European Union Risk Assessment Report

TERTIARY BUTYL HYDROPEROXIDE

CAS No: 75-91-2

EINECS No: 200-915-7

RISK ASSESSMENT

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RISK ASSESSMENT

May, 2008

The Netherlands

Rapporteur for the risk assessment of TERTIARY BUTYL HYDROPEROXIDE is the Ministry of Housing, Spatial Planning and the Environment (VROM) in consultation with the Ministry of Social Affairs and Employment (SZW) and the Ministry of Public Health, Welfare and Sport (VWS). Responsible for the risk evaluation and subsequently for the contents of this report, is the rapporteur.

The scientific work on this report has been prepared by the Netherlands Organization for Applied Scientific Research (TNO) and the National Institute of Public Health and the Environment (RIVM), by order of the rapporteur.

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Final report: **2008**

Foreword

This Draft Risk assessment Report is carried out in accordance with Council Regulation (EEC) 793/93[1] on the evaluation and control of the risks of "existing" substances. "Existing" substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.

There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.

The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94[2], which is supported by a technical guidance document[3]. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a Meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) which gives its opinion to the European Commission on the quality of the risk assessment.

This Draft Risk Assessment Report is currently under discussion in the Competent Group of Member State experts with the aim of reaching consensus. During the course of these discussions, the scientific interpretation of the underlying scientific information may change, more information may be included and even the conclusions reached in this draft may change. The Competent Group of Member State experts seek as wide a distribution of these drafts as possible, in order to assure as complete and accurate an information basis as possible. The information contained in this Draft Risk Assessment Report does not, therefore, necessarily provide a sufficient basis for decision making regarding the hazards, exposures or the risks associated with the priority substance.

This Draft Risk Assessment Report is the responsibility of the Member State rapporteur. In order to avoid possible misinterpretations or misuse of the findings in this draft, anyone wishing to cite or quote this report is advised to contact the Member State rapporteur beforehand.

Contact Details of the Rapporteur(s)

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[1] O.J. No L 084, 05/04/199 p.0001 – 0075

[2] O.J. No L 161, 29/06/1994 p. 0003 – 0011

[3] Technical Guidance Document, Part I – V, ISBN 92-827-801 [1234]

OVERALL RESULTS OF THE RISK ASSESSMENT[4]

CAS Number: 75-91-2
EINECS Number: 200-915-7
IUPAC Name: Tert-butyl hydroperoxide

Environment

<mark>[keep only appropriate conclusion(s)]</mark>

Conclusion (i)   There is a need for further information and/or testing.

Conclusion (ii)   There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion ( ) applies to [click here to insert text in accordance with conclusion(s)]

Human health

Human health (toxicity)

Workers

Conclusion (i)   There is a need for further information and/or testing.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (i) is reached because:
local carcinogenicity can not be excluded as TBHP is considered mutagenic to the sites of first contact and useful data on the potential local carcinogenic effects of TBHP are not available. This conclusion applies to all scenarios.

Conclusion (iii) is reached because:
- systemic effects cannot be excluded after acute inhalation exposure in the scenarios 'Production and use of TBHP containing hardeners of plastics' and 'Use of products containing <1% TBHP';
- respiratory tract irritation cannot be excluded after inhalation exposure in all scenarios;
- skin sensitisation cannot be excluded after dermal exposure in all scenarios;
- systemic effects cannot be excluded after repeated inhalation exposure in the scenario 'Use of products containing <1% TBHP - Manual application and cleaning of equipment';

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4   Conclusion (i)   There is a need for further information and/or testing.
    Conclusion (ii)   There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
    Conclusion (iii)   There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

- mutagenic effects after dermal and inhalation exposure cannot be excluded in all scenarios.

It might be possible that in some workplaces adequate worker protection measures are already being applied.

Consumers

Not applicable because there is no consumer exposure.

Humans exposed via the environment

Conclusion (i)   There is a need for further information and/or testing.

Conclusion (ii)   There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (iii)   There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (i) is reached because:
local carcinogenicity can not be excluded as TBHP is considered mutagenic to the sites of first contact and useful data on the potential local carcinogenic effects of TBHP are not available. This applies to all local sites and for the regional scenario.

Conclusion (ii) applies to the endpoint reproductive toxicity for all local sites and for the regional scenario, and to the endpoint repeated dose toxicity for the sites not mentioned below.

Conclusion (iii) applies to the endpoint mutagenicity for all local sites and for the regional scenario, and to the endpoint repeated dose toxicity for the local sites I-c and II-b2.

Combined exposure

A risk characterisation for combined exposure was not performed because the conclusions already made for each scenario will not be changed by adding the exposure via the environment.

Human health (physico-chemical properties)

Conclusion (ii)   There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

TBHP is flammable and oxidizing and is labelled with respect to these physico-chemical properties. However, it is assumed that existing controls (i.e., engineering controls and personal protective equipment based on classification and labelling with R7 and R10) are applied for exposure situations. Therefore, in the case that engineering controls and personal protective equipment are effectively used, it is concluded that TBHP is of no concern with

regard to physico-chemical properties (**conclusion ii**). There is no need for further information and/or testing.

CONTENTS

EUSES Calculations can be viewed as part of the report at the website of the European Chemicals Bureau:
http://ecb.jrc.it

TABLES

1   GENERAL SUBSTANCE INFORMATION

1.1   IDENTIFICATION OF THE SUBSTANCE

CAS-No.:   75-91-2
EINECS-No.:   200-915-7
IUPAC name: tert-Butyl hydroperoxide
Synonyms: TBHP, 2-Hydroxyperoxy-2-methylpropane, (1,1-)Dimethylethyl
                  hydroperoxide, tert-Butyl hydrogen peroxide,
Trade names: TBHP-70 (T-Hydro), Cadox TBH, Trigonox AW70, Perbutyl H
Molecular formula: $C_4H_{10}O_2$
Structural formula:
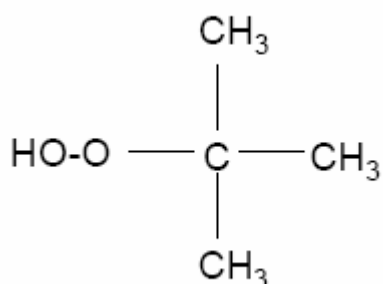
Molecular weight: 90.1

1.2   PURITY/IMPURITIES, ADDITIVES

Purity/impurities and additives for TBHP-70 (T-Hydro):

Purity: 68.4-69.6%

Impurity:	2-Methylpropano-2-ol	< 0.5%
	Dialkyl peroxide	≤ 0.1%
	Ketones	≤ 0.2%
	Other hydroperoxides	≤ 1%
	Other organics	≤ 0.4%
Additives:	Water	≤ 30%

1.3   PHYSICO-CHEMICAL PROPERTIES

In **Table 1.1** the physico-chemical properties for 70% THBP and, where indicated, for 100% TBHP are summarised.

Table 1.1 Summary of physico-chemical properties of 70% TBHP and, where indicated, of 100% TBHP

Property	Value / result	Note	References
Physical state	liquid		
Melting point	-8 to -3°C 3 to 5.5°C (crystals)	1	MSDS, 1996, Merck Index, 1989, Beilstein, 1990, HSDB, 1999
Boiling point	96°C at 760 mm Hg 35°C at 35 mm Hg (TBHP-pure)	1	MSDS, 1994, Merck Index,1989, Beilstein, 1990, HSDB, 1999
(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	1	MSDS, 1994, MSDS, 1996, Beilstein, 1990, HSDB, 1999
Vapour pressure	2.7 kPa at 20°C (experimental) 3.07 kPa at 21°C 0.73 kPa at 25°C	1,2	Van Hooidonk, 1992 MSDS, 1994 SRC PhysProp, 2001 HSDB, 1999
Surface tension	56 dynes/cm		ARCO, 1994
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	1,3,4	MSDS, 1996 SRC PhysProp, 2001 ChemFinder, 2001 ARCO, 1994 OECD/SIDS, 1995
Solubility in other solvents	Soluble in ethanol, ether, chloroform; very soluble in alkali metal hydroxyl solution		HSDB, 1999
Dissociation constant (pKa)	12.8 at 20°C (experimental)		SRC PhysProp, 2001; HSDB, 1999
Partition coefficient n-octanol/water (log Kow)	0.7 at 25°C (experimental) 0.94 (estimate)	2	Hooidonk, 1992
Henry's Law constant (H)	2.43 Pa*m^3/mole (estimate) 1.63 Pa*m^3/mole at 25°C (estimate)	5	EUSES calculation Howes et al, 1995
Atmospheric OH rate Constant	3E-12 cm^3/molecule*second at 25 °C (experimental)	1,6	Anastasi et al, 1978
Flash point	43°C 62°C		MSDS, 1994 Chemfinder, 2001
Flammability	Flammable		MSDS, 1994
Autoflammability temperature	238°C		MSDS, 1994
Explosive properties	Not explosive Explosive (TBHP-pure)		Peroxid-Chemie, 1993
Oxidising properties	Oxidising		ARCO, 1994
Granulometry	Not applicable, liquid		-

Property	Value / result	Note	References
Conversion factors	1 ppm = 3.75 (mg/m^3)		-
	1 ppm = 3.68 (mg/m^3)		

[1] The same or similar results were found in several literature sources.

