National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

di-tert-butyl peroxide (dTBP)
EC No 203-733-6
CAS No 110-05-4

Evaluating Member State(s): The Netherlands

Dated: 1 June 2020

Evaluating Member State Competent Authority

Bureau REACH on behalf of the Ministry of Infrastructure and the National Institute for Public Health and the Environment
P.O.Box 1
3720 BA Bilthoven
The Netherlands

Year of evaluation in CoRAP: 2016

Member State concluded the evaluation without any further need to ask more information from the registrants under Article 46(1) decision.

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

Di-tert-butyl peroxide (dTBP) was originally selected for substance evaluation in order to clarify concerns about:

- CM(R)
- Consumer use
- High (aggregated) tonnage
- Wide dispersive use

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

One testing proposal on human health hazard was submitted by the registrants. ECHA's decision on the test proposal was agreed in 2012 and contained (for human health) an inhalation sub-chronic toxicity test (90-day) and a pre-natal developmental toxicity test. The testing with regard to these studies is finished and the new studies are included in the registration dossier.

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in the table below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION Conclusions	Tick box
Need for follow-up regulatory action at EU level	
Harmonised Classification and Labelling	
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	X

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

Not applicable.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

Table 2

REASON FOR REMOVED CONCERN The concern could be removed because	Tick box
Clarification of hazard properties/exposure	X
Actions by the registrants to ensure safety, as reflected in the registration dossiers (e.g. change in supported uses, applied risk management measures, etc.)	

The substance has a harmonised classification as Muta 2. However, new data were generated after the RAC opinion and the available mutagenicity summaries lacked details and were not conclusive for a decision on germ cell mutagenicity. Details on the studies were provided during the SEv process. In addition, new data became available for two related substances, including a comet assay study via inhalation performed with tert-butyl hydroperoxide (TBHP) and an in vitro micronucleus (MN) test with di-tert-pentyl peroxide (dTPP), which both gave negative results. Also an *in vitro* study was performed to evaluate the reactive oxygen species (ROS) production of dTBP. The results confirmed the hypothesis that dTBP induced mutagenicity is related to ROS production, has a threshold mode of action and occurs only at high concentrations. The provided details of the available studies and the new data demonstrated that there is no concern for germ cell mutagenicity and that the substance is not considered Muta 1B.

The substance is classified as Muta 2, but no carcinogenicity data are available for the substance, which led to a concern on the carcinogenic potential of the substance. Assessment of the data showed that the substance is not irritating via dermal or inhalation exposure. The sub-chronic repeated dose toxicity study via inhalation shows no signs of pre-carcinogenic effects or local effects in the respiratory system. Available dermal tumor promotion studies are negative. Recently, a carcinogenicity study with TBHP became available as a result of a Compliance Check. The study demonstrated that carcinogenic effects are induced by TBHP, however, these effects were most likely initiated by irritation/corrosive injury to nasal tissues. These local effects were not induced by dTBP and therefore these carcinogenic effects are not expected for dTBP. It is concluded that there is no concern for carcionogenicity based on the current data and that no new data are needed.

For consumer uses, several process categories and product categories were described but no exposure scenarios or estimates were provided, which raised concerns. During the process, the dossier was updated and consumer uses and professional uses were removed. Therefore, the concern on consumer exposure is removed.

5.2. Other actions

Not applicable.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Not applicable.

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

Di-tert-butyl peroxide (dTBP) was originally selected for substance evaluation in 2016 in order to clarify concerns about:

- CM(R)
- Consumer use
- High (aggregated) tonnage
- Wide dispersive use

The substance has a harmonised classification as Muta 2. However, new data were generated after the RAC opinion and the available mutagenicity summaries lacked details and were not conclusive for a decision on germ cell mutagenicity.

The substance is classified as Muta 2, but no carcinogenicity data are available for the substance, which led to a concern on the carcinogenic potential of the substance.

For consumer uses, several process categories and product categories were described but no exposure scenarios or estimates were provided, which raised concerns.

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Germ cell mutagenicity	Concern not substantiated. No further action. dTBP has the potential to induce micronuclei systemically at high doses after intraperitoneal and oral administration. No such aberrations were detected in the bone marrow after inhalation. No local mutagenicity is expected based on the lack of local effects and the negative Comet assay for TBHP. Overall, mutagenicity including germ cell mutagenicity is only expected above a threshold and at high concentrations. The data do not provide justification to request further information related to germ cell mutagenicity. Based on the available mutagenicity information and the negative germ cell test, classification as Muta 1B is considered not appropriate.
Carcinogenicity	Concern not substantiated. No further action. There is no concern for carcinogenic potential of dTBP via the inhalation route. DTBP is most likely genotoxic and possibly carcinogenicity above a threshold and only at high concentrations which are unlikely to occur. The data do not provide any justification to request further information relating to carcinogenicity.
Consumer exposure	Concern not substantiated. No further action. During the process, the dossier was updated and consumer uses and professional uses were removed.
Wide dispersive use	Concern not substantiated. No further action.

7.2. Procedure

dTBP was included in the Community Rolling Action Plan (CoRAP) for substance evaluation in 2016 by the competent authority of the Netherlands. The scope of the evaluation was

human health, targeted to concerns for mutagenicity and carcinogenicity, consumer use, high tonnage and wide dispersive use. Other human health hazard endpoints (e.g. repeated dose toxicity, irritation) were only evaluated in relation to the primary concerns and were therefore not fully assessed. Environmental fate properties and environmental hazard were not assessed.

The evaluation was based on the updated registration dossier from [30-11-2016]. In addition, informal interaction with the Registrant via e-mail and a meeting (29 June 2016) took place during the evaluation period. During the process, NL was also requested by the Registrant to review a position paper and to submit a new CLH dossier for removing the harmonized Muta 2 classification. As explained in section 7.9.5, NL still believe that this classification is still applicable.

The evaluating Member State considered that further information was required to clarify the human health concern. Therefore, it prepared a draft decision pursuant to Article 46(1) of the REACH Regulation to request further information. It submitted the draft decision to ECHA on 23 March 2017. The registrant provided comments on the Draft Decision.

During the process, new data for dTBP and for related substances were generated and were used for the evaluation of dTBP. Based on all available data, it was decided that no further information was required anymore to clarify the concerns.

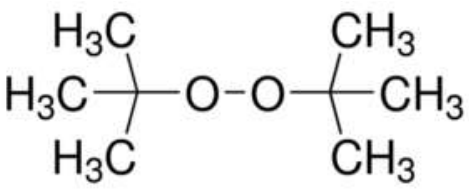
7.3. Identity of the substance

Table 4

SUBSTANCE IDENTITY	
Public name:	Di-tert-butyl peroxide
EC number:	203-733-6
CAS number:	110-05-4
Index number in Annex VI of the CLP Regulation:	617-001-00-2 CLP00
Molecular formula:	$C_8H_{18}O_2$
Molecular weight range:	146.2
Synonyms:	2,2'-dioxybis(2-methylpropane) (IUPAC) DTBP Trigonox B

Type of substance ☒ Mono-constituent ☐ Multi-constituent ☐ UVCB

Structural formula:



Multiconstituent/UVCB substance/others

Two structural analogues of dTBP were used during the evaluation of dTBP. Information on these analogues is included in Table 5 and 6.

