CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Tert-butyl hydroperoxide

EC Number: **200-915-7**

CAS Number: **75-91-2**

Index Number:

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***tert-butyl hydroperoxide***
EC number:	***200-915-7***
CAS number:	***75-91-2***
Annex VI Index number:	
Degree of purity:	68.4-69.6%***% (w/w)***
Impurities:	***Not relevant for classification***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	-
Current proposal for consideration by RAC	Muta 2, H341
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Muta 2, H341

No classification is proposed according to 67/548/EC as this is no longer required.

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives				
2.2.	Flammable gases				
2.3.	Flammable aerosols				
2.4.	Oxidising gases				
2.5.	Gases under pressure				
2.6.	Flammable liquids				
2.7.	Flammable solids				
2.8.	Self-reactive substances and mixtures				
2.9.	Pyrophoric liquids				
2.10.	Pyrophoric solids				
2.11.	Self-heating substances and mixtures				
2.12.	Substances and mixtures which in contact with water emit flammable gases				
2.13.	Oxidising liquids				
2.14.	Oxidising solids				
2.15.	Organic peroxides				
2.16.	Substance and mixtures corrosive to metals				
3.1.	Acute toxicity - oral				
	Acute toxicity - dermal				
	Acute toxicity - inhalation				
3.2.	Skin corrosion / irritation				
3.3.	Serious eye damage / eye irritation				
3.4.	Respiratory sensitisation				
3.4.	Skin sensitisation				
3.5.	Germ cell mutagenicity	**Muta 2, H341**	**-**	**none**	
3.6.	Carcinogenicity				
3.7.	Reproductive toxicity				
3.8.	Specific target organ toxicity –single exposure				
3.9.	Specific target organ toxicity – repeated exposure				
3.10.	Aspiration hazard				

4.1.	Hazardous to the aquatic environment				
5.1.	Hazardous to the ozone layer				

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Warning
Hazard statements: H341: suspected of causing genetic defects
Precautionary statements: Not part of an Annex VI entry.

Proposed notes assigned to an entry: -

Table 4: Proposed classification according to DSD

Not required.

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

The Netherlands was rapporteur for TBHP under the Existing Substances Regulation A RAR was produced and a proposal for harmonized classification and labelling. Recommendations by the Technical Committee on Classification and Labelling (**TC-C&L**) in October 2006 and September 2007 for physical/chemical and human health endpoints (see annexes) and in April 2006 for environmental effects were:

Classification:

O; R7

R10

Muta Cat 3; R68

C; R34

T; R23

Xn; R21/22

R43

N; R51/53

Labelling:

Symbol: O, T, N

R-phrase: 7, 10, 21/22, 23, 34, 43, 68, 51/53

S-phrase: 3/7, 14, 26, 36/37/39, 43, 45, 61

Specific concentration limits were concluded with R37 between 5% < C ≤ 10% and R43

above C ≥ 0.1%.

This classification and labelling would be included in Annex I of EU 67/548 as tert-butyl

hydroperoxide 70% in water because TBHP with less than 30% water is probably explosive.

TBHP has been evaluated by the TC-C&L resulting in a recommendation for classification with Muta Cat.3; R68. The TC-C&L recommendation has not been included in an ATP due to the partial replacement of DSD by CLP. Therefore, a new proposal according to CLP is required. This proposal is limited to the mutagenic properties of TBHP. A re-evaluation by RAC is explicitly requested because of the differences between the DSD and CLP criteria with respect to mutagenicity and because new information regarding mutagenic properties is available. In our opinion TBHP is a local mutagen. However, the CLP states that this hazard class is primarily concerned with substances that may cause mutations in the germ cells of humans that can be transmitted to the progeny. However, the criteria for category 3 are based on effects observed in somatic cells. In this case there seems to be a difference between the concern and the criteria. Therefore, re-evaluation by the RAC is requested.

2.2 Short summary of the scientific justification for the CLH proposal

TBHP is positive in several *in vitro* studies. The *in vivo* dataset is limited and in most studies negative. However, TBHP is positive in a dominant lethal assay in mice after intraperitoneal exposure. This could be a local effect of TBHP because substances can migrate from the abdominal cavity through the inguinal channel to the testis. However, TBHP has been shown to be unstable in blood in *in vivo* ADME studies. Therefore, it is unlikely that TBHP will reach the testis after exposure via normal routes. Therefore, TBHP is considered a local mutagen fulfilling the requirements for Muta 2 (CLP).

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

None

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

None

2.4 Current self-classification **and labelling**

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

The self-classification of the registrant is provided in the table below. This classification was only used by 2 out of more than 600 notifiers.

Table 5. Self-classification by the registrant.

Flam. Liq. 3	H226	H226	GHS02 GHS06 GHS09 GHS05 GHS08 Dgr	Eye Dam. 1: C ≥ 1% STOT SE 3: 5% ≤ C ≤ 10% Skin Sens. 1: C ≥ 0,1%
Org. Perox. F	H242	H242		
Acute Tox. 4	H302	H302		
Acute Tox. 3	H311	H311		
Skin Corr. 1C	H314	H314		
Skin Sens. 1	H317	H317		
Eye Dam. 1	H318			
Acute Tox. 2	H330	H330		
Muta. 2	H341	H341		

Aquatic Chronic 2	H411	H411		

An copy of the self-classification as available in the C&L inventory on 8 November 2012 is provided in Annex C. A summary is provided in the table below.

Table 6. Summary of CLP self-classifications

Type of hazard	Hazard class	Number of notifiers classifying in the hazard class (percentage of total notifications)
No classification		181 (27%)
Physical hazards	Flam. Liq. 3	446 (67%)
	Flam. Liq. 2	4 (0.6%)
	Organic Perox. C	157 (24%)
	Ox. Liq. 1	52 (8%)
	Ox. Liq. 3	38 ((6%)
	Self-react. F	72 (11%)
	Organic Perox. F	35 (5%)
	Self-react. C	9 (1.3%)
	Organic Perox. A	5 (0.7%)
Health hazards	Acute Tox. 4 (H302)	400 (60%)
	Acute Tox. 3 (H311)	213 (32%)
	Acute Tox. 4 (H312)	187 (28%)
	Acute Tox. 2 (H330)	247 (37%)
	Acute Tox. 3 (H331)	54 (8%)
	Acute Tox. 4 (H332)	99 (15%)
	Skin Corr. 1B	334 (50%)
	Skin Corr. 1C	35 (5%)
	Eye Dam. 1	224 (34%)
	Skin Sens. 1	254 (38%)
	Muta. 2	67 (10%)

	STOT SE 3	1 (0.1%)
Environmental hazards	Aquatic Chronic 2	341 (51%)
	Aquatic Chronic 3	4 (0.6%)

2.4.2 Current self-classification and labelling based on DSD criteria

R7 O: Oxidising

R10: flammable

Health hazards

Xn; R21/22; harmful in contact with skin and if swallowed

T; R23: toxic by inhalation

C; R34: Corrosive causes burns

Xi; R41; Risk of serious damage to eyes

R43; May cause sensitisation by skin contact

Muta cat 3, R68; Possible risk for irreversible effects

Environmental hazards

N; R51/53

Based on the available registrations (8 November 2012).

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Classification with Muta 2; H341 is normally subject to harmonised classification (CLP article 36.1.b). Other hazard classes were not included, as there is currently no need for harmonisation of these hazard classes.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

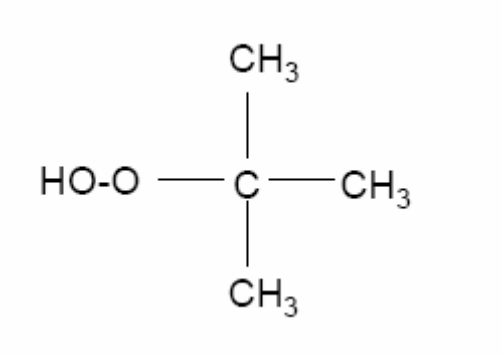
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 7: Substance identity

EC number:	200-915-7
EC name:	tert-butyl hydroperoxide
CAS number (EC inventory):	
CAS number:	75-91-2
CAS name:	tert-butyl hydroperoxide
IUPAC name:	hydroperoxide, 1,1-dimethylethyl
CLP Annex VI Index number:	
Molecular formula:	$C_4H_{10}O_2$
Molecular weight range:	90.1

Structural formula:



1.2 Composition of the substance

Table 8: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
TBHP	70%	68.4-69.6%	RAR

Current Annex VI entry: none

Table 9: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
• 2-Methylpropano-2-ol • Dialkyl peroxide • Ketones • Other hydroperoxides • Other organics	• < 0.5% • ≤ 0.1% • ≤ 0.2% • ≤ 1% • ≤ 0.4%		RAR

Current Annex VI entry: not relevant

Remark:

There are two registrations. The composition of the currently registered substances is deviating from the one mentioned in the RAR. TBHP purity is close to 100% w/w (exact purity is confidential) when the purity is calculated excluding water. The typical water concentration in the solution is 30 % (w/w). Information on the impurities in the registered substances is confidential and will be included in IUCLID. The presence of water as a stabilizer is important because TBHP without stabilizer is explosive (see Table 11).

Table 10: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
Water		≤ 30%		RAR

Current Annex VI entry: -

1.2.1 Composition of test material

The purity of the test material is indicated in the individual test descriptions and is considered relevant for TBHP.

1.3 Physico-chemical properties

The physico-chemical properties for 70% THBP and, where indicated, for 100%

TBHP (= TBHP pure) are presented in Table 9. This table is copied from the RAR.

Table 11: Summary of physico - chemical properties of TBHP[1]

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	liquid	RAR	
Melting/freezing point	-8 to -3˚C	MSDS, 1996; Merck Index, 1989; as summarised in RAR	
Boiling point	96 at 760 mmHg 35 at 35 mm Hg (TBHP pure)	MSDS, 1994; Merck Index,1989; Beilstein,1990; HSDB, 1999. as summmarised in RAR	
Relative density	Liquid: 935-964 kg/m^3 at 25°C Liquid: 791-902 kg/m^3 at 20°C (TBHP pure) Vapour: 3.1	MSDS, 1994; MSDS, 1996; Beilstein, 1990; HSDB, 1999. as summarized in RAR	
Vapour pressure	2.7 kPa at 20°C (experimental) 3.07 kPa at 21°C (unknown) 0.73 kPa at 25°C (experimental)	Hooidonk, 1992. MSDS, 1994. SRC PhysProp, 2001; HSDB, 1999 as summarised in RAR	
Surface tension	56 dynes/cm	ARCO, 1994. as sumarised in RAR	
Water solubility	≥ 100 mg/l at 25°C and pH 4.3 20,000 mg/l at 20°C (estimate) ≥ 100,000 mg/l at 22°C ca. 100,000 - 150,000 mg/l at 0-50°C 700,000 mg/l	MSDS, 1996. SRC PhysProp, 2001. ChemFinder, 2001. ARCO, 1994. OECD/SIDS, 1995. As summarized in RAR	
Partition coefficient n-octanol/water	0.7 at 25°C (experimental) 0.94 (estimate)	Hooidonk, 1992. SRCPhysProp, 2001. As summarized in RAR	
Flash point	43°C 62°C	MSDS, 1994. Chemfinder, 2001 as summarized in RAR	
Flammability	Flammable	MSDS, 1994.as summarized in RAR	
Explosive properties	Not explosive Explosive (TBHP pure)	RAR	Conclusion for non-explosive for 70% TBHP is based on theoretical, and/or structural considerations.
Self-ignition temperature	238˚C	RAR	

Oxidising properties	Oxidising	ARCO, 1994 as summarised in RAR	Conclusion based on theoretical, and/or structural considerations.
Granulometry	Not applicable (TBHP is liquid)		
Stability in organic solvents and identity of relevant degradation products	-		
Dissociation constant	12.8 at 20°C (experimental)	SRC PhysProp, 2001; HSDB, 1999 as summarized in RAR	
Viscosity	-		

1 70% TBHP and, where indicated, of 100% TBHP

2 MANUFACTURE AND USES

2.1 Manufacture

2.2 Identified uses

Tert-butyl hydroperoxide (TBHP) is primarily used in the chemical industry. TBHP is used as starting material (or intermediate) and as a reactive ingredient (catalyst, initiator or curing agent) (RAR).

According to the registrations there is also professional use including roller applicaton or brushing (PROC 10) and non industrial spraying (PROC 11).

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not relevant as this proposal is limited to classification for mutagenicity.

4 HUMAN HEALTH HAZARD ASSESSMENT

This CLH proposal is based on the risk assessment report (RAR) and the additional information in the Annex XV transitional report of TBHP which is available at:

http://echa.europa.eu/web/guest/information-on-chemicals/transitional-measures/annex-xv-transitional-reports

Also the REACH registration as available at the ECHA website was used (last visit at 3 April 2012).

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

The text below is a direct copy from the paragraph on Toxicokinetics from the RAR of TBHP. Also the references can be found in the RAR.

4.1.1 Non-human information

Toxicokinetics

In general, hydroperoxides are known to be reductively metabolized (Casarett and Doull, 1996). The main detoxification pathway is a two-electron reduction by glutathione peroxidase using glutathione to the corresponding alcohol (for TBHP this is 2-methylpropan-2-ol (= *tert*-butanol)). But 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 (Davies, 1988). Subsequent fragmentation of the *tert*-butoxyl radical results in the formation of carbon-centred radicals (CH_3; Greenley and Davies, 1992). The generation of such radicals has been demonstrated in several *in vitro* systems, such as human endothelial cells (O'Donnell and Burkitt, 1994), intact skin samples of the mouse (Timmins and Davis, 1993), rat liver microsomes (Greenley and Davies, 1992), and isolated rat liver nuclei (Greenley and Davies, 1994).

It is known from intracerebroventricular research that TBHP easily diffuses across biological membranes (Chang *et al.*, 1995; Di Meo *et al.*, 1997).

Studies in animals

In vivo studies

Inhalation

No information after inhalation exposure was provided because this route of exposure was considered unacceptable on safety grounds (explosion risk after evaporation). The subcutaneous route was tested as a potential surrogate.

