# Committee for Risk Assessment

# RAC

## Opinion

proposing harmonised classification and labelling
at EU level of

## p-tert-butylphenol

**EC number: 202-679-0**
**CAS number: 98-54-4**

ECHA/RAC/CLH-O-0000002629-66-01/F

.

**Adopted**

**12 June 2012**

Annankatu 18, P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

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

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

**Substance Name: p-tert-butylphenol**

**EC Number: 202-679-0**

**CAS Number: 98-54-4**

The proposal was submitted by **Norway**
and received by RAC on **7 January 2011**.

**The proposed harmonised classification**

| | **CLP Regulation (EC) No 1272/2008** | **Directive 67/548/EEC** |
|---|---|---|
| **Current entry in Annex VI of CLP Regulation (EC) No 1272/2008** | - | - |
| **Proposal by dossier submitter for consideration by RAC** | STOT SE 3; H335<br>Skin Irrit. 2; H315<br>Eye Dam. 1; H318<br>Repr. 2; H361f | Xi: R37/38 R41,<br>Repr. Cat 3; R62 |
| **Resulting harmonised classification (future entry in Annex VI of CLP Regulation) as proposed by dossier submitter** | STOT SE 3; H335<br>Skin Irrit. 2; H315<br>Eye Dam. 1; H318<br>Repr. 2; H361f | Xi: R37/38 R41,<br>Repr. Cat 3; R62 |

## PROCESS FOR ADOPTION OF THE OPINION

***Norway*** 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/web/guest/harmonised-classification-and-labelling-previous-consultations*** on **07 January 2011**. Parties concerned and MSCAs were invited to submit comments and contributions by **21 February 2011**.

## ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Eugenio Vilanova**
Co-rapporteur, appointed by RAC: **Helmut Greim**

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

The RAC opinion on the proposed harmonised classification and labelling has been reached on **12 June 2012**, in accordance with Article 37 (4) of the CLP Regulation, giving parties concerned the opportunity to comment.

The RAC Opinion was adopted by **consensus.**

## OPINION OF RAC

RAC adopted the opinion that **p-tert-butylphenol** should be classified and labelled as follows:

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

| Index No | International Chemical Identification | EC No | CAS No | Classification | | Labelling | | | Specific Conc. Limits, M-factors | Notes |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Hazard Class and Category Code(s) | Hazard statement Code(s) | Pictogram, Signal Word Code(s) | Hazard statement Code(s) | Suppl. Hazard statement Code(s) | | |
| 604-090-00-8 | **p-tert-butylphenol** | 202-679-0 | 98-54-4 | Skin Irrit. 2<br>Eye Dam. 1<br>Repr. 2 | H315<br>H318<br>H361f | GHS05<br>GHS08<br>Dgr | H315<br>H318<br>H361f | | | |

**Classification and labelling in accordance with the criteria of Directive 67/548/EEC**

| Index No | International Chemical Identification | EC No | CAS No | Classification | Labelling | Concentration Limits | Notes |
|---|---|---|---|---|---|---|---|
| 604-090-00-8 | **p-tert-butylphenol** | 202-679-0 | 98-54-4 | Xi; R38-41<br>Repr. Cat. 3; R62 | Xn<br>R: 38-41-62<br>S: (2-)26-36/37-39-46 | | |

**SCIENTIFIC GROUNDS FOR THE OPINION**

The opinion relates only to those hazard classes that have been reviewed in the proposal for harmonised classification and labelling, as submitted by ***Norway***.

## Irritation

***Skin irritation*:**

In the most recent study (Sandoz Chemicals, 1991), p-tert-butylphenol was found to be highly irritating to skin. In this guideline study (OECD TG 404), following GLP, 500 mg of p-tert-butylphenol was moistened with distilled water and applied (semi-occluded), to the intact skin of three New Zealand rabbits (1 male and 2 females), for 4 hours. Skin reactions (erythema, eschar formation, oedema) were scored according to Draize at one hour, 24, 48 and 72 hours, as well as 7 and 14 days after treatment. The mean scores for erythema were as follows: 24 hours, score 4; 48 hours, score 4; 72 hours, score 3.3; 14 days, score 0. Average score for erythema over 24-48-72 hours was 3.8. Mean scores for oedema were: 24 hours, score 2; 48 hours, score 1.3; 72 hours, score 1.7; 14 days, score 0. Average score for oedema over 24-48-72 hours was 1.7.

Other adverse skin reactions noted were: small areas of white-coloured necrosis and well-defined erythema surrounding scabs in all exposed animals at 24 and 48 hours and in two exposed animals at 72 hours; hardened light brown-coloured scabs and thickening of the skin at two treated skin sites at day 7, and reduced re-growth of fur at these sites at day 14; crust formation at one treated skin site at day 7. No irreversible skin alterations were reported after 14 days, and as no further information on the nature of the white coloured necrosis is provided, it is considered that p-tert-butylphenol is not corrosive according to classification criteria in the CLP Regulation (EC) No 1272/2008 and Directive 67/548/EEC (DSD) (full thickness destruction of the skin).

