, the mating, fertility, conception and gestation indexes, number of corpora lutea, implantations and duration of gestation were unaffected at all doses

Developmental toxicity: The number of live births was not different between groups, but 6.6% and 12.8% of pups died during four days after birth in the 10 and 30 mg/kg bw/d groups. The body weight of male and female pups in the 10 and 30 mg/kg bw/d groups at postnatal day 1 and postnatal day 4 (the end of observation period in this study) were statistically reduced by ca. 10% and ca. 20%, respectively. Single pups (1-3) in control and in the 3 and 10 mg/kg bw/d groups had blue spots on snout, head, back or neck but were not dose related. Pallor was observed in 3 pups in 10 mg/kg bw/d and in one pup in 30 mg/kg bw/d and at the top group: one pup with absence of milk in stomach, one pup with missing tail, two pups with tail point and 2 pups with dehydrated appearance were noted.

Noting that 2,4,6-tri-tert-butylphenol did not affect fertility parameters in the submitted study but affected the development of pups by leading to significant reduction of early postanal viability of pups, the DS concluded that the substance should be classified as Repr. 2, H361d: Suspected of damaging the unborn child.

Comments received during general consultation

Two MSCA disagreed with classification of 2,4,6-tri-tert-butylphenol as Repr. 2, H361d as proposed by DS. Considering the significant and dose-related increase in postnatal mortality of pups and reduced body weight of pups in 10 and 30 mg/kg bw/d groups, which were not considered to be secondary non-specific consequence of maternal toxicity, both MSCAs proposed classification Repr. 1B, H360D: May damage the unborn child, which was thereafter agreed by the DS.

Assessment and comparison with the classification criteria

In the combined repeated dose toxicity study with reproduction/developmental toxicity screening test (GLP, OECD TG 422, Anonymous 12, 2015) Wistar rats (10/sex/dose) were exposed by gavage to 2,4,6-tri-tert-butylphenol at doses 0, 3, 10 and 30 mg/kg bw/d: males - 2 weeks before start of mating till the end of mating; females - during 2 weeks before mating, during gestation and until day 4 of lactation. There were no mortality or clinical symptoms throughout the study period, and body weight changes were minor.

Adverse effects on sexual function and fertility

The fertility index was unaffected in all treated groups (90, 100, 100 and 100% respectively at 0, 3, 10 and 30 mg/kg bw/d). No dose related changes were observed concerning corpora lutea, implantation and duration of gestation. No information about the oestrus cycle, resorptions, and pre and post implantation loss were available.

It is noted that the only results available are from a TG 422 screening test with small number of animals, short duration of exposure and lacking the evaluation of effects on parental gonads and on sexual function and fertility of offspring generation. Considering the limited available data, the **classification for effects on sexual function and fertility is not warranted based on inconclusive data**.

Adverse effects on development

2,4,6-tri-tert-butylphenol at doses 10 and 30 mg/kg bw/d caused dose dependent increase in pup mortality (6.6 and 12.8% respectively) during the first four days of life in spite of the fact that maternal care and body weight of dams during pregnancy was not affected.

The body weight of pups in these groups was significantly reduced at birth (11.3% and 9.7% lower than in control group) indicating 2,4,6-tri-tert-butylphenol affected the development of pups *in utero*. Taking into account that effects in pups were considerably more severe (mortality) than those in dams, in which no clinical symptoms nor severe changes in maternal body weight during pregnancy were observed, and moderate effects found in liver are not expected to affect development of foetuses, RAC considers that developmental effects are not due to a secondary consequence of maternal toxicity, **therefore 2,4,6-tri-tert-butylphenol warrants classification as Repr. 1B; H360D: May damage the unborn child**.

Effects on or via lactation

Classification to category for lactation effects is not warranted since, due to design of the screening test, only the first short period of lactation was covered and the data provided do not allow comparison with classification criteria, therefore they are inconclusive for classification in this category.

10.11 Specific target organ toxicity-single exposure

See chapter 10.1 and 10.2 for the summary of animals studies on STOT SE

No human data and no other studies available

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

In the acute toxicity studies via oral and dermal routes, no specific effects on target organs were observed.

10.11.2 Comparison with the CLP criteria

Criteria for STOT SE 1	Criteria for STOT SE 2	Results of available studies
"Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure. Substances are classified in category 1 for specific target organ toxicity (single exposure) on the basis of : ▪ Reliable and good quality evidence from human cases or epidemiological studies; or ▪ Observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations."	Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following single exposure. Substances are classified in category 2 for specific target organ toxicity (single exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations."	Since no human studies are available and no significant or severe effects were seen in acute experimental animals, classification in STOT SE 1 or 2 is not appropriate.

Criteria for STOT SE 3	Results of available studies
Transient target organ effects This category only includes narcotic effects and respiratory tract irritation.	Since no narcotic effects or respiratory tract irritation were observed, a classification as

These are target organ effects for which a substance does not meet the criteria to be classified in Category 1 or 2 indicated above. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function.	STOT SE 3 is not appropriate.

10.11.3 Conclusion on classification and labelling for STOT SE

Based on the available information, no classification as STOT SE is warranted

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The DS is of the opinion that 2,4,6-tri-tert-butylphenol does not require classification to STOT SE, since no specific effects in target organs were observed in the acute toxicity studies.

Comments received during general consultation

No comments were received.