[2] Full test report available.

[3] Range of concentrations derived from the phase diagram for TBHP-70. The diagram shows one liquid phase up to 100,000-150,000 mg/l (solubility), two liquid phases at ca. 100,000-150,000 mg/l to ca. 650,000 mg/l (above water solubility, but TBHP and water not miscible) and one liquid phase above ca. 650,000 mg/l (TBHP completely miscible with water).

[4] Based on composition of TBHP-70 (70% TBHP and 30% water). Concentration is above the water solubility, see above.

[5] Henry's Law Constant of 2.43 Pa*m^3/mole: EUSES calculation, from a vapour pressure of 2700 Pa and a water solubility of 100,000 mg/l (see also TGD Chapter 3 – section 2.3.5). These values have been used in the further EUSES calculations underlying the environmental exposure assessment (section 3.3). The Henry's Law Constant of 1.63 Pa*m^3/mole was calculated with the "Henry's Law Constant Program", using the "bond contribution method" (Howes et al., 1995).

[6] The atmospheric OH rate constant of 3E-12 cm3/molecule*second (Anastasi et al., 1978, also cited in Atkinson 1989, 1990 and SRC PhysProp, 2001), has been used in the further EUSES calculations underlying the environmental exposure assessment (section 3.3).

Conclusion:

TBHP is commercially available and used mostly as TBHP-70 (T-Hydro), an aqueous solution of approximately 70 weight percent TBHP and 30 weight percent water. The physico-chemical properties listed in **Table 1.1** include also some values for the pure substance. TBHP-70 is a highly reactive peroxide with an active oxygen content of about 12%.

Detailed experimental data on explosive properties was not submitted by industry. However, general transport classification data submitted by industry (Peroxid-Chemie, 1993) indicate that TBHP-70 has no explosive properties in the sense of 92/69/EEC. This is also in line with the current transport classification of TBHP-70.

All other required physico-chemical data were submitted by industry. Most of these data are based on information from databases, material safety data sheets (ARCO, 1994; MSDS, 1994, 1996) or general published information summarising experimental or estimated physico-chemical properties. Only the vapour pressure of 2700 kPa at 20°C and the log Kow of 0.7 at 25°C are based on full test reports (Van Hooidonk, 1992). Nevertheless, the available data on the physico-chemical properties of TBHP and TBHP-70 are considered to meet the Annex VIIA requirements.

The substance is flammable, but does not need to be classified as flammable according to the criteria. However, the flashpoint indicates labelling with R10. In addition, the substance (70% dilution of TBHP) should be classified as oxidising (symbol O) and labelled with the R-sentence R7, because it is an organic peroxide. Furthermore, the following S-sentences are applicable based on the physico-chemical properties: S3, S7, S14, S43.

1.4 CLASSIFICATION

1.4.1 Current classification

Classification according to Annex I: none

1.4.2   Proposed classification

Provisional classification by the manufacturer/importer, according to material safety data sheet; C, O, Xn, N, R7-10-20/21/22-34-51-53, S3/7/9-14-15-16-24/25-33-36/37/39-45

Decisions by the Technical Committee on Classification and Labelling (TC-C&L) in October 2006 and September 2007 for physical/chemical and human health endpoints and in April 2006 for environmental effects:

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 $5\% < C \leq 10\%$ and R43 above $C \geq 0.1\%$.

This classification and labelling will 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.

2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION

The production of tert-butyl hydroperoxide (hereafter referred to as TBHP) is located at three sites in the European Union (**Table 2.1**). The total EU production volume is around 14,500 tonnes/year (based on the combined 2000 data of the three production companies). The total EU processing volume is around 14,200 tonnes/year (based on the submitted data for 1996-2000). Import into and export outside the EU are 143 and 164 tonnes/year, respectively. The difference of about 300 tonnes/year between production volume and processing volume is thought to be caused by the difference in the year of record of the reported amounts.

The annual market growth in the European Union is expected to be below 3 percent in the near future as indicated by industry.

Table 2.1. Production sites of TBHP in the EU in the year 1996.

Company	Location	Tonnage (tpa)
Arco Chemical Nederlands LTD	Rotterdam, Netherland	Confidential
Peroxid-Chemie GmbH	Pullach, Germany	Confidential
ATOFINA	Günzburg, Germany	Confidential

2.1.1 Production processes

The production of TBHP takes place in a closed batch or closed continuous process. The main types of production of TBHP are:

Direct reaction of isobutane and liquid oxygen.

(Used by one of the three EU producers. Overall reaction: $(CH_3)_3CH + O_2 \rightarrow (CH_3)_3CO\text{-}OH$. This reaction produces numerous minor by-products such as t-butyl alcohol and di-butyl peroxide which are removed during the purification by distillation which involves a TBHP-water azeotrope. Decanting of the aqueous phase of the distillation process leaves an organic phase containing around 70% TBHP and 30% water.).

Preparation from tertiary-butyl alcohol and 30% hydrogen peroxide in presence of sulphuric acid

(Used by one of the three producers. Overall reaction: $(CH_3)C\text{-}OH + H_2O_2 \rightarrow (CH_3)CO\text{-}OH$.)

Oxidising of tertiary-butylmagnesium chloride.

Epoxidation of propylene catalysed by a molybdenum complex.

Oxidation of t-butyl alcohol in a 50% hydrogen peroxide solution with a reaction catalyst of silicotungstic acid.

2.2   USES

Tert-butyl hydroperoxide (TBHP) is primarily used in the chemical industry (HEDSET, 1997). TBHP is used as starting material (or intermediate) and as a reactive ingredient (catalyst, initiator or curing agent). Applications are:

the epoxidation of propylene to propylene oxide (intermediate);

free radical initiator for polymerisations, copolymerisations, graft polymerisations and curing of polymers (plastic industry);

free radical initiator to polymerise unsaturated monomers, usually to high polymers. Mainly used by manufacturers of synthetic lattices or water borne dispersions. Also used as a component of catalysts systems for unsaturated polyester resins (resin industry; see Annex 3 for additional data on the use of TBHP in the resin industry);

the synthesis of other organic peroxy molecules (as a precursor of initiators) such as perester, persulphate, dialkyl peroxide and perketal derivatives;

the preparation of speciality chemicals required by fine chemical and performance chemical industries, such as pharmaceuticals and agrochemicals (fungicide).

the use as an ingredient of hardeners for plastics. These products contain 5 - 20 % TBHP. Hardeners for plastics are also used in the plastic industry.

According to the Danish product register TBHP is used in several products. Only the most important product types and industry groups are listed in descending order according to substance quantity. Product types are paint, lacquer and varnishes, adhesives and binding agents. Industry groups are chemical industry, real estate, renting and other business services (The Danish Product Register, 1997). The numbers of products containing TBHP within a certain concentration interval are summarised in **Table 2.2**.

Table 2.2. Results from Danish Product Register (1997).

Concentration interval	Number of products	Quantity (tons per annum)
< 1 %	38	<1
>1% - <80 %	4	<2
Total	43	<3

Some sources give information about the use of TBHP in bleaching and deodorising operations as an oxidation and sulfonation catalyst (Hawley, 1977) or in cooling systems as an anti-slime agent and as a settling agent in the precipitation of various mineral tailing in aqueous slurries (Brink, 1968 and Hamer, 1977). There is no indication that TBHP is (still) used for these purposes in Europe.

Table 2.3 shows the industrial and use categories of TBHP for the European market in IC/UC terminology being relevant for the environmental exposure assessment. The quantitative distribution for the processing stage tonnage's is around 20% for IC/UC 3-33 and 80% for IC/UC 11-43, based on the data submitted by industry.

Table 2.3. Industrial and use categories of TBHP.