The lesions reported indicate that p-tert-butylphenol is severely irritating to skin.

In a skin irritation study by Klonne *et al* (1988), 4 hours of occluded application of 0.5 g p-tert-butylphenol produced no irritation in 4 out of 6 rabbits. Minor, transient erythema developed in one rabbit by day 1, with desquamation evident during days 10-17 post exposure. One of 6 rabbits exhibited slight oedema on days 1-3, dermal necrosis on days 1-10, scab formation on days 7-10, desquamation on days 10-14. The skin of this rabbit appeared normal on day 17 post exposure.

In a skin irritation study from Huels (1985b), 4 hours of (semi-)occluded application of 0.5 g p-tert-butylphenol produced signs of irritation in all 6 rabbits. The test substance was put in a patch test on the clipped back skin. The average score for erythema over 24-48-72 hours was 2.4, for oedema this was 1.6. Scab or scale formation was observed on days 6, 8, 10 and 14 post exposure (in 4, 4, 5 and 3 animals, respectively), and detaching of the skin on days 8, 10 and 14 post exposure (in 2 animals per time point).

In a skin irritation study conducted according to US DOT regulation 173.1300 (Schenectady, 1982), 500 mg p-tert-butylphenol moistened with saline was applied for 4 hours (semi-occluded) to the intact skin of New Zealand rabbits (1 female and 5 males). Skin reactions were observed after removal of the patch and approximately 48 hours thereafter. Mean scores for the effects seen were as follows: Erythema: 4 hours, score 2; 48 hours, score 2.3. Oedema: 4 hours, score 1.5; 48 hours, score 1.7. One male showed necrosis at 48 hours. No further details are provided.

Two studies with prolonged exposure during 24 hours are available.

In a percutaneous toxicity study with rabbits (Klonne *et al*, 1988), signs of severe skin irritation were reported in all exposed animals after prolonged skin contact (24 hours) with doses of 2, 8 and 16 g/kg. The effects seen were erythema, oedema, fissuring,

desquamation and/or necrosis in both sexes in all dose groups. For animals dosed with 8 and 16 mg/kg signs of skin irritation generally persisted at 14 days post exposure. For rabbits dosed with 2 mg/kg bw, signs of erythema, necrosis and fissuring was seen through day 7, and desquamation and scabs were still present at day 14. No information related to the nature of the corrosivity and necrosis reported is available.

In a skin irritation study (Shell, 1980) with occlusive patch testing according to the method of Draize, intact and abraded skin of rabbits were exposed for 24 hours to 500 mg of ptBP. Mean scores at each observation time (24, 48, 72 h, and 7 days) were registered for erythema, oedema only. The primary irritation score according to the method of Draize was 2.04 and in the study report it was concluded that ptBP was to be regarded as mildly irritating to rabbit skin based on the effects seen. It was also mentioned that three of the six animals in the study had small white areas of skin similar in appearance to a burn, and it is stated that this may be due to a protein denaturing effect of the compound.

Summary of skin irritation studies

| **Species** | **Method** | **Exposure** | **Result** | **Reference** |
|---|---|---|---|---|
| Rabbit | OECD 404, GLP | 4 hours | Severely irritating | Sandoz Chemicals, |
| Rabbit (male/female) | | 24 hours | Non- to moderately irritating. Severely irritating/corrosive to 1/6 animals | Klonne *et al*., 1988/UCC 1985 |
| Rabbit (male/female) | OECD 404 | 4 hours | Irritating | Huels, 1985b |
| Rabbit (male/female) | US DOT regulation 173.1300 | 4 hours | Irritating. Severely irritating/corrosive to 1/6 animals | Schenectady, 1982 |
| Rabbit (male/female) | | 24 hours | Mildly irritating | Shell, 1980 |

Comparison with criteria

| **Criteria for Skin Irrit. 2; H315** | **Data fulfilling the criteria** |
|---|---|
| (1). 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<br>(2) 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, | Sandoz Chemicals, (1991): Erythema: 4 hours, score 4; 72 hours, score 3.4; with average score 3.8. No irreversible skin alterations were reported after 14 days and the substance was judged to be non-corrosive.<br>Klonne et al (1988), Percutaneous toxicity study: 2, 8 and 16 g/kg bw p-tert-butylphenol for 24 hours produced severe irritation and dermal necrosis. Severe skin irritation (including erythema, oedema, fissuring, desquamation and necrosis) were noted in both sexes of all treatment groups. For the middle and high dose groups necrosis generally persisted through the 14-days post-exposure period. For the low dose animals (2 mg/kg bw) signs of erythema, necrosis and fissuring were present through day 7, whereas desquamation and scabs were present at day 14.<br>Klonne et al (1988), Skin irritation study: 6 animals. One rabbit developed transient erythema (grade 1; day 1) and persisting desquamation (day 10-17), and one rabbit showed erythema (grade 1-2; day 1-10), minor oedema |