Table 5. Structural analogue tert-butyl hydroperoxide (TBHP)

Substance identity	
EC number:	200-915-7
EC name:	tert-butyl hydroperoxide
CAS number:	75-91-2
CAS name:	tert-butyl hydroperoxide
IUPAC name:	hydroperoxide, 1,1-dimethylethyl
Molecular formula:	$C_4H_{10}O_2$
Molecular weight range:	90.1

Structural formula:

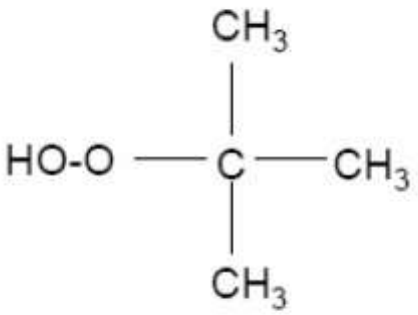
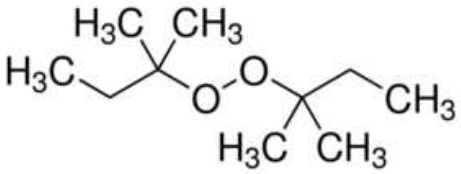


Table 6. Structural analogue di-tert-pentyl peroxide

Substance identity	
EC number:	234-042-8
EC name:	Di-tert-pentyl peroxide Di-tert-amyl peroxide
CAS number:	10508-09-5
CAS name:	Peroxide, bis(1,1-dimethylpropyl)
IUPAC name:	2-methyl-2-[(2-methylbutan-2-yl)peroxy]butane
Molecular formula:	C10H22O2
Molecular weight range:	174.2805

Structural formula:



7.4. Physico-chemical properties

Table 7

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Clear colourless liquid
Vapour pressure	3500 Pa at 20°C Calculated vapour pressure is 9650 Pa at 40°C
Water solubility	171 mg/l at 20°C and pH 8.1, purity 99.0%
Partition coefficient n-octanol/water (Log Kow)	3.2 at 22°C and pH 7.2 (shake flask method)
Flammability	No test available, but labelled as H225: Highly flammable liquid and vapour.
Explosive properties	Non explosive. No test available, but labelled as H225: Highly flammable liquid and vapour and H242: Heating may cause a fire.
Oxidising properties	Non oxidising Study technically not feasible. No test available, but the substance is an peroxide with a harmonised classification as Org. Perox. Type E.
Granulometry	Not applicable, the substance is a liquid
Stability in organic solvents and identity of relevant degradation products	Study technically not feasible
Dissociation constant	Study technically not feasible
Flash point	6°C at ca. 1 013 mBar

7.5. Manufacture and uses

7.5.1. Quantities

Table 8

AGGREGATED TONNAGE (PER YEAR)

☐ 1 – 10 t	☐ 10 – 100 t	☐ 100 – 1000 t	☒ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

7.5.2. Overview of uses

DTBP is used in the production of polyolefins, for crosslinking/grafting polyethylene, for crosslinking rubber (ethylene propylene diene monomer (EPDM), ethylene-vinyl acetate (EVA)), for polypropylene degradation, and for emulsion polymerization. It may also be used in acrylic resin manufacturing (OECD 2012a).

Table 9

USES	**Use(s)**
Uses as intermediate	
Manufacture	Manufacture of the substance
Formulation	- Formulation and (re)packing of organic peroxides and mixtures and distribution - Formulation of organic peroxides in materials - Formulation of preparations (generic)
Uses at industrial sites	- Industrial open process spray use of organic peroxides as polymerization initiators, crosslinking agents or curing agents - Industrial open process non-spray use of organic peroxides as polymerization initiators, crosslinking agents or curing agents - Other industrial uses of organic peroxides (eg. AISE, COLIPA) - Industrial open process use of organic peroxides as polymerization initiators, crosslinking agents or curing agents - Industrial closed process use of organic peroxides as polymerization initiators, crosslinking agents or curing agents - Other Industrial open process uses of organic peroxides - Industrial closed process non-spray use of organic peroxides as polymerization initiators, crosslinking agents or curing agents - industrial use in polymerisation as initiator - Industrial open process non-spray use of organic peroxides as polymerization initiators, crosslinking agents, curing agents, as radical source for organic synthesis - Industrial closed process spray use of organic peroxides as polymerization initiators, crosslinking agents or curing agents - Industrial closed process non-spray use of organic peroxides as polymerization initiators, crosslinking agents or curing agents - Industrial open process spray use of organic peroxides as polymerization initiators, crosslinking agents or curing agents

	- Industrial closed process spray use of organic peroxides as polymerization initiators, crosslinking agents or curing agents - Industrial use of organic peroxides as polymerization initiators, crosslinking agents or curing agents (eg. wdk, SRM, PEST, EPDLA, PPRM, ETRMA, UPR, CEPE, FEICA, EFCC) - Other Industrial closed process uses of organic peroxides - Other Industrial process non-spray uses of organic peroxides
Uses by professional workers	-
Consumer Uses	-
Article service life	-

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

Table 10 (ATP Inserted / Updated: CLP00/ATP03)

HARMONISED CLASSIFICATION ACCORDING TO ANNEX VI OF CLP REGULATION (REGULATION (EC) 1272/2008)							
Index No	**International Chemical Identification**	**EC No**	**CAS No**	**Classification**		**Spec. Conc. Limits, M-factors**	**Notes**
				Hazard Class and Category Code(s)	**Hazard statement code(s)**		
617-001-00-2	di-tert-butyl peroxide	203-733-6	110-05-4	Flam. Liq. 2 Org. Perox. E Muta. 2	H225 H242 H341		

7.6.2. Self-classification

In the registration(s)*:*

H412: Aquatic Chronic 3 (Harmful to aquatic life with long lasting effects.)

The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory*:*

H302: Acute Tox. 4 (Harmful if swallowed)
H312: Acute Tox. 4 (Harmful in contact with skin)
H332: Acute Tox 4 (Harmful if inhaled)
H319: Eye Irrit. 2 (Causes serious eye irritation)
H412: Aquatic Chronic 3 (Harmful to aquatic life with long lasting effects.)

7.7. Environmental fate properties

Not evaluated.

7.8. Environmental hazard assessment

Not evaluated.

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

No toxicokinetic studies are available on dTBP in the registration dossier. Therefore, for this endpoint, relevant data were collected from other sources.

Based on physicochemical properties (e.g. water solubility and octanol-water partition coefficient), absorption would be expected by the dermal route. Clinical signs suggested that absorption of dTBP occurred in an acute inhalation study and in a 90-day repeated dose toxicity study. This is supported by other acute toxicity data. Based on the log Kow, dTBP can be regarded as lipophilic and may be taken up by micellular solubilisation by the oral route of exposure (OECD, 2012a). Absorption is confirmed by the systemic effects observed in an oral screening study for reproduction toxicity (OECD 422), including effects on body weight, food consumption, signs of discomfort, histopathological effects in liver, kidneys and forestomach at 1000 mg/kg/day (Unnamed study report, 2008).

In vivo, glutathione peroxidases are expected to catalyze the reduction of organic peroxides to the corresponding stable alcohols and water using cellular glutathione as the reducing agent (OECD, 2012a). The metabolism of dTBP produces *t*-butyl alcohol (CAS No. 75-65-0).