Industrial category	EC no.	Use category	EC no.	Main category
Chemical industry: used in synthesis	3	Intermediates	33	I b Intermediates stored on site
Chemical industry: used in synthesis	3	Oxidising agents	37	III Multi-purpose equipment
Polymers industry	11	Process regulators	43	Type III, "Wet"

3   ENVIRONMENT

See final draft of April 2004.

4   HUMAN HEALTH

4.1   HUMAN HEALTH (TOXICITY)

4.1.1   Exposure assessment

4.1.1.1   General discussion

Text below is identical to contents of section 2.2.

TBHP is produced and used as a liquid which contains approximately 70 - 80 % TBHP and water. The 70 % aqueous solution has a moderate vapour pressure (3.1 kPa at 21 °C).

Tert-butyl hydroperoxide (TBHP) is primarily used in the chemical industry (HEDSET, 1997). TBHP is used as starting material (or intermediate) and as a reactive ingredient (catalyst, initiator or curing agent). Applications are:

*   the epoxidation of propylene to propylene oxide (intermediate);

*   free radical initiator for polymerisations, copolymerisations, graft polymerisations and curing of polymers (in the plastic industry);

*   the synthesis of other organic peroxy molecules (as a precursor of initiators) such as perester, persulphate, dialkyl peroxide and perketal derivatives;

*   preparation of specialty chemicals required by fine chemical and performance chemical industries, such as pharmaceuticals and agrochemicals (fungicide).

TBHP is also used as an ingredient of hardeners for plastics. These products contain 5 - 20 % TBHP. Hardeners for plastics are also used in the plastic industry.

According to the Danish product register TBHP is used in several products. Only the most important product types and industry groups are listed in descending order according to substance quantity. Product types are paint, lacquer and varnishes, adhesives and binding agents. Industry groups are chemical industry, real estate, renting and other business services (The Danish Product Register, 1997). The number of products containing TBHP within a certain concentration interval are summarized in **Table 4.1.1.A**.

Table 4.1.1.A   Results from Danish Product Register (1997).

	number of products	Quantity (tons per annum)
0 - 1 %	38	< 1
1 - 10 %	1	1
10- 80 %	3	< 1
80 - 100 %	-	-
n.d.	1	
Total	43	1

Some sources give information about the use of TBHP in bleaching and deodorizing operations as an oxidation and sulfonation catalyst (Hawley, 1977) or in cooling systems as an anti-slime agent and as a settling agent in the precipitation of various mineral tailing in aqueous slurries (Brink, 1968; Hamer and Petzen, 1981). There is no indication that TBHP is (still) used for these purposes in Europe.

No occupational limit values could be retrieved for TBHP.

4.1.1.2 Occupational exposure

Occupational exposure is possible in industries where TBHP is produced, used as a chemical agent or used as an ingredient (of hardeners for plastics, paint, lacquer and varnishes, adhesives and binding agents). Besides exposure during production and use of TBHP in the industry, occupational exposure to TBHP is possible, when TBHP-containing products are used.

The use of TBHP and products containing TBHP may include:

- transfer of liquids by means of a transfer line and pumping;

- manual transfer of liquids (in drums);

- manual or automated adding to (chemical) processes;

- manual and automated filling (into packaging);

    manual use of paint, varnishes and lacquers, adhesives and binding agents.

Routes of exposure to TBHP in all the mentioned situations are by inhalation and in a minority of situations also by skin contact.

TBHP is a labelled corrosive substance. For the handling of corrosive substances and formulations, immediate dermal contacts occur only occasionally and it is assumed that repeated daily dermal exposure can be neglected. Therefore according to the TGD dermal exposure to pure TBHP will not be assessed. Dermal exposure to dilutions of TBHP that result in a substance or formulation which has no corrosive labelling (dilutions containing <10% TBHP, according to EU classification and labelling commission), will be taken into account. Repeated dermal exposure cannot be neglected for these substances and formulations.

Relevant populations potentially exposed are workers in the chemical industry, plastic industry and workers using products containing TBHP. More specifically those workers that may have more or less direct contact with the substance, being:

- workers involved in the production, drumming and transferal of TBHP;

- workers using TBHP as a chemical in the chemical industry;

- workers producing and drumming products containing TBHP (e.g. paint, lacquer and varnishes, adhesives and binding agents);

- maintenance and (specialised) cleaning workers in production facilities;

    workers using products containing TBHP in the real estate, renting and other business services.

The exposure is assessed using the available information on substance, processes and work tasks. Information on the process and measured data have only been provided for the production and use of TBHP in the chemical industry.

The following data (if available) are used for occupational exposure assessment:

- physico-chemical data of TBHP and products containing the substance: physical appearance, vapour pressure at room temperature, percentage of TBHP in products;

- data regarding methods of use and use pattern of the substance and products potentially containing TBHP and exposure control pattern in the relevant industries (from the HEDSET or other sources);

- exposure data for TBHP from the HEDSET and other sources (literature, exposure databases);

results from exposure models if applicable (EASE model); in the exposure models the above mentioned types of data are used.

For the occupational exposure assessment the exposure situations can be clustered into 4 scenarios based on the type of use of TBHP. In the first scenario production and use of TBHP in the chemical industry is considered. The second scenario considers the production and use of TBHP containing hardeners of plastic and the third scenario considers the production of products containing less than 1% TBHP. The fourth scenario indicates possible exposure levels in situations where product are used containing <1% TBHP.

Occupational scenario 1   Production and use of TBHP in the chemical industry

Occupational scenario 2   Production and use of TBHP containing hardeners of plastics

Occupational scenario 3   Production of products containing <1% TBHP

Occupational scenario 4   Use of products containing <1% TBHP

In this report for each occupational exposure scenario the general description of exposure will be followed by measured data (if available), and results from similar substances in comparable exposure scenarios. This will be followed by suitable inhalation models. The methods of estimation for inhalation exposure will be compared using expert judgement and a choice for the best applicable estimates will be made.

Dermal exposure will be described and assessed by means of EASE and other approaches as described in the TGD.

The following parameters of exposure are assessed for each (sub)scenario:

- *full shift reasonable worst case inhalation exposure level*: the inhalation exposure level considered representative for a high percentile (90 percentile) of the distribution of full shift exposure levels;

- *short term inhalation exposure level*: the inhalation exposure level considered representative for a high percentile (90 percentile) of the distribution of short term exposure levels; short term exposure is for this purpose considered to be exposure for up to one hour, with typical duration of approximately 15 minutes;

- *dermal exposure level*: the dermal exposure level considered representative for a high percentile (90 percentile) of the full shift dermal exposure levels.

4.1.1.2.1   Occupational scenario 1: Production and use of TBHP in the chemical industry.

This scenario includes all activities concerning the production and use of TBHP in the chemical industry. In Europe TBHP is produced at two production sites, continuously in closed systems. During production exposure to TBHP is possible during certain activities, such as sampling for analytical purposes, drumming, transfer activities involving the coupling and uncoupling of a road tanker or connection or disconnection of pipes, cleaning and maintenance and repair work. According to information in the HEDSET, the use of inert gas, to ensure removal of oxygen, is a guarantee that there is no point source emission in the production process.

Transferral and subsequent use as raw material or as an intermediate (initiator, catalyst or curing agent) occurs also in closed systems (continuous and batch wise). Transferral may involve flexible hosing, pumping for emptying drums and coupling and uncoupling of a road tanker that may lead to some leakage of TBHP. For certain activities, such as checking the level of TBHP catalyst and sampling for analytical purposes, valves may have to be opened manually and some TBHP will evaporate. When TBHP is consumed or reacted in the process (use as a reactive intermediate or raw material for other organic peroxy molecules) exposure will be limited to the activities during transfer, filling of the reactor, sampling and analysis and repair work.

When TBHP is not consumed or reacted in the process, exposure may also occur while filling TBHP into packaging, as well as during cleaning and maintenance and repair work. According to information from one producer, filling of TBHP in packaging takes place both manual and automated. During manual filling local exhaust ventilation (LEV) is present and automated filling takes place in separated rooms.

Inhalation exposure

Measured data

A small number of exposure data regarding this type of use has been provided by the producers. No additional data on exposure were found in literature. The measured data concern only inhalation. Data are summarised in **Table 4.1.1.B**.