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| hyperplasia, and scaling; or (3) 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. | (grade 1; day 1-3), desquamation (day 10-14), scab formation (day 7-10) and necrosis (day 1-10). This study indicates that p-tert-butylphenol can be severely irritating and possible also corrosive to skin.<br>Huels (1985): Erythema was well defined in 2 of 6 animals and moderate to severe in 4 of 6 animals, with an average score of 2.4. Oedema was very slight in 4 of 6 animals, and moderate in 2 of 6 animals at 24 hours. Erythema and oedema was present in some animals through day 10. Scab or scale formation and detaching of skin was observed in some animals from day 6 post exposure.<br>Schenectady (1982): Erythema: 4 hours, score 2; 48 hours, score 2.3. Oedema: 4 hours, score 1.5; 48 hours, score 1.7. One male showed necrosis at 48 hours |

Classification as skin corrosive (H314) is discarded because:

(i) Skin corrosion should be applied to substances where irreversible skin damage is seen after up to 4 hour exposure. PtBP did not induce any irreversible skin lesions or full skin destruction in a skin irritation study according to OECD test guidelines and GLP, with exposure for 4 h in semi-occluded intact skin in rabbits. There are reports of necrosis in several studies, but there are doubts concerning the interpretation of these effects as the skin was reported to look normal at the end of the observation period, and necrosis is per definition not reversible;

(ii) in the 1 of 6 animal with indication of necrosis in the study of Klonne *et al* (1988), normal skin was observed at day 17 post exposure; and

(iii) in the 1 animal of 6 in the study by Schenectady *et al* (1982) for which necrosis at 48 h was indicated, no details are reported.

On the basis of the effects seen and the arguments listed above, RAC agreed that p-tert-butylphenol should be classified as Skin Irritant, Category 2, according to the CLP Regulation .

**Based on the animal data available, RAC concluded on classification according to CLP criteria with Skin Irrit. 2; H315 (Xi; R38 according to DSD).**

***Eye irritation:***

In three studies p-tert-butylphenol was shown to be highly irritating to rabbit eyes, and the severe irritating effects persisted during the 7- and 21-day observation period.

Comparison with criteria

| **Criteria for Eye Dam. 1; H318** | **Data fulfilling the criteria** |
|---|---|
| - at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days;<br>and/or<br>- at least in 2 of 3 tested animals, a positive response | (Klonne 1988). Corneal opacity of grade 1 (1 h) to 3.2 (7 d), iris lesion grade 1, conjunctival redness of grade 1.8 (1 h) to 2.2 (72 h), and chemosis of grade 2.3 (1 h) to 3.8 (72 h). Due to corneal opacity, the scoring of iris lesions after 4 h was not possible in many animals and thus reversibility could not be established. The corneal opacity was significant 21 days after exposure (mean score 2.5; range 0-4).<br>(Shell 1980) corneal opacity grade 0 (1 h) to grade 1.4 (48 h-7 d), iris lesions grade 0 (1 h) to 0.5 (48 h-7 d), |

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| of:<br>- corneal opacity ≥ 3 and/or<br>- iritis > 1,5 calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material. | conjunctival redness grade 2 (1h-48 h) to 1.2 (7 d), chemosis grade 2.2 (24 h) to 0.3 (7 d).<br>(Basf 1971). Severe irritation and probably corrosive effects were mentioned in another test. |

**Based on the above information RAC regarded p-tert-butylphenol as severely irritating to eyes and a classification according to CLP criteria with Eye Dam. 1; H318 (Xi; R41 according to DSD).**

## Sensitisation

***Skin sensitisation:***

Of the three animal studies reported, two are negative and one is positive. The negative studies used the GPMT test, and were performed according to current test guidelines and GLP. The positive study is an older study and the protocol is not well described. No firm conclusions can be drawn based on the animal studies. However, based on the scientific quality of the studies it appears more likely that p-tert-butylphenol does not cause skin sensitisation in animals.

P-tert-butylphenol has been reported to be the first allergen identified in ptBP-FR (p-tert-butylphenolformaldehyde resin) (Zimerson and Bruze in Kanerva *et al*.; Handbook of Occupational Dermatology, 2000). There are several sensitisation studies performed using patch tests of patients with either work related contact allergy or general allergy. Furthermore, many case reports were found in the literature. Many of them used ptBP-FR and are of limited value in evaluating a possible sensitisation potential for p-tert-butylphenol. The results from these studies/reports give a very variable picture of human sensitisation to p-tert-butylphenol. In Contact Dermatitis of Fisher, 1986, (p. 649) it is stated that in the 1950s and 1960s an excess of free p-tert-butylphenol was present in the resin.