Assessment and comparison with the classification criteria

Taking into account that there are no human data on toxic effects after single exposure and toxic symptoms were only observed in rats administered 2,4,6-tri-tert-butylphenol by gavage at a lethal dose of 2000 mg/kg bw, but not at the lower dose of 200 mg/kg bw and noting that specific target organ toxicity (single exposure) is defined in CLP Regulation as specific non-lethal target organ toxicity arising from a single exposure to a substance, RAC is of the opinion that 2,4,6-tri-tert-butylphenol **does not warrant classification in STOT SE**.

10.12 Specific target organ toxicity-repeated exposure

Table 31 : Summary table of animal studies on STOT RE

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

Combined repeated dose toxicity study with reproduction/developmental toxicity screening test OECD TG 407, 421 and 422 Rat (Wistar) 10/sex/dose GLP	2,4,6-TTBP Vehicle : corn oil Oral (gavage) 0, 3, 10 and 30 mg/kg bw/d Exposure : 29D for males (beginning 2w before mating) and 41 to 56D for females (from 2w prior mating until day 4 of lactation)	No mortality and no clinical signs observed Bw : slight changes (see table 23) Some slight changes in the haematological and clinical biochemistry parameters were observed (lower neutrophils count, higher lymphocyte count and RBC count at the highest dose, and at the 2 highest dose level lower MCV and MCH) Necropsy examination : Liver : enlargement in 3 males and 1 female at 30 mg/kg bw/d. Increase abs. and rela. Weight Hepatocellular hypertrophy in males and females exposed to 10 and 30 mg/kg bw/d Hepatocellular necrosis in 1 male and 1 female at the high dose Cecum : mucosal hypertrophy in males at 10 and 30 mg/kg bw/d Spleen : decreased hematopoiesis in females at 10 and 30 mg/kg bw/d NOAEL : 3 mg/kg bw/d	Anonymous 12, 2015
Chronic toxicity study OECD TG 452 Rat (Wistar) 40/sex/dose GLP : not specified	2,4,6-TTBP Vehicle: no information available Oral (feed) 0, 30, 100, 300 and 1000 ppm (approx. 0, 2.51, 8.35, 25.05 and 83.5 mg/kg bw/d) Exposure : 24m	No treatment-related mortality and clinical signs Sign. lower bw in females exposed to 1000 ppm after 12m of exposure and thereafter Necropsy : only liver, kidneys and adrenals were examined Liver : sign. increase liver weight at 300 and 1000 ppm in males and in all treated groups in females + swelling, focal necrosis and vacuolisation of hepatocytes after 6m of exposure and thereafter Kidneys : higher relative kidneys weight at the highest dose Adrenals : higher relative adrenals weight at the highest dose	Matsumoto K. *et al*., 1991
Subacute toxicity study No guideline followed Rat (SD) 10 males GLP : not specified	2,4,6-TTBP Vehicle : unchanged Oral (feed) Dose : 1.98 mmol/kg/d Exposure : 3w	All animals died during the exposure period (between D5 and D11) Gross pathology examination : haemothorax, haematocoelia, intracranial haematoma, intranasal haemorrhage, intramuscular haematoma, intratesticular haematoma and intraepididymis haemorrhage. LT50 : 7.4D	Takahashi O. and Hiraga K., 1978

No human data and other studies available

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

In a combined 28-d repeated dose toxicity study with the reproduction/developmental toxicity screening test (Anonymous 12, 2015), following OECD TG 407, 421 and 422, groups of 10 male and 10 female Wistar rats were given, by gavage, 2,4,6-TTBP at a concentration of 0, 3, 10 or 30 mg/kg bw/d. Exposure of males began 2 weeks before mating and continues for a total exposure of 29 days. Females were exposed 2 weeks prior mating and until lactation day 4 (for a total of 41-56days).

No mortality and no clinical signs were noted during the study.

Examination of body weight revealed slight increases in females (see table 23). Some slight changes in haematology and clinical biochemistry parameters were also observed (see table 24 and 25).

At the end of the study, animals were necropsied. The microscopic examination was performed in 5 selected animals/sex/group. In the control and high dose level of both sexes, all collected tissues were microscopically examined. For the low and mid dose group in males, microscopic examination was only performed on liver and cecum whereas in females the examination was performed only on liver and spleen.

Macroscopic examination revealed an enlargement of the liver in 3 males and in 1 female exposed to 30 mg/kg bw/d. The liver weight was significantly increased at the mid dose in females and at the high dose in both sexes (see table 32). The microscopic liver evaluation showed slight to moderate hepatocellular hypertrophy at the mid and high dose levels. Additionally, hepatocellular necrosis was noted in 1 male and 1 female exposed to 30 mg/kg bw/d. Mucosal hypertrophy in caecum was present in males at the mid and high dose levels. Moreover, a lower hematopoiesis was observed in females at the 2 highest dose levels.