Dermal

In a preliminary study (de Bie, 2003), two male Wistar rats were dermally exposed on 10 cm^2 shaved skin to 0.5 mg uniformly labelled [^{14}C]-TBHP at a concentration of 5% in water for 8 hours using semi-occlusive conditions. Samples of expired air, blood, urine and faeces were taken at several time points for the determination of total radioactivity. Urine samples were also used for metabolic profiling using HPLC. Further, a skin wash sample was taken after 8 hours and a cage wash sample at the end of the collection period. At the necropsy at 8 and 48 hours after the start of treatment, tissues and organs were examined for residual radioactivity.

The total recovery of radioactivity was only 30 and 40%. According to the author, this was probably caused by evaporation during the application of the substance to the skin because the odour of TBHP was noticeable during application. Only 1 to 3% radioactivity was recovered in urine, 0 to 2% in faeces and 22 to 34% in the cage air. This could be due to exhalation and/or evaporation. In the animal sacrificed after 48 hours, 33% radioactivity was found in the air after 8 hours and only 1% between 8 and 48 hours. The amount of radioactivity remaining in the body was 3.5% after 8 hours and 2% after 48 hours. The amount of radioactivity in cage wash and skin wash was approximately 1%. If it is assumed that all radioactivity in the air was due to evaporation, then the total absorption was only 5 to 7% of the total applied amount. This absorption value cannot be used in the risk characterisation because of the (uncontrolled) evaporation. As a result of evaporation, exposure of TBHP via inhalation cannot be excluded. Exposure via inhalation would result in an overestimation of the actual dermal absorption figure. The Cmax, found at 8 hours, was approximately 1 µg/g blood for radioactive equivalents. Radioactivity levels in other organs were highest in the kidneys and liver and approximately 3 times lower in other tissues like testes and lungs. No TBHP but 3 unknown metabolites (U1, U2 and U3) and probably 2-methylpropan-2-ol were found in urine using HPLC. No U2 was found in urine from 0-8 hours but this shifted to only U2 in urine from 24 to 48 hours.

The abstract of a dermal study including the determination of free radicals was provided by the NTP. TBHP was dermally administered in 50 percent aqueous acetone to male Fischer 344 rats at a dosage of 0 or 175 mg/kg bw (Ritchie *et al*., 2005c). The study animals were administered TBHP 12 times on weekdays only during the 17-day study period. There were eight rats per group and they received 0.5 ml/kg bw. On the last study day, each rat was dosed with one of two spin trap agents (PBN (N-*tert*-butyl-α-phenylnitrone): i.p.; 250 mg/mL; 0.1 mL/100 g body weight, or POBN (α-(4-pyridyl 1-oxide)-N-*tert*-butylnitrone): i.p.; 0.5 g/mL; 0.2 mL/100 g body weight) to allow post-termination assessment of free radical presence in the blood, urine, and in selected organs. The results of the free radical determination were not provided in the abstract but some results were available in a review of the study. There were

no increases in free radical formation measured in the lipid extracts of liver, kidney, blood, lung and heart from the animals.

Comparison of oral and subcutaneous exposure

In a preliminary study (de Bie, 2003), single male fasted Wistar rats were exposed by gavage (PO) or by subcutaneous injection (SC) to 10 (low) or 100 (high) mg/kg bw uniformly labelled [^{14}C]-TBHP. All doses were diluted in saline and provided at 20 ml per kg bw. Samples were collected for total radioactivity determination from expired air, blood, faeces and urine at several time points up to necropsy at 48 hours. At necropsy, residual radioactivity was determined in several organs. Further, a cage wash was performed to determine residual radioactivity. Urine samples were also analysed by HPLC to profile the metabolites with and without enzymatic digestion. Metabolites were identified using LC-MS/MS.

Only the animal exposed to 100 mg/kg bw via subcutaneous exposure showed drowsiness and slow respiration between 10 and 60 minutes after dosing. After 48 hours, 55 to 69% of the applied radioactivity was excreted in the urine, 1 to 2% in the faeces and 9 to 15% exhaled. The cage wash accounted for 1 to 3% and the radioactivity retained in the body for 10 to 31%. This resulted in a recovery of 94 to 97% of radiolabel. The main difference between the treatments was the increase in retained radioactivity in SC treated animals compared to the oral treated animals. The results indicate (n=1) that TBHP is almost completely absorbed after oral exposure. The blood kinetics are shown in **Table 4.1**. The results indicate that SC exposure resulted in somewhat longer terminal half-lives and higher AUC values for total radioactivity.

Table 4.1 ^{14}C Blood kinetics in male rats (preliminary study)

Parameter		PO low	SC low	PO high	SC high
Cmax	µg/ml	9.2	11.1	68.5	91.7
C at 48 hours	µg/ml	2.6	4.7	13.3	50.3
Tmax	h	8	8	8	24
Terminal half-life	h	21.8	32.1	17.1	27.7
AUC 0-48 h	µg/ml.h	270	278	1573	3056
AUC 0-infinity	µg/ml.h	351	597	1901	5060

The highest tissue radioactivity concentrations at 48 hours after oral and subcutaneous administration were found in the kidney followed by liver and blood. Somewhat lower concentrations were found in lungs, testes and carcass but the difference between the highest and the lowest value for each treatment was a factor 3 at most, indicating an almost equal distribution over the body. No TBHP but 4 unknown metabolites and probably 2-methylpropan-2-ol were detected in the urine. During the first 8 hours mainly U1 was excreted in the low dose animals and U3 in the high dose animals. This shifted at later sampling times to almost only U2. Treatment with beta-glucuronidase/aryl-sulphatase did not change the metabolic profiles. The metabolites could not be identified.

The provided preliminary ADME study indicated only minor differences between oral and SC exposure.

Oral

Male Wistar rats (n unknown) were treated with a spin trapping agent (PBN (phenyl-N-tert-butylnitrone) or POBN (α-(4-pyridyl-1-oxide)-N-tert-butylnitrone)) and with TBHP through gavage at dose levels of 0.5 or 1.0 ml/kg bw for 70% TBHP (solvent unknown) or 0.4 ml/kg bw for 90% [^{13}C]-TBHP (solvent unknown) (Hix *et al*., 2000). Bile was collected via bile duct cannulation at 20, 40 and 60 minutes after TBHP exposure. At 60 minutes, the animals were sacrificed and samples from blood, liver and stomach collected. The radicals that reacted with the spin trap were determined using electron paramagnetic resonance. Products of the reaction of the radicals with the spin trap (adduct) were found in the three tissues studied but no quantitative information was provided. The adduct in the blood was mainly confined to the erythrocytes and was identified as the PBN-thiylhemoglobin radical adduct. The EPR spectra of the kidney and liver were characteristic of PBN-alkyl radical adducts. The EPR signal in the bile increased from the first sample (0-20 minutes) to the second sample (20-40 minutes) but then decreased. According to the authors, this was probably due to extensive haemoglobin oxidation and impaired urine excretion by all treated rats. One adduct in the bile was characterised using [^{13}C]-TBHP as PBN-$^{\cdot}$CH3 radical adduct or POBN-$^{\cdot}$CH3 radical adduct. The identity of the main adduct in the bile remained unknown. Also methyl DNA adducts were found in liver and stomach (see B.5.7.2). It should be noted that the tested dose levels were above or comparable to the LD50.

The abstract of an oral study including the determination of free radicals was provided by the NTP. TBHP in 0.5 percent aqueous methylcellulose was delivered by gavage to male Fischer 344 rats at dosages of 0 or 175 mg/kg bw at a volume of 5 mL/kg bw (Ritchie *et al*., 2005a). Animals were dosed daily 12 times (weekdays only) during the 17-day study period. There were eight rats in each treatment group. On the last study day, each rat was treated with one of two spin-trap agents (PBN: i.p.; 250 mg/mL; 0.1 mL/100 g body weight, or POBN: i.p.; 0.5 g/mL; 0.2 mL/100 g body weight) to allow post-termination assessment of free radical presence in the blood, urine, and in selected organs (liver, right kidney, heart, and lung). However, the results of this study were not provided in the abstract but some results were available in a review of the study. A two to five fold increase in free radical formation in the lipid extracts of the tissues was found which was statistically significant in liver, kidney and blood. Liver had the highest (about five fold) increase in free radical formation.

Subcutaneous versus intravenous

Based on the comparable absorption, distribution, metabolism and excretion after oral and subcutaneous exposure in the preliminary study (de Bie, 2003), the main study (de Bie and Grossouw, 2004) was performed using the subcutaneous route. These results were expected also to be relevant to the dermal and inhalatory route.

Groups of 4 to 5 male and sometimes female Wistar rats were subcutaneously exposed to a single dose of 5 or 50 mg/kg bw/day [^{14}C]-TBHP in saline. The TBHP contained approximately 5% 2-methylpropan-2-ol. As a reference, a group of male rats was treated intravenously with 5 mg/kg bw [^{14}C]-TBHP. Also one group was treated subcutaneously with 5 mg/kg bw after an oral 14 day pre-treatment with 50 mg/kg bw/day unlabelled TBHP. In all groups, samples from blood, expired air, urine and faeces were collected at several time points for total radioactivity determination and metabolic profiling with or without acid or

alkali hydrolyses. At necropsy on day 7 after treatment, samples of several tissues and organs were collected for the same determinations. Also, the GSH and GSSG concentration was determined in selected tissues. Further, a cage wash sample was collected at the end of the collection period.

Blood kinetics

The kinetic parameters for total radioactivity in male rats after a single SC and IV exposure were almost comparable (**Table 4.2**), indicating almost complete absorption after SC exposure. Cmax, Tmax and AUC for total radioactivity were somewhat lower in females compared to males which is probably caused by the higher VD and lower T1/2 in females. No effect of pre-treatment was seen on the kinetic parameters. Also, no dose dependent differences were seen. Seen the quick metabolism of TBHP to 2-methylpropan-2-ol, the AUC and T1/2 of TBHP itself is probably much lower than for total radioactivity.

Table 4.2 ^{14}C-Blood kinetics in rats (main study)

Route	**IV**	**SC**	**SC**	**SC with oral pre-treatment**	**SC**	**SC**
Sex	male	male	female	male	male	female
Dose (mg/kg)	5.39	5.12	5.16	5.16	49.7	47.2
Cmax (µg/g)	8.6	9.0	8.2	8.6	79.8	72.4
Tmax (h)	4	6	4	6	6	2
Plateau time (h)	0-8	2-12	2-8	2-12	2-15	2-12
T1/2 (h)	19.2	21.6	16.2	24.0	24.4	18.3
AUC 0-168 (µg/g.h)	235	296	151	322	3335	1793
AUC 0-inf (µg/g.h)	236	298	151	325	3367	1797
VD (L/kg bw)	0.63	0.54	0.80	0.55	0.52	0.69

Absorption and excretion

In all groups between 79 and 81% of the injected radioactivity was excreted in the urine mainly within the first 48 hours. Only in females receiving a single dose of 50 mg/kg bw, 68% was excreted in urine. Faeces accounted for 1 to 2% of the injected radioactivity, cage wash for approximately 0.5% and approximately 1% was retained in the body after 7 days. Tissue residues in females were somewhat lower compared to man. Further, approximately 2% was exhaled as carbon dioxide and 5 to 9% was exhaled as volatiles. Only in the females receiving a single dose of 50 mg/kg bw, 15% volatiles were exhaled. Total recovery was between 88 and 93% of the injected amount of radioactivity. The results indicate no difference between IV and SC treatment, no effect of pre-treatment, proportionality to dose and only some minor differences between males and females.

Tissue distribution and elimination

The highest residue levels (total radioactivity) were found in the kidney. Residue levels in most other organs were approximately 50% of the kidney levels but clearly lower in fat. A two-phase residue elimination of radioactivity was seen. In the first phase between 2 and 36

hours, an elimination with a half-life of approximately 12 hours was seen. The half-life in the second phase between 36 and 96 hours was slower with a half-life of approximately 50 hours. The half-lives were somewhat higher in high dose males compared to low dose males resulting in proportionally somewhat higher residue levels at the high dose after 7 days. Somewhat higher residue levels (total radioactivity) were also found in the pre-treated group. Further, residue levels were lower in the females compared to the males. The final residue levels after IV treatment were somewhat lower compared to SC treatment.

Metabolite profiling

No TBHP was found in the urine collected over 96 hours but 3 major metabolites (U1, U2 and U3) and 6 minor metabolites could be detected. The minor metabolite 2-methylpropan-2-ol was mainly found at the earlier collection times. Also, all three major metabolites were found at the earlier time points shifting to only U2 at later time points after the low dose and mainly U2 after pre-treatment and the high dose. Comparable metabolic profiles over 96 hours were seen between IV and SC treatment, males and females and pre-treatment and no pre-treatment. In high dose animals lower percentages of U1 were found and higher percentages of U3.

No TBHP was found in the exhaled air after IV treatment and small amounts after SC treatment. It was assumed that some TBHP leaked away from the SC injection site. 2-methylpropan-2-ol was the main metabolite in exhaled air accounting for approximately 97% of the material found. The other metabolite in exhaled air was not identified.

Within 15 minutes (first measurement) after IV injection no TBHP but mainly 2-methylpropan-2-ol was found in plasma. The 2-methylpropan-2-ol levels were steady for almost an hour and then quickly disappeared from the plasma. The metabolites P1, P2 and P3 (in the metabolite codes for the various matrices comparable numbers indicate the same retention time) increased and reached Tmax after 2, 8 and 2 hours, respectively. P2 was the main metabolite after 2 hours and the only one after 12 hours.

Also in plasma and tissues collected after 2 and 12 hours from male rats treated subcutaneously with 5 or 50 mg/kg bw, no TBHP was detected. At 2 hours, 2-methylpropan-2-ol was the main metabolite in all tested tissues accept for the liver where L3 was the main metabolite at 2 hours. Substantial amounts of K3 were also found in the kidney at this time point. At 12 hours, metabolite 2 was the only metabolite in all organs of the low dose animals and the main metabolite at the high dose. The results indicated saturation of metabolism at the high dose.