Sensitisation studies indicate that an allergic reaction to the resin is frequently caused by a reaction to both the resin itself (PTBPFR) and to the free p-tert-butylphenol. It was also recommended to eliminate the excess of free p-tert-butylphenol in the resin by Malten *et al.* (1958) based on a study on shoemakers exposed to ptBP-FR/ptBP resin containing glue. Thus, earlier human exposure was more likely to have higher levels of free ptBP than current exposure, which consists of lower levels of free ptBP and more of the intermediate and degradation products (Fisher, 1986). Accordingly, patients now allergic to ptBP-FR commonly do not react to free ptBP and rarely to free formaldehyde (F). Studies performed before changing the production process are expected to reflect allergic reaction to free p-tert-butylphenol and are of more importance when assessing the sensitisation potential of ptBP than studies performed later with ptBP-FR (Rudner, 1977; Romaguera *et al.*, 1981).

It is concluded that human data on p-tert-butylphenol on skin sensitisation was derived from an old test protocol with a significant risk of misdiagnosis. Other studies according to modern protocols and standards showed no effect.

The database for assessing skin sensitisation for p-tert-butylphenol has limitations. The animal data are of varying reliability and are not sufficient to draw any conclusions of p-tert-butylphenol as a sensitiser. The human data are also of limited value since most of the studies shows very few positive results and they are mainly performed on patients with former skin allergy or other skin diseases or there is limited information about the exposure substance.

**RAC concluded that the data does not fulfil the classification criteria and no classification is proposed.**

***Respiratory sensitisation:***

**RAC concluded that the available data are not sufficient to propose classification for respiratory sensitisation.**

## Repeated dose toxicity

No repeated dose toxicity study according to current Guidelines, OECD 407 (Repeated dose 28-day oral toxicity study in rodent) or OECD 408 (Repeated dose 90-day oral toxicity study in rodent) is available for p-tert-butylphenol. The only study available is an OECD combined repeated dose and reproductive/developmental toxicity screening test (OECD Guideline 422). The highest dose tested in the study was 200 mg/kg bw/day, and was considered a LOAEL value from this study for systemic toxicity. The NOAEL was 60 mg/kg bw/day. The NOAEL/LOAEL values were based on respiratory distress in exposed females and on effects on several blood parameters in males.

Long-term exposure to high doses of p-tert-butylphenol in the diet induced moderate effects on relative kidney and liver weights.

**Based on the available data, RAC concluded that no classification for repeated dose toxicity is warranted**.

## Mutagenicity

P-tert-butylphenol was shown to be non-mutagenic in all available bacterial tests. The mouse lymphoma TK+/-locus assays have given both negative and positive results, apparently depending upon duration of exposure. However, it is important to be aware that the positive *in vitro* TK+/- test was not GLP-certified, whereas the negative *vitro* TK+/- test was p-tert-butylphenol induced chromosomal aberrations with exogenous metabolic activation and polyploidy with and without exogenous metabolic activation in two studies with Chinese hamster lung cells but was negative in a study with rat lymphocytes, and in a study with a cultured rat-liver cell line. Thus, the overall results regarding mammalian cell mutagenicity *in vitro* is inconclusive.

No response was reported in preliminary results from an unpublished *in vivo* micronucleus test with mice. These *in vivo* studies have, however, limited value due to the absence of cytotoxicity in the target tissue or lack of information in this aspect.

**Based on the available data, RAC concluded not to propose classification for mutagenicity.**

## Carcinogenicity

Based on the results from the Hirose (1988) rat study where only one papilloma of the forestomach was found, and the uncertain mutagenic effects, it is considered unlikely that p-tert-butylphenol is a human carcinogen. However, its ability to increase the frequency of squamous cell carcinomas in the rat forestomach following initiation with MNNG indicates that p-tert-butylphenol may act as a tumour promoter in rats. Whether or not it may be a promoter in humans needs to be clarified. Though p-tert-butylphenol apparently is not a mutagen, the underlying database is not very solid.

The data available does not indicate a carcinogenic activity for p-tert-butylphenol. However, the available information is not sufficient to address its carcinogenic properties.

**Based on the available data, RAC concluded not to propose classification for carcinogenicity.**

## Reproductive toxicity

### Fertility:

The results from the Combined Repeated Dose and Reproductive/Developmental Toxicity Screening Test (OECD 422) indicated that p-tert-butylphenol had no effect on fertility at the dose levels tested (0, 20, 60 and 200 mg/kg bw/day).

However, in the 2-generation reproduction study, the following effects were reported: At 7500 ppm a decreased number of implantation sites and live pups born were reported as well as slightly smaller litter size compared to controls. At 7500 ppm also an increase in atrophy of the vaginal epithelium with 12/28 rats affected in the F1 generation and 14/24 rats affected in the F2 generation were seen. Furthermore, in the F0 females at 7500 ppm an increase in the incidence of primordial follicles with a concurrent decrease in the incidence of growing follicles were reported.