Table 4.1.1.B. TBHP concentrations at workplaces during production and use of TBHP in the chemical industry

Company/ reference	Year of measure-ments	Activities/circumstances	Number of measure-ments	Duration of measurement (min)	(Range of) measurements data (mg/m^3)	Personal (P) or Stationary (S) measurement
IRPTC data profile	Unknown	Limited access: peak concentration	1	unknown	24	P
		"worst case"	1	unknown	1.44	P
		mean	1	unknown	0.37	P
A	1993	Routine activities	7	full shift	0.08-0.23	P
		Drumming operation with extraction ventilation	4	full shift	< 0.04*	P
	1994/1995	Filling of drums with local exhaust ventilation	5	80 - 180	< 0.07 - < 0.19	P
	1995	Coupling/decoupling trucks with vapour return system	7	5-15	< 0.22 - < 1.85	P
	1999	Filling drums with local exhaust ventilation and coupling /decoupling trucks with vapour return system	3	120 – 230	0.22 – 0.33	P
		Putting drums on pallets, transfer with fork-lift truck	2	219 – 228	0.04 – 0.07	P
I	1989-1991	Operation of drums emptying by means of pumping:				
		1 m from the top of the drum	4	unknown	2.70 -15.11	S
		5 m from the top of the drum	3	unknown	0.16 - 1.51	S
		Near process tanks				
		1 m from tank venting	1	unknown	26.6	S
		5 m from tank venting	1	unknown	1.35	S
J	2005	TBHP Bulk unloading	2	360-480	0.32-0.64	P
		Other tasks	7	360-480	0.21-0.45	P
H	2000	Coupling/decoupling trucks	2	55	<10	S
	2005	TBHP (70%) rounds, samples,	1	480	< 0.1	S
		TBHP (70%) loading, disconnecting ISO container	1	15	< 3.32	S
		TBHP (55%) unloading, disconnecting ISO container	1	15	<3.32	P
	2005	Close	4	180-240	1.3-4.7	S
M	2000	At TBHP unloading station	1	55	< 10	S

* mean

Data from company A show an exposure level during production ('routine activities') between 0.08 and 0.23 mg/m^3 and for drumming a mean of < 0.04 mg/m^3 (n=4, with extraction ventilation). Additional data from company A gives more information about several activities. Filling of drums with local exhaust ventilation gives exposure levels between <0.07 and 0.33 mg/m^3 (n=5 and n=3). Coupling and decoupling of trucks with vapour return system presents non detectable exposure between <0.22 and <1.85 mg/m^3 with

a duration of 5-15 minutes (n=7). Company H performed further measurements in 2005 on TBHP. The results of personal measurements are below the detection limit of 3.32 mg/m³ TBHP. Some stationary sampling was done at a possible point of emission. This is not considered to be relevant for personal exposure. The stationary measurement by Company M may be relevant, but resulted in non-detected levels. No information is available about the activities during the measurements from the IRPTC data profile. It is also not clear, whether these measurements are from a production facility where TBHP is produced or from a facility where TBHP is used. At one plant (company I) concentration TBHP in the air was measured when emptying drums by means of pumping. Concentrations at 1 m from the top of the drum when emptying drums were between 2.7 and 15.1 mg/m³ (n=4), at 5 m distance of the top of the drum the concentration vary from 0.16 to 1.51 mg/m³ (n=3). Near process tanks, concentrations were measured of 26.6 and 1.35 mg/m³. The data from Company I are considered not to be representative for worker exposure.

Modelled data

For inhalation exposure to vapours of moderately volatile substances in closed systems the ease model (version 2.0 for windows) estimates (reasonable worst case estimates):

- without breaching: 0-0.1 ppm (0-0.4 mg/m³);

- with breaching (non dispersive use with LEV): 10-20 ppm (38-76 mg/m³);

- with breaching (non dispersive use, direct handling and dilution ventilation): 100-140 ppm (380-532 mg/m³).

Summary/statement of the exposure level

There is some measured data available. Not all data are presented with sufficient contextual information and not all data are relevant for worker exposure. Nevertheless the data can be used as an indication for estimating the reasonable worst case.

The reported data for personal exposure (0.08-0.64 mg/m³) are in the same range as the results from the EASE model for 'closed system, without breaching' (0-0.4 mg/m³). It is assumed that these values are representative for workers exposure in production facilities where TBHP is produced and used.

The EASE model results for "non dispersive use with LEV" (38-76 mg/m³) and for "non dispersive use, direct handling and dilution ventilation" (380-532 mg/m³) appear to be excessively high, in comparison with the exposure data found for drumming with extraction ventilation (< 0.04 mg/m³) or even in comparison with the worst case (1.44 mg/m³), the concentrations for coupling and decoupling with vapour return system (<1.85 mg/m³) or the latter activity without LEV (<10 mg/m³) and the peak concentrations (24 mg/m³). This is due to the fact that the use of LEV in this specific situation is very effective and/or that breaching occurs only for very short periods of time (according to a representative of Company A: no more than 5 minutes).

Duration of inhalation exposure due to specific activities (drumming etc.) is estimated to be 1-2 hour/day, whereas exposure to background concentrations is 4-8 hours per day. Frequency of exposure depends on work patterns. In Company A, drumming only takes place 2 hrs per week but daily exposure is possible during loading of trucks/ isocontainers.

The worst case full shift exposure is estimated to be 0.5 mg/m^3, which is at the high end of the measured exposure levels reported by Company J and slightly higher than the highest personal exposure levels in Company A.

Reasonable worst case short term exposure level is estimated to be 5 mg/m^3. This value is clearly higher than measured personal exposure levels (including short term levels), but substantially lower than the highest values in older measurements (Company I and IRPTC) of unknown duration. In the estimation of this value, the higher detection limits (Company H and Company A) for short term values are taken into account and a slightly higher value than those is used to account for the older, higher measured values.

Typical full shift exposures are estimated to be in the order of 0.2 mg/m^3, based on the data from Company A and Company J.

Dermal exposure

Measured data

There are no measured data available

Modelled data

Dermal exposure to TBHP during the production and use of TBHP in the chemical industry is possible during sampling, drumming and filling (automated and by hand), coupling and uncoupling of road tankers, cleaning and maintenance and repair work. Although there are no workers needed for automated filling and the process takes place in separated rooms, dermal exposure will be possible when contact with package or machine is necessary due to disturbance in the process.

On account of the corrosive effect of TBHP and taking into consideration the highly accepted use of suitable protective equipment within the large scale chemical industry, it can be assumed that, as a rule, daily repeated immediate contact is avoided by using suitable personal protective equipment (PPE; gloves and eye protection). During activities like drumming, sampling, filling and cleaning and maintenance potential exposure is assumed only on single days and by single contacts. The corresponding exposure level is estimated with the EASE model. Potential dermal exposure is estimated to be 0-0.1 mg/cm^2/day (non-dispersive use with direct handling and incidental contact).

The area potentially exposed depends on the activity. It is assumed that during sampling, drumming, filling and coupling and uncoupling of road tankers the exposed area will be half of two hands. This corresponds with an exposed area of 420 cm², which results in a reasonable worst case estimate of 0-42 mg/day.

Summary/statement of the exposure level

Dermal exposure to TBHP during the production and use of TBHP in the chemical industry is possible during sampling, drumming and filling (automated and by hand), coupling and uncoupling of road tankers, cleaning and maintenance and repair work.

On account of the corrosive effect of TBHP it is assumed that repeated daily contact is avoided by the use of PPE. Single day contact to TBHP in the chemical industry is estimated to be up to 42 mg/day.

Conclusions scenario 1

The number of companies providing exposure data is rather limited but nevertheless the data provided was considered sufficient to estimate exposure levels.

The following exposure levels will be used for further risk assessment for scenario 1.

-      Inhalation exposure: reasonable worst case, full shift: 0.5 mg/m^3;

-      Inhalation exposure: reasonable worst case, short term: 5 mg/m^3;

-      Dermal exposure: reasonable worst case (single day):   42 mg/day.

4.1.1.2.2   Occupational scenario 2: Production and use of TBHP containing hardeners of plastics

In this scenario the exposure of workers involved in the formulation and use of hardeners for plastic is assessed. For this purpose a small percentage (ca. 0.05%) of the total amount of TBHP produced is used.

Limited information is available about the production and use of TBHP containing hardeners for plastics. The formulation (mixing) of the hardeners takes place in batches in a closed system. The hardeners contain <1-20 % TBHP. The use of hardeners in the plastic industry probably involves (manual or automated) adding of the hardener to the process. Handling in the plastic industry may be more open than during production and use in the chemical industry.