Acid hydrolyses of urine samples resulted in a strong decrease in U3 and a comparable increase in 2-methylpropan-2-ol indicating that U3 is a conjugate (probably the glucuronide or sulphate) of 2-methylpropan-2-ol. This is supported by the observation that U3 was mainly found in the liver and also in the kidney. 2-Methylpropan-2-ol was identified based on co-elution. U2 co-eluted with 2-hydroxy-isobutyric acid, which is also a metabolite of 2-methylpropan-2-ol. U1 was not identified but based on literature data on the metabolism of 2-methylpropan-2-ol it is postulated that this is 2-methyl-1,2-propanediol. The proposed metabolic pathway is that TBHP is converted to 2-methylpropan-2-ol which is either conjugated or further oxidised to 2-methyl-1,2-propanediol. 2-methyl-1,2-propanediol is oxidised to 2-hydroxyisobutyric acid probably via 2-hydroxyisobutyraldehyde.

No changes in total glutathione and GSSG were found in kidney and testes of high and low dosed rats at 2 and 36 hours after SC injection. A reduction of total GSH was found in the liver at 2 hours after SC injection with 50 mg/kg bw and an increase in total GSH at 36 hours.

Metabolism of 2-methylpropan-2-ol

The metabolism of 2-methylpropan-2-ol was studied in several species. The major metabolites identified with ^{13}C-NMR in the urine of rats (n=3) orally treated with 250 mg/kg bw ^{13}C-2-methylpropan-2-ol were tert-butyl sulfate, 2-hydroxyisobutyric acid and 2-methyl-1,2-propanediol. Minor metabolites were acetone and tert-butyl glucuronide. 2-Methylpropan-2-ol was found as a minor fraction in the urine (Bernauer *et al*., 1998).

The major metabolites identified with ^{13}C-NMR in the urine of one male volunteer orally treated with 5 mg/kg bw ^{13}C-2-methylpropan-2-ol were 2-hydroxyisobutyric acid and 2-methyl-1,2-propanediol. Minor metabolites were 2-methylpropan-2-ol and tert-butyl glucuronide. Only a trace of tert-butyl sulfate was found (Bernauer *et al*., 1998).

In the urine of untreated rats low amounts of 2-methylpropan-2-ol, 2-hydroxyisobutyric acid and 2-methyl-1,2-propanediol were found (Amberg *et al.*, 2000).

In vitro studies

Homogenates of stomach and small intestinal contents from two fasted and two non-fasted male Wistar rats (20 g) were diluted (1:4) and incubated with 100 mg [^{14}C]-TBHP at 37°C in wash bottles continuously flushed with nitrogen. The effluent gas was led through washing bottles to trap CO_2 and volatile organic compounds. Samples were taken from the effluent and the incubation mixture at several time points up to 4 hours to determine the total amount of radioactivity, TBHP and metabolites.

Up to 45% of the applied amount of TBHP evaporated from the incubation bottles over 4 hours. Over 90% of the radioactivity remaining in the incubation mixture was TBHP, indicating that TBHP is reasonably stable in stomach and small intestine contents. No formation of 2-methylpropan-2-ol (tributyl alcohol) or other unknown metabolites was found after incubation with stomach contents. A small increase in 2-methylpropan-2-ol was found after incubation with small intestine contents (de Bie, 2003).

The percutaneous absorption was determined using an *in vitro* method with rat skin membranes according to OECD 428 (Maas, 2004). [^{14}C]-TBHP (6.4 µl) was applied on top of the rat skin membranes (surface 0.64 cm^2, 0.6 mm thickness, non-fresh) in a flow-through diffusion cell. Concentrations of 1.0, 10.0 and 60% were used as these were considered relevant for workers. Exposure times were 1, 4 and 8 hours for 1.0 and 10% and 1, 2 and 4 hours for 60% using 4 membranes per group. A preliminary test with semi-occlusion demonstrated poor recovery. Therefore, the *in vitro* test was done using either a charcoal filter or glass slide to cover the exposure site. The receptor fluid was saline containing 0.01% azide and 3% bovine serum albumin. The integrity of the membranes was checked using tritiated water. Receptor fluid samples were taken every hour and samples of all other materials at the end of the exposure period for radioactivity determination.

The mean total recovery of radioactivity was 88 ± 3% for cells with a charcoal filter and 81 ±9% for cells with glass coverings. At 1 and 10% TBHP with charcoal covering penetration was seen without a lag time with maximal penetration between 0 and 2 hours. At the higher concentration with charcoal and all concentrations with glass slides, a short lag time of less than 1 hour was seen with maximal penetration between 1 and 3 hours. Strong differences in flux and Kp values were seen between the concentrations and coverings (**Table 4.3**). The total penetration of TBHP and/or metabolites (sum of radioactivity from receptor fluid, cell wash and skin membrane (total skin digest) as a percentage of the applied radioactivity) was constant after 1 hour for the 1.0% and 10% concentrations using the charcoal filter (**Table 4.4**). This might indicate that after 1 hour most TBHP was bound to the charcoal. Skin surface damage was often seen at the end of the exposure period especially with the higher dose levels and under occlusive conditions. The damage can explain the increase in penetration at higher concentrations and under occlusion.

Table 4.3 **In vitro penetration parameters of TBHP (as total radioactivity)**

covering	**charcoal**			**glass**		
exposure time	8	8	4	8	8	4
dose (mg/cm^2)	0.1	1.0	6.0	0.1	1.0	6.0
mean flux constant (slope)(µg/cm^2.h)	0.788	8.215	280.84	6.884	128.15	800.82
Kp value * 10-3 (cm/h)	0.079	0.082	0.472	0.695	1.279	1.345
lag time (h)	0.0	0.0	0.3	0.8	0.5	0.6

Table 4.4 **Total absorption (as total radioactivity) in rat skin in vitro (%) and between brackets the percentage determined in the skin**

exposure period (h)	**charcoal**			**glass**		
	0.1 mg/cm^2	**1.0 mg/cm^2**	**6.0 mg/cm^2**	**0.1 mg/cm^2**	**1.0 mg/cm^2**	**6.0 mg/cm^2**
1	4.6 (1.8)	7.5 (1.5)	12.6 (5.3)	9.4 (4.8)	26.2 (8.5)	35.2 (16.0)
2	3.7 (1.2)	5.0 (1.2)	14.4 (4.9)	13.5 (3.5)	40.0 (4.2)	35.2 (8.1)
4			14.1 (1.7)			36.9 (5.3)
8	3.5 (1.3)	3.1 (1.0)		36.9 (5.4)	51.2 (2.1)	

Full thickness skin was obtained from untreated male Lac a mice, stored at 4°C and used within 2 hours (Timmins and Davis, 1993). Circular skin samples with a diameter of 5 mm were placed in custom-made EPR sample cells and treated with 2 µl 0.05 - 1 M TBHP with or without pre-treatment with the spin trapping agent DMPO. Treatment with 0.1 M TBHP in acetone without a spin trapping agent resulted in the formation of the ascorbyl radical. A maximum level was found within 3 to 5 minutes after which the radical level was stable. The ascorbyl radical level increased dose dependently up to 0.25 M followed by a decrease that can be explained by the oxidation of the ascorbyl radical to dehydroascorbate. The use of acetone as solvent compared to water and tape stripping of the skin resulted in an increase of the ascorbyl radical. This indicates that the reaction takes place below the surface of the skin. Incubations of skin samples pretreated with DMPO with TBHP resulted in EPR signals which

were assigned to carbon centred and alkoxyl radicals based upon their hyperfine coupling constants and comparison with previous data. This indicates that "*in vivo*", one electron reduction is the main dermal metabolic pathway for radical formation from TBHP in mice.

Derivation of a dermal absorption figure based on the available data

No dermal absorption figure can be derived from the dermal *in vivo* study (de Bie, 2003) because of the low recovery in this study and possible co-exposure via inhalation as a result of evaporation. The mean total recoveries in the *in vitro* dermal absorption studies (Maas, 2004) were 88 ± 3% and 81 ± 9% for cells with a charcoal filter and for cells with glass coverings, respectively. These values are just below the minimum recovery as specified in OECD guideline 428 (90%). However, these values are considered acceptable as TBHP may evaporate to a relatively large extent. In these studies, the tested dermal area doses were 0.1, 1.0 and 6.0 mg/cm^2 (concentrations of 1%, 10% and 60% TBHP, respectively) under (semi-) occlusive conditions.

When establishing the dermal absorption figures which will be taken forward to the risk characterisation, the actual dermal exposure levels used in the risk characterisation should also be taken into consideration, as the percentage dermal absorption may depend amongst others on the dermal area dose (often being inversely related to the percentage absorption, see TGD, 2003, Appendix IV B Dermal absorption). The dermal exposure levels which are considered in the risk characterisation are 0.001-0.01 mg TBHP/cm^2 (occupational exposure scenario 3, contact with products containing <1% TBHP) and 0.032 mg TBHP/cm^2 (occupational exposure scenario 4, use of products containing <1% TBHP)) (see **Table 9.4** in section B.9.5).

The retrieved dermal absorption parameters for the lowest tested dermal area dose (0.1 mg/cm^2) in the studies of Maas (2004) are relevant because this dermal area dose is comparable to the estimated dermal exposure loading of workers in occupational exposure scenario 4 (0.032 mg/cm^2) and because the *in vitro* data do not show an inverse relationship between area dose and percentage absorption. Because it is not likely that occlusion will occur (contamination of hands in occupational exposure scenarios 3 and 4 is estimated assuming no gloves), the relative absorption parameters using a charcoal cover (semi-occlusive condition) are considered relevant.

In the studies by Maas (2004), skin damage was observed with the higher levels and under occlusive conditions (using a glass cover). At the level of 0.1 mg/cm^2 under semi-occlusive conditions, skin damage was not observed in the *in vitro* study. The comparable Kp-values retrieved from Maas' experiments for the dermal area doses of 0.1 mg/cm^2 and 1.0 mg/cm^2 in the presence of a charcoal cover showed that there was also no skin damage at a level of 1.0 mg/cm^2. Therefore, the absorption results from the dermal area dose of 0.1 mg/cm^2 can be used for the risk characterisation.

The total absorption (sum of radioactivity from receptor fluid, cell wash and skin membrane (total skin digest) as a percentage of the applied radioactivity) of TBHP using a charcoal cover at the dermal area dose of 0.1 mg/cm^2 was 3.5% after 8 hours. The relative absorption of the potentially absorbed amount of about 3.5% determined at 8 hours using a charcoal cover will be taken forward to the risk characterisation regarding occupational exposure scenario 4.

With respect to dermal exposure to products containing <1% TBHP in occupational scenario 3, the dermal exposure values are somewhat lower compared to the dermal exposure values in occupational exposure scenario 4. However, as in the study by Maas (2004) the absorption figures are comparable for the dermal area doses of 0.1 mg/cm^2 and 1.0 mg/cm^2 after 8 hours in the presence of a charcoal cover (see **Table 5.4**), it is expected that for a dermal area dose of 0.01 mg/cm^2 (estimated skin exposure level in scenario 3) a relative absorption figure comparable to that of the dermal area doses of 0.1 mg/cm^2 and 1.0 mg/cm^2 may be applicable. Therefore, 3.5% as dermal absorption value will also be taken forward to the risk characterization for this scenario.

4.1.2 Human information

None

4.1.3 Summary and discussion on toxicokinetics

The text below is a direct copy from the Summary of the toxicokinetics, metabolism and distribution, from the RAR of TBHP.

TBHP is stable in the stomach and intestine and completely absorbed after single and repeated SC and oral exposure at levels between 5 and 50 mg/kg bw. The absorbed TBHP is rapidly converted to 2-methylpropan-2-ol and distributed over the body. The significant reduction of GSH at 2 hours after exposure in liver is consistent with a first-pass metabolism. 2-Methylpropan-2-ol is either excreted in exhaled air, conjugated and eliminated in the urine or oxidised to and excreted in the urine as 2-methyl-1,2-propanediol and 2-hydroxyisobutyric acid (Figure 1). 2-hydroisobutyric acid was the main metabolite in all tissues at 12 hours after treatment. Female rats showed an increased metabolism resulting in lower tissue residues. The results at both dose levels were almost proportional but indicated some saturation of metabolism.

More specific in vivo and in vitro studies show that besides the major detoxification route TBHP can also form tertiair-butyl peroxyl radicals, tertiair-butoxyl radicals and carbon centered radicals. These radicals can react with many other molecules resulting in many different reaction products.