Comparison with the criteria

| **Criteria for Repr. 2 H361f** | **Data fulfilling the criteria** |
|---|---|
| CLP Regulation 3.7.2. Hazard categories for reproductive toxicants.<br><br>Category 2. Suspected human reproductive toxicant (Label H361: Suspected of damaging fertility or the unborn child)<br>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 Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.<br>Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.<br><br>Guide 3.7.1.3: Adverse effects on sexual function and fertility<br>Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects | There are evidences of adverse effects in fertility in experimental animals in a two generation reproduction toxicity study:<br>Decreased number of implantation sites and live pups born were reported as well as slightly smaller litter size compared to controls. At 7500 ppm an increase in atrophy of the vaginal epithelium with 12/28 rats affected in the F1 generation and 14/24 rats affected in the F2 generation Furthermore, in the F0 females at 7500 ppm an increase in the incidence of primordial follicles with a concurrent decrease in the incidence of growing follicles were reported.<br>As the observed effects occur only at the high dose and there is not obvious severe alteration of reproductive performance, the data does not support category 1 but category 2 is considered appropriate. |

| | |
|---|---|
| on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behavior, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems. | |

During specific critical periods of the life of the exposed animals, the actual average dose of the group was higher than the "limit dose" of 1000 mg/kg bw/day. This occurred for the groups at the high dose (7500 ppm) as follows:

- Males in F1 generation: weeks 5 and 6 (1221 and 1013 mg/kg bw/day)
- Females in F0 generation: last 2-3 weeks of lactation (1353 and 1788 mg/kg bw/day)
- Females in F1 generation: first 5-6 weeks (1220, 1033 mg/kg bw/day)
- Females in F1 generation: last 2-3 weeks of lactation (1525 and 1814 mg/kg bw/day)

Moreover, values in the range of 800-988 mg/kg bw/day were observed in F1 males during week 7 and in F0 females during week 3 of gestation and week 1 of lactation, as well as in F1 females during week 7 of gestation and week 1 of lactation.

In the groups exposed to 800-1000 and 2500 ppm, respectively, the average doses did not exceed the limit dose of 1000 mg/kg bw/day.

As the most critical effects are observed only at the high dose (7500 ppm) at which the limit dose is exceeded during critical periods, the proposal for classification could be considered inappropriate.

However, RAC considered that classification cannot be excluded by the argument that the limit dose is exceeded as:

(1) the limit dose is exceeded only during lactation,

(2) the classification should be based on the severity of the effects seen,

(3) the limit dose is a guideline for testing and there is no cut-off value for classification according to the CLP Regulation.

Therefore based on the effects seen on fertility, RAC supports classification of p-tert-butylphenol for reproductive toxicity, effects on sexual function and fertility, Category 2, (Repr. 2; H361f), according to the CLP Regulation.

**Based on the data from the 2-generation reproduction study in rats p-tert-butylphenol, RAC proposed to be classified for fertility according to CLP criteria with Repr. 2; H361f (corresponding to Repr. Cat. 3; R62 according to DSD).**

**<u>Developmental toxicity:</u>**

The results from the Combined Repeated Dose and Reproductive/Developmental Toxicity Screening Test (OECD 422) indicated that p-tert-butylphenol induced no embryotoxicity or teratogenicity at the dose levels tested (0, 20, 60 and 200 mg/kg bw/day).

In the 2-generation reproduction study, the following effects were reported: A decrease in pup body weights and litter weights in the F1 generation from 2500 ppm, and a smaller litter size as well as an increase in pup mortality in the F1 generation at 7500 ppm. A delay in vaginal opening and preputial separation in the F1 generation was reported at 7500 ppm.

**RAC concluded that the available data are not sufficient to propose classification for developmental toxicity.** This is based on the fact that no embryotoxicity or teratogenicity was induced at the tested doses in a Combined Repeated Dose and Reproductive/ Developmental Toxicity Screening Test (OECD 422), and the fact that the doses causing significant fertility effect in the 2-generation reproduction study did not caused significant developmental toxicity effects supporting classification.

## Specific target organ toxicity- single exposure

### Respiratory irritation:

The dossier submitter is proposing classification of p-tert-butylphenol as severely irritating to the respiratory tract (STOT SE 3 - H335 according to the CLP Regulation). The data used by the dossier submitter as justification for the proposal comes from the following studies:

Acute inhalation limit test (non-guideline) (Klonne et al., 1988)

- Repeated dose test: Combined Repeated Dose and Reproductive Toxicity Screening test (OECD Test Guideline 422). (MHW, Japan 1996)

Other available data are also considered in this evaluation:

- Two generation reproduction study OECD Test Guideline 416, US EPA OPPTS 870.3800
- Possible human data

*Inhalation studies in humans*

No studies examining acute inhalation toxicity of p-tert-butylphenol in humans were found. Occupational biomonitoring of urine metabolites has been described in several studies (described in the RAR) and urine metabolites has been detected and identified. In one study, average exposure was estimated to be 0.39 mg/m3 (n=15) in one group and 0.10 mg/m3 (n = 5) in another group. The urine excreted mean urine concentration of p-tert-butylphenol was 5.07 µg/ml and 3.03 µg/ml.