Table 32 : Organ weight data

		Males				Females			
Dose level (in mg/kg bw/d)		0	3	10	30	0	3	10	30
FBW		351 (n=10)	363 (n=10)	371* (n=10)	367 (n=10)	235	249	246	246
Brain	Abs (g)	1.97	2.01	2.08*	1.99	1.91	1.87	1.89	1.93
Epididymides	Abs (g)	1.091	1.134	1.156	1.122				
	Rela (%)	0.310	0.314	0.311	0.307				
Liver	Abs (g)	8.07	8.68	9.24	11.38**	7.09	7.98	8.95**	12.08**
	Rela (%)	2.25	2.40	2.52	3.13**	3.01	3.20	3.64**	4.91**
Prostate	Abs (g)	0.542	0.570	0.663	0.623				
	Rela (%)	0.151	0.158	0.181	0.171				
Seminal vesicles	Abs (g)	1.485	1.472	1.420	1.644				
	Rela (%)	0.413	0.410	0.388	0.450				
Spleen	Abs (g)	0.531	0.563	0.667*	0.586	0.587	0.567	0.518	0.511
	Rela (%)	0.148	0.156	0.182*	0.160	0.251	0.227	0.211	0.208
Testes/ovaries	Abs (g)	3.31	3.55	3.66	3.41	0.125	0.140	0.190	0.135
Thyroid	Abs (g)	0.016	0.018	0.013	0.017	0.012	0.014	0.013	0.017
Uterus	Abs (g)					0.721	0.687	0.658	0.655
	Rela (%)					0.307	0.276	0.268	0.268

* : p<0.05 ; ** : p<0.01

Table 33 : Microscopic data

			Males				Females			
Dose level (in mg/kg bw/d)			0	3	10	30	0	3	10	30
Cecum	Hypertrophy, mucosa	Grade 1	0	0	1	1	0	0	0	0
		Grade 2	0	0	0	2	0	0	0	0
Liver	Hypertrophy hepatocellular	Grade 1	0	0	2	1	0	0	0	0
		Grade 2	0	0	0	4	0	0	5	0
		Grade 3	0	0	0	0	0	0	0	5

	Necrosis hepatocellular	Grade 1	0	0	0	1	0	0	0	1
Spleen	Haematopoiesis	Grade 1	2	/	1 (n=1)	2	0	0	3	2
		Grade 2	0	/	0	0	1	2	1	3
		Grade 3	0	/	0	0	4	3	1	0

Grade 1 : minimal/very few/very small ; Grade 2 : slight/few/small ; Grade 3 : moderate/moderate number/moderate size

For the information on the range finding study of the combined study see table 22 and chapter 10.10.2

In a chronic toxicity study (Matsumoto K. *et al.*, 1991), groups of 40 male and 40 female Wistar rats were given 2,4,6-TTBP at a concentration of 0, 30, 100, 300 or 1000 ppm during 24 months. The dose levels were equivalent approximately to 0, 2.51, 8.35, 25.05 and 83.5 mg/kg bw/d. After 6, 12, 18 and 24 months of exposure, haematology, clinical chemistry, organ weight (only liver, kidneys and adrenals) and histopathology examinations (only liver, kidneys and adrenals) were performed in some animals per groups.

A significant lower body weight was observed in females exposed to 1000 ppm after 12 months of exposure and thereafter. No treatment-related mortality or clinical signs were observed at any dose level.

Already after 6 months of exposure, some haematology or clinical biochemistry variations were observed (see table 34).

Table 34 : Clinical chemistry and haematology data

		Males					Females				
Dose level (in ppm)		0	30	100	300	1000	0	30	100	300	1000
After 6m of exposure	Hb (g/dl)	16.6	16.3	16.4	15.9*	15.7**	16.2	15.8	15.7	15.1**	14.9**
	MCV (fl)	48.3	48.1	47.9	47.2**	46.6**	52.3	51.3**	50.6**	49.9**	47.1**
	Plt ($x10^3$/µl)	741	733	723	784	916**	715	762	801	831*	891**
	BUN (mg/d)	15.8	15.7	16.8	15.4	15.9	18.2	16.1**	17.1	16.5	16.6*
	GOT (mU/ml)	87	83	74	79	73	111	83*	76**	77**	72**
	PL (mg/dl)	167	172	186	184*	206**	204	232**	255**	256**	304**
	T-chol (mg/dl)	82	86	90	102**	157**	137	184**	199**	220**	427**
	Gamma-GTP (mU/ml)	0.8	1.4	1.3	0.8	0.9	0.5	1.4	0.7	1.2	4.3**
After 12m of exposure	Hb (g/dl)	15.5	15.7	15.7	15.4	14.3**	15.3	15.2	15.1	14.8*	14.2**
	MCV (fl)	49.1	47.7*	48.6	47.1**	45.5**	52.9	52.8	52.3**	50.6**	47.3**
	Plt ($x10^3$/µl)	745	770	762	818*	925**	683	804	794*	815*	908**
	BUN (mg/d)	13.4	13.6	14.5	16.1**	17.3**	16.0	16.4	16.8	16.2	17.5
	GOT (mU/ml)	90	89	84	77	69	141	57**	59**	59**	58**
	PL (mg/dl)	174	172	157	181	213**	219	225	252**	261**	325**
	T-chol (mg/dl)	122	119	103	141	210**	167	196*	232**	284**	518**
	Gamma-GTP (mU/ml)	2.1	1.5*	1.2**	1.6	2.3	1.8	1.6	1.5*	1.7	3.4
After 18m of	Hb (g/dl)	16.0	15.8	15.6	15.9	15.1	15.7	15.1*	14.9	14.5**	13.1**
	MCV (fl)	50.5	50.5	51.0	49.8	46.7**	54.5	53.1**	52.5**	51.6**	48.2**
	Plt ($x10^3$/µl)	778	786	834	862	897	682	705	795	833*	929**
	BUN (mg/d)	13.5	15.0	18.0**	16.7**	17.7**	13.9	14.0	15.8	17.1	17.3**
	GOT	63	65	122	62	57	86	62*	82	60**	130