An oral absorption of 100% was determined for TBHP based on the comparable kinetic parameters after IV and SC exposure for total radioactivity, the high urinary excretion compared to the total recovery and the stability of TBHP in stomach and small intestine contents. However, the bioavailability (presence of substance in the systemic circulation) of

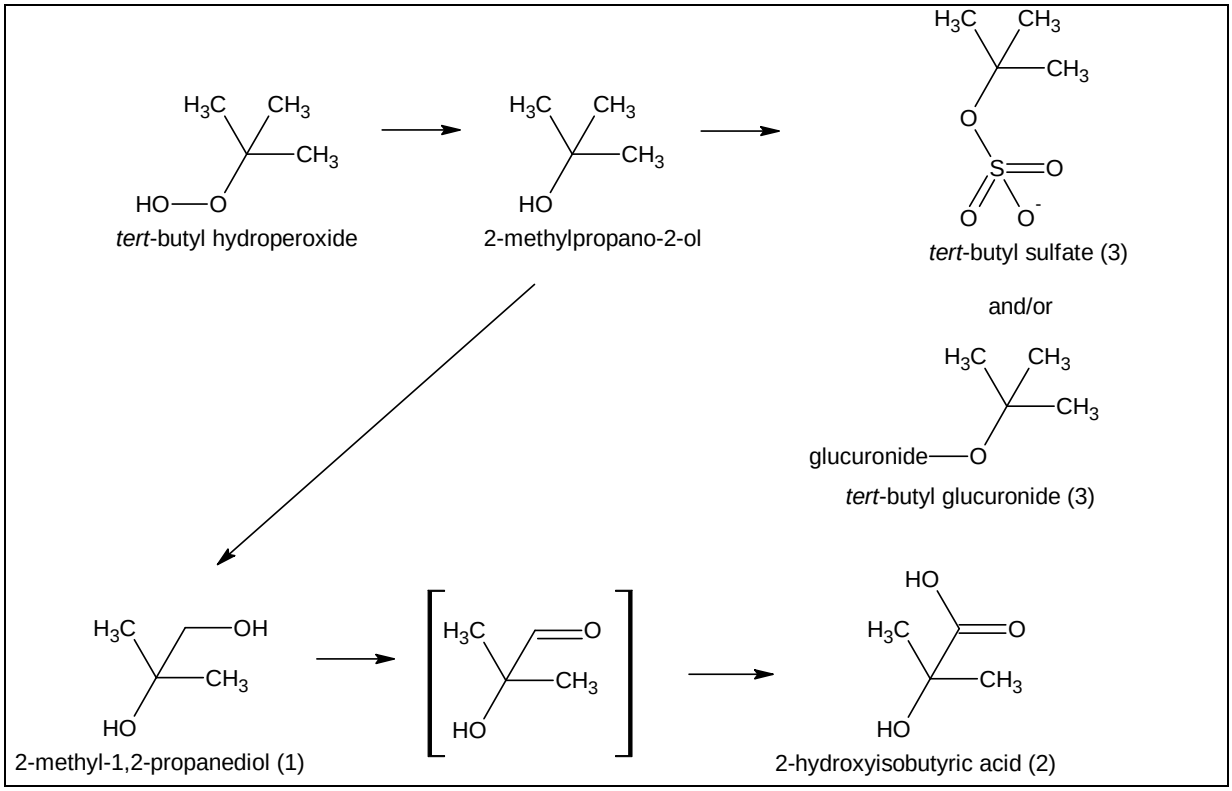
TBHP is very low or absent due to the reactivity of TBHP and the rapid conversion to 2-methylpropan-2-ol as shown by the absence of TBHP at 15 minutes after IV injection.

An increase in free radicals in some organs was observed by Ritchie et al. (2005a) after oral exposure but not after dermal exposure (Ritchie et al., 2005c). The increase in free radicals in the liver and blood after oral exposure is considered evidence of a local formation of free radicals. The increase in free radicals in the kidney could be interpreted as an indication of the presence of TBHP in the systemic circulation. However, it is unclear why this increase was not found in the heart or the lung. Overall, the information on the free radical formation from the studies by Ritchie et al., (2005a,c) are too limited to make a firm conclusion on the bioavailability of TBHP. The absence of systemic bioavailability, as observed in the i.v. study, is confirmed by the pattern of toxicology which showed only local toxicity and no systemic toxicity. Overall, systemic availability of TBHP and radical formation in organs beyond the site of first contact are not expected because of the corrosive properties of TBHP which will prevent such high exposures to occur.

No information on the inhalatory absorption is available. However, given the good absorption after oral exposure indicating good membrane diffusion, the good water solubility and high vapour pressure, 100% absorption after inhalatory exposure is expected and taken forward to the risk characterisation.

Based on the available in vitro dermal absorption study and taking into consideration the actual dermal exposure levels used in the risk characterisation, a dermal absorption value of 3.5% is taken forward to the risk characterisation for exposure without occlusion to products containing concentrations below 1% TBHP.

Figure 1 Proposed metabolic pathway of TBHP in rats


tert-butyl hydroperoxide
2-methylpropano-2-ol
tert-butyl sulfate (3)
and/or
glucuronide
tert-butyl glucuronide (3)
2-methyl-1,2-propanediol (1)
2-hydroxyisobutyric acid (2)

4.2 Acute toxicity

Not relevant

4.3 Specific target organ toxicity – single exposure

Not relevant

4.4 Irritation

Not relevant

4.5 Corrosivity

Not relevant

4.6 Sensitisation

Not relevant

4.7 Repeated dose toxicity

The provided data is limited to the new studies available after publication of the RAR. The old studies are available in the RAR.

Table 4.5: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
subacute 5-day range finding inhalation study	overall NOAEC of 20 mg/m^3		Ma-Hock et al., 2010a
subacute 28-day inhalation study	overall NOAEC of 7.2 mg/m^3		Ma-Hock et al., 2010b

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Not relevant.

4.7.1.2 Repeated dose toxicity: inhalation

The studies summarised in this paragraph are inserted in this CLH report only for information and not for classification. The text below is copied from the Annex XV transitional report of TBHP.

In a 5-day range-finding inhalation study (Ma-Hock et al., 2010a), groups of 5 male rats (Wistar) per dose were exposed for 6 hours a day on 5 consecutive days to a vapour of TBHP. Additional groups of 5 male rats were treated the same way and were used for the Comet assay. The actual exposure levels were 0, 20, 91 and 179/377 mg TBHP/m^3 (presented as 100% TBHP). These concentrations were generated by heating TBHP-70 containing 69.8% TBHP and dilution with air. The observations included: clinical observations, body weight, Comet assay under alkaline conditions in lung cells (control and high dose only), FACS analyses of the type of cells present in the isolated lung cells (control and high dose only), gross pathology, organ weight for a limited number of organs and histopathological examination of the respiratory tract, thymus and adrenals.
Two animals out of 10 in the highest dose group died during the exposure period (on day 1 and day 2). The exposure concentration in the highest dose group was therefore reduced from 377 mg/m^3 to 179 mg/m^3 for the remaining treatment period. The animals in the highest dose group showed abdominal respiration, apathy, eyelid closure of both eyes, gasping, labored respiration, red nose crust formation, piloerection and respiration sounds on all exposure days. The body weight of the animals in the highest dose group was significantly reduced on day 4. Body weight gain was significantly reduced in the intermediate and high dose group. The absolute and relative lung weight in the intermediate dose group, were significantly reduced whereas the relative lung weight in the high dose group was significantly increased. The

adrenal weight showed a dose related increase (absolute and relative) which was significant at the high dose. The thymus weight was dose dependently reduced (absolute and relative) which was significant for the high dose group. Histopathological changes were observed in the nasal cavity, the larynx and the trachea in the mid and high dose group. The severity of the effects was the highest in the upper parts of the respiratory tract and decreased towards the lower parts. Changes in the lung bronchi on the bronchiolar epithelium were observed in 3 out of 6 animals of the high dose group. Bronchiolar necrosis was observed in one animal and two others showed hyperplasia of the bronchiolar epithelium. In the adrenal cortex fatty change (lipid-like vacuoles within the cytoplasm) was observed in one animal of the mid dose and three animals of the high dose group. In the thymus, a decrease of the cellular density of the cortex and an increase in starry sky cells (macrophages containing apoptic lymphocytes within the cytoplasm) were present in three out of five animals of the high and mid dose group and one animal of the low dose group. The Comet assay in lung cells showed comparable values for tail moment, length and relative intensity for the high dose group (n=4) and the control group (n=5). The FACS analyses for the type of cells present in the long cell suspension was limited to three animals in the control group and two animals in the high dose group. Apparently not all parameters could be determined for these animals (the part on FACS analyses was only limitedly described in the report). The results indicate an increase in CD45-positive cell stated as meaning leukocytes from the blood or interstitial space or free lung cells in the high dose group compared to the controls. This also means a reduction in the percentage of epithelial and endothelial cells in the lung cell preparation. There was no increase of CD11 positive cells indicating no relevant inflammation occurred in the lung.

Based on the absence of effects at 20 mg/m^3 and several effects at 91 mg/m^3, including histopathological changes in the respiratory tract indicative of respiratory tract irritation, target dose levels of 7.4, 22.2 and 77.7 mg/m^3 were chosen for the 28-day inhalation study.

In a subacute inhalation study (Ma-Hock et al., 2010b), groups of 6 rats (Wistar) per sex and dose were exposed for 6 hours a day, 5 days a week for in total 28 treatment days to a vapour of TBHP. The actual exposure levels were 0, 7.2, 22.6 and 67.0 mg TBHP/m^3 (presented as 100% TBHP). These concentrations were generated by heating TBHP-70 containing 69.8% TBHP and dilution with air. The study was performed according to OECD 412 and B.8 of the EU Test Method Regulation (EC 440/2008), according to GLP and including extensive histopathology of the nasal cavity, larynx and lungs and cell proliferation at two sites of level I of the nasal cavity. The choice of the exposure levels was based on a 5 day range finding study in male rats which showed mortality and irritation of the upper respiratory tract at 179/377 mg/m^3, moderate irritation at 91 mg/m^3 and no adverse effects at 20 mg/m^3.

No deaths were recorded throughout the study. Body weight and body weight gain were reduced in the males at the highest dose on day 7 and day 14 (Table 4.6). A reduction in relative kidney weights was observed in all treated females compared to the controls. Although this decrease was statistically significant in the mid and high dose groups, the decrease was less than 10%, there was no dose response relationship and there were no histopathological findings that could explain the weight reduction. Therefore, this effect is considered to have no biological significance. An increase in hyperplasia/metaplasia of the transitional epithelium in nasal cavity level 1 sections was observed in 5 males and 3 females at the high dose level. This occurred mainly at the tip of the maxilloturbinate and the lateral wall of the nasal cavity as a minimal to mild increase in thickness of the transitional epithelium and a slight change towards squamous epithelium. The unit length labelling index (ULLI) of the affected regions of the maxilloturbinate and the lateral wall, measured as the incorporation of BrdU, was increased in males and females at the highest dose. A slight but

significant increase in ULLI of the affected regions of the maxilloturbinate was also observed in males at the mid dose. An increase in ULLI indicates an increase in the proportion of cells undergoing replicative DNA synthesis. The increase in the males at the mid dose was not considered substance related by the study author because it was minimal (1.5-fold) and mainly caused by a single animal. In our opinion this effect is probably substances related because there was a dose response relationship and because the increase was statistically significant. No histopathological changes were observed in other organs including the larynx and the lung.
An overall NOAEC of 7.2 mg/m^3 is derived from this study based on an increase in ULLI in males at a dose level of 22.6 mg/m^3 (LOAEC).

Table 4.6.

	0 mg/m^3		7.2 mg/m^3		22.6 mg/m^3		67.0 mg/m^3	
Parameter	male	female	male	female	male	female	male	female
Body weight day 7	270.9	177.7	260.5	174.5	264.3	174.3	247.4 *	174.6
Body weight day 14	288.7	183.1	278.4	179.5	284.5	181.4	262.4 *	183.2
Body weight gain day 7	20.8	5.8	13.3	4.8	16.4	2.1	6.1 **	2.6
Body weight gain day 14	38.5	11.2	31.2	9.8	36.5	9.2	21.1 *	11.2
Relative kidney weight	0.658	0.706	0.675	0.673	0.660	0.646 *	0672	0.651 *
Hyperplasia/metaplasia of the transitional epithelium in nasal cavity level 1 sections								
Grade 1	0/6	0/6	0/6	0/6	0/6	0/6	4/6	3/6
Grade 2	0/6	0/6	0/6	0/6	0/6	0/6	1/6	0/6
ULLI maxilloturbinate	6.69	9.04	7.45	8.97	10.19 *	10.81	111.8 **	75.4 *
ULLI lateral wall	5.89	5.21	7.19	5.79	6.56	6.77	83.6 **	31.6 **

No increase in lung cell DNA damage was observed in the Comet assay at the highest dose tested compared to the control group. Changes of the bronchiolar epithelium were observed in some animals of the high dose group. The absence of DNA damage at a dose level inducing some bronchiolar epithelial changes may indicate that TBHP at this dose level does not induce local DNA damage in the lung detectable with the Comet assay. However, it can not be excluded that possible DNA damage to bronchiolar epithelium was diluted by the presence of other cell types not affected by TBHP. An increase in other cell types in the high dose group was indicated by the FACS analyses.

No information was provided regarding DNA damage in the upper respiratory tract where the most severe toxicological effects were observed. Determination of DNA damage in nasal and bronchial epithelial cells was not possible in the COMET assay using the techniques described in the feasibility study (Schulz, 2009).

4.8 Germ cell mutagenicity (Mutagenicity)

The information in this chapter is based on the RAR of TBHP plus the additional information available in the transitional report. The information in the RAR was available for the TC-C&L in their discussion in 2006 and 2007 (see Annex A and B). However, the information from the transitional report was not available for the TC-C&L. The main additional information regarding mutagenicity in the transitional report is the Comet assay in the lung.

The results of available genotoxicity studies are summarized in Table 4.7 for *in vitro* prokaryotic test systems, Table 4.8 for *in vitro* eukaryotic test systems, Table 4.9 for *in vivo* genotoxicity tests with insects, and Table 4.10 for *in vivo* genotoxicity tests with mammals.