In general, hydroperoxides are known to be reductively metabolized. Metabolism of TBHP (TBHP, EC number: 200-915-7, CAS number: 75-91-2), which is a structural analogue of dTBP being a dimer of TBHP, has been studied in detail. The main detoxification pathway is a two-electron reduction by glutathione peroxidase using glutathione to the corresponding alcohol (for TBHP this is t-butyl alcohol, the same as is produced by dTBP). When these reducing equivalents have been depleted (i.e. at high concentrations of TBHP), TBHP undergoes a one-electron oxidation generating the peroxyl radical (t-BuOO·) or a one-electron reduction generating the tert-butoxyl radical (t-BuO·), the latter being the major process. Subsequent fragmentation of the tert-butoxyl radical results in the formation of carbon-centred radicals (CH_3). The generation of such radicals has been demonstrated in several in vitro systems, such as human endothelial cells, intact skin samples of the mouse, rat liver microsomes, and isolated rat liver nuclei. Formation of free radicals is a proposed mechanism of mutagenicity of TBHP (ECHA, 2014).
DTBP is expected to have the same metabolic route as TBHP. It is assumed that the mutagenicity of dTBP is caused via the formation of radicals. Radical formation from dTBP can occur by splitting the electronic bond between the two oxygen atoms or by splitting the bond between one oxygen atom and the carbon atom (ECHA, 2014).

Summary and evaluation:
DTBP is assumed to be metabolised by glutathione peroxidase, producing t-butyl alcohol as metabolite at low doses. Even though there is no toxicokinetics data for dTBP itself, there are reliable studies with the close structural analogue TBHP. Based on chemical similarity, it can be assumed that dTBP is similarly detoxified by glutathione peroxidases forming the same alcohol. The available kinetic information on dTBP and TBHP indicate that the formation of free radicals is limited to concentrations exceeding the detoxifying capacity and indicates the presence of a threshold.

7.9.2. Acute toxicity and Corrosion/Irritation

These endpoints were not evaluated, however, the results were used as supportive information for reaching conclusions on mutagenicity and carcinogenicity. DTBP has a low acute toxicity (oral LD50 greater than 2000 mg/kg body weight, acute inhalation LC50 (4 hr) greater than 22 mg/l (22000 mg/m^3)) and is not a skin or eye irritant. It may be concluded, that dTBP has a low potency for acute toxicity and reactivity.

7.9.3. Sensitisation

This endpoint was not evaluated, however, the results were used as supportive information for reaching conclusions on mutagenicity and carcinogenicity. DTBP is not a skin sensitizer.

7.9.4. Repeated dose toxicity

This endpoint was not evaluated, however, the studies relevant for reaching conclusions on mutagenicity and carcinogenicity are described here. The summary represents the evaluation as reported in the registration dossier.

Sub-chronic Inhalation Toxicity: 90-Day (OECD Guideline 413)

The toxicity of dTBP upon repeated exposure by inhalation was studied in a sub-chronic (90-day) study with Wistar Hannover rats, according to OECD Guideline 413. The target concentrations for this study of 100, 300 and 1000 mg/m^3 in vapour form (as low-, mid- and high-concentration, respectively) were selected based on a range-finding study. In the range-finding study (2 weeks, 5 days/week and 6 hours/day) at dose levels up to 10,000 mg/m^3, only limited effects on kidney and liver weight and signs of discomfort were observed but no histopathological changes of the respiratory tract.

The sub-chronic (main) study included four groups of 10 rats/sex. The animals were exposed nose-only, 6 hours/day, 5 days/week for 13 consecutive weeks (resulting in 65 exposure days in total) to the above target concentrations or to clean air for the control group.

The measured concentrations were 101 (± 3), 299 (± 3) and 993 (± 10) mg/m^3 for the low-, mid- and high concentration level respectively.

All animals survived until scheduled sacrifice. Clinical and ophthalmoscopic observations, growth and food consumption results, haematology values, most clinical chemistry values, most organ weights, and necropsy and histopathology findings showed no treatment-related changes. No microscopic changes were seen in the respiratory tract.

Clinical chemistry values showed slight but statistically significant changes in the plasma levels of cholesterol in males (increased) and creatinine in females (decreased) at the high concentration. These findings are considered to be of limited toxicological significance.

The relative weights of the liver and kidneys were slightly (about 10%) but statistically significantly increased in male rats of the high-concentration group. In female rats of this group relative liver weight was increased to about the same extent but the difference from controls was not statistically significant. Though these organ weight changes were related to treatment, they were considered not to represent adverse effects of the test material because of the modest magnitude of the increases and the absence of corroborative histopathological alterations or clinical chemical indicators of organ damage. There were no hyperplasia or any neoplastic findings observed.

Under the conditions of this study exposure to dTBP resulted in a few modest changes at the highest concentration tested (increases in liver and kidney weight and altered plasma levels of cholesterol and creatinine). No treatment-related changes were observed at the lower concentrations (Unnamed study report, 2013).

Combined repeated dose toxicity study with the reproduction / developmental toxicity screening test (OECD Guideline 422)

The effects of repeated exposure to dTBP and potential effects on male and female reproductive performance were investigated in a repeated dose toxicity study combined with screening for reproduction and developmental toxicity (according to OECD 422). Wistar rats were treated with 0, 100, 300, 1000 mg/kg bw day by oral gavage throughout the pre-pairing, the pairing, the gestation and the lactation periods until day 4 post partum (last dosing). Mean body weight and food consumption were decreased at 1000 mg/kg bw day in males, but not affected in females. Discomfort was shown in males and females of the high dose group by movement of their heads through the bedding material after the daily administration of test item and ruffled fur of some animals. DTBP caused adverse effects in the liver in males and females and the kidney and forestomach in males at 1000 mg/kg bw/day. Liver weights were increased and showed minimal centrilobular and diffuse hepatocellular hypertrophy with association of a consequent increase in diffuse follicular cell hypertrophy in thyroid glands. Kidney weights were increased in males and moderate diffuse tubular degeneration/regeneration with slight multifocal single cell necrosis and hyaline casts as well as hyaline droplets was observed. Minimally increased incidence and severity of diffuse hyperkeratosis was seen in the forestomach. No effects were noted on reproduction data, for the parameters during the clinical laboratory investigations, or for macroscopic findings during necropsy.

Notes by evaluating MSCA:

DTBP did cause limited toxicologically relevant effects after 90 days of exposure via inhalation at dose levels up to 1000 mg/m^3. Slight changes in liver and kidney weight and plasma levels of cholesterol and creatinine indicate that dTBP or its metabolites have reached the systemic circulation. No local effects (signs of irritation or hyperplasia) were observed on the upper (larynx and nasal tissue) and lower (lung) respiratory tract, which have undergone specific investigation. However, based on the very limited effects at the highest tested concentration and the limited effects in the range finding study at a 10-fold higher concentration it is questioned whether this study has been performed at sufficiently high concentrations.

No local carcinogenicity of the respiratory tract, caused by damage of the tissues and higher rate of cell division is expected at dose levels up to 10000 mg/m^3, taking into account the range-finding study.

7.9.5. Mutagenicity

In vitro mutagenicity

Three reliable in vitro tests were available for the evaluation, i.e. two Ames tests (OECD Guideline 471) and an in vitro mammalian cell gene mutation test (OECD Guideline 476). It is noted that due to the high volatility of the test substance, some may have evaporated during the experiment. No information is available on measures taken to prevent evaporation in any of the studies described below.