It is assumed that during production of TBHP containing hardeners handling of both 'pure' TBHP and TBHP containing hardeners may lead to exposure. 'Pure' TBHP contains 70-80% TBHP and is handled during transfer activities which may involve flexible hosing, coupling and uncoupling of a road tanker, connection or disconnection of pipes or pumping for emptying drums that may lead to some leakage of TBHP. TBHP containing hardeners (<1-20% TBHP) are handled during the following activities: quality control sampling, manual or automated filling of the product into packaging, cleaning and maintenance and repair work of the production facility. Some information was received from two downstream user companies. In one of the companies TBHP is added by pumping and some manual addition to reservoirs and finishing vessels. The addition takes up to 20 minutes per shift and is done daily. The produced product contains approximately 0.1% TBHP (Company L, 2006). Another company uses TBHP on a few sites, where it is pumped into closed systems. Duration of this task is 60-90 minutes and it is done twice per week. The produced products contain 0.2-1% TBHP (Company O, 2006).

Exposure to TBHP is also possible during use of the hardeners in the plastic industry. Adding of TBHP containing hardeners to the production process may involve manual handling.

Inhalation exposure

Measured data

There are some measured data of exposure levels during formulation of hardeners or use of the hardeners. No detailed information is presented. Average short term exposure levels are reported to be approximately 2.7 mg/m^3, while average long term exposure levels are reported to be approximately 0.07 mg/m^3 (Company L, 2006). These data do not provide sufficient information for estimating reasonable worst case levels. Therefore exposure is also estimated with the EASE model.

Modelled data

Exposure is estimated with the EASE model.

For inhalation exposure to vapours of moderately volatile substances in closed systems the ease model (version 2.0 for windows) estimates (reasonable worst case estimates):

- without breaching: 0-0.1 ppm (0-0.4 mg/m^3);

- with breaching (non dispersive use with LEV): 10-20 ppm (38-76 mg/m^3).

- with breaching (non-dispersive use, direct handling and dilution ventilation): 100-140 ppm (380-532 mg/m^3).

Inhalation exposure due to evaporation of TBHP from 'pure' TBHP is expected during transfer activities, such as, coupling and uncoupling of road tankers and adding 'pure' TBHP to the process by connection or disconnection of pipes or emptying drums by means of pumping. The exposure level during these activities in well equipped companies is expected to be of the same level as during similar activities of scenario 1 (production and use of TBHP in the chemical industry: transfer activities, drumming etc). Values in the order of the levels measured close to emission points at Company I (Table 4.1.1.B) are expected to be possible, i.e. up to more than 20 mg/m^3. These values are somewhat lower than the range of the EASE model. The average measured values in one company are substantially lower. However, it is not sure whether these values are representative for all situations in this scenario.

Exposure to lower concentrations of TBHP is expected when hardeners containing TBHP are handled. The following activities may lead to inhalation exposure: quality control sampling, filling of packaging (automated or by hand) of the product and use of the product in the plastic industry.

Lower exposure levels will occur during the rest of the day. Breaching of the system will lead to background concentrations of TBHP. Background concentrations are estimated with EASE to be 0-0.1 ppm (0-0.4 mg/m^3), assuming no aerosol formation and full containment.

Duration of inhalation exposure during production and use of TBHP containing hardeners due to specific activities (transfer activities and filling of packaging, etc.) is estimated to be 1-2 hour/day, whereas exposure to background concentrations is 4-6 hours per day. Frequency of exposure is expected to be limited and is estimated to be up to 50 days/year.

Summary/statement of the exposure level

Exposure to TBHP is possible during the production and use of TBHP containing hardeners for plastics. Exposure may occur in the formulation and plastic industry.

Inhalation exposure to TBHP during production of TBHP containing hardeners for plastics is possible due to evaporation of TBHP from 'pure' TBHP and from the hardeners containing

<1-20 % TBHP. Exposure is expected during transfer activities, quality control sampling, filling of the hardeners into packages and cleaning and maintenance and repair work of the production facility. Lower exposure levels will occur during the rest of the day. Exposure to TBHP in the plastic industry is possible when adding the hardener to the production process.

The measured exposure levels are reported in too limited detail to be useful for drawing conclusions on reasonable worst case levels and furthermore, they are only from one downstream user. For normal working conditions a short term exposure level of 10 mg/m^3 will be used as a reasonable worst case estimate for this scenario. This value is twice the estimated short term exposure value for scenario 1, because it is assumed that at downstream users slightly less well controlled closed equipment may be used, leading to higher short term values. The value is also less than four times the "average" short term values reported by one company and therefore do not seem to be unreasonable for reasonable worst case assumptions. The total duration of such short term exposures may be different from that in scenario 1, because of several short term activities being combined on a workday. Full shift exposure levels will be lower. They are estimated to be up to about 3.2 mg/m^3 (calculated from a concentration of 10 mg/m^3 during 2 hours and 0.4 mg/m^3 during 6 hours exposure during the remainder of the day (background values modelled through EASE)) which may be reached if several activities that lead to exposure occur on one day. If the estimated concentration of 10 mg/m^3 is a true reasonable worst case estimate for short term exposure and if the duration of short term exposure of 2 hours is a reasonable worst case estimate of the duration, the resulting calculated full shift exposure levels is an overestimate of the full shift reasonable worst case value. This conservative approach is taken because of the limited basis for both values (concentration and duration).

Dermal exposure

Dermal exposure to TBHP during the production and use of TBHP containing hardeners is possible due to contact with 'pure' TBHP and TBHP containing hardeners.

Measured data

There are no measured data available.

Modelled data

On account of the corrosive effect of 'pure' TBHP and TBHP containing hardeners for plastics (<1-20 % TBHP) and taking into consideration the highly accepted use of suitable protective equipment within the chemical and plastic industry, it can be assumed that, as a rule, daily repeated immediate contact is avoided by using suitable personal protective equipment (PPE; gloves and eye protection). During activities like coupling and uncoupling of transfer lines, emptying drums, sampling, filling and cleaning and maintenance potential exposure is assumed only on single days and by single contacts. The corresponding exposure levels are estimated with the EASE model. For activities where contact with 'pure' TBHP is possible, potential dermal exposure is estimated to be 0-0.1 mg/cm^2/day (non-dispersive use with direct handling and incidental contact). For handling of TBHP containing hardeners potential dermal exposure is estimated to be 0-0.02 mg/cm^2/day (0.2 * (0-0.1 mg/cm^2/day)).

The area potentially exposed depends on the activity. It is assumed that during transfer activities, sampling, filling and adding the product to the production process the exposed area will be half of two hands. This corresponds with an exposed area of 420 cm^2, which results in a reasonable worst case estimate of 42 mg/day for handling 'pure' TBHP and 8 mg/day for handling TBHP containing hardeners.

Frequency of exposure is expected to be limited: up to 50 days/year.

Summary/statement of the exposure level

Dermal exposure to TBHP is possible during production and use of TBHP containing hardeners. The following activities may lead to exposure: coupling and uncoupling of road tankers, quality control sampling, filling of packages, cleaning and maintenance and repair work of the production facility and adding of the TBHP containing hardeners to the production process of plastics.

On account of the corrosive effect of TBHP it is assumed that repeated daily contact is avoided by the use of PPE. Single day contact to TBHP is estimated to be 42 mg/day.

Conclusions scenario 2

The following exposure levels will be used for further risk assessment for scenario 2.

-     Inhalation exposure: reasonable worst case, full shift: 3.2 mg/m^3;

-     Inhalation exposure: reasonable worst case, short term: 10 mg/m^3;

-     Dermal exposure (single day contact): reasonable worst case: 42 mg/day.

4.1.1.2.3   Occupational scenario 3: Production of products containing < 1 % TBHP

According to the Danish product register TBHP is present in some products used in Denmark. About 38 product like paints, lacquers and varnishes, adhesives and binding agents contain less than 1% of TBHP. The amount of TBHP in these products is less than one ton per year. The downstream users that provided information that was used for scenario 2 actually produce products (emulsion polymers and or polymeric dispersions) that contain up to 1% TBHP. In this scenario the production of products containing up tot 1% of TBHP is considered.

There is no specific information available about the production of products containing less than 1% TBHP.

TBHP or a preparation, such as a polymeric dispersion containing TBHP, is mixed with other ingredients in production facilities which may be only partially closed. Liquids (paint, lacquers) may be drummed in drums, cans or even smaller packaging. The use of very good local exhaust ventilation cannot be assumed in all facilities that mix chemical products. Some of these facilities are relatively small and not very modern. Products are assumed to contain up to 1% TBHP and are probably drummed in cans with a volume up to 10 L. Only mixing and drumming of liquid products will be considered here.

