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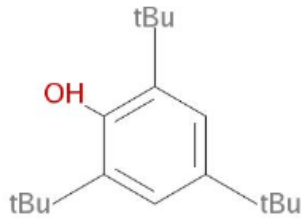
Version number: 2 **Date: 14 February 2019**

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 and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No existing entry in Annex VI of CLP										
Dossier submitters proposal	TBD	2,4,6-tri-tert-butylphenol	211-989-5	732-26-3	Repr. 2 Acute Tox. 4 STOT RE 1 Skin Sens. 1B	H361d H302 H372 (liver) H317	GHS07 GHS08	H361d H302 H372 (liver) H317		Oral: ATE = 500 mg/kg bw	
Resulting Annex VI entry if agreed by RAC and COM	TBD	2,4,6-tri-tert-butylphenol	211-989-5	732-26-3	Repr. 2 Acute Tox. 4 STOT RE 1 Skin Sens. 1B	H361d H302 H372 (liver) H317	GHS07 GHS08	H361d H302 H372 (liver) H317		Oral: ATE = 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

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.

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.

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."	"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."	SI or 1.7, 3.3 and 4.6 respectively at 10, 25 and 50% EC3 of 22.2%

EC3 value ≤ 2	EC3 value > 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**.**

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 chromosome aberration test Japabese guideline (for screening mutagenicity) GLP	2,4,6-TTBP Purity : unknown Vehicle : DMSO	Chinese hamster ovary (CHO) 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	Genotoxicity : negative Cytotoxicity : yes	Anonymous 11, 1998

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 transmission to progeny."	"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.

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.

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).

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.

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. 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	Since no narcotic effects or respiratory tract irritation were observed, a classification as STOT SE 3 is not appropriate.

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.	

10.11.3 Conclusion on classification and labelling for STOT SE

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

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 exposure	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 (mU/ml)	63	65	122	62	57	86	62*	82	60**	130
	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)	24 months	3.09 mg/kg bw/d	STOT RE 1

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
	observed at : 25.05 mg/kg bw/d (300 ppm)			

10.12.2 Comparison with the CLP criteria

<table>
<tr><td>Criteria for STOT RE 1</td><td>Criteria for STOT RE 2</td></tr>
<tr><td>"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 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"
<table><tr><td>Route of exposure</td><td>Units</td><td>Guidance value</td></tr><tr><td>Oral (rat)</td><td>mg/kg bw/d</td><td>C≤10</td></tr></table></td><td>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 to occur within the guidance value range as indicated in table 3.9.3"
<table><tr><td>Route of exposure</td><td>Units</td><td>Guidance value range</td></tr><tr><td>Oral (rat)</td><td>mg/kg bw/d</td><td>10 < C ≤ 100</td></tr></table></td></tr>
</table>

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.

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