The first Ames test was performed with test concentrations 0, 10, 33, 100, 333, 666, 1000, 3333, 6666 and 10000 µg dTBP /plate. The tested strains of Salmonella typhimurium were TA1535, TA100, TA97, TA1537 and TA98. DTBP did not increase the rate of reverse mutation and was not mutagenic under the conditions of this assay (Zeiger et al., 1988).

In the second Ames test the test item dTBP was assessed for its potential to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, and TA 102, with and without S9 at the following concentrations. Experiment I was performed with 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate; experiment II with

33, 100, 333, 1000, 2500, and 5000 µg/plate. No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation. Only in experiment II a minor reduction in the number of revertants (below the indication factor of 0.5) was observed in strain TA 98 at 5000 µg/plate in the absence of metabolic activation (Unnamed study report, 2010a). DTBP did not increase the rate of reverse mutation and was not mutagenic under the conditions of this assay.

The in vitro mammalian cell gene mutation test was performed with dTBP in two independent experiments, using two parallel cultures each. The first main experiment was performed with and without liver microsomal activation and a treatment period of 4h. The second experiment was performed with a treatment period of 4h with and 24h without metabolic activation. The maximum tested concentration was equal to about 10 mM. Both main experiments were evaluated at the following concentrations:

- without S9 mix: 92.5, 185, 370, 740 and 1480 µg/mL
- with S9 mix: 92.5, 185, 370, 740, and 1480 µg/mL

No relevant toxic effects indicated by a relative total growth of less than 50% of survival in both parallel cultures were observed up to the maximum concentration with and without metabolic activation, following 4 and 24 hours of treatment. No substantial and reproducible dose dependent increase of the mutation frequency was observed in both experiments. The threshold of 126 colonies per 10^6 cells above the corresponding solvent control was not exceeded in any of the experimental parts. It was concluded that dTBP did not increase of the relative quantity of small versus large induced colonies (Unnamed study report, 2010b).

No chromosomal damage in vitro test test is available for dTBP. A chromosomal aberration in human lymphocytes cultures and a micronucleus in TK6 lymphoblastoid human cells performed with dTPP according to OECD TG 473 and 487, respectively, had negative results.

Summary and evaluation

Two negative Ames test (OECD Guideline 471) and one mouse lymphoma L5178Y (OECD Guideline 476) performed with dTBP were available. Based on these study results, it is concluded that dTBP does not induce gene mutations in vitro.

Genetic toxicity in vivo

Mammalian Erythrocyte Micronucleus Test, inhalation route (OECD Guideline 474)

This micronucleus test was part of a sub-chronic (13-week) inhalation toxicity study in which Wistar Hannover rats were exposed nose-only to target concentrations of 0 (control, clean air), 100, 300 and 1000 mg/m^3 of dTBP vapour for 6 hours/day, 5 days/week for 13 consecutive weeks (resulting in 65 exposure days in total).

The micronucleus test was conducted in accordance with the OECD Guideline 474. At scheduled necropsy at the end of the 13-week study period, bone marrow cells of one of the femurs per rat of five male rats per group (negative control, low, mid and high concentration) were collected, processed into smears and examined microscopically. The study included a positive control group of five male rats treated with the mutagen Mitomycin C (single intraperitoneal injection; 1.5 mg/kg body weight) and sacrificed 24 hours after administration of the mutagen.

The target concentrations were accurately achieved as demonstrated by the results of total carbon analysis of the test atmospheres. The overall mean actual concentrations (± standard deviation of the daily mean concentration) were 101 (± 3), 299 (± 3) and 993 (± 10) mg/m^3 for the low-, mid- and high concentration level respectively.

The mean number of MPE/2000 PE in the negative control (group 1) was within the historical range. The mean number of MPE/2000 PE in the positive control group treated with Mitomycin C (group 5) was within the historical positive control range and statistically significantly increased (p value: 0.0097) compared to the concurrent negative control (group 1). This indicates that the positive control substance Mitomycin C reached the bone marrow and induced damage to the chromosomes and/or to the spindle apparatus of the bone marrow cells under the conditions of this study. These results, together with the normal MPE/PE ratio in the negative control group, demonstrate the validity of the test system.

The mean numbers of MPE/2000 PE in the groups exposed to the test material (groups 2-4) did not differ statistically significantly from the mean MPE/2000 PE in the negative control group (group 1). This indicates that treatment with the test material under the conditions of this study did not result in damage to the chromosomes and/or to the spindle apparatus of the bone marrow cells.

DTBP did not adversely affect the general health, appearance or body weight development of the animals. Microscopic examination of bone marrow smears of male rats revealed no signs of toxicity to the bone marrow and no evidence of chromosomal damage and/or damage to the mitotic apparatus of bone marrow erythrocytes. There was no reason to assume that the negative bone marrow response was due to lack of systemic exposure because treatment-related systemic effects (including increases in liver and kidney weight) occurred in male rats of the high-concentration group.

Under the conditions of this study, exposure to dTBP up to 1000 mg/m^3 did not induce chromosomal damage or damage to the mitotic apparatus of bone marrow erythrocytes of male rats. However, based on the very limited effects at the highest tested concentration and the limited effects in the range-finding study, testing at higher concentrations would have been possible. Further, this dose is provided over a 6 hour period per day likely resulting in a lower peak level compared to intraperitoneal and oral gavage exposure (Unnamed study report, 2014).

Mammalian Erythrocyte Micronucleus Test, intraperitoneal route (OECD Guideline 474)

Three groups of five male and five female Swiss mice were given intraperitoneal administrations of di-tert-butyl peroxide at dose-levels of 500, 1000 and 2000 mg/kg/day, over a 2-day period. One group of five males and five females received the vehicle (corn oil) under the same experimental conditions, and acted as control group. One group of five males and five females received the positive control test item (cyclophosphamide) once by oral route at the dose-level of 50 mg/kg. The animals of the treated and vehicle control groups were killed 24 hours after the last treatment and the animals of the positive control group were killed 24 hours after the single treatment. Bone marrow smears were then prepared. For each animal, the number of the micronucleated polychromatic erythrocytes (MPE) was counted in 2000 polychromatic erythrocytes. The polychromatic (PE) and normochromatic (NE) erythrocyte ratio was established by scoring a total of 1000 erythrocytes (PE + NE).

No clinical signs and no mortality were observed in the animals of both sexes given 500 mg/kg/day. At the dose-levels of 1000 and 2000 mg/kg/day, no mortality was noted. Piloerection was observed in the animals from 24 hours following the first treatment. Statistically significant and dose-related increases in the frequency of MPE were observed in male and female mice of the test item treated groups (Table 11). Historical control data are provided in Table 12. Cyclophosphamide induced a highly significant increase ($p < 0.01$) in the frequency of MPE, indicating the sensitivity of the test system under these experimental conditions. The study was therefore considered valid.

Under these experimental conditions, the test item di-tert-butyl peroxide induced damage to the chromosomes or the mitotic apparatus of mice bone marrow cells after two intraperitoneal administrations, at a 24-hour interval, at the dose-levels of 500, 1000 and 2000 mg/kg/day. This positive result at the high doses is mostly likely explained by the

saturation of the detoxification mechanism (by glutathione peroxidases resulting in the formation of corresponding alcohol), and resulting in radical formation (Unnamed study report, 2005a; ECHA, 2010).