It is assumed that during production of products containing TBHP handling of both 'pure' TBHP and products containing TBHP may lead to exposure. 'Pure' TBHP contains 70-80% TBHP and is handled during transfer activities which may involve flexible hosing, coupling and uncoupling of a road tanker, connection or disconnection of pipes or pumping for emptying drums that may lead to some leakage of TBHP. Exposure to TBHP from products containing TBHP (<1%) is possible due to the following activities: mixing of ingredients,

quality control sampling, manual or automated filling of the product into packaging, cleaning and maintenance and repair work of the production facility.

<u>Inhalation exposure</u>

Inhalation exposure is expected due to evaporation of TBHP especially if production takes place in partially closed systems.

Measured data

The workplace measurements from Company L (2006) may be relevant for this scenario. They report average short term exposure levels of 2.7 mg/m^3 and average long-term exposure levels of 0.07 mg/m^3. These exposures are due to the pumping of TBHP to reservoirs and manual addition to finishing tanks (see also scenario 2).

Modelled data

Because the measured exposure data are too limited in number and quality, exposure assessment is performed applying the EASE model.

For inhalation exposure to vapours of moderately volatile substances ('pure' substance) the EASE model (version 2.0 for windows) estimates:

- non dispersive use with LEV: 10-20 ppm (38-76 mg/m^3);

- non-dispersive use, direct handling and dilution ventilation: 100-140 ppm (380-532 mg/m^3).

For inhalation exposure to vapours of low volatile substances (assuming a concentration of 1% TBPH, and a vapour pressure of 0.01*3.1=0.031 kPa) the EASE model (version 2.0 for windows) estimates:

- non dispersive use with LEV: 0.5-1 ppm (2 -4 mg/m^3);

- non-dispersive use, direct handling and dilution ventilation: 10-20 ppm (38-76 mg/m^3).

Inhalation exposure due to evaporation of TBHP from 'pure' TBHP is expected during transfer activities, such as, coupling and uncoupling of road tankers and adding 'pure' TBHP to the process by connection or disconnection of pipes or emptying drums by means of pumping. Because very limited information is available, the same values as presented for scenario 2 will be used for these activities: 10 mg/m^3, which is twice the value for scenario 1, because it is assumed that exposure may not be controlled .as effectively as in production and less than 4 times the "average" short term value reported by a downstream user.

Exposure to lower concentration of TBHP is expected when the products containing TBHP are handled. The following activities may lead to inhalation exposure: mixing, quality control sampling and filling of product into drums or cans (automated or by hand). Because of the low concentration of TBHP (< 1%) in the products a partial vapour pressure of 31 Pa (3.1 kPa * 0.01) is assumed. Inhalation exposure due to evaporation of TBHP from products containing <1% TBHP during production is estimated with EASE to be 2-4 mg/m^3 (0.5-1 ppm) assuming no aerosol formation, non dispersive use with LEV. The upper range of the EASE estimate will be used as a reasonable worst case estimate.

Duration of inhalation exposure during production of products containing <1% TBHP due to mixing, filling of cans and drums etc. is estimated to be 4-6 hour/day. Higher exposure levels

are expected to occur, due to transfer activities of 'pure' TBHP and cleaning and maintenance of the production facility, for about half an hour a day. Frequency of exposure is expected to be up to 100 days/year.

Summary/statement of the exposure level

If TBHP is used as an ingredient in paint, lacquers and varnishes and adhesives and binding agents exposure to TBHP is possible during the production of these products. Inhalation exposure to TBHP during production of products containing less than 1% TBHP is possible due to evaporation of TBHP from 'pure' TBHP and from the products. Exposure is expected during transfer activities, mixing of ingredients, quality control sampling, filling of the products into cans or drums and cleaning and maintenance and repair work of the production equipment.

During production of the products (mixing and filling into packaging) an exposure level of 4 mg/m^3 will be used as a reasonable worst case. For normal working conditions a short term exposure level of 10 mg/m^3 will be used as a reasonable worst case estimate for this scenario (during transfer activities with the 'pure' substance). Full shift exposure levels will be lower. They are estimated to be up to about 3.6 mg/m^3 (calculated from a concentration of 10 mg/m^3 during half an hour, and an exposure level of 4 mg/m^3 (based on the modelled data for mixing and filling) during 6 hours and negligible exposure during the remainder of the day) which may be reached if several activities that lead to exposure occur on one day. If the estimated concentrations of 10 and 4 mg/m^3 are true reasonable worst case estimates for short term exposure and if the durations of short term exposure of 0.5 and 4 hours are reasonable worst case estimates of the duration, the resulting calculated full shift exposure levels is an overestimate of the full shift reasonable worst case value. This conservative approach is taken because of the limited basis for both values (concentration and duration).

Dermal exposure

Dermal exposure to TBHP during the production of products containing TBHP is possible due to contact with 'pure' TBHP and the products.

Measured data

There are no measured data available.

Modelled data

On account of the corrosive effect of 'pure' TBHP, it can be assumed that, as a rule, daily repeated immediate contact is avoided by using suitable personal protective equipment (PPE; gloves and eye protection). During activities like coupling and uncoupling of transfer lines and emptying drums potential exposure is assumed only on single days and by single contacts. The corresponding exposure levels are estimated with the EASE model. For activities where contact with 'pure' TBHP is possible, potential dermal exposure is estimated to be 0-0.1 mg/cm2/day (non-dispersive use with direct handling and incidental contact).

TBHP is a corrosive substance, but if a preparation or a product contains less than 10% of a corrosive substance it doesn't have to be labelled as corrosive according to Council Directive 67/548/EEC. The products used in this scenario (containing less than 1% TBHP) are therefore considered as non corrosive and exposure is estimated for repeated contact. Potential dermal exposure for activities where contact with the products is possible like sampling and filling of packaging is estimated with EASE to be 0.1-1 mg/cm^2/day (non-dispersive use with direct

handling and intermittent contact). If the amount of TBHP is considered (1%) potential dermal exposure will be 0.001-0.01 mg/cm^2/day.

The area potentially exposed depends on the activity. It is assumed that during transfer activities and adding of the product to the production process the exposed area will be half of two hands. This corresponds with an exposed area of 420 cm², which results in a single day reasonable worst case estimate of 42 mg/day for handling 'pure' TBHP.

During sampling and filling of the products into drums and cans the exposed area is expected to be half of two hands. This corresponds with an exposed area of 420 cm², which results in a reasonable worst case estimate for repeated contact with products containing less than 1% TBHP of 4 mg/day.

Frequency of exposure is expected to be limited: up to 100 days/year.

Summary/statement of the exposure level

If TBHP is used as an ingredient in the production of paint, lacquer and varnishes, adhesives and binding agents dermal exposure to TBHP is possible during production. Exposure is expected during transfer activities, quality control sampling, filling of the product into cans or drums and cleaning and maintenance and repair work of the production equipment.

On account of the corrosive effect of TBHP it is assumed that repeated daily contact with pure TBHP is avoided by the use of PPE. Single day contact to 'pure' TBHP is estimated to be 42 mg/day.

Products containing less than 10 % TBHP are considered as non corrosive and the reasonable worst case estimate for repeated exposure is estimate to be 4 mg/day.

Conclusions scenario 3

This scenario is based upon information from the Danish product register. According to this information 38 products like paints, lacquers and varnishes, adhesives and binding agents contain less than 1% of TBHP. It is not known whether TBHP is used as an ingredient or TBHP is a contaminant of one of the other ingredients. If TBHP is a contaminant this exposure scenario 'Production of products containing <1% TBHP' is not relevant.

The following exposure levels will be used for further risk assessment for scenario 3.

-       Inhalation exposure: reasonable worst case, full shift: 3.6 mg/m^3;

-       Inhalation exposure: reasonable worst case, short term: 10 mg/m^3;

-       Dermal exposure (single day contact): reasonable worst case: 42 mg/day;

-       Dermal exposure (repeated exposure): reasonable worst case: 4 mg/day.

4.1.1.2.4   **Occupational scenario 4: Use of products containing < 1 % TBHP**

According to the Danish product register TBHP is present in some products in Denmark which are used in the chemical industry, real estate, renting and other business services. About 38 products, paints, lacquers and varnishes, adhesives and binding agents, contain less

than 1% of TBHP. The total amount of TBHP used in these products less than one ton per year.

There is no specific information available about the use of products containing less than 1% TBHP.

Because there is not sufficient information available to estimate exposure in all possible use scenarios of products containing less than 1% TBHP, one estimated 'representative' use scenario is considered. Exposure of TBHP due tot the use of paints containing less than 1% TBHP in the real estate and renting business is considered as being representative for the scenario 'Use of products containing less than 1% TBHP'. Exposure to TBHP is estimated for manual application of paint by rolling and brushing and cleaning of the material. Spray application is not expected to be common in this scenario and will not be assessed.

