Committee for Risk Assessment

RAC

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

Adopted

8 October 2020

P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

8 October 2020

CLH-O-0000006909-58-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **2,4,6-tri-*tert*-butylphenol, [1]; Reaction mass of 2,6-di-*tert*-butylphenol and 2,4,6-tri-*tert*-butylphenol, [2]; Reaction mass of 2-*tert*-butylphenol and 2,6-di-*tert*-butylphenol and 2,4,6-tri-*tert*-butylphenol, [3]**

EC Number: **211-989-5 [1]; - [2]; - [3]**

CAS Number: **732-26-3 [1]; - [2]; - [3]**

The proposal was submitted by **Belgium** and received by RAC on **14 February 2019.**

PROCESS FOR ADOPTION OF THE OPINION

Belgium has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **1 April 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **31 May 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Bogusław Barański**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **8 October 2020** by **consensus.**

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	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 current Annex VI entry										
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 H317		Oral: ATE = 500 mg/kg bw	
RAC opinion	TBD	2,4,6-tri-tert-butylphenol	211-989-5	732-26-3	Repr. 1B Acute Tox. 4 STOT RE 2 Skin Sens. 1B	H360D H302 H373 (liver) H317		H360D H302 H373 H317		Oral: ATE = 500 mg/kg bw	
Resulting Annex VI entry if agreed by COM	TBD	2,4,6-tri-tert-butylphenol	211-989-5	732-26-3	Repr. 1B Acute Tox. 4 STOT RE 2 Skin Sens. 1B	H360D H302 H373 (liver) H317	GHS08 GHS07 Dgr	H360D H302 H373 (liver) H317		Oral: ATE = 500 mg/kg bw	

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD EVALUATION

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 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

<u>Oral route</u>

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.

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**.

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**.

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**.

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.**

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 a 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 a 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 an 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 via feed at a dose of approximately 520 mg/kg bw/d, thus below the guidance value 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 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.**

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 warrant classification for germ cell mutagenicity**.

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**.

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 due to 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.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in "RAC boxes".

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).