Table 4.7

In vitro **prokaryotic test systems**

Type of test	Species	Method	Concentrations	Remarks	Results	Reference
Bacterial gene mutation	Salmonella typh. TA98, TA100, TA1537, Salmonella typh. TA1535, TA1538	Ames test	0 – 300 µg/plate 70% TBHP	-/+ S9 (Aroclor induced rat or hamster liver)	+ (+ S9) - (- S9) - (-/+ S9)	Haworth *et al.*, 1981; Haworth *et al.*, 1983; SIDS 1995
Bacterial gene mutation	Salmonella typh. TA98, TA100	Ames test	50 µg/plate 75% TBHP	+ S9 (PCB-induced rat liver)	+	Yamaguchi & Yamashita, 1980
Bacterial gene mutation	Salmonella typh. TA102	Ames test	2.5 mM TBHP	No metabolic activation	+	Minnunni *et al*., 1992
Bacterial gene mutation	Salmonella typh. MX100 TA102	Ames test	0.11 - 0.55 µmol/plate TBHP	No metabolic activation	+	Kranendonk *et al*., 1996
Bacterial gene mutation	Salmonella typh. TA102, TA2638A Escherichia coli WP2/pKM101, WP2 uvrA/pKM101	Ames test	20-1250 µg/plate TBHP	No metabolic activation	+	Watanabe *et al*., 1998
Bacterial gene mutation	several strains of Escherichia coli and Salmonella typh.	Ames test	5 - 100 µg/plate TBHP	No metabolic activation	+	Ohta *et al*., 2000
Bacterial gene mutation	Salmonella typh. TA102, TA2638A	Ames test	45-401 µg/plate TBHP*	No metabolic activation	+	Ryden *et al*., 2000
Bacterial gene mutation	several strains of Escherichia coli	Ames test	12.5 - 100 µg/plate TBHP	No metabolic activation	+	Blanco *et al*., 1998

Table 4.7

***In vitro* prokaryotic test systems**

Type of test	Species	Method	Concentrations	Remarks	Results	Reference
Bacterial gene mutation	Escherichia coli IC3789 and IC3821	Ames test	10 - 200 µg/plate TBHP	No metabolic activation	+	Blanco *et al*., 1995
Bacterial gene mutation	Escherichia coli IC188 and IC203	Ames test	25 - 100 µg/plate TBHP	IC188 only without and IC203 with and without metabolic activation	+ (with and without metabolic activation)	Martinez *et al*., 2000
Bacterial gene mutation	Salmonella typh. BA9 and BA13	L-arabinose forward mutation assay	55-222 nmol/ml	No metabolic activation	+	Ruiz-Rubio *et al*., 1985
Bacterial DNA damage	Salmonella typh. TA1535/pAQ1, TA1978/pAQ1	Measurement of repair endonucleases in supercoiled DNA	0 – 42 mM	No metabolic activation	+ (>10 mM)	Epe *et al.*, 1990
Bacterial DNA damage	Escherichia coli PQ 37 and PQ300	SOS-chromotest	1.8 - 460 µM	No metabolic activation	+	Muller and Janz, 1992
Bacterial DNA damage	Escherichia coli PQ 37	SOS-chromotest	0.3 mM	No metabolic activation	+	Mersch-Sundermann *et al.*, 1994
Bacterial DNA damage	Escherichia coli PQ 37	SOS-chromotest	0.3 mM	No metabolic activation	+	von der Hude *et al.*, 1988
Bacterial DNA damage	Escherichia coli KY946 KY945	SOS-chromotest	1 - 50 µg/ml	No metabolic activation	+ -	Nunoshiba and Nishioka, 1991
Bacterial DNA damage	Escherichia coli PQ 37	SOS-chromotest -/+ plasmid pKM101	0, 3, 10, 30 µg/plate*	No metabolic activation	+	Kato *et al*., 1994

* purity of TBHP not specified

Table 4.8
***In vitro* eukaryotic test systems**

Type of test	Species	Method	Concentrations	Remarks	Results	Reference
Recombination	Saccharomyces cerevisiae D4	Gene conversion	0 – 5.6 mM TBHP	No metabolic activation	+	Callen & Larson, 1978; Zimmermann *et al.*, 1984
Reverse mutation	Neurospora crassa	Back mutations at the adenine-less locus	89 mM TBHP	- S9	+	Dickey *et al.,* 1949; Brockman *et al.*, 1984; SIDS 1995
Forward gene Mutation	Mouse Lymphoma L5178Y	$TK^{+/-}$ assay 4 hour exposure with TBHP, expression time 48 hours	- S9: 1.3 – 18.0 ng/ml 70% TBHP + S9: 24 – 320 ng/ml 70% TBHP	-/+ S9 (Aroclor induced rat liver)	+ +	Kirby *et al.*, 1981; SIDS 1995
Chromosomal aberration	Chinese Hamster V79 cells	OECD 473-like, but 1h incubation with TBHP, and directly harvested thereafter	0.15, 0.2 and 0.5 mM TBHP	No metabolic activation	+	Ochi, 1989
Chromosomal aberration	CHO K-1 cell line	OECD 473; 3 hour incubation, harvesting time 21 hours	- S9: 1, 5, 10 µg/ml 70% TBHP + S9: 20, 30, 40 µg/ml 70% TBHP	-/+ S9 (Aroclor induced rat liver)	+ +	De Vogel, 1992; SIDS 1995
Chromosomal damage	Chinese hamster V79 lung cell line	Aneuploidy	34 µM TBHP	No metabolic activation	+	Önfelt, 1987
Cell transformation	C3H/10T½ CL8 cell line (derived from primary mouse embryo cells)	Transformation assay	0.0003 – 0.0049 µl/ml 70% TBHP	No metabolic activation	_	Thilagar *et al.*,1981; SIDS 1995
DNA base damage	SP2/0 derived murine hybridoma cells	Isotope-dilution mass spectrometry	0.01 – 10 mM TBHP	No metabolic activation	+ (0.01-0.1 mM TBHP) - (1.0 – 10 mM TBHP	Altman *et al.*, 1994

Table 4.8
***In vitro* eukaryotic test systems**

Type of test	Species	Method	Concentrations	Remarks	Results	Reference
DNA fragmentation	Rat hepatocytes	Fluorimetric analysis of alkaline DNA unwinding	0 – 0.5 mM TBHP		+	Latour *et al.*,1995
DNA strand breaks	Rat hepatocytes	Comet assay	250 µM during 20 minutes		+	Lee *et al*., 2004

Table 4.9

***In vivo* genotoxicity tests with insects**

Type of test	Species	Method	Concentrations	Remarks	Results	Reference
Dominant lethal mutation	Drosophila melanogaster, adults ♀ eggs	vapour immersed or in vapour	5 ml 50 Molar percent solution* various dilutions of 50 Molar percent solution*	Sifter technique	– +	Altenburg, 1954
Sex linked recessive lethal mutation	Drosophila melanogaster, adults	oral injection	2000 and 2500 ppm 100% TBHP in feed (nominal) 0.2 - 0.3 ul of 1000 and 2000 ppm 100% TBHP in water		– +	Woodruff e*t al.*, 1985; SIDS 1995
Reciprocal translocation	Drosophila melanogaster, adults	injection	0.2 - 0.3 ul of 1000 and 2000 ppm 100% TBHP in water		–	Woodruff *et al.*, 1985; SIDS 1995
Somatic mutation and recombination test	Drosophila melanogaster, adults	Oral	1, 2.5 and 5 mM TBHP in the medium	Toxic and highly toxic dose levels	+	Gaivao *et al*., 1999

*exact concentration not clear

Table 4.10

***In vivo* genotoxicity tests with mammals**

Type of test	Species	Method	Concentrations	Remarks	Results	Reference

Table 4.10

***In vivo* genotoxicity tests with mammals**

Type of test	Species	Method	Concentrations	Remarks	Results	Reference
DNA adducts (7-methylguanine and 8-methylguanine) in liver and stomach	Male Wistar rats	Single gavage	1 ml/kg bw (70% TBHP in unknown solvent)	It should be noted that this is a very high dose, exceeding the oral LD50.	+	Hix *et al*., 2000
Comet assay for DNA breaks and oxidized pyrimidines and altered purines	Male Fisher rats	Single subcutaneous injection at 2 hours before sacrifice	1.3 mmol/kg bw (117 mg/kg bw)	Liver cells Non-OECD Effects on other parameters (4.1.2.10) indicated that the TBHP had reached the liver.	_	Farombi *et al*., 2004
Bone marrow micronucleus test	Swiss mice ♀♂ (Charles River CD-1 strain)	Single intravenous injection	100 mg/kg bw 70% TBHP	OECD-474	_	Van Delft & de Vogel, 1995
Bone marrow aberration assay	Rats, ♀♂ adult Sprague-Dawley	5 day inhalation	9, 30, 74 and 94 ppm (= 0, 33, 111, 273 and 347 mg/m³ 100% TBHP)	OECD-like	_	Ben-Dyke and Hogan, 1981; SIDS, 1995
Bone marrow cytogenetic assay	Rats, ♂ Mongrel albino	Single inhalation Repeated inhalation (2.5 and 4 months)	118 and 360 mg/m³* 0, 17 and 107 mg/m³*	Study translated and limited data available	inconclusive	Katosova *et al.,* 1978; SIDS 1995
Dominant lethal assay	CFT-Swiss mice	5 day i.p. injection	300 µmol/kg bw/day (= 27 mg/kg bw/day 100% TBHP)	OECD-like	+	Kumar and Muralidhara, 1999
Dominant lethal assay	CFT-Swiss mice	5 day i.p. injection	300 µmol/kg bw/day (= 27 mg/kg bw/day 100% TBHP) 600 µmol/kg bw/day (= 54 mg/kg bw/day 100% TBHP)	OECD-like	+ +	Kumar *et al*., 2002

Table 4.10

***In vivo* genotoxicity tests with mammals**

Type of test	Species	Method	Concentrations	Remarks	Results	Reference
Sperm morphology assay	CFT-Swiss mice	5 day i.p. injection	300 µmol/kg bw/day (= 27 mg/kg bw/day 100% TBHP) 600 µmol/kg bw/day (= 54 mg/kg bw/day 100% TBHP)	Non-OECD	+ +	Kumar *et al*., 2002
Strand breaks in testis and epididymal sperm	CFT-Wistar rats	14 day i.p. injection	75 µmol/kg bw/day (= 6.8 mg/kg bw/day 100% TBHP) 150 µmol/kg bw/day (= 13.5 mg/kg bw/day 100% TBHP) 300 µmol/kg bw/day (= 27 mg/kg bw/day 100% TBHP)	Non-OECD	- + +	Kumar and Muralidhara, 2007
Dominant lethal mutations	Tetrahybrid mice ♂	Repeated inhalation (2 months)	2, 17 and 107 mg/m³*	Study translated and limited data available	inconclusive	Katosova *et al.,* 1978; SIDS, 1995
Dominant lethal assay	ICR/Ha Swiss mice	Single i.p. injection	15 and 75 mg/kg bw*	Insufficiently reported study	–	Epstein *et al.*,1972; SIDS, 1995
COMET assay in the lungs	Male Wistar rats	Repeated inhalation for 5 consecutive days during 6 hours per day	0, 20, 91 and 179/377 mg TBHP/m^3	The negative result may be caused by dilution as the main irritation of the lungs was only in the upper parts.	(-)	Ma-Hock et al., 2010a

* purity of TBHP not specified

4.8.1 Non-human information

4.8.1.1 *In vitro* data

In prokaryotic cells TBHP causes gene mutations (Haworth *et al*., 1981; Haworth *et al*.,1983; Yamaguchi and Yamashita, 1980, Ryden *et al.,* 2000, Blanco *et al*., 1995, Blanco *et al*., 1998, Minnunni *et al.,* 1992, Kranendonk *et al*., 1996, Watanabe et al., 1998; Ohta *et al*., 2000) and DNA damage (Epe *et al*., 1990; Mersch-Sundermann *et al*., 1994; von der Hude *et al*., 1988; Kato *et al.;* 1994, Nunoshiba and Nishioka, 1991, Muller and Janz, 1992).

In eukaryotic cells TBHP can induce gene mutations (Callen & Larson, 1978; Zimmermann *et al*., 1984; Dickey *et al*., 1949; Brockman *et al*., 1984; Kirby *et al*., 1981), chromosomal aberrations (Ochi, 1989; de Vogel, 1992) and changes in chromosome number (Önfelt, 1987). Also, TBHP can induce DNA strand breaks (Lee *et al*., 2004) and DNA fragmentation (Latour *et al*., 1995) in *in vitro* test systems.

In the study of Altman *et al*. (1994) treatment of mammalian cells with low concentrations of TBHP resulted in DNA base damage. However, high concentrations of TBHP inhibited DNA base damage. The authors suggest that this may be the result from scavenging of the intermediate tert.-butoxyl radical by TBHP giving rise to the formation of tert.-butyl peroxyl radical which is further oxidized. It indicates that TBHP exposure by itself may not directly cause the DNA base damage. Hazlewood and Davies (1995) showed with the technique of EPR spin trapping that the tert.-butoxyl radical generated from TBHP is capable of damaging DNA and RNA *in vitro*.

TBHP did not induce cell transformations in Chinese hamster cells (Thilagar *et al*., 1981).

4.8.1.2 *In vivo* data

Insects

Genotoxicity studies with Drosophila melanogaster indicate that TBHP *in vivo* can induce lethal mutations after exposure of the eggs to the vapour or dilutions of TBHP, but no mutagenic effect was detected when the males were treated in the adult stage (Altenburg, 1954). Sex–linked recessive lethal mutations in germ cells of Drosophila melanogaster after injection of the adults with TBHP indicate that TBHP can have a genetic effect (Woodruff et al., 1985). Also a mutagenic effect was found in the w/w+ SMART assay after oral exposure to toxic and highly toxic dose levels (Gaivao *et al.,* 1999).

Mammals

Fasted male Wistar rats (n=2-3 but not clearly stated) were treated by gavage with a single dose of TBHP (70%) at 1 ml/kg bw (approximately 700 mg/kg bw). The rats were sacrificed after 4 hours and samples of liver and stomach were collected for DNA isolation and analysis of 7-methylguanine and 8-methylguanine using HPLC with UV and electrochemical detection after acid hydrolysis. The 7-methylguanine levels in the stomach (135±15 ug/g guanine) were significantly increased compared to control rats (n=3)(85±10 ug/g guanine). No significant increase was found in the liver (n=2). 8-methylguanine was not detectable in the liver and stomach from control

animals. In treated rats, the 8-methylguanine level was higher in liver (63 ug/g guanine) compared to stomach (35±7 ug/g guanine) (Hix *et al.,* 2000). Other tissues were not tested. It should be noted that this is a very high dose, exceeding the oral LD50.

Groups of 6 male Fisher rats received a single subcutaneous injection with TBHP at a dose of 117 mg/kg bw (Farombi *et al*., 2004). The rats were sacrificed at 2 hours after injection. The Comet assay was performed on liver cells using no enzymatic digestion, digestion with endonuclease III to determine oxidized pyrimidines and digestion with formamidopyrimidine glycosylase to determine altered purines including 8-oxo guanine. No increase in tail DNA was seen with or without digestion of the liver cells. Effects on other parameters, namely increases in 2-amino adipic semialdehyde (a biomarker of protein oxidation) and malondialdehyde (a biomarker of lipid peroxidation) in liver, indicated that the TBHP had reached the liver. Numerical values are not available (only figures).