Table 11

SUMMARY OF STUDY RESULTS – *IN VIVO* I.P. MICRONUCLEUS ASSAY							
Group	Doses (mg/kg/d)	MPE/1000PE mean	(sd)		PE/NE ratio mean	(sd)	Time of sacrifice
Males							
Vehicle	-	0.5	(1.1)		0.3	(0.1)	24h
Test item	500	1.9	(0.9)		0.5	(0.1)	
	1000	2.9	(1.5)	*	0.4	(0.2)	
	2000	5.4	(3.2)	**	0.4	(0.1)	
CPA	50	26.3	(8.1)	**	0.5	(0.1)	
Females							
Vehicle	-	1.4	(1.6)		0.6	(0.1)	24h
Test item	500	4.2	(1.3)	*	0.8	(0.3)	
	1000	4.9	(3.3)		0.9	(0.2)	
	2000	6.0	(2.7)	*	0.9	(0.2)	
CPA	50	18.0	(7.5)	**	0.8	(0.2)	

*: $p<0.05$; **: $p<0.01$ comparing the treated group with its concurrent vehicle control group based on a chi-square test value when homogeneity or based on a Mann-Whitney test if heterogeneity. Homogeneity within groups was tested based on the heterogeneity chi-square test value.

Table 12

HISTORICAL CONTROL DATA FROM 8 MICRONUCLEUS STUDIES ON BONE MARROW IN MOUSE PERFORMED BETWEEN MARCH 2002 AND JULY 2004				
Group	MPE/1000PE		PE/NE ratio	
	mean	range	mean	range
Males				
Vehicle	0.66	0.3-1.7	0.44	0.3-0.6
CPA	23.9	17.0-33.4	0.61	0.4-0.8
Females				
Vehicle	0.59	0.0-1.1	0.64	0.4-1.0
CPA	19.24	12.7-25.6	0.8	0.6-1.1

Mammalian Erythrocyte Micronucleus Test, oral route (OECD Guideline 474)

In the initial micronucleus assay, male and female mice were dosed with 1250, 2500 or 5000 mg/kg body weight of di-tert-butyl peroxide via oral gavage. No mortality occurred in either sex in the initial study. Clinical signs observed in males and females after dose administration included diarrhoea at 2500 and 5000 mg/kg. Bone marrow cells, collected 24 hours after treatment, were examined microscopically for micronucleated polychromatic erythrocytes. No apparent reductions in the ratio of polychromatic erythrocytes to total erythrocytes were observed in the test article-treated groups relative to the vehicle control groups. Statistically significant increases in micronucleated polychromatic erythrocytes relative to the respective vehicle control group were observed in female mice at 1250 and 5000 mg/kg at 24 h only ($p \leq 0.05$ based on Kastenbaum-Bowman tables), with no evidence of a dose response (Table 13).

To confirm the results, the micronucleus study was repeated with a 24 hour harvest only. For the repeat micronucleus assay, male and female mice were dosed with 1250, 2500 or 5000 mg/kg body weight of di-tert-butyl peroxide. In this repeat assay, a statistically significant increase was observed at 2500 and 5000 mg/kg in female mice and at 5000 mg/kg in male mice. The number of micronucleated polychromatic erythrocytes induced in both assays were within the historical solvent control range (0-8 MPE/1000 PE per animal) and within the criteria for the determination of a valid test for negative control (5/1000) with the exception of 1/5 female mice at 1250 and 5000 mg/kg in the initial assay and 1/5 male mice at 5000 mg/kg in the repeat assay (9 MPE/1000 PE). This positive result at the high doses is most likely explained by the saturation of the detoxification mechanism (by glutathione peroxidases resulting in the formation of corresponding alcohol) and radicals formation (Unnamed study report, 1996; ECHA, 2010).

Table 13

SUMMARY OF STUDY RESULTS – *IN VIVO* GAVAGE MICRONUCLEUS ASSAY							
Group	**Doses (mg/kg)**	**MPE/1000PE mean**	**(sd)**		**PE/NE ratio mean**	**(sd)**	**Time of sacrifice**
	Initial study						
Males							
Vehicle	-	1.4	(1.14)		0.54	(0.07)	24h
Test item	1250	1.6	(1.34)		0.55	(0.06)	
	2500	2.6	(1.52)		0.51	(0.13)	
	5000	3.6	(2.07)		0.52	(0.12)	
CPA	60	32.8	(8.58)	*	0.47	(0.10)	
Females							
Vehicle	-	0.6	(0.55)		0.55	(0.03)	24h
Test item	1250	3.6	(3.71)	*	0.56	(0.08)	
	2500	1.8	(2.05)		0.59	(0.04)	
	5000	3.4	(3.36)	*	0.55	(0.06)	
CPA	60	29.6	(7.37)	*	0.52	(0.07)	
Males							
Vehicle	-	1.4	(2.61)		0.55	(0.04)	48h
Test item	1250	1.2	(1.30)		0.55	(0.08)	
	2500	1.4	(0.55)		0.59	(0.05)	
	5000	2.6	(2.30)		0.47	(0.11)	
Females							

Vehicle	-	1.4	(0.89)		0.51	(0.06)	48h
Test item	1250	0.8	(0.45)		0.57	(0.04)	
	2500	1.2	(0.84)		0.58	(0.04)	
	5000	2.8	(2.17)		0.45	(0.17)	
Males							
Vehicle	-	1.0	(1.41)		0.53	(0.11)	72h
Test item	1250	0.2	(0.45)		0.52	(0.08)	
	2500	1.2	(1.10)		0.55	(0.13)	
	5000	1.4	(1.14)		0.51	(0.09)	
Females							
Vehicle	-	0.8	(0.45)		0.59	(0.05)	72h
Test item	1250	0.4	(0.55)		0.57	(0.06)	
	2500	0.8	(0.84)		0.59	(0.04)	
	5000	1.0	(0.71)		0.56	(0.06)	
Repeated study							
Males							
Vehicle	-	0.2	(0.45)		0.53	(0.04)	24h
Test item	1250	1.6	(0.89)		0.55	(0.03)	
	2500	1.4	(1.67)		0.52	(0.04)	
	5000	4.8	(2.49)	*	0.53	(0.07)	
CPA	60	19.4	(5.86)	*	0.45	(0.12)	
Females							
Vehicle	-	0.4	(0.55)		0.53	(0.03)	24h
Test item	1250	1.6	(0.89)		0.55	(0.05)	
	2500	2.4	(0.89)	*	0.52	(0.06)	
	5000	2.6	(1.34)	*	0.58	(0.07)	
CPA	60	14.8	(6.98)	*	0.43	(0.09)	

*: p<0.05 based on Kastenbaum-Bowman tables.

Mammalian Spermatogonial Chromosome Aberration Test (OECD Guideline 483)

The test article, dTBP, was tested in the mammalian spermatogonial chromosome aberration test using male ICR mice. The chromosome aberration assay was designed to evaluate the potential of the test article to induce chromosome aberrations in spermatogonial cells. The chromosome aberration assay consisted of five groups, each containing 5 male ICR mice. Animals in these groups were intraperitoneally exposed to the controls (negative or positive) or to di-tert-butyl peroxide at a dose of 200, 1000 or 2000 mg/kg/day. The test article was formulated in corn oil. Corn oil was used as the negative control (vehicle) and Mitomycin C (MMC), at a dose of 4 mg/kg, as the positive control article.