	(mU/ml)										
	PL (mg/dl)	218	227	214	219	267	196	246**	261**	273**	330**
	T-chol (mg/dl)	245	277	183	206	344	163	247**	253**	304**	497**
	Gamma-GTP (mU/ml)	1.6	1.8	2.4	2.6	4.4**	1.7	0.7**	3.2	1.0	4.5
After 24m of exposure	Hb (g/dl)	14.9	13.0	11.9	14.9	12.8	14.7	14.6	14.0	14.2	13.0
	MCV (fl)	53.5	54.7	56.3	47.9	45.1*	53.4	53.3	51.4	49.9**	48.2*
	Plt ($x10^3$/µl)	726	951	1032	912	1311**	684	792	830	825	1252**
	BUN (mg/d)	22.8	17.1	34.7	25.7	37.0	14.0	14.2	14.1	19.1	37.5
	GOT (mU/ml)	64	57	100	77	51	83	66	58	75	63
	PL (mg/dl)	201	209	257**	254*	315**	214	244**	255**	275**	285**
	T-chol (mg/dl)	167	194	285**	336*	595**	187	266**	362*	443**	516**
	Gamma-GTP (mU/ml)	6.0	3.9	5.0	5.7	5.6	2.2	1.5	0.5*	3.7	2.4

* : p<0.05; ** : p<0.01

At necropsy, the liver was modified. The relative liver weight was significantly higher (see table 35). Additionally, swelling, focal necrosis and vacuolisation of hepatocytes were noted at 300 and 1000 ppm after 6 months of exposure and thereafter (no more information available). Moreover, the relative kidney and adrenal weights were modified in few groups. No information on the other organs were available.

Table 35 : Relative organ weights

		Males					Females				
Dose level (in ppm)		0	30	100	300	1000	0	30	100	300	1000
After 6m of expo	liver w (g%)	2.40	2.29	2.39	2.56*	3.14**	2.42	2.72**	2.90**	3.36**	5.02**
	kidney w (g%)	0.49	0.50	0.48	0.48	0.52	0.63	0.61	0.59	0.63	0.65
	adrenal w (mg%)	11	12	12	11	14*	25	26	25	24	26
After 12m of expo	liver w (g%)	2.30	2.39	2.32	2.76**	3.40**	2.19	2.19	2.53**	3.02**	5.39**
	kidney w (g%)	0.49	0.51	0.50	0.51	0.56**	0.55	0.52*	0.56	0.57	0.76**
	adrenal w (mg%)	11	11	11	12	13*	18	17	17	18	22**
After 18m of expo	liver w (g%)	2.47	2.57	2.63	2.85**	3.86**	2.09	2.44**	2.61**	3.22**	5.26**
	kidney w (g%)	0.53	0.54	0.51	0.52	0.58	0.56	0.57	0.57	0.65	0.78**
	adrenal w (mg%)	11	12	11	12*	15	17	18	16	19	20
After 24m of expo	liver w (g%)	2.76	2.72	3.46*	3.62**	5.58**	2.44	2.80**	3.40**	4.65**	6.61**
	kidney w (g%)	0.61	0.59	0.86	0.65	0.89*	0.59	0.65	0.73**	0.89**	1.12**

	adrenal w (mg%)	16	14	21	15	18	19	17	20	22	26*

* : p<0.05; ** : p<0.01

The Takahashi O. and Hiraga K.'s article (1978) investigate the relationship between haemorrhage induced by butylated hydroxytoluene and its antioxidant properties or structural characteristics. 10 male SD rats were given 1.98mmol/kg/d of 2,4,6-TTBP by diet during 3w.

All animals died during the exposure period (between D5 and D11). Necropsy was performed in all dead animals and showed haemothorax, haematocoelia, intracranial haematoma, intranasal haemorrhage, intramuscular haematoma, intratesticular haematoma and intraepididymis haemorrhage.

Table 36: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Combined repeated with reproduction screening test (Anonymous 12, 2015)	Liver effects (higher liver weight and microscopic changes) observed at : Males : 30 mg/kg bw/d Females : 10 mg/kg bw/d	Males : 29D Females : 41-56days	Males : approx. 10 mg/kg bw/d Females : approx. 5 mg/kg bw/d	STOT RE 1
Chronic toxicity study (Matsumoto K. *et al.*, 1991)	Liver effects (higher relative liver weight + microscopic changes) observed at : 25.05 mg/kg bw/d (300 ppm)	24 months	3.09 mg/kg bw/d	STOT RE 1

10.12.2 Comparison with the CLP criteria

Criteria for STOT RE 1	Criteria for STOT RE 2
"Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substance are classified in category 1 for target organ toxicity (repeat exposure) on the basis of : ▪ Reliable and good quality evidence from human cases or epidemiological studies; or ▪ Observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally	Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations." "Classification in category 2 is applicable, when significant toxic effects observed in a 90-day repeated dose study conducted in experimental animals are seen

low exposure concentrations."