RAC concluded that the exposure by inhalation in human has been demonstrated by detecting and identifying metabolites in urine but no data about respiratory effects are described in these studies.

*Acute inhalation study in animals*

No acute inhalation toxicity study fulfilling current test guidelines is available. However, a non-guideline acute inhalation study is published (described in Klonne *et al.*, 1988). This paper includes several studies that have also been considered for dermal and eye irritation. Two inhalation experiments are described in the paper:

Exposure to saturated vapour for 6 h

Rats were exposed in a static generated vapour. It was prepared putting 100g of the substance and leaving it to statically saturate the chamber. The actual concentration in the air of the chamber is not indicated. We can assume it to be around the concentration determined by its relative vapour pressure (0.5 Pa at 20 °C) in the air close to the site of deposit of the substance and somewhat lower in the whole chamber. No effects were observed in the rat exposed for 6 hours in this chamber. There were no effects on body weight and no signs of toxicity following clinical observation and necropsy. No respiratory effects are indicated.

Another similar study is mentioned in the RAR with an 8 h exposure period (BASF 1971) with no observed effects. No details on this study are indicated.

A limit test at dynamically generated dust aerosol (5600 mg/m3) for 4 hours

Five male and five female rats (Sprague-Dawley) were exposed in a 120 liter chamber for 4 hours to p-tert-butylphenol as dust aerosol of 5600 mg/m3 (median particle diameter of 3.6 µm) with additional vapour component of 30 mg/m3. Dust aerosol was generated by leading vapour from melted p-tert-butylphenol (110 °C) to the exposure chamber where the vapour condensed in air to fine powder.

Clinical signs observed on the day of exposure and up to 7 days post exposure included signs of mucosal irritation (perinasal, perioral, and periocular encrustation) and signs of respiratory distress (audible respiration, gasping, and a decreased respiration rate). It is unclear if the mucosal irritation is cased by the "dust" particles or by the substance as animals were exposed to fine powder suspended in the air. No further details on severity of clinical signs of toxicity or number of animals affected are given in the report. Within one to two days following exposure, one rat of each sex died. The dead animals showed dark red or purple discoloration. This study demonstrates irritating properties of p-tert-butylphenol when administered as dust aerosol. It is unclear if this is due to the dust of or specific effect of the substance. The high dose of 5600 mg/m3 is over the cut off of 5000 mg/m3 indicated in the guide for STOT single exposure by inhalation for dust aerosols as STOT SE 2. Therefore there are objective criteria for this to be considered a high dose.

*Repeated dose study*

A Combined Repeated Dose and Reproductive Toxicity Screening test (OECD Test Guideline 422; MHW, Japan 1996)

Sprague-Dawley rats were dosed by oral gavage ("oral intubation"). Specific details of the technical procedure are not described in the available unofficial translation of the report (i.e: flexible tubing or rigid feeding needles, animal immobilization, rate of liquid administration)

The test substance, p-tert-butylphenol [CAS No.; 98-54-4, Purity; 99.9 % (wt %)] is a white flaky substance that is stable at room temperature. A 4 % suspension (for 200 mg/kg dose group) was prepared in 0.5 % methylcellulose solution in water (1500cp, Wako Junyaku Co., Lot No.: DSG 1980; Japanese Pharmacopoeia; San-a Seiyaku Co., Manufacture No.: DH004)]. The suspension was diluted gradually for the other doses. It was shown that the suspension was stable at room temperature for eight days, under dark light conditions. The suspension was used within seven days.

A preliminary range finding study (5 rats/sex/group) was carried out in order to determine the doses of p-tert-butylphenol for the main test. Five male and female eight-week old Sprague-Dawley rats per dose group were administered by oral gavage ("oral intubation") p-tert-butylphenol at daily doses of 0, 250, 500 and 1000 mg/kg for two weeks, after which they were weighed and examined for toxic effects. At 1000 mg/kg, two females and one male died. Decreases in weight gain and abnormal respiratory sounds accompanying difficult breathing were observed in three females. At 500 mg/kg, the number of animals with abnormal respiratory sounds, the same type as at 1000 mg/kg, increased gradually during the treatment period, and at the end of treatment these symptoms were observed in three males and three females.