The test and negative control article were administered on two consecutive days, separated by approximately 24 hours. Each administration was conducted at a dose volume of 20 mL/kg body weight. Animals were observed following each dose administration and during

the course of the study. Colchicine was given 4-5 hours prior to being euthanized to arrest cells in metaphase. Twenty-four hours after the last dose, animals were euthanized and testes were removed from animal body cavity. Spermatogonial cells were isolated from the tubules and smeared onto the microscope slide. The smears were stained with Giemsa stain. One hundred metaphase cells per each animal were scored for structural chromosome aberrations. A statistically significant difference between the test article treated groups relative to the concurrent negative (vehicle) control was determined using Fisher's exact test for level of significance of pS 0.05. Mitotic index (MI) was calculated for each animal as the ratio of spermatogonial cells in mitosis per 1000 cells observed.

No mortality or clinical signs were observed in any of the mice during the course of the study. No statistically significant increase in the percentage of aberrant cells and no dose-related decrease of the mitotic index were observed in the test article-treated groups relative to the vehicle control ($p > 0.05$ Fisher's exact test). The results of the study indicate that under the conditions described in this report, di-tert-butyl peroxide, when intraperitoneally administered on two consecutive days (at doses up to 2000 mg/kg/day), did not induce a significant increase in the percentage of spermatogonial cells with structural chromosome aberrations. Therefore, di-tert-butyl peroxide was concluded to be negative in the spermatogonial chromosome aberration test (Unnamed study report, 2005b).

Mutagenicity of related substances

An in vivo mammalian alkaline comet assay with the structural analogue tert-butyl hydroperoxide (TBHP; EC 200-915-7) according to OECD Guideline 489 became available during the substance evaluation process. The objective of this study was to assess the potential of the test substance to cause DNA damage in rat nasal tissue when administered via nose-only inhalation to Sprague Dawley rats for 6 hours per day for 3 consecutive days.

The exposure concentrations were 7.4, 15.6, and 30.5 ppm. A concurrent control group was exposed to humidified, filtered air on a comparable regimen. A positive control group received a single oral gavage dose of 200 mg/kg ethyl methanesulfonate (EMS) on study day 2. On study day 2, between 2 and 4 hours after completion of the final exposure, animals were euthanized and subjected to collection of nasal tissue. The nasal tissues from 5 rats in each group were collected and processed for comet assay evaluation.

There were no test substance-related effects on survival or clinical observations. Test substance-related microscopic findings included minimal to mild subacute inflammation of the respiratory and/or transitional epithelium in the 15 ppm group in nasal section II; marked subacute inflammation of the respiratory and transitional epithelium in the 30 ppm group in nasal section II; and mild to marked degeneration of olfactory epithelium of the dorsal meatus in the 30 ppm group in nasal sections III and IV.

The test substance gave a negative response (non-DNA damaging) in the comet assay in the nasal tissues of the male rats. None of the test substance-exposed animals had significant increases in the % tail DNA compared to the filtered air control group. The filtered air control group's % tail DNA was within the historical range and the positive control group had a statistically significant increase in % tail DNA compared to the filtered air control group. Additionally, it was concluded that the test system was exposed up to the maximum feasible concentration, based on evidence of tissue cytotoxicity noted in the nasal cavity in the 30 ppm group. Therefore, TBHP was concluded to be negative in in vivo mammalian alkaline comet assay (Unnamed study report, 2016).

Mechanistic in vitro studies on reactive oxygen species (ROS) production and lipid peroxidation

The potential for dTBP to elicit production of reactive oxygen species (ROS), or lipid peroxidation was evaluated in primary rat hepatocytes (Report, 2019). ROS were quantified using the ROS-sensitive dye CellROX Green, which is primarily a sensor of

hydroxyl free radicals and superoxide. Menadione was used as a positive control for ROS experiments. Lipid peroxidation was evaluated using the Click-iT Lipid Peroxidation Imaging Kit. Cumene hydroperoxide was used as a positive control for lipid peroxidation. Preliminary cytotoxicity experiments were performed.

The cytotoxicity experiments demonstrated that dTBP did not induce significant cytotoxicity in rat hepatocytes up to 100 µM after 24 or 48 hours of culture. Based on this outcome, it was decided to increase the dTBP concentration to 400 µM in the ROS and lipid peroxidation assays (Unnamed study report, 2019a).

Di-tert butyl peroxide caused a statistically significant, dose-dependent, elevation in ROS production (maximum of 2.6 fold-control). The positive control robustly elevated ROS levels in rat hepatocytes by 2 – 4 fold-control in all three independent experiments carried out (Unnamed study report, 2019b).

Di-tert butyl peroxide showed weak, statistically-significant, evidence for dose-dependent effects on lipid peroxidation (up to 2 fold-control). The positive control did not reproducibly increase peroxidation across three independent experiments. On one occasion, there was no statistically-significant, dose-dependent, increase in peroxidation. On the other two occasions, there were statistically-significant, dose-dependent effects on lipid peroxidation. However, the increases in peroxidation was mild (1.7 – 2.2 fold-control). Therefore, it was not possible to reach a conclusion on the ability of dTBP to affect lipid peroxidation (Unnamed study report, 2019c).

Summary and evaluation

The three in vitro studies (two Ames tests and an mammalian gene mutation assay) were all negative and demonstrated that dTBP does not induce gene mutations.

Three in vivo micronucleus studies (OECD Guideline 474) were performed with dTBP: one via the intraperitoneal (IP) route, one via the inhalation route, and one via the oral route by gavage.

The intraperitoneal study was evaluated to be positive. The oral study showed significant increases in micronucleated cells, but without a clear dose response. The numbers of micronucleated polychromatic erythrocytes induced in the oral micronucleus test were within the historical solvent control range. This study was considered by RAC to be positive and was used as supporting evidence for mutagenic potential. The micronucleus study via the inhalation route, which became available after the RAC opinion was made, was evaluated to be negative. The study was part of a 90-day repeated dose toxicity study by inhalation, where no local effects such as irritation or hyperplasia were reported. This study did not lead to other conclusions on the mutagenicity of dTBP.

Positive results in the intraperitoneal and oral in vivo micronucleus tests for dTBP have been found at high doses (500 – 5000 mg/kg bw/day). These positive results are most likely explained by the saturation of the detoxification mechanism (by glutathione peroxidases resulting in the formation of corresponding alcohols) causing dTBP to undergo another metabolism pathway. This results in the production of ROS either in situ in the bone marrow and/or after hepatic metabolism, which is confirmed by recent in vitro data. These radicals can damage critical cellular macromolecules like DNA and/or modulate gene expression pathways. This would also suggest that genotoxicity is induced via a threshold mechanism.

Inhalation exposure will result in much lower systemic peak exposure compared to gavage or intraperitoneal exposure making it likely that the capacity of the antioxidant and DNA repair mechanisms in the cell are not exceeded. This provides evidence that dTBP induces systemic mutagenicity only after exposure to very high concentrations.

The related substance TBHP is much more reactive than dTBP, however, it was negative in an in vivo mammalian alkaline comet assay in the nasal tissue, thereby indicating that TBHP does not induce local mutagenicity and supporting the conclusion that also dTBP is not mutagenic to the nasal tissue after exposure via inhalation.

An intraperitoneal mammalian spermatogonial chromosome aberration test in male ICR mice (200 – 2000 mg/kg bw/day) with dTBP was evaluated to be negative.