A bone marrow micronucleus test described by van Delft and de Vogel (1995) was performed according to OECD 474 guideline. Male and female Swiss mice (fifteen/sex/dose) were given a single intravenous dose of 100 mg/kg bw TBHP in saline, the maximum tolerated dose as determined in an earlier range-finding study. Intravenous injection of TBHP should result in adequate exposure of the bone marrow target cells. At 24, 48, and 72 hours after treatment, ten vehicle controls (five/sex) and ten test-animals (five/sex) were sacrificed. PCE:NCE ratios were not statistically significantly different in male mice treated with 70% TBHP than those found in vehicle controls. In female mice at 48 and 72 hours sacrifice times the PCE:NCE ratios were weakly significantly higher than those found in controls. At all sacrifice times, the incidences of micronucleated polychromatic erythrocytes per 1000 polychromatic erythrocytes in mice treated with TBHP were not increased, indicating that an intravenous injection of 100 mg/kg bw TBHP did not result in chromosomal damage and/or damage to the mitotic apparatus in bone marrow cells of mice.

In a bone marrow test described by Ben-Dyke and Hogan (1981) rats were exposed for five days to TBHP by inhalation (0, 33, 111, 273, and 347 mg/m³ 100% TBHP). Cytotoxicity of bone marrow cells was measured after exposure of the animals to 369 mg/m³ 100% TBHP using the mitotic index: in female rats the mitotic index increased from 2.2% after exposure to untreated air to 3.7% after exposure to 369 mg/m³ 100% TBHP, whereas in male rats a decrease was observed of 3.5% (control) to 2.8% (369 mg/m³ 100% TBHP). TBHP (up to and including 347 mg/m³ 100% TBHP) did not induce any chromosomal aberrations in rats of either sex.

A third bone marrow test is described by Katosova (1978). It should be noted that only a translation of this study is available and the description of the methodology and results are very limited. Mongrel albino male rats (no group sizes given) were exposed by inhalation to a single concentration of 118 or 360 mg/m³ TBHP, the latter stated by the authors as the toxic action threshold (purity of TBHP not specified). Another group of male mongrel albino rats were exposed by inhalation for a longer term (2.5 or 4 months) to 0 or 17 mg/m³ TBHP (stated by the authors as chronic action threshold) or 107 mg/m³ TBHP. In addition to the effects on chromosome aberrations the gonadal functions were studied. No effects of TBHP on spermatogenesis were observed but the findings were not substantiated by any numerical data. An increased number of chromosomal aberrations was found in rats exposed to the highest concentration of TBHP both after 2.5 months (4.6, 4.5, 9.0% cells with aberrations for the control, 17 and 107 mg/m³ TBHP, respectively) and 4 months (5.0, 5.4, and 8.5% cells with aberrations for control, 17 and 107 mg/m³ TBHP, respectively). Given the rapid metabolism of TBHP, the pattern of results obtained appears implausible. No details of general toxicity are given. Because of the fact that this study deviates

markedly from the OECD guideline 474, and the poor description of the methodology and results, the rapporteur considers this study as not valuable for risk assessment.

In a dominant lethal study (Kumar and Muralidhara, 1999) male Swiss mice were given intraperitoneal 300 µmol/kg bw for five days (= 27 mg/kg bw/day 100% TBHP, which is 1/10th of the determined LD_{50}-value in this study (see section 5.2.1). Fifteen males from each treatment group were mated with virgin females (1:2) each week sequentially for a period of 8 weeks. Successful mating was ascertained by the presence of vaginal plugs and all the pregnant females were killed 16-17 days thereafter. The percentage of induced pregnancies ranged from 83.3-100% (for TBHP and the positive control ethyl methane sulphonate) and was comparable to those of negative controls. Total number of implantations per male was similar after treatment with TBHP. A statistically significant decrease was found in the number of live embryos resulting from TBHP treated male mice during the first 4 weeks, and it normalized at week 5. TBHP treated males produced a 3- and 2-fold increase in dead implantations (DI) during the first and second week, respectively. Nearly a 4-fold increase of DI was observed during the third and fourth week compared to the control group.The numerical percentage of dominant lethality for week 1 to 5 were 8.6%, 8.6%, 11.3%, 8.5% and 8.5% for controls and 23.1%, 18.5%, 50.0%, 37.5% and 11.5% for TBHP. This was statistically significant from week 1 to 4 ($p<0.001$).

In a follow-up of the dominant lethal study by Kumar and Muralidhara (1999), male Swiss mice were given intraperitoneal 300 or 600 µmol/kg bw for five days (= 27 or 54 mg/kg bw/day 100% TBHP) or 150, 300 or 600 µmol/kg bw for one day (Kumar *et al*., 2002). Samples from testis and epididymal sperm were collected at 24 hour and 1, 2, 3 and 5 weeks after treatment. Males with repeated exposure were mated with untreated females for one week during five weeks. No effects were found on mortality, clinical toxicity, body weight, testis weight and testis histopathology at 1, 2, 3 and 5 weeks after repeated exposure. No or only a marginal increase in lipid peroxidation, as measured by the formation of thiobarbituric acid reactive substances, in testis and epididymal sperm cells was found at 24 hours. However, an increase in lipid peroxidation of approximately 30 to 40% was found at 24 hours and 1 week after repeated exposure at both dose levels. An increase of 15 to 20% was seen in week 2 and levels returned to control levels on week 3 and week 5. The percentage double stranded DNA measured using the fluorimatic analysis of DNA unwinding, was decreased in the testis at both dose levels at 2 hours after repeated exposure. In epididymal sperms, this effect was only seen at the highest dose. Caudal sperm counts were not affected by repeated treatment but an increase in abnormal sperms was seen during the first 3 weeks followed by a normalisation at week 5. Repeated treatment at both dose levels resulted in a 20-30% decrease in pups/litter after mating during the first three weeks but not in week 4 and 5. No numerical values available.

This study was recently followed by a study on the underlying biochemical mechanisms for the effects on the testis (Kumar and Muralidhara, 2007). However, in this study rats were used instead of mice. Groups of 4 male CFT-Wistar rats received a single intraperitoneal injection with 0, 6.8, 13.5 or 27 mg/kg bw TBHP. The rats were killed at 24 hours after exposure. In a second experiment, the male rats (n=4) were exposed daily to the same dose levels for 1 or 2 weeks. The rats were killed at 24 hours or 1 or 2 weeks after treatment. Single and repeated treatment did not induce mortality, clinical effects, body weight effects or testis weight effects. Also no histopathological changes were found in the testis and epididymis. Caudal sperm counts were significantly decreased at the highest dose level after 2 weeks of treatment. A single exposure did not affect the malondialdehyde level (MDA, a marker for lipid peroxidation) nor reactive Oxygen Species (ROS) in the testis. However, repeated exposure for 1 or 2 weeks induced a dose and

exposure duration dependent increase in MDA and ROS which were significant from 13.5 mg/kg bw. Comparable effects on both parameters were seen in epididymal sperms. The increase in ROS was also significant at the lowest dose after 2 weeks of exposure. The protein carbonyl and iron content of the testis was increased from 13.5 mg/kg bw after two weeks of exposure. The non-enzymatic antioxidant levels in the testis (GSH, ascorbic acid and alpha-tocopherol) were reduced. The reduction was significant at 13.5 mg/kg bw/day for ascorbic acid and alpha-tocopherol and at 27 mg/kg bw/day for GSH. The activity of 3 out of 5 tested antioxidant enzymes in the testis (GPX, GST and CAT) was increased after 2 weeks of treatment. The activity of GR(not specified) was reduced. The changes were significant for some endpoints already at the lowest dose level. The SOD activity was not affected. The activity of four different dehydrogenases (LDH-x, SDH, G6PDH and ICDH) was significantly increased at the highest dose level. The percentage of dsDNA, as determined by fluorimetric analysis of DNA unwinding, in testis and epididymal sperm showed a dose dependent decrease which was significant from 13.5 mg/kg bw/day, indicating increased strand breaks.

This study indicates that oxidative stress and DNA effects can occur after TBHP exposure to levels which also induce a change in some but not all enzymatic and non-enzymatic antioxidants. However, some effects like an increase of ROS and an increase in antioxidant enzyme activity were found at levels without a reduction in non-enzymatic antioxidant levels. These effects were seen at levels which did not induce effects on testis weight or testis histopathology.

A dominant lethal test is described by Katosova (1978). It should be noted that only a translation of this study is available and the description of the data is very limited. Male tetrahybrid mice (n=20/dose) were exposed by inhalation to 2, 17, or 107 mg/m³ TBHP (purity not specified) for two months, and mated with females. Incidence of pregnancies, total implantations and live embryos were determined. Number of pregnant females was 70 for the control group and 60 for each exposed group. The study authors state that in the group treated with 17 mg/m³ TBHP the embryonal mortality before implantation was increased (but not the embryonal mortality after implantation), and an exposure of 107 mg/m³ increased the postimplantation mortality of the embryos. However, the results given in the additional table could not be derived from the available raw data. The rapporteur considers this study not valuable for risk assessment.

In another dominant lethal study (Epstein et al., 1972), male ICR/Ha Swiss mice received a single intraperitoneal injection with subtoxic concentrations of 15 mg/kg bw (n=7) or 75 mg/kg bw (n=9) TBHP (purity not specified), and were subsequently caged with 3 untreated females, which were replaced weekly for 8 consecutive weeks. Females were sacrificed 13 days after the midweek of their caging, without being checked for vaginal plugs. The study authors report that no differences were observed in early foetal deaths and preimplantation losses, but no data are given.

Another study on the biochemical mechanism of the effects of TBHP on the testis was performed by Kaur *et al*. (2006). Male balb/c mice (n=6) were treated i.p. with a dose of 76 mg/kg bw/day (dosimetry confirmed by study authors in writtten correspondence) for 2 weeks and killed or bred to normal dams. Treatment significantly reduced the percentage of dams giving birth by 50% and the litter size in any dam giving birth as were epididymal sperm count and sperm motility. Lipid peroxidation (MDA) was significantly induced as were the enzyme activities for GSH-Px, GST and SOD. RNA levels, determined with PCR, of the stress related transcription factor NF-κB and of the anti-oxidant enzymes were increased. It is speculated that the effect on the sperm cells is directly or indirectly regulated by NF-κB. It is unclear whether the reduced fertility is due to a mutagenic

effect (resulting in non-viable embryos) or a direct effect on fertility (killing of sperm cells, resulting in no embryos at all) as no information on number of implantation sites was available.

The possibility of determining DNA damage via the COMET assay in nasal epithelium and epithelium of the upper airways was determined in a feasibility study by Schulz (2009). The technique used for cell isolation was based on several procedures described in the public literature. The epithelial layer was scraped from the nasal turbinates and septum and from the trachea using a scalpel. The collected tissues were incubated with residual turbinates and septum or trachea in several digesting enzymes. Only a small number of viable cells could be recovered from trachea. For nasal epithelium approximately $1 – 3.5 * 10^6$ viable cells could be isolated per rat. Single cell gel electrophoresis under alkaline conditions of nasal epithelial cells of untreated rats showed a limited percentage of cells with intact DNA. Approximately 44-49% Hedgehog cells were observed in the isolations with the highest viability of approximately 80%. The tail intensities of the non-Hedgehog cells were high and exceeded the expected values for tissue preparations from untreated animals. The values were in the range normally associated with positive controls. Therefore, it was concluded that it is not possible to isolate good quality cells from nasal epithelium and trachea of the rat for the performance of the COMET assay.

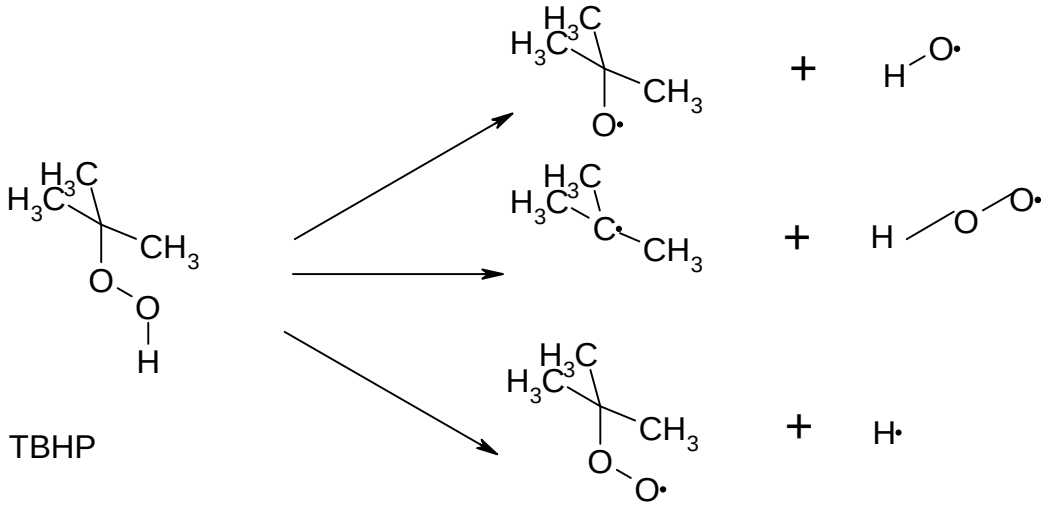
The COMET assay on lung tissues of rats exposed to TBHP via inhalation showed no increase in tail moment, length and relative intensity (see summary in 4.7.1.2). However, the main effects were observed in the upper respiratory tract with no changes at the alveolar level and some bronchiolar changes. This indicates that only a limited part of the TBHP reached the lower respiratory tract. The absence of effects in the Comet assay may therefore be caused by the very limited exposure and dilution of possible genotoxic effects with unaffected tissues. The Comet assay could not be performed using epithelial tissues from the nose and upper airways due to technical problems.