Based on these results, daily doses of 500 and 1000 mg/kg were considered to exceed the maximum tolerable dose. At 250 mg/kg, no significant effect on weight gain was observed. However, abnormal respiratory sounds were observed in one female. Considering that the number of animals with abnormal respiratory sounds increased progressively during treatment with 500 and 1000 mg/kg of p-tert-butylphenol and that the treatment period of the main study would be longer than the preliminary study, 250 mg/kg was also considered to slightly exceed the maximum tolerance dose. Thus, it was decided to use 200 mg/kg/day for the high dose, and 60 and 20 mg/kg/day for middle and low dose, respectively.

In the main study, 13 rats/sex/group were dosed by oral gavage with 0, 20, 60, 200 mg/kg bw/day. Approximately 4 weeks of exposure in males, and from 14 days before mating to day 3 of lactation in females. At 200 mg/kg bw/day, one female was found dead on day 43; however, this was considered to be caused by an administration mistake. Some females of the highest dose group showed stridor, associated with dyspnea (abnormal respiration). Further, in the F0 generation at 200 mg/kg some females showed abnormal respiratory sound after the 3rd administration and a total of four animals showed abnormal respiratory sound at the end of the experiment.

According to the study the respiratory sound observed in the repeated dose study might be caused by irritation of the respiratory tract during administration. However, histopathological examinations did not reveal signs of irritation of the respiratory tract. The mean plasma concentration of albumin in the males was slightly lower in the 60 and 200 mg/kg dose groups (6 % and 13 %), accompanied by decrease in plasma protein in the 200 mg/kg bw/day males (6 %). A significant lower mean red blood cell count (5 %), and higher mean white blood cell count (38 %) in males in the 200 mg/kg bw/day dose group was also reported. No compound related morphological changes were observed during pathological examination of parental animals. In males there was a slight (less than 5 %) increase in mean relative liver weight. Based on respiratory distress in exposed females and effects on several blood parameters in males, the NOAEL in parental animals is considered to be 60 mg/kg bw/day. Admittedly, the severity of the systemic toxicity observed is questionable.

However this is not confirmed by actual observations of irritation as histopathological examinations did not reveal signs of irritation of the respiratory tract. In the available report of the original study (unofficial translation) it is stated that *"Irritation of the oral cavity or the trachea caused by oral administration of the tested substance might be involved in the abnormal respiratory sounds observed in 200 mg/kg dose group in the present study."* In fact, another study showed abnormal respiratory sounds in rats caused by chemical substance that is irritating, and it is recognised that *"However, pathological examination was not able to support this"*. In this study, animals were dosed by "oral gavage" suspended at 4% concentration in a 5% methylcellulose suspension in water.

Oral gavage is an exposure way that is not expected to occur in humans and how the "physical" manipulation of the procedure has been contributing to the respiratory problems observed is unclear. The substance is suspended at 4% concentration in a 5% methylcellulose solution in water and administered into the stomach by gavage and so probably using a tube-needle from the mouth to the stomach. The concentrated preparation and also the needle may hence be in direct contact with the upper respiratory tract. In fact, one female animal died and this was considered to be due to "an administration mistake" in which "gross necropsy showed sub involution and change in colour (red or black) in the lungs" and "histopathological examinations revealed congestion in lungs".

Another available study for oral repeated exposure is the following:
Two generation reproduction study, OECD Test Guideline 416, US EPA Guideline OPPTS 870.3800 (Clubb and Jardine, 2006). Sprague Dawley rats (F0: 28/sex/group, F1: 24 sex/group, oral exposure in the diet)

In the *two generation reproduction study*, p-tert-butylphenol was administered orally, by a mixture with the diet at concentration of 0, 800, 2500 and 7500 ppm. The diet contained a constant concentration of test item and was available continuously to the animals. The average doses estimated from the food consumption were 70, 200, 600 mg/kg bw/day. In some periods, at initiation of the F0 and F1 generations, in the group of high dose, the actual intakes were higher than 700 and 1300 mg/kg/day for males and females, respectively. p-tert-butylphenol intakes over 1300 and 1700 mg/kg/day were observed during the second and third weeks of lactation for F0 and F1 females, respectively. No observations of respiratory noise and respiratory irritation were indicated.

*Human data*
No human data are available on respiratory effect of p-tert-butylphenol

Based on the noisy respiratory sound observed in the combined repeated dose toxicity study (OECD 422) and the respiratory effects observed in the rat acute inhalation study (limit test), p-tert-butylphenol was proposed to be regarded as severely irritating to the respiratory system and classification according to CLP criteria with STOT SE 3 - H335 was proposed by the dossier submitter. This was also *agreed at TC C&L in March 2006 (Xi; R37 according to DSD).*

However there are arguments for no classification as follows:

The data of the respiratory noise observed in the combined oral repeated dose toxicity study have uncertainties and the value of it for classification is questioned by RAC. The study was done by oral gavage which is a non-expected way of exposure in humans and this might have caused dust to enter the respiratory tract. Moreover the physical manipulation of the daily intubation may cause additional physical/mechanical effects. In fact, one female animal died and this was considered to be due to "an administration mistake" in which "gross necropsy showed sub involution and change in colour (red or black) in the lungs". The effect is not confirmed by the histopathological examination and it is not confirmed in the two generation study at higher doses by oral intake in the food.