Overall, it is concluded that dTBP has the potential to induce chromosome aberrations systemically in the bone marrow at high doses after intraperitoneal and oral administration. No such aberrations were detected in the bone marrow after inhalation. No chromosome aberrations were detected in spermatogonial cells after intraperitoneal administration which gives the strongest induction of chromosome aberrations in the bone marrow. No local mutagenicity is expected based on the lack of local effects after inhalation of dTBP and the negative Comet assay in nasal epithelium for TBHP. Based on the negative germ cell test classification as Muta 1B is considered not appropriate. As mutagenic effects are expected to only occur after exceeding a threshold and at high concentrations, no concern remains and no further information is required for this endpoint.

NL still believe that classification with category 2 is still applicable, as no new information has become available that contradicts the information which warranted classification in category 2 for mutagenicity according to RAC. The difference in mutagenic effects between the i.p. and oral tests versus the inhalatory test can probably be explained by the lower peak concentration after inhalation exposure.

7.9.6. Carcinogenicity

No carcinogenicity study is available for dTBP. However, a carcinogenicity study for the related substance TBHP became available as a result of a compliance check. This study was not fully evaluated as we had no acess to the full study report. However, the results of the study based on the robust study summary provided within the registration were taken into account in the assessment of the carcinogncity of dTBP. Further, tumor promotion studies were available for dTBP and summarized in this section.

Carcinogenicity study TBHP

TBHP was administered as a vapour via whole-body inhalation to Wistar Han rats for up to 24 months. The inhalation exposure schedule was 6 hours per day on a 5-day per week basis. 50 males and 50 females were exposed to concentrations of 0 ppm (filtered air), 4 ppm, 15 ppm, or 60 ppm TBHP (Unnamed study report, 2019d).

Statistically significant lower survival was not associated with test substance exposure at 4, 15, or 60 ppm. In fact there was a statistically significant increase in survival (males) for the 4 ppm group when compared to the 0 ppm control group (p=0.0036). The survival in males at concentrations of 0 ppm, 4 ppm, 15 ppm, and 60 ppm were 28/50, 42/50, 25/50, and 29/50; whilst for females the survival was 29/50, 31/50, 34/50, and 38/50 respectively. There were no test substance-related effects on clinical observations or palpable masses. Test substance-related effects on organ weights resulted from lower final body weights in the 60 ppm group males.

Test substance-related macroscopic findings included increased incidence of white areas in the lungs in the 60 ppm group males and females and nasal masses (bone or subcutis) in the 60 ppm group males.

Test substance-related causes of death were noted in males and females at 60 ppm. Nasal squamous cell carcinoma was observed in 6 males and 3 females that were euthanized in extremis and for which the carcinoma was considered to have the

strongest causative relationship with the clinical condition of each animal. These animals were euthanized between Weeks 67 and 97 for males and Weeks 56 and 102 for females.

Test substance-related nasal squamous cell carcinoma was observed for 9 of 50 males and 5 of 50 females in the 60 ppm group and a single nasal chondroma was observed in one 60 ppm group female (for all animals whether found dead or euthanized in extremis or at scheduled terminal necropsies). There was a statistically significant increase in the incidence of nasal level II tumor in males and females when comparing the 60 ppm group with the 0 ppm control group. Although the p-values for some trend tests, including nasal level III in males, indicated statistical significance, there were no nasal carcinomas in the 4 or 15 ppm groups. These results led to analysis of pairwise comparisons and provided additional support for the conclusion that the nasal squamous cell carcinoma was a test substance-dependent finding at 60 ppm only.

Test substance-related microscopic non-neoplastic findings included adverse changes in nasal levels I through VI, lungs, and eyes in the 60 ppm group males and females and adverse changes in nasal levels I and II and eyes (males only) in the 15 ppm group males and females. Test substance-related changes in nasal levels I through VI included degeneration/regeneration and/or necrosis of squamous, transitional, respiratory, and olfactory epithelium; squamous mucous, and/or respiratory metaplasia; mucous cell hyperplasia; mixed cell inflammation; inflammatory exudate; and/or adhesion. Test substance-related changes in the lung included alveolar macrophage, granulomas, interstitial and pleural fibrosis, and mononuclear cell inflammation only in the 60 ppm group males and females. Test substance-related changes in the eye included corneal ulceration, neutrophil inflammation, hyperplasia (60 ppm group only), and neovascularization (60 ppm group only) in the 15 and 60 ppm group males and corneal epithelium hyperplasia in a single 60 ppm group female.

The no-observed-effect concentration (NOEC) for carcinogenicity and the NOEC for systemic toxicity was 15 ppm.

The no-observed-adverse effect concentration (NOAEC) for non-neoplastic changes was 4 ppm, because non-neoplastic nasal findings were considered adverse at 15 and 60 ppm. Nasal carcinogenicity was limited to the group exposed to the 60 ppm TBHP concentration that was higher than the maximum tolerated dose based on body weight and that resulted in excessive irritation/corrosive injury to nasal tissues including respiratory, transitional, and olfactory epithelium, general toxicity and early deaths.

Specific investigations - tumor promotion

The potential for tumor promotion was studied in a two-stage initiation promotion study with SENCAR mice (30 per sex per dose). The mice were initiated with a single topical application of 10 nmol DMBA (7,12-dimethylbenz(a)anthracene) followed two weeks later by twice-weekly application of dTBP. The number and incidence of skin tumors were determined after 60 weeks. dTBP did not promote tumour formation on initiated skin (Gimenez-Conti, 1998).

Two short-term dermal studies were performed to determine the effect of dTBP on a series of markers of tumor promotion.

In the first study, hyperplasia, induction of dark basal keratinocytes and induction of ornithine decarboxylase activity were evaluated. Female Sencar mice (1-9 weeks) were treated topically once with dTBP (three different doses) in acetone solution or acetone alone. Following a single application on the dorsal skin, four or five animals were killed 0.25, 1, 2, 4 or 6 days after treatment. In the second part of this study, each animal was treated topically twice weekly for two weeks with three different doses. Groups of four to

five animals were killed at 0.25, 1, 2, 4 or 7 days after the last application. After the single application, dTBP induced non-dose response, transient epidermal hyperplasia (slight inflammatory alteration in the dermis) when compared to the control acetone. dTBP showed no hyperplastic effect at any dose level following multiple treatments. Further, dTBP did not increase the percentage of dark basal keratinocytes compared to acetone. Ornithine decarboxylase activity was stimulated by dTBP, but the activity was low. Overall, it is concluded that dTBP is not active in inducing the tested short-term markers for tumor promotion (Gimenez-Conti 1991).

In the second study, sustanied epidermal hyperplasia, dermal inflammation and oxidative DNA damage were examined. Sencar mice were exposed topically for 4 weeks to 100 and 200 µmol of dTBP. Treatment with dTBP did not exhibit significant increases in all three biomarkers associated with tumour promoting activity (Hanausek, 2004).

Summary and conclusion on carcinogenicity

One of the concerns identified for dTBP was carcinogenicity via inhalation, based on the suspected carcinogenic properties and expected exposure route.

No carcinogenicity study is available for dTBP. There are three dermal promotion studies available. DTBP did not increase biomarkers for tumour promotion after 4 weeks of dosing and did not induce tumour formation on the initiated skin. DTBP induced transient epidermal hyperplasia after a single application, but not after multiple application. DTBP did not increase the percentage of dark basal keratinocytes. However, used models have not been fully validated for the identification of tumour promotors or carcinogenic substances.