"Classification in category 1 is applicable, when significant toxic effects observed in a 90-day repeated dose study conducted in experimental animals are seen to occur at or below the guidance value (C) as indicated in table 3.9.2"

Route of exposure	Units	Guidance value
Oral (rat)	mg/kg bw/d	C≤10

to occur within the guidance value range as indicated in table 3.9.3"

Route of exposure	Units	Guidance value range
Oral (rat)	mg/kg bw/d	10 < C ≤ 100

In the combined repeated dose toxicity study with reproduction/developmental toxicity screening test (Anonymous 12, 2015), liver toxicity was observed. The liver weight was significantly higher at 10mg/kg bw/d in females and at 30 mg/kg bw/d in both sexes. Moreover, the microscopic liver evaluation showed slight to moderate hepatocellular hypertrophy at 10 and 30 mg/kg bw/d. Additionally, hepatocellular necrosis was observed in 1 male and 1 female of the highest dose (see table 33). These effects are confirmed by the chronic toxicity study (Matsumoto K. *et al.*, 1991) which revealed significant higher relative liver weight and microscopic changes, such as focal necrosis and vacuolisation, at 300 and 1000 ppm (approx. 25.05 and 83.5 mg/kg bw/d).

Since these severe effects were seen in 2 studies and at very low dose (< 10 mg/kg bw/d when extrapolate to an exposure period of 90 days), a classification as STOT RE 1 is more appropriate than a classification as STOT RE 2 (see table 36).

10.12.3 Conclusion on classification and labelling for STOT RE

Based on the available studies, liver toxicity at low dose was observed. A classification as **STOT RE 1 H372 (causes damage to organs (liver) through prolonged or repeated exposure)** is warranted.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The results of three animal studies were provided to assess STOT RE: 1) Subacute toxicity study; 2) OECD TG 422 the combined repeated dose toxicity study with reproduction/developmental toxicity screening test; 3) OECD TG 452 the chronic toxicity study.

1. In the subacute toxicity study (Takahashi and Hiraga, 1978; no guideline, no GLP) 10 male Wistar rats were exposed to 2,4,6-tri-tert-butylphenol in feed at dose of 1.98 mmol/kg bw/d (519.6 mg/kg bw/d). All rats died between the 5th and 11th day of exposure. In gross pathology examination the following findings were found: haemothorax, haematocele, intracranial haematoma, intranasal haemorrhage, intramuscular haematoma, intratesticular and intraepididymis haematoma.

2. In the range finding study for the combined repeated dose toxicity study with reproduction/developmental toxicity screening test (Anonymous 12, 2015) 2,4,6-tri-tert-butylphenol was given by gavage to Wistar rats (3 females/dose) at doses of 50, 100 and

250 mg/kg bw/d for 10 days. At the dose of 250 mg/kg bw/d, 2 animals were sacrificed in extremis on day 10 and one animal was found dead on day 9 of exposure. The following symptoms were observed:

- at ≥ 50 mg/kg bw/d: hunched posture
- at ≥ 100 mg/kg bw/d: lethargy, piloerection and uncoordinated movements
- at 250 mg/kg bw/d: abnormal gait, labored respiration, ventro-lateral recumbency, deep respiration, mortality.

Body weight was slightly reduced at doses of 100 and 250 mg/kg bw/d. Liver weight was increased at 50 and 100 mg/kg bw/d but was not determined at 250 mg/kg bw/d because all animals were sacrificed/found dead before scheduled necropsy. The histopathological examinations were not performed.

In the main combined repeated dose toxicity study with reproduction/developmental toxicity screening test (GLP, OECD TG 422, Anonymous 12, 2015) Wistar rats (10/sex/dose) were exposed by gavage to 2,4,6-tri-tert-butylphenol dissolved in corn oil at doses 0, 3, 10 and 30 mg/kg bw/d: males - 2 weeks before start of mating till the end of mating; females - during 2 weeks before mating, during gestation and until day 4 of lactation.

No mortality or clinical symptoms were observed. Minor increases in body weight (less than 5%) were observed at the beginning of the mating period (i.e. within 2 weeks of exposure) in females at doses of 10 and 30 mg/kg bw/d and in males exposed to 10 mg/kg bw/d (but not in males exposed to 30 mg/kg bw/d). It was also observed a dose dependent decrease in total bilirubin in males and females of 10 and 30 mg/kg bw/d dose groups and, only in females of both groups, an increase in cholesterol level.

There was an increase of absolute and relative liver weight in females at 10 mg/kg bw/d and in both sexes at 30 mg/kg bw/d.

In histopathological examination it was observed slight to moderate hepatocellular hypertrophy in both sexes at doses of 10 and 30 mg/kg bw/d. Furthermore, in the 30 mg/kg bw/d group, hepatocellular necrosis of very small area (grade 1) was observed in 1 male and 1 female.

3. In the OECD TG 452 chronic toxicity study (GLP not specified, Matsumoto *et al*., 1991) Wistar rats (40/sex/dose) were given 2,4,6-tri-tert-butylphenol in feed at concentrations of 0, 30, 100, 300 and 1000 ppm (approx. 0, 2.51, 8.35, 25.05 and 83.5 mg/kg bw/d) for 24 months. No treatment-related mortality or clinical signs were reported. The body weight of females exposed to 1000 ppm (ca. 83.5 mg/kg bw/d) after 12 months of exposure and thereafter was significantly decreased. After 24 months of exposure at 1000 ppm the body weight of females amounted to ca. 65% of the control females. The body weight of males in the 25.05 and 83.5 mg/kg bw/d groups was significantly increased to 112% and 111% respectively after 6 months of exposure, but after 18 and 24 months of exposure the body weight of exposed males did not differ from that of control males. After 24 months of exposure the relative liver weight was significantly increased in males exposed at 8.35, 25.05 and 83.5 mg/kg bw/d and in females exposed at all doses. In histopathological examinations swelling, focal necrosis and vacuolisation of hepatocytes after 6 months of exposure at dose levels of 25.05 and 83.5 mg/kg bw/d (300 and 1000 ppm) were observed.