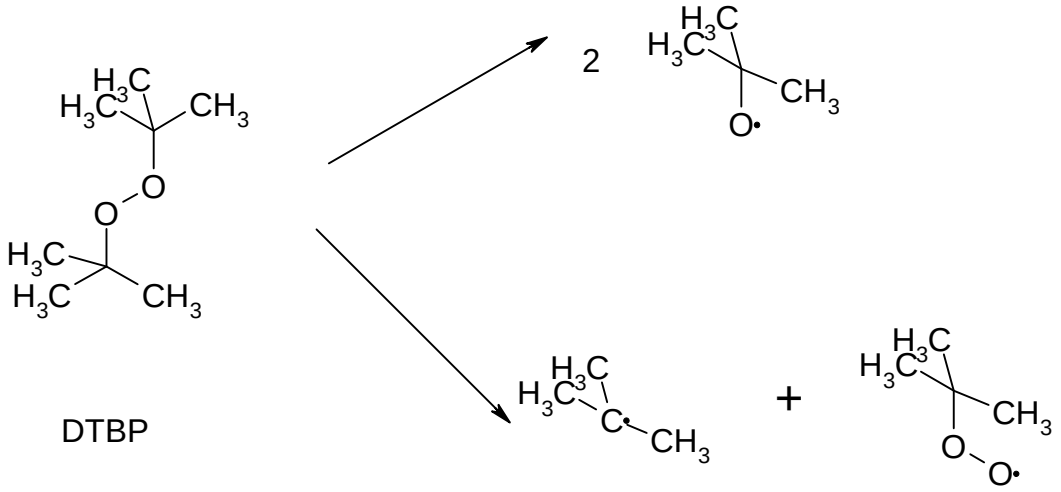
4.8.2 Human information

4.8.3 Other relevant information: read-across

The substance di-tert-butyl-peroxide (DTBP) has been discussed for harmonised classification with with Muta. Cat 3; R68 by the RAC. RAC concluded that classification as Muta Cat. 3; R68 was justified based on clear positive results in two well conducted *in vivo* micronucleus assays (RAC opinion 2010). It is assumed that the mutagenicity of DTBP is caused via the formation of radicals from DTBP. 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. The same radicals plus other radicals can be formed from TBHP (see figure 2). As all radicals that can be formed from DTBP can also be formed from TBHP, it can be assumed that TBHP can also induce mutagenicity. The absence of a positive result in the *in vivo* micronucleus assay with TBHP seems to contradict this conclusion. However, as TBHP is very unstable it probably cannot reach the bone marrow to induce micronuclei as the probably more stable DTBP can. However, TBHP is expected to have the same mutagenic effect as DTBP in cells which can be reached by TBHP.

Figure 2 Possible radicals of TBHP and DTBP





4.8.4 Summary and discussion of mutagenicity

Based on the positive effects in the bacteriological gene mutation tests, a positive result in a *tk*$^{+/-}$ assay with mammalian cells, and the fact that TBHP induces chromosomal aberrations and aneuploidy it is concluded that TBHP is mutagenic *in vitro*. Moreover, the fact that TBHP induces DNA base damage and DNA fragmentation indicates that TBHP is genotoxic *in vitro*.

The data set on genotoxicity of TBHP *in vivo* towards somatic cells is limited. Consequently, it is difficult to reach a conclusion on the genotoxicity *in vivo* of TBHP. The available *in vivo* studies indicate that TBHP induces DNA adducts in the liver and stomach after oral exposure to a dose exceeding the oral LD50. Since lower dose levels were not tested it is impossible to make a

statement on this effect at lower levels. Therefore, the worst case assumption is made that mutagenicity will occur at all dose levels including the levels to which humans are exposed.

Other *in vivo* data show that TBHP does not induce chromosomal aberrations in bone marrow *in vivo* and was negative in several other tests on the bone marrow as well. A limited Comet assay in rat liver after subcutaneous exposure was negative.

TBHP induces dominant and recessive lethal mutations in Drosophila when eggs are exposed or adults are injected, but no mutagenic activity is detected in adults upon oral exposure or exposure by inhalation. TBHP is positive in a dominant lethal assay in mice after intraperitoneal exposure and induces changes in sperm morphology. Comparable effects on fertility were found in additional tests on rats and mice after intraperitoneal exposure. This could be a local effect of TBHP on the testis because substances can migrate from the abdominal cavity through the inguinal channel to the testis.

The substance di-tert-butyl-peroxide (DTBP) was shown to be mutagenic to the bone marrow in an *in vivo* assay. As DTBP forms only radicals also formed by TBHP, it is likely that TBHP is also mutagenic.

The ADME study (de Bie and Grossouw, 2004, see paragraph 4.1.1) shows that TBHP is rapidly converted *in vivo* to 2-methylpropan-2-ol. After intravenous injection, no TBHP but mainly 2-methylpropan-2-ol was found in blood at the earliest measurement of 15 minutes after injection. Also after subcutaneous injection, no TBHP but mainly 2-methylpropan-2-ol was found in blood and tissues at the earliest measurement of 2 hours after injection. Based on the rapid conversion of TBHP to 2-methylpropan-2-ol after parenteral administration, no detectable levels of TBHP will also be expected after oral, dermal and inhalatory exposure due to the slower absorption and the first pass effect in the liver after oral exposure. 2-Methylpropan-2-ol was tested for mutagenicity by the NTP in 1995 and all *in vitro* and *in vivo* results were negative.

As mentioned above, TBHP is clearly genotoxic and mutagenic *in vitro*. TBHP was negative in several mutagenicity tests in the bone marrow. However, seen the rapid conversion of TBHP to the non-mutagenic compound 2-methylpropan-2-ol, it is very likely that TBHP did not reach the bone marrow. There are no local mutagenicity tests with TBHP available. TBHP is mutagenic in germ cells after *in vivo* exposure (changes in sperm morphology and an increase in dominant lethal mutations) after intraperitoneal exposure. This positive rodent dominant lethal mutation test would normally fulfil the criteria for classification in category 1B. The *in vivo* mutagenicity of TBHP is confirmed by the positive *in vivo* results with DTBP. However, it is unlikely that TBHP will reach the gonads through relevant routes of exposure in view of the rapid conversion to 2-methylpropan-2-ol. Therefore, the positive results of these germ cell tests are considered evidence for a local mutagenic effect. Consequently, the *in vivo* mutagenicity of TBHP through relevant routes is likely limited to somatic cells in the tissues of first contact and could possibly result in local carcinogenicity. The conclusion is that TBHP is mutagenic. However, as TBHP will not reach the germ cells after oral, inhalation and dermal exposure, exposure to TBHP is unlikely to result in inheritable genetic damage.

The mutagenic effects of TBHP are probably due to the formation of TBHP-derived radicals after one-electron oxidation or one-electron reduction and their reaction with DNA. This mechanism would theoretically lead to no threshold for the mutagenicity. However, radical formation and their reaction with DNA will probably depend on the antioxidant levels of the cell, with an increase in DNA adducts at TBHP levels which induce a reduction in the antioxidant levels. This would indicate a sub-linear dose-effect relation but could also indicate a threshold. No information is

available on the dose-effect relation within the sites of first contact. A study to determine the feasibility of the COMET assay to detect genotoxicity in epithelial cells of the nose and trachea showed that it is not possible to isolate good quality cells. The available studies on the testis after intraperitoneal exposure indicate that DNA effects were found at or around TBHP levels which also reduce the antioxidant level but at levels without histological changes. However, an increase in ROS and the activity of enzymatic antioxidants (which can be seen as secondary to the increase in ROS) was found at levels without a decrease in non-enzymatic antioxidants like GHS. The limited studies on the testis do not provide sufficient evidence that the formation of free radicals and possible DNA effects including mutations cannot occur at levels without a reduction in non-enzymatic antioxidants, nor do the *in vivo* metabolism data (e.g. de Bie and Grossouw (2004)) exclude the occurrence of radical formation before glutathione is depleted. Further, no information is available on the extrapolation to other tissues including the sites of first contact. Based on the available data it is assumed that TBHP is a non-threshold mutagen.

4.8.5 Comparison with criteria

Classification with Muta 1A; H340 is not justified as there are no human data. Classification with Muta 1B; H340 could be considered as TBHP is positive in a rodent dominant lethal mutagenicity test. However, this test was positive after intraperitoneal exposure whereas the kinetic data show that TBHP does not reach the systemic circulation, and thus does not reach germ cells, after oral, inhalation and dermal exposure. Classification with Muta 1B; H340 is not justified because TBHP will not induce germ cell mutagenicity via normal routes of exposure. However, classification with Muta 2; H341 is proposed because it is shown in the dominant lethal mutagenicity test that TBHP is mutagenic to cells with which it comes into direct contact. Classification of local mutagens as Cat 2 is also in line with the guidance in chapter 3.5.1 of the Guidance on the application of Regulation (EC) No 1272/2008. Classification with Muta Cat. 3; R68 was recommended by the TC-C&L.

4.8.6 Conclusions on classification and labelling

TBHP is a mutagen. However, the substance will only be mutagenic at the sites of first contact in somatic cells. Classification with Muta 2; H341 (CLP) is proposed.

4.9 Carcinogenicity

Not relevant

4.10 Toxicity for reproduction

Not relevant

4.11 Other effects

Not relevant

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not relevant

6 OTHER INFORMATION

Not relevant

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8 ANNEXES

Annex A Extract from the Summary record of the TC-C&L meeting of October 2006.

L015	**TBHP; Tert-butyl hydroperoxide (NL)** *Issue for discussion: Complete classification proposal for new Annex I entry*	EC:200-915-7 CAS: 75-91-2	**ECBI/03/06 Rev. 2,** Add.1 Rev. 1, 2, 3, 4, 5, 7 **and 8** ECBI/87/06

Currently not in Annex I.

Classification proposal: O; R7, R10, Xn; R21/22, T; R23,C; R34, R43, Muta Cat3; R68, N; R51/53

Documents:

ECBI/03/06 Rev. 2	**NL, Rev. 2 C&L proposal**
ECBI/03/06 Add.1 Rev. 1	**NL, RAR**
ECBI/03/06 Add. 2	**IND, Comment**
ECBI/03/06 Add. 3	**D, Comment**
ECBI/03/06 Add. 4	**D, Comment**
ECBI/03/06 Add. 5	**F, Comment**
ECBI/03/06 Add. 7	**NL, response to comments to RAR**
ECBI/03/06 Add. 8	**NL, response to comments to C&L proposal**
ECBI/87/06	**F, Comment**

The Chair introduced the classification proposal from NL, and mentioned the comments from D and F received prior the meeting.

NL said that the first issue to discuss was the actual name of the substance since it was mentioned in a comment that the substance containing less than 30% of water should also be classified as explosive. However, the substance was not marketed in such a form. The TC C&L agreed that the

substance will be covered in a single entry namely "Tert-butyl hydroperoxide (containing > 30% water)".

The TC C&L then agreed to classify TBHP with O; R7 and R10 and to apply also S43

IND asked whether in regard to inhalational toxicity the values obtained should be re-calculate to 100% of the substance **NL** answered that that was not necessary since it was the vapour that was tested. **IND** after reflecting agreed to the proposed classification with T; R23 that was then agreed by the TC C&L.

Also Xn; R21-22 and R34 and the application of R37 with SCLs at 10 % were agreed by **the TC C&L.**

NL reported that there was a negative Buehler test and a positive GPMT. Thus they proposed R43 and based on the potency considerations also SCLs with C ≥ 0.1% (instead of the 1% default value).
.

UK and **B** said that they were not so sure to support specific concentration limits as suggested by the NL. **UK** also stressed that the guidance document for potency of sensitisation (which was employed by when proposing SCLs the NL) had not formally been adopted by the TC C&L. **D** did not agree to the application of SCLs. While the GMPT indicated a borderline effect up to strong sensitization the Buehler test was negative. **NL** responded to that saying that in the expert report (draft guidance) it was clearly stated that in case of differing results from different tests the most severe result should be used.

The Chair then asked all other MS to give their opinion on the issue and a majority agreed to the application of SCLs for R43 as proposed by NL.

NL further went on saying that while for repeat dose effects there was no classification warranted based on data there were clearly positive in vitro mutagenicity tests available. *In vivo* the results were mainly negative. Thus **NL** suggested Muta. Cat. 3; R68. **D,** however, was of the opinion that the *in vivo* data did not support a classification for mutagenicity. **F** and **UK** agreed to Muta. Cat. 3; R68 adding that possibly the in vivo test were negative because of the site where the substance was applied. There was a data gap regarding *in vivo* mutagenicity tests. The in vivo tests were done with high doses. A test with skin application of the substance to the skin would have to be done in order to get a clear picture. After an extensive summary given by **IND** on the mutagenicity database and the reasons for their proposal not to classify TBHP for mutagenicity (their arguments can be found in ECBI/03/06 Add. 2) the **Chair** asked all MS to give their opinion on the issue and a majority agreed to the application of Muta. Cat. 3; R68. **UK** suggested because of the split opinions on that matter to give the TC C&L further possibility to reflect on the issue. Following that suggestion the

Chair stated that the recommendation taken was only provisional and that it was still possible to come back to the issue within the follow-up period.

The TC C&L furthermore agreed without discussion to follow the **NL** proposals not to classify for carcinogenicity (based on lack of data), fertility and development.

Conclusion:

The TC C&L agreed that the name of the substance should be "TBHP in 30% water" and that a splitting of entries (suggested by DE) was not necessary since the substance was marketed only in this form.

Although a majority of the TC C&L agreed to apply Muta. Cat. 3; R68 the recommendation is only provisional in order to give MS the time to reflect further on the issue within the follow up period.

Follow-up:

BE sent in a written comment where they favoured no classification for mutagenicity. NL in response to that submitted document ECBI/03/06 Add. 9 (still supporting Muta Cat. 3 R68). **DE** reiterated also their position.**UK** suggested further discussion of the issue. It was concluded to re-discuss mutagenicity at the meeting in March 2006.