In the limit test with inhalation exposure (described in Klonne *et al.*, 1988), animals were exposed to dust aerosol ("fine powder"). It can not be excluded that the irritating effect could be an unspecific consequence of the inhalation of particles rather than a specific effect of the test substance, but after evaluating the data and recognizing also the irritating effects on skin and eyes, it is considered by RAC that the respiratory effects are most likely caused by the substance itself. The dose at which the effects were seen is however very high (5600 mg/m3). For STOT SE 3, there is no cut-off value for classification indicated neither in the CLP Regulation, nor in the CLP guidance since the classification is primarily based on human data. It can however be compared with the cut-off values used for classification as STOT SE 2 for inhalation by dust aerosols of 5 mg/l/4h (correlating to 5000 mg/m3). The dose at which effects were seen is hence higher than this cut-off value.

Taken together, there are therefore some doubts whether the data available provide enough evidence for classification for respiratory tract irritation under the CLP Regulation.

In the criteria for STOT SE 3 – H335 in the CLP Regulation it is stated that the classification is mainly based **on human data**, but it is also stated that animal data may be used as supportive in a weight of evidence evaluation. There are no human data for supporting the classification. However RAC considers that from a scientific point of view the lack of human data is secondary for the justification for no classification.

The animal data in the repeated dose study do not provide clear evidences for supporting the classification for this hazard class due to the uncertainty in the way of dosage by gavage and histopathology data not supporting it. The data of acute respiratory studies showed some respiratory effect when exposed to high concentration of dust aerosol but the concentration is considered too high to be relevant. No effects are indicated in two studies in saturated atmosphere of p-tert-butylphenol. Therefore, although general irritating properties of p-tert-butylphenol were demonstrated (see skin irritation and eye damage) the experimental data in repeated dose toxicity and respiratory exposure does not support classification for specific target organ toxicity.

Comparison with criteria

| **Criteria for Category 3 for respiratory tract irritation** | **Data fulfilling the criteria** |
|---|---|
| (a) respiratory irritant effects (characterised by localised redness, oedema, pruritis and/or pain) that | No human data is available. Respiratory noise observed in |

| | |
|---|---|
| impair function with symptoms such as cough, pain, choking, and breathing difficulties are included. **This evaluation will be based primarily on human data**; | the Combined repeated dose study by oral gavages. There are doubts about how careful the oral intubation was done as one animal died due to a mistake in the intubation. Effects in mucosa were not confirmed by the histopathological examination and it is not confirmed in the two generation study at higher doses by oral intake in the food. |
| (b) **subjective human observations** could be supported by objective measurements of clear respiratory tract irritation (RTI) (such as electrophysiological responses, biomarkers of inflammation in nasal or broncho alveolar lavage fluids); | No human data is available. |
| (c) **the symptoms observed in humans** shall also be typical of those that would be produced in the exposed population rather than being an isolated idiosyncratic reaction or response triggered only in individuals with hypersensitive airways. **Ambiguous reports simply of 'irritation' shall be excluded** as this term is commonly used to describe a wide range of sensations including those such as smell, unpleasant taste, a tickling sensation, and dryness, which are outside the scope of classification for respiratory irritation;<br>d) there are currently no validated animal tests that deal specifically with RTI, however, useful information may be **obtained from the single and repeated inhalation toxicity tests.** For example, animal studies may<br>provide useful information in terms of clinical signs of toxicity (dyspnoea, rhinitis etc) and histopathology (e.g. hyperemia, edema, minimal inflammation, thickened mucous layer) which are reversible and may be reflective of the characteristic clinical symptoms described above. **Such animal studies can be used as part of weight of evidence evaluation**;<br>(e) this special classification would occur only when more severe organ effects including in the respiratory system are not observed. | No human data is available.<br><br>No specific unequivocally irritation data are described in repeated dose toxicity study. Noisy respiration is indicated. Dosing by gavage might have produced physico-mechanical disturbances. Histopathology examination is not supporting specific tract irritation.<br><br>No repeated inhalation study available.<br><br>A limit test, exposure via inhalation at a dose of 5600 mg/m3 and a vapour component showed signs of mucosal irritation and respiratory distress. The concentration is however considered high, (higher than for example 5000 mg/m3 used as cut off for STOT SE 2). |

**Based on the available data, RAC proposed no classification for STOT RE.**

**Additional information**

The Background Document, attached as Annex 1, gives the detailed scientific grounds for the Opinion.

**ANNEXES:**

Annex 1 Background Document (BD)[1]
Annex 2 Comments received on the CLH report, response to comments provided by the dossier submitter and RAC comments (excl. confidential information)

[1] The Background Document (BD) gives detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by a dossier submitter.