There was a significant reduction of haemoglobin level in blood of males and females (but less than 10%) in the 25.05 and 83.5 mg/kg bw/d groups after 6 months of exposure but it

was not observed after 24 months of exposure. There was a significant increase in level of cholesterol in blood of male rats in the 25.05 and 83.5 mg/kg bw/d groups and in females of all exposed groups after 6 months of exposure. After 24 months of exposure, in the dose groups of 8.35, 25.05 and 83.5 mg/kg bw/d, males/females reached 170/193%, 201/237% and 356/275% of the cholesterol level in control animals. After 24 months of exposure the number of platelets per microliter of blood was increased in males and females in the 83.5 mg/kg bw/d group.

No neoplastic lesions were observed. Only liver, kidneys and adrenals were examined. No additional information was available.

Based on the results of the combined repeated dose toxicity study with reproduction /developmental toxicity screening test (Anonymous 12, 2015) and the OECD TG 452 chronic toxicity study (mainly higher liver weight and microscopic changes in the liver observed at low doses), the DS proposed to classify 2,4,6-tri-tert-butylphenol as STOT RE 1, H372: Causes damage to organs (liver) through prolonged or repeated exposure.

Comments received during general consultation

One MSCA disagree with classification of 2,4,6-tri-tert-butylphenol as STOT RE 1 and using a different approach for extrapolation of doses used in the studies for comparison with the guidance values, considered that classification STOT RE 2 would be more appropriate. The DS agreed with this approach and with classification of 2,4,6-tri-tert-butylphenol as STOT RE 2.

Assessment and comparison with the classification criteria

In the subacute toxicity study (Takahashi and Hiraga, 1978; no guideline, no GLP) serious toxic effects such as lethality and massive haemorrhages were observed in 10 rats exposed in feed at dose of approximately 520 mg/kg bw/d, thus below the guidance value threshold of 1000 mg/kg bw/d. Therefore, the results support classification as STOT RE Category 2. Still it should be noted that the study is of limited reliability due to study design and poor reporting.

In the range finding study for the combined 28-d repeated dose toxicity study with the reproduction/developmental toxicity screening test at the highest dose of 250 mg/kg bw/d 2 animals were sacrificed in extremis on day 10 and one animal was found dead on day 9. The mortality occurred after more than 4 days exposure, thus this effect is attributable to repeated exposure and not to a single exposure. This adverse effect occurred within the GV for STOT RE 2 for a 10-day exposure of 90–900 mg/kg bw/d, thus it should be taken into account for classification Category 2. The LD_{50} of 2,4,6-tri-tert-butylphenol is much closer to 2000 mg/kg bw/d than to 250 mg/kg bw/d. No symptoms were observed in the 10 rats administered a single dose by gavage of 200 mg/kg bw of 2,4,6-tri-tert-butylphenol. On the other hand, 5 out of 5 females and 1 out of 5 males were killed by a single dose of 2000 mg/kg bw. Thus, a dose level of 250 mg/kg bw is considered as being well below the acute oral LD_{50}. The lethality observed for a daily dose of 250 mg/kg bw/d during 10 days justify classification as STOT RE 2, although it is noted that only three animals were exposed, thus limiting the reliability of this range finding study.

In the main combined 28-d repeated dose toxicity study with reproduction/developmental toxicity screening test (Anonymous 12, 2015; GLP, OECD TG 422) no mortality or clinical

symptoms were observed in animals exposed via the oral route. Males were exposed for 29 days (beginning 2 weeks prior to mating and during mating). Females were exposed for approximately 45-50 days (starting at 2 weeks prior to mating, during mating and throughout pregnancy and until at least day 4 of lactation). Minor alterations in body weight, biochemical parameters in blood of exposed animals and increases in liver weight, are not considered as sufficiently adverse for classification. As noted in point 3.9.2.8. of the CLP Regulation small changes in bodyweight gain, in clinical biochemistry, haematology or urinalysis parameters, changes in organ weights with no evidence of organ dysfunction are not considered to support classification for specific target organ toxicity following repeated exposure. However, in males exposed at 30 mg/kg bw/d and in females exposed at 10 and 30 mg/kg bw/d the increase in the absolute and relative liver weight was above 15% of the negative control values. See table below:

		Males (n-10)				**Females (n-10)**			
Dose level (in mg/kg bw/d)		0	3	10	30	0	3	10	30
Liver weight	Absolute in g	8.07 100%	8.68 107.6%	9.24 114.5%	11.38** 141.0%	7.09 100%	7.98 112.5%	8.95** 126.2%	12.08** 170.4%
	Relative (%)	2.25	2.40	2.52	3.13** 139%	3.01	3.20	3.64** 129.9%	4.91** 163.1%