Inhalation exposure

Measured data

Neither workplace measurements nor exposure data in literature are available.

Modelled data

Exposure assessment is performed applying the EASE model. Because of the low concentration of TBHP (<1%) in the products a partial vapour pressure of 31 Pa (3.1 kPa * 0.01) is assumed.

For inhalation exposure of low volatile substances the EASE model (version 2.0 for windows) estimates:

- non dispersive use with LEV: 0.5-1 ppm (2 - 4 mg/m^3);

- non-dispersive use, direct handling and dilution ventilation: 10-20 ppm (38-76 mg/m^3);

- wide dispersive use, direct handling and dilution ventilation: 100-140 ppm (380-532 mg/m^3).

The highest exposure is expected during manual application of paints by rolling or brushing. These activities are modelled by wide-dispersive use, direct handling and dilution ventilation. Exposure is estimated with EASE to be 100-140 ppm (380-532 mg/m^3). Considering the reactive properties of TBHP and the function of TBHP as a hardener in plastics it is very likely that TBHP will react with one of the ingredients of paint and form to some extent a chemical bond. Due to the chemical bond evaporation of TBHP will be limited and therefore the lower exposure range of the EASE estimate could be used as the reasonable worst case (expert judgement).

Duration of inhalation exposure during painting is estimated to be 2-4 hours/day. Frequency of exposure is expected to be up to 50 days/year.

Analogous data for this scenario is available from the RAR of toluene (see Table 4.1.1C). The vapour pressures of TBHP and toluene are comparable.

Table 4.1.1C. Average toluene exposure levels per shift during brush/roller application of paint (Bock et al., 1999).

Company type	Measurements from analogue scenario				
Work ares	*Measurements*	*Companies*	*50% value*	*90% value*	*95% value*

	number	number	mg/m³	mg/m³	mg/m³
Brush/roller application of paint – toluene exposure (construction industry) – All measurements without local exhaust ventilation	285	78	< 0.3	4	25

The paint used in the construction industry is comparable with the paint used in the real estate and renting business. No information is given on the amount of toluene in the paint, but it's assumed that over 285 measurements the average toluene concentration in the product will be more than 1%. This measurement data will give an overestimation of exposure to TBHP due to the fact that the percentage of toluene in the paint is probably higher compared to that of TBHP. Furthermore TBHP will react with one of the ingredients of paint and form to some extent a chemical bond and will not evaporate as much as toluene does. The 90% value of 4 mg/m³ is taken to risk characterization as RWC value for full shift measurements. As reasonable worst case scenario for short term exposure 8 mg/m³ (2 times the value of the shift exposure) is taken.

Summary/statement of the exposure level

The use of paints containing less than 1% TBHP in the real estate and renting business is considered as being representative for the scenario 'Use of products containing less than 1% TBHP'.

Inhalation exposure to TBHP during painting is possible due to evaporation of TBHP. Exposure is expected during manual application of paint by rolling and brushing and cleaning of the equipment. Analogous data from toluene is used to make an estimation of exposure to TBHP. These data is preferred above the estimations made by EASE. For manual application of paint an exposure level of 8 mg/m³ will be used as a reasonable worst case estimate. Full shift exposure levels will be lower. They are estimated to be up to 4 mg/m³. These values are expected to overestimate exposure to TBHP because there is probably more toluene in the paint and TBHP forms a chemical bond in the paint while toluene does not.

Dermal exposure

TBHP is a corrosive substance if a preparation or a product contains less than 10% of a corrosive substance it doesn't have to be labelled as corrosive according to Council Directive 67/548/EEC. The products used in this scenario (containing less than 1% TBHP) are therefore considered as non-corrosive and exposure is estimated for repeated contact.

Measured data

There are no measured data available.

Modelled data

Analogous dermal exposure data for application of the paint (1% substance) is given in the TGD (2003). The activity "brushing and rolling of liquids" (**Table 2**, Appendix I E) leads to a

reasonable worst case estimate of 100 mg/day (TGD estimate of 10000 mg/day x 1% substance). Assuming an exposed skin surface of 840 cm^2, the concentration is 0.12 mg/cm^2/day (100 mg/day/840 cm^2). The typical estimate is 17 mg/day (TGD estimate of 1700 mg/day x 1%), *i.e.* 0.02 mg/cm^2/day (17 mg/day/840 cm^2).

Reasonable worst case dermal exposure is estimated with EASE 2.0 to be 0.05 mg/cm^2/day (0.01 x (1-5)) assuming inclusion into a matrix, direct handling with an extensive contact level. With an estimated exposed area of 840 cm^2 (two hands) the daily dose is assumed to be 42 mg (0.05 x 840).

The TGD 2003 estimate is based on a few data sets. However, after the TGD was made, new dermal exposure data have become available from the RISKOFDERM project. These have been summarised in Marquart *et al.* (2006). In this paper, the authors conclude a default reasonable worst case estimate, based on a number of data sets for "brushing and rolling of (relatively viscous) liquid products on surfaces" of 6500 mg (8 mg/cm^2). This estimate combines the earlier TGD estimate with the new data.
However, the earlier TGD estimates are for consumer application of paints and are partly extrapolated. Therefore, the relevance of these earlier estimates is questionable.

RIVM has produced a "fact sheet paint" (Bremmer and Van Veen, 2000) in which they describe the same data sets that were used for the TGD estimates, as well as some work (without reference) in which hands were actually contaminated with paint (low, medium and high contamination). Based on estimated thickness of paint coat and paint density, they estimate that fully painted hands would contain 4,300 mg (on 600 cm^2), whereas their experiments with high contamination resulted in 800 mg paint on the hand(s), with surface area not presented, but assumed to be also 600 cm^2. As default values for consumer exposure, RIVM suggests (based on all the presented information) in their "fact sheet":
- Rolling and brushing solvent based paint on large surfaces: 2,700 mg;
- Rolling and brushing solvent based paint on small surfaces: 1,400 mg;
- Rolling and brushing water based paint on large surfaces: 900 mg;
- Rolling and brushing wall paint on small surfaces: 1,800 mg

The most relevant data from the RISKOFDERM project are those for rolling of recently applied styrene in polyester boat building (described in detail in Eriksson and Wiklund, 2004) and for painting window frames or doors using a brush (described in detail in Gijsbers *et al.*, 2004), while the few data points from brushing on graffiti remover (RISKOFDERM DL 42, 2003) may also be relevant.
The results from these measurements on approximately 840 cm^2 of hands are (Marquart *et al.,* 2006):
- rolling of recently applied styrene in polyester boat building:
    - reasonable worst case = 2200 mg (2.68 mg/cm^2);
    - typical case = 1100 mg (1.34 mg/cm^2);
- painting window frames and doors using a brush:
    - reasonable worst case = 400 mg (0.48 mg/cm^2);
    - typical case = 100 mg (0.12 mg/cm^2);
- brushing on graffiti remover: too limited number of measurements to derive a reasonable worst case value.

Considering the available information, and considering the various products that may contain < 1% TBHP, it is assumed that relatively large volumes may be rolled and brushed with these

products. The values for small surfaces (including the measurements by Gijsbers *et al.* for window frames and doors) are lower than for larger surfaces. The values from rolling applied styrene and the default from the RIVM fact sheet for rolling and brushing solvent based paint on large surfaces are in the same order of magnitude. The larger of the two will be used for the risk characterization: 2700 mg of paint on a skin surface area of 840 cm^2.

The fact that TBHP will on the one hand evaporate from the product and on the other hand react with other components in the product may lead to lower values than for non-evaporating and non-reacting substances. On the other hand, the measured data include measurements on styrene (from high styrene content material) that also evaporates from and reacts (polymerises) in the product. The exposure levels from styrene are in the same order of magnitude as those estimated above. The effect resulting from evaporation and reaction of the substance is therefore considered to be partly integrated in the estimate already.

Frequency of exposure is expected to be up to 50 days/year.

Summary/statement of the exposure level

The use of paints containing less than 1% TBHP is considered as being representative for the scenario 'Use of products containing less than 1% TBHP'.

Dermal exposure is expected during manual application of paint by rolling and brushing and cleaning of the equipment.

Assuming a maximum concentration of TBHP in the paint of 1% leads to the following reasonable worst case exposure level, based on the combined data from the RIVM fact sheet and the RISKOFDERM measurements on styrene:

Dermal exposure: reasonable worst case: 27 mg/day.

Conclusions scenario 4

The scenario 'Use of products containing <1% TBHP' is based upon very limited information from the Danish product register. According to this information 38 products like paints, lacquers and varnishes, adhesives and binding agents contain less than 1% of TBHP.