In the available 90-day repeated dose toxicity inhalation study, no local effects - signs of irritation or hyperplasia, which can be the onset of higher cell turn over, resulting in fixation of spontaneous mutations as a first step in the formation of neoplastic changes- were observed on the upper (larynx and nasal tissue) and lower (lung) respiratory tract, which have undergone specific investigation, up to 1000 mg/m^3. Based on the observation of the shorter range finding studies, no local effects on the respiratory tract, such effects are also not expected at up to 10,000 mg/m^3. In addition, the substance is not irritating for skin and eyes based on available studies. It can be concluded, that di-tert-butyl peroxide does not cause effects which may lead to local mutagenicity via an increase of cell turn over.

A carcinogenicity study with tert-butyl hydroperoxide demonstrated that carcinogenic effects are induced by this substance, however, the effects were initiated by irritation/corrosive injury to nasal tissues as there was no increase in effects in the Comet assay. Local effects are not induced by dTBP and these carcinogenic effects are not expected for dTBP.

Taking into account all evidence, including the absence of local mutagenicity of TBHP after inhalation, there is no concern for carcinogenic potential of dTBP via the inhalation route. DTBP is most likely genotoxic and possibly carcinogenicity only at high concentrations above a threshold. Based on the provided exposure information in the registration such concentrations are unlikely to occur with the current use even when taking the usual extrapolation factors into account. The data do not provide any justification to request further information relating to carcinogenicity.

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

Not evaluated.

7.9.8. Hazard assessment of physico-chemical properties

Not evaluated.

7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

Not evaluated.

7.9.10. Conclusions of the human health hazard assessment and related classification and labelling

Not evaluated.

7.10. Assessment of endocrine disrupting (ED) properties

Not evaluated.

7.11. PBT and VPVB assessment

Not evaluated.

7.12. Exposure assessment

7.12.1. Human health

Worker

This section was not evaluated.

Consumer

In the initial registration dossier, consumer uses were included. However, during an informal meeting with the registrant the uses were explained and the registration dossier was updated. According to the currently available registration dossier there is no consumer use of dTBP.

7.12.2. Environment

Not evaluated.

7.12.3. Combined exposure assessment

Not evaluated.

7.13. Risk characterisation

Not evaluated.

7.14. References

Author	Date	Publication/source details	Title
ECHA	2010	https://echa.europa.eu/documents/10162/13579/rac_opinion_bd_dtbp_en.pdf	Background Document To The Opinion Of The Committee For Risk Assessment On A Proposal For Harmonised Classification And Labelling Of DI-TERT-BUTYL

			PEROXIDE, EC number: 203-733-6, CAS number: 110-05-4
ECHA	2014	https://echa.europa.eu/documents/10162/1b270252-8535-4f5d-bbcb-803cf34f8e60	Background document to the Opinion proposing harmonised classification and labelling at Community level of Tertbutyl hydroperoxide (EC number: 200-915-7, CAS number: 75-91-2) CLH-O-0000001412-86-27/F
Gimenez-Conti et al.	1998	Toxicology And Applied Pharmacology, volume 149, Issue 1, p73–79. Doi: 10.1006/taap.1997.8355	Comparison of the Skin Tumor-Promoting Potential of Different Organic Peroxides in SENCAR Mice
Gimenez-Conti, I.	1991	Carcinogenesis, volume 12, Issue 4, p563-569. Doi: 10.1093/carcin/12.4.563	Induction of short-term markers of tumor promotion by organic peroxides
Hanausek et al.	2004	Carcinogenesis, volume 25, Issue 3, p 431-437. Doi: 10.1093/carcin/bgh022	Exposure of mouse skin to organic peroxides: subchronic effects related to carcinogenic potential
OECD	2012a	SIDS Initial Assessment Profile for CoCAM 3	Di-tert-butyl peroxide (dTBP)
OECD	2012b	SIDS Initial Assessment Report For CoCAM 3	Alkyl and Aryl Substituted Dialkyl Peroxides Category
Unnamed study report	1996	Dissemination site, https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14549, viewed 17-08-2016	OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test), oral gavage
Unnamed study report	2005 a	Dissemination site, https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14549, viewed 17-08-2016	OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test), intraperitoneal
Unnamed study report	2005 b	Dissemination site, https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14549, viewed 17-08-2016	OECD Guideline 483 (Mammalian Spermatogonial Chromosome Aberration Test)
Unnamed study report	2008	Dissemination site, https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14549	OECD Guideline 422 (Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity

			Screening Test)
Unnamed study report	2010 a	Dissemination site, https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14549, viewed 17-08-2016	OECD Guideline 471 (Bacterial Reverse Mutation Assay)
Unnamed study report	2010 b	Dissemination site, https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14549, viewed 17-08-2016	OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test)
Unnamed study report	2013	Dissemination site, https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14549, viewed 17-08-2016	OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day)
Unnamed study report	2014	Dissemination site, https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14549, viewed 17-08-2016	OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test), inhalation
Unnamed study report	2016	Dissemination site, https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14549	OECD Guideline 489 (In vivo Mammalian Alkaline Comet Assay)
Unnamed study report	2019a	Dissemination site, https://echa.europa.eu/registration-dossier/-/registered-dossier/15822	Investigation the potential of di-tert amyl peroxide (DTA) and di-tert butyl peroxide (DTB) to elicit cytotoxicity in primary rat hepatocytes.
Unnamed study report	2019b	Dissemination site, https://echa.europa.eu/registration-dossier/-/registered-dossier/15822	Investigation the potential of di-tert amyl peroxide (DTA) and di-tert butyl peroxide (DTB) to elicit production of reactive oxygen species (ROS) in primary rat hepatocytes.
Unnamed study report	2019c	Dissemination site, https://echa.europa.eu/registration-dossier/-/registered-dossier/15822	Investigation the potential of di-tert amyl peroxide (DTA) and di-tert butyl peroxide (DTB) to elicit lipid peroxidation in primary rat hepatocytes.
Unnamed study report	2019d	Dissemination site, https://echa.europa.eu/registration-dossier/-/registered-dossier/13623	OECD Guideline 451 - Carcinogenicity Study
Zeiger et al.	1988	Environmental and Molecular Mutagenesis, Volume 11, Supplement 12: 1-158 (1988). Doi: 10.1002/em.2850110602	Salmonella mutagenicity tests:IV Results from the testing of 300 chemicals.

7.15. Abbreviations

dTBP	di-tert-butyl peroxide
eMSCA	evaluating Member State Competent Authority
ROS	reactive oxygen species

7.16. Comparison of di-tert-butyl peroxide (dTBP), tert-butyl hydroperoxide (TBHP) and Di-tert-pentyl peroxide

Public name:	Di-tert-butyl peroxide	Tert-butyl hydroperoxide	Di-tert-pentyl peroxide Di-tert-amyl peroxide
EC number:	203-733-6	200-915-7	234-042-8
CAS number:	110-05-4	75-91-2	10508-09-5
Molecular formula:	$C_8H_{18}O_2$	$C_4H_{10}O_2$	$C_{10}H_{22}O_2$
Molecular weight range:	146.2	90.1	174.2805
Synonyms:	2,2'-dioxybis(2-methylpropane) (IUPAC) DTBP Trigonox B	TBHP	Di-tert-pentyl peroxide di-tert-pentyl peroxide
Structural formula