**: $p < 0.01$

The slight to moderate hepatocellular hypertrophy was noted in both sexes at 10 and 30 mg/kg bw/d. In addition, in the 30 mg/kg bw/d group, hepatocellular necrosis of very small area (grade 1) was observed in 1 male and 1 female. The degree of necrosis does not clearly meet the criterion for classification as an adverse effect which is defined in point 3.9.2.7.3. of the CLP Regulation as multi-focal or diffuse necrosis in vital organs with regenerative capacity. However, slight hepatocellular hypertrophy was observed in all 5 examined histopathologically females out of 10 exposed at 10 mg/kg bw/d, and a moderate hepatocellular hypertrophy was also observed in all 5 examined histopathologically females out of 10 exposed at a dose of 30 mg/kg bw/d. This hypertrophy in exposed female rats could be potentially linked to induction of microsomal enzymes by 2,4,6-tri-tert-butylphenol, however since no measurement of liver microsomal enzyme activity was made, such a link cannot be considered as demonstrated. Therefore, observed hypertrophy cannot be explained by metabolic adaptation to exposure to toxic substance. Taking into account all toxic effects occurring in liver of females exposed at a repeated dose of 30 mg/kg bw/d, which is within GV for STOT RE 2 (45 day exposure 20 – 200 mg/kg bw/d), these effects can be considered as adverse effects warranting classification. So, in conclusion, this study results provided evidence in support of classification as STOT RE 2 with liver as a target organ.

In the OECD TG 452 the chronic toxicity study (GLP not specified) male and female rats were exposed for 6, 12, 18 and 24 months at dose level of 0, 30, 100, 300 and 1000 ppm (approx. 0, 2.51, 8.35, 25.05 and 83.5 mg/kg bw/d). The guidance values for classification to STOT RE 1 would be in case of 6, 12, 18 and 24-months exposure: ≤ 5.0, 2.5, 1.67 and 1.25 mg/kg bw/d, respectively. The lowest and upper guidance values for classification for STOT RE 2 due to oral exposure would be in case of 6, 12, 18 and 24-months exposure: 5 - 50 mg/kg bw/d, 2.5 – 25 mg/kg bw/d, 1.67 – 16.7 mg/kg bw/d and 1.25 – 12.5 mg/kg bw/d, respectively.

In this study no treatment-related mortality and clinical signs were reported. The body weight of females and males were only affected at exposure levels of 25.05 and 83.5 mg/kg bw/d. The relative liver weight in males was significantly increased only at exposure levels of 25.05 and 83.5 mg/kg bw/d, while in females the relative liver weight was increased in all treated groups (2.51, 8.35, 25.05 and 83.5 mg/kg bw/d) after 12, 18 and 24 months of exposure.

At necropsy only liver, kidneys and adrenals were examined. In the histopathological examinations, only in animals exposed to 25.05 and 83.5 mg/kg bw/d (sex and number of examined animals not given) swelling, focal necrosis and vacuolisation of liver cells were noted from 6 months after start of exposure. No other noteworthy histopathological changes were observed in other organs throughout the experiment. No adverse effects were found in histopathological examinations after exposure to 2,4,6-tri-tert-butylphenol at dose levels of 2.51 and 8.35 mg/kg bw/d after 6, 12, 18 and 24 months of exposure. However, at the higher dose of 25.05 mg/kg bw/d (within guidance values for STOT RE 2 after 6 and 12 months of exposure), a swelling, focal necrosis and vacuolization of hepatocytes was observed. The adversity of these effects does not meet the criteria given in point 3.9.2.7.3., however, lack of a more detailed characterisation of the observed histopathological changes makes the interpretation uncertain. The histopathological changes in the liver were accompanied by significant changes in biochemical parameters in blood (two fold increase in blood levels of cholesterol after 18 and 24 months of exposure at 83.5 mg/kg bw/d and in the level of γ-GTP after of 18 months exposure at 83.5 mg/kg bw/d.

Table. Summary of effects observed in the OECD TG 452 the chronic toxicity study within the STOT RE 2 guidance values are given in the table below:

<u>Effects observed after 6 months:</u> Males: Increased of relative liver weight at 25.05 and 83.5 mg/kg bw/d by 6.6% and 30%, respectively, but not at lower doses of 2.51 and 8.35 mg/kg bw/d. Females: Increased of relative liver weight at 2.51, **8.35, 25.05** and 83.5 mg/kg bw/d by 12.4%, **19.8%, 38.8%,** and 107.4%, respectively. Swelling, focal necrosis and vacuolization of hepatocytes at **25.05** and 83.5 mg/kg bw/d and thereafter, no additional information available. No 2-fold increase or decrease in biochemical parameters at doses below 83.5 mg/kg bw/d.	GV for STOT RE 2 for 6 months: 5 – 50 mg/kg bw/d	Classification STOT RE 2