Annex B Follow-up III of the TC-C&L of September 2007

(no summary records exist of this meeting)

L015 **TBHP; Tert-butyl hydroperoxide [(containing > 30% water)] (NL)** **CAS: 75-91-2** **EC No: 200-915-7** **Not in Annex I**	*In October 2006* the substance was discussed for the first time based on the NL proposal. NL had sent in a second revision of their C&L proposal (ECBI/03/06 Rev.2) and reactions to the written comments received during the preparation period in ECBI/03/06 Add. 8. At the meeting in October 2006 the TC C&L agreed that the name of the substance should be "TBHP in 30% water" and that a splitting of entries (suggested by D) was not necessary since the substance was marketed only in this form. Although a majority of the TC C&L agreed to apply Muta. Cat. 3; R68 the recommendation is only provisional in order to give MS the time to reflect further on the issue within the follow up period. All other endpoints were agreed as proposed by the NL rapporteur.
Classification: O; R7 *Agreed* *1006* R10 *Agreed* *1006* Muta. Cat. 3; R68 *Agreed* *0907* T; R23 *Agreed* *1006* Xn; R21/22 *Agreed* *1006* C; R34 *Agreed* *1006* R43 *Agreed* *1006* N; R51-53 *Agreed* *0406* **Specific concentration limits:**	*Member States not agreeing to Muta. Cat. 3; R68 were asked to react during Follow-up period else the provisionally classification will be regarded as a final classification proposal from the TC C&L.* **BE** did not support classification with R68 because, typically the substance is only a local mutagen and not a systemic mutagen. It is not the first time we have such a case: for example, the pesticide dichlorvos is also a local mutagen and not a systemic mutagen and it is not classified for mutagenicity. Could it be possible to raise the question in the pesticide group (meeting November)? It seems us very important to have the same approach in the two groups when classifying substances to avoid any inconsistency. **NL** responded to the BE comment in document ECBI/03/06 Add. 9 The **DE** position for not to classify with R68 is still the same and was explained in written before.

Xi; R37: 5% ≤ C < 10% *Agreed 1006* **R43: C ≥ 0.1%** ***Agreed 1006*** **Labelling:** O, T, N R: 7-10-21/22-23-34-43-68-~~50~~51/53 S: 3/7-14-26-36/37/39-45-~~60~~61 **Classification assigned in accordance with the CLP Regulation:** Org. Perox. EF; H242 Flam. Liq. 3; H226 Muta. 2; H341 Acute Tox. 2; H330 Acute Tox. 3; H311 Acute Tox. 4; H302 Skin Corr. 1C; H314 Skin Sens. 1; H317 Eye dam. 1; H318 Aquatic Chronic 2; H411 **Specific concentration limits:** Skin Sens. 1; H317: C ≥ 0.1% (Xi; R37: 5% ≤ C < 10%] Translation of this SCL not necessary as the new GCL for Corrosive substances is 5 %.)	**UK** agreed with BE that it is important to ensure consistency in classification in these cases. TBHP is an in vitro mutagen but has tested negative in standard in vivo tests. The concern, leading to R68, was that because of the reactivity of TBHP these negative findings may be a false negative due to insufficient exposure of the tissues examined (bone marrow) and TBHP might still be a mutagen at the local site of contact (e.g. in the lungs following inhalation or skin) and given that local mutagenicity has not been investigated this is a remaining concern. It may be appropriate to have a discussion on the general issue of how such substances should be classified for mutagenicity. **NL** sent a revised proposal for TBHP, including the GHS classification in document ECBI/03/06 Rev. 3. The rationale for the presented GHS classification can be found there. In addition NL proposes to use STOT 1 or 2 for "Corrosive to the respiratory tract". Furthermore NL confirmed that the GHS classification should be Org. Perox. EF for O; R7. **DK** sent their position in support of classification mutagenicity in document ECBI/03/06 Add. 10 distributed with Rev. 2 of the September agenda. In addition they suggest classifying with Carc. Cat. 3; R40. **Conclusion Follow-up:** Based on the comments by BE, DK, DE and UK, mutagenicity should be re-discussed at the September 2007 meeting. *MS were invited to send further comments/positions within the deadlines for the September meeting to facilitate the discussions. In addition MS were asked to react in written prior the meeting in case they supported to further discuss carcinogenicity as suggested by DK.* There was no additional support for further discussion of carcinogenity. NL presented their position on mutagenicity and carcinogenicity together with a summary of new studies in document ECBI/03/06 Add. 11 distributed with Revision 5 of the agenda. They support Muta.Cat.3; R68 (and Muta. 2 H341) and state that the data available is insufficient for classification for carcinogenicity. ***In September 2007*** the TC C&L agreed to confirm the provisional classification for Muta. Cat. 3; R68 (Muta. 2 H341) from the last meeting, and not to classify with STOT 1 or 2 under the CLP Regulation as the

	effects were already covered by the agreed classification. **NL:** There is a difference in the follow-up document I for the environmental classification between the classification and the labelling. Could you please check this? Does this affect the S-sentences? **ECB**: Yes, thanks this is correct. It should be R51-53 and S61. ⇨ **Next ATP**

Annex C. Overview of the self-classification according to CLP according to the C&L inventory.

Notified classification and labelling according to CLP criteria

Classification		Labelling			Specific Concentration limits, M-Factors	Notes	Number of Notifiers	Joint Entries	View
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms Signal Word Code(s)					
Not Classified							181		
Flam. Liq. 3	H226	H226		GHS02 GHS06 GHS09 GHS05 Dgr			86		
Org. Perox. C	H242	H242							
Acute Tox. 4	H302	H302							
Acute Tox. 3	H311	H311							
Skin Corr. 1B	H314	H314							
Eye Dam. 1	H318	H318							
Acute Tox. 2	H330	H330							
Aquatic Chronic 2	H411	H411							
Flam. Liq. 3	H226	H226		GHS01 Dgr			60		
Org. Perox. C	H242	H242							
Acute Tox. 4	H302	H302							
Acute Tox. 4	H312	H312							

Skin Corr. 1B	H314	H314							
Skin Sens. 1	H317	H317							
Eye Dam. 1	H318	H318							
Acute Tox. 2	H330	H330							
Aquatic Chronic 2	H411	H411							
Flam. Liq. 3	H226	H226		GHS02 GHS05 GHS03 Dgr			52		
Ox. Liq. 1	H271	H271							
Acute Tox. 4	H302	H302							
Acute Tox. 4	H312	H312							
Skin Corr. 1B	H314	H314							
Acute Tox. 3	H331	H331							
Flam. Liq. 3	H226	H226		GHS02 Wng			51		
Flam. Liq. 3	H226	H226		GHS06 GHS05 GHS03 Dgr			38		
Ox. Liq. 3	H272	H272							
Acute Tox. 4	H302	H302							
Acute Tox. 4	H312	H312							
Skin Corr. 1B	H314	H314							
Skin Sens. 1	H317	H317							

Eye Dam. 1	H318								
Acute Tox. 4	H332	H332							
Aquatic Chronic 2	H411	H411							
Skin Sens. 1	H317	H317		GHS07 Wng			35		
Flam. Liq. 3	H226	H226		GHS02 GHS06 GHS09 GHS05 GHS08 Dgr			30		
Self-react. F	H242	H242							
Acute Tox. 4	H302	H302							
Acute Tox. 3	H311	H311							
Skin Corr. 1B	H314	H314							
Skin Sens. 1	H317	H317							
Acute Tox. 4	H332	H332							
Muta. 2	H341	H341							
Aquatic Chronic 2	H411	H411							
Flam. Liq. 3	H226	H226		GHS06 GHS02 GHS09 Dgr			28		
Acute Tox. 4	H302	H302							
Acute Tox. 3	H311	H311							
Acute Tox. 2	H330	H330							
Aquatic Chronic 2	H411	H411							

Flam. Liq. 3	H226	H226		GHS02 GHS06 GHS09 GHS05 Dgr			26		
Org. Perox. F	H242	H242							
Acute Tox. 4	H302	H302							
Acute Tox. 3	H311	H311							
Skin Corr. 1C	H314	H314							
Skin Sens. 1	H317	H317							
Eye Dam. 1	H318								
Acute Tox. 2	H330	H330							
Aquatic Chronic 2	H411	H411							
Flam. Liq. 3	H226	H226		GHS02 GHS06 GHS09 GHS05 GHS08 Dgr			23		
Org. Perox. F	H242	H242							
Acute Tox. 4	H302	H302							
Acute Tox. 3	H311	H311							
Skin Corr. 1B	H314	H314							
Skin Sens. 1	H317	H317							
Acute Tox. 2	H330	H330							
Muta. 2	H341	H341							
Aquatic Chronic 2	H411	H411							

		H302+H312+H332		GHS07 GHS02 GHS09 GHS05 Dgr			19		
Flam. Liq. 3	H226	H226							
Org. Perox. F	H242	H242							
Acute Tox. 4	H302								
Acute Tox. 4	H312								
Skin Corr. 1B	H314	H314							
Skin Sens. 1	H317	H317							
Acute Tox. 4	H332								
Aquatic Chronic 2	H411	H411							
Flam. Liq. 3	H226	H226		GHS07 GHS02 GHS06 GHS09 GHS05 Dgr			9		
Self-react. C	H242	H242							
Acute Tox. 4	H302	H302							
Acute Tox. 4	H312	H312							
Skin Corr. 1B	H314	H314							
Acute Tox. 2	H330	H330							
Aquatic Chronic 2	H411	H411							
Flam. Liq. 3	H226	H226		GHS07 GHS02 GHS09			8		
Org. Perox. C	H242	H242							
Acute Tox. 4	H302	H302							

Acute Tox. 4	H312	H312		GHS05 Dgr					
Skin Corr. 1B	H314	H314							
Skin Sens. 1	H317	H317							
Acute Tox. 4	H332	H332							
Aquatic Chronic 2	H411	H411							
Flam. Liq. 3	H226	H226		GHS02 GHS06 GHS09 GHS05 GHS08 Dgr	Skin Sens. 1: C ≥ 0,1%		5		
Org. Perox. F	H242	H242							
Acute Tox. 4	H302	H302							
Acute Tox. 3	H311	H311							
Skin Corr. 1C	H314	H314							
Skin Sens. 1	H317	H317							
Eye Dam. 1	H318								
Acute Tox. 2	H330	H330							
Muta. 2	H341	H341							
Aquatic Chronic 2	H411	H411							
Flam. Liq. 2	H225	H225		GHS01 GHS06 GHS09 GHS05 GHS08			4		
Org. Perox. A	H240	H240							
Acute Tox. 4	H302	H302							
Acute Tox. 3	H311	H311							

Skin Corr. 1B	H314	H314		Dgr					
Skin Sens. 1	H317	H317							
Eye Dam. 1	H318	H318							
Acute Tox. 2	H330	H330							
Muta. 2	H341	H341							
Aquatic Chronic 2	H411	H411							
		H311		GHS02 GHS06 GHS05 Dgr			2		
		H226							
		H314							
		H242							
		H331							
		H302							
		H412							
Flam. Liq. 3	H226	H226		GHS02 GHS06 GHS05 Dgr			2		
Org. Perox. C	H242	H242							
Acute Tox. 4	H302	H302							
Acute Tox. 3	H311	H311							
Skin Corr. 1B	H314	H314							
Acute Tox. 4	H332	H332							

Aquatic Chronic 3	H412	H412							
Flam. Liq. 3	H226	H226		GHS02 GHS06 GHS09 GHS05 GHS08 Dgr	Eye Dam. 1: C ≥ 1% STOT SE 3: 5% ≤ C ≤ 10% Skin Sens. 1: C ≥ 0,1%		2	✔	
Org. Perox. F	H242	H242							
Acute Tox. 4	H302	H302							
Acute Tox. 3	H311	H311							
Skin Corr. 1C	H314	H314							
Skin Sens. 1	H317	H317							
Eye Dam. 1	H318								
Acute Tox. 2	H330	H330							
Muta. 2	H341	H341							
Aquatic Chronic 2	H411	H411							
		H334		GHS07 GHS01 GHS05 Dgr			1		
		H312							
		H241							
		H200							
		H332							
		H302							
Flam. Liq. 3	H226	H226		GHS02 GHS06			1		
Acute Tox. 4	H302	H302							

Acute Tox. 3	H311	H311		GHS05 Dgr					
Skin Corr. 1B	H314	H314							
Acute Tox. 2	H330	H330							
Flam. Liq. 3	H226	H226		GHS02 GHS06 GHS05 GHS08 Dgr			1		
Org. Perox. F	H242	H242							
Acute Tox. 4	H302	H302							
Acute Tox. 3	H311	H311							
Skin Corr. 1B	H314	H314							
Skin Sens. 1	H317	H317							
Eye Dam. 1	H318								
Acute Tox. 2	H330	H330							
Muta. 2	H341	H341							
Flam. Liq. 3	H226	H226		GHS02 GHS06 GHS09 GHS05 Dgr			1		
Org. Perox. F	H242	H242							
Acute Tox. 4	H302	H302							
Acute Tox. 3	H311	H311							
Skin Corr. 1B	H314	H314							
Skin Sens. 1	H317	H317							
Acute Tox. 4	H332	H332							

Aquatic Chronic 2	H411	H411							
Flam. Liq. 3	H226	H226		GHS02 GHS06 GHS09 GHS05 GHS08 Dgr			1		
Org. Perox. C	H242	H242							
Acute Tox. 4	H302	H302							
Acute Tox. 3	H311	H311							
Skin Corr. 1C	H314	H314							
Skin Sens. 1	H317	H317							
Eye Dam. 1	H318								
Acute Tox. 2	H330	H330							
STOT SE 3	H335	H335							
Muta. 2	H341	H341							
Aquatic Chronic 2	H411	H411							
Flam. Liq. 3	H226	H226		GHS01 GHS06 GHS09 GHS05 GHS08 Dgr			1		
Org. Perox. A	H240	H240							
Acute Tox. 4	H302	H302							
Acute Tox. 3	H311	H311							
Skin Corr. 1C	H314	H314							
Skin Sens. 1	H317	H317							
Eye Dam. 1	H318	H318							

Acute Tox. 2	H330	H330							
Muta. 2	H341	H341							
Aquatic Chronic 2	H411	H411							

Number of Aggregated Notifications: 25