<u>Effects observed after 12 months:</u> Males: Increased relative liver weight at **25.05** mg/kg bw/d and 83.5 mg/kg bw/d by **20%** and 47.8%, respectively but no increase observed at lower doses (2.51 and 8.35 mg/kg bw/d). Females: Increased relative liver weight at **8.35, 25.05** and 83.5 mg/kg bw/d by **15,5 %, 37.9%,** and 146.1%, respectively, but no increase observed at the lower dose (2.51 mg/kg bw/d). Swelling, focal necrosis and vacuolization of hepatocytes at **25.05** and 83.5 mg/kg bw/d, no additional information available. No 2-fold increase or decrease in biochemical parameters in blood at dose of 25.05 mg/kg bw/d or at lower doses.	GV for STOT RE 2 for 12 months: 2.5 – 25 mg/kg bw/d	Classification supported STOT RE 2
<u>Effects observed after 18 months:</u> Males: Increased relative liver weight at 25.05 mg/kg bw/d and 83.5 mg/kg bw/d by 15% and 56%, respectively, but not at lower doses of 2.51 and 8.35 mg/kg bw/d. Females: Increased relative liver weight at 2.51, **8.35**, 25.05 and 83.5 mg/kg bw/d by 10.7%**, 24.8%,** 54, 1% and 151.7% respectively. Swelling, focal necrosis and vacuolization of hepatocytes at 25.05 and 83.5 mg/kg bw/d, no additional information available. 2-fold increase in blood in activity of γ-GTP in males and females, and in level of cholesterol in female rats at 83.5 mg/kg bw/d.	GV for STOT RE 2 for 18 months: 1.6 – 16.5 mg/kg bw/d	Classification supported STOT RE 2
<u>Effects observed after 24 months:</u> Males: Increased relative liver weight at **8.35,** 25.05 and 83.5 mg/kg bw/d by **25%,** 31% and 102%, but not at lower dose of 2.51 mg/kg bw/d. Females: Increased relative liver weight at 2.51**, 8.35**, 25.05 and 83.5 mg/kg bw/d by 14.7%, **39.3%,** 90.6% and 170%. Swelling, focal necrosis and vacuolization of hepatocytes at 25.05 and 83.5 mg/kg bw/d, no additional information available. 2-fold increase in level of cholesterol in blood of males and females at 83.5 mg/kg bw/d.	GV for STOT RE 2: 1.25 – 12.5 mg/kg bw/d	Classification supported STOT RE 2

The description of the results in this study is poor, e.g. it does not at all report on type and intensity of liver effects at the 12, 18 and 24 months of exposure. Nevertheless, the

reported results provided some evidence of adverse effects supporting classification as STOT RE 2.

RAC notes that no adverse effects, such as considerable increase in weight of liver (above 15%) or histopathological changes in liver or other examined organs, were reported in male or female rats exposed at doses within GV for STOT RE 1 in the combined 28-d repeated dose toxicity study with reproduction/developmental toxicity screening test (GLP, OECD TG 422, Anonymous 12, 2015) or in the OECD TG 452 chronic toxicity study. Therefore, classification as STOT RE 1 is not warranted.

However, other adverse effects were reported. There was more than a 60% increase in the relative weight of liver and moderate hepatocellular hypertrophy in all examined female rats exposed by gavage for ca. 45 days to a dose of 30 mg/kg bw/d. For the same dose level, in 1/10 males and in 1/10 females, it was also observed hepatocellular necrosis of very small area (grade 1). In addition, in rats exposed for 6 months to 25.05 mg/kg bw/d it was observed a ca. 40% increase in relative weight of liver in females rats and swelling, focal necrosis and vacuolisation of liver cells accompanied by increased level of cholesterol and γ-GTP in blood of females rats. Should also be noted the mortality observed in rats exposed for 10 days to 2,4,6-tri-tert-butylphenol in feed at dose of ca. At 520 mg/kg bw/d or by gavage at dose of 250 mg/kg bw/d. Highlighting that all these adverse effects have occurred at exposure levels within the guidance values for classification to STOT RE 2, RAC is of the opinion that **classification of 2,4,6-tri-tert-butylphenol as STOT RE 2 is warranted** with the hazard statement H373: Causes damage to organs (liver) through prolonged or repeated exposure.

10.13 Aspiration hazard

Not evaluated in this CLH dossier

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in this CLH dossier.

12 ADDITIONAL LABELLING

NA

13 LIST OF ABREVIATIONS

2,4,6-TTBP : 2,4,6-tri-tert-butylphenol

Abs. : absolute

Approx.. : approximately

ASAT : aspartate aminotransferase

ATE : acute toxicity estimate

BUN : blood urea nitrogen

Bw : body weight

Cat. : category

CE : cloning efficiency

DMSO : dimethyl sulfoxide

DS : dossier submitter

E. Coli : Escherichia coli

EC3 : estimated test substance concentration that will give a SI of 3

F : female

FBW : final body weight

Gamma-GTP : gamma-glutamyl transpeptidase

GLP : good laboratory practice

GOT : glutamate oxaloacetate transaminase

Hb : haemoglobin

Irrit. : irritation

LD50 : lethal dose 50

LLNA : local lymph node assay

LT50 : lethal time 50

M : male

MCH : mean corpuscular haemoglobin

MCV : mean corpuscular volume

Met. act. : metabolic activation

NA : not applicable

Nb. : number

Neg. : negative

NZW : New Zealand White

OECD : Organisation for Economic Co-operation and development

Pl : phospholipid

Plt : platelet

Pos. : positive

Pt : prothrombin time

RBC : red blood cell

Rel. : reliability

Rela. : relatif

RS : relative survival

RSG : relative suspension growth

RTG : relative total growth

S. Typh : Salmonella typhimurium

SD : Sprague-dawley

Sem. Ves. : seminal vesicle

Sens. : sensitisation

SI : simulation index

Sign. : significant

STOT RE : specific target organ toxicity (repeated exposure)

T-Chol : total cholesterol

TG : test guideline

Tot. : total

Tox. : toxicity

W. : weight

WBC : white blodd cell

14 REFERENCES

Anonymous 1-12 : see confidential annex to the CLH report.

Matsumoto K et al., 1991, Chronic toxicity of 2,4,6-tri-tert-butylphenol in rats, The J. of Toxicological sciences, 16, 167-179.

Takahashi O. and Hiraga K., 1978, The relationship between Hemorrhage Induced by Butylated Hydroxytoluene and its Antioxidant Properties or Structural Characteristerics, Toxicol. Appl. Pharm., 46, 811-814.

15 ANNEXES

Confidential annex to CLH report