The following exposure levels will be used for further risk assessment for scenario 4.

-          Inhalation exposure: reasonable worst case, full shift: 4 mg/m^3;

-          Inhalation exposure: reasonable worst case, short term: 8 mg/m^3;

-          Dermal exposure: reasonable worst case: 27 mg/day.

The inhalation exposure levels are expected to overestimate the real exposure levels.

4.1.1.2.5 Summary of occupational exposure

Conclusions

Table 4.1.1D Conclusions of the occupational exposure assessment

Scenario / subscenario	Activity	Frequency (day/year)	Duration (hr/day)	Estimated inhalation exposure level: RWC		Estimated skin exposure level		
				(mg/m³)	Method	(mg/cm²/day)	dose (mg/day)	Method
1: Production and use of TBHP in the chemical industry	peak activities: drumming, sampling, cleaning and maintenance, repair work	100-200	1 – 2	5	measured	0-0.1	42 (single day)	EASE, expert judgement
	Full shift*	100-200	7 – 8	0.5	measured			
2: Production and use of TBHP containing hardeners of plastics	Peak activities: transfer activities, emptying drums, sampling, packaging, cleaning and maintenance, repair work	10-50	1 – 2	10	EASE, expert judgement	0-0.1	42 (single day)	EASE, expert judgement
	full shift*	10-50	7 – 8	3.2	Calculated			
3: Production of products containing <1% TBHP	peak activities: emptying drum and cleaning and maintenance	10-50	0 – 1	10	EASE, expert judgement	0-0.1	42 (single day)	EASE, expert judgement
	full shift**	10-50	6 – 8	3.6	Calculated	0.001-0.01	4	EASE, expert judgement
4: Use of products containing <1% TBHP	Manual application and cleaning of equipment	10 – 50	2 – 4	8	Measured (analogous data)	0.032***	27	EASE, expert judgement
	full shift	10 – 50	7 – 8	4	Measured (analogous data)			

*   Full shift exposure calculated from 2 hours at 10 mg/m³ and an exposure of 0.4 mg/m³ during the remaining 6 hours.

**   Full shift exposure calculated from 0.5 hour at 10 mg/m³ and an exposure of 4 mg/m³ during 6 hours and negligible during the rest of the day.

***   Calculated from the dose assuming an exposed surface area of 840 cm².

4.1.1.3   Consumer exposure

TBHP is mainly used in the chemical industry as starting material (or intermediate) and as a reactive ingredient (catalyst, initiator or curing agent).

When used as an intermediate, it is not likely that TBHP will be present in the end product, as TBHP will be totally converted. Also for the use as process regulator it is expected that no TBHP will be present after polymerisation (see Note in section 2.2).

Although this use does not indicate consumer exposure, a search in several databases indicated that TBHP is used in consumer products. According to Ritchie *et al.*, (2005), TBHP is used in home and commercial bleaching and disinfectant products. The SPIN database indicated that TBHP was used in consumer products in Sweden from 1999 to 2003 and in Norway from 2001 to 2003 (no later data available). The total amount of TBHP used in these products is less then one ton per year. The Swiss product database contained two products, Parmetol H 92 and Parmetol T 94, produced by Schulke and Mayr in Germany. These products are used as a can preservative in different products which could be consumer products like cleaning products, dish washing liquid, softener, liquid detergent, glues and paints, in concentrations ranging from 0.05-0.20%. As they contain 22% TBHP, the maximum concentration TBHP in the endproducts is 0.04%. TBHP is only identified and not notified according to the Biocidal Product Directive. Therefore, the production of this product will be ended after 1 September 2006 (Schulke & Mayr, 2005).

4.1.1.4   Humans exposed via the environment

TBHP is completely soluble in water (> 100 g/L), is moderately volatile (2700 Pa) and has a low octanol-water partition coefficient (log K_{ow} of 0.7). In an STP TBHP is degraded for around 90% into the primary metabolite TBA (tertiary butyl alcohol, see also section 3.5.1.3). Based on the low log K_{ow}, the bioaccumulation and sorption potential of TBHP is considered to be very low. The biocentration factor for fish was estimated with the QSAR in EUSES (BCF of 1.41 for fish).

Local exposure

The environmental emissions of TBHP for the production and processing sites are summarized in Table 3.1.16 (section 3.1.5). On the basis thereof the estimated TBHP concentrations in air, drinking water and food for all relevant life-cycle steps are presented in **Table 4.1.1.4A** (EUSES calculation), and the total daily intakes in **Table 4.1.1.4B**.

Table 4.1.1.4A TBHP concentrations in various environmental compartments relevant for exposure man indirectly via the environment (local scale, all relevant scenarios)

	Conc. Air [μg/m³]	Conc. Drinking water [mg/l]	Conc. Wet fish [mg/kg]	Conc. Root tissue [mg/kg]	Conc. Leaves [mg/kg]	Conc. Meat [mg/kg]	Conc. Milk [mg/kg]
Production							
I-a [1]	3.56E-02	2.1E-03	2.97E-03	4.36E-05	3.45E-05	9.71E-08	9.71E-07
I-b [1]	3.34E-03	2.23E-04	3.15E-04	1.01E-05	3.25E-06	1.02E-08	1.02E-07
I-c	30.8	8.31	0.0833	8.26	0.0389	3.68E-04	3.68E-03

	Conc. Air [µg/m³]	Conc. Drinking water [mg/l]	Conc. Wet fish [mg/kg]	Conc. Root tissue [mg/kg]	Conc. Leaves [mg/kg]	Conc. Meat [mg/kg]	Conc. Milk [mg/kg]
Processing							
II-a1	See scenario I-b						
II-a2	1.91	0.0599	6.06E-04	0.0596	1.91E-03	2.91E-06	2.91E-05
II-a3	13.3	0.361	2.35E-03	0.36	0.0133	1.78E-05	1.78E-04
II-b1	2.29	0.438	0.437	0.435	2.69E-03	1.95E-05	1.95E-04
II-b2	See scenario I-c						
II-b3	4.6E-01	0.0875	0.0876	0.0871	5.41E-04	3.9E-06	3.9E-05
II-b4	2.62E-02	0.0544	4.68E-03	0.0541	8.47E-05	2.39E-06	2.39E-05
II-b5	1.29E-02	0.0218	2.13E-03	0.0217	3.62E-05	9.55E-07	9.55E-06
II-b6	9.13E-03	2.23E-04	3.15E-04	1.59E-05	8.86E-06	1.11E-08	1.11E-07
II-b7	2.7E-01	5.47E-04	3.31E-04	5.44E-04	2.62E-04	6.42E-08	6.42E-07
II-b8	5.51E-03	5.34E-03	5.66E-03	5.31E-03	1.12E-05	2.35E-07	2.35E-06
II-b9	1.1E-02	4.3E-04	6.07E-04	3.08E-04	1.1E-05	2.04E-08	2.04E-07
II-b10	1.86E-02	3.61E-03	3.59E-04	3.59E-03	2.19E-05	1.61E-07	1.61E-06
II-b11	2.24E-02	3.66E-03	3.95E-03	3.64E-03	2.57E-05	1.63E-07	1.63E-06
II-b12	9.47E-03	1.18E-03	1.49E-03	1.18E-03	1.05E-05	5.32E-08	5.32E-07
II-b13	3.61E-03	2.59E-04	3.66E-04	6.45E-05	3.57E-06	1.19E-08	1.19E-07
II-b14	8.67E-03	1.23E-03	1.33E-03	1.23E-03	9.75E-06	5.53E-08	5.53E-07
II-b15	1.1E-02	8.5E-04	3.57E-04	8.45E-04	1.15E-05	3.88E-08	3.88E-07
II-b16	7.15E-03	2.59E-04	3.18E-04	2.57E-04	7.21E-06	1.24E-08	1.24E-07
II-b17	2.23E-02	2.87E-04	4.06E-04	1.2E-04	2.17E-05	1.59E-08	1.59E-07
II-b18	1.9E-01	3.74E-04	3.26E-04	3.72E-04	1.84E-04	4.47E-08	4.47E-07
II-b19	7.19E-02	6.56E-03	7.89E-03	6.52E-03	7.68E-05	2.98E-07	2.98E-06
II-b20	3E-02	2.31E-04	3.27E-04	1.7E-04	2.92E-05	1.46E-08	1.46E-07

Table 4.1.1.4B Daily doses [mg/kg bw/day] of TBHP through intake of food and air (local scale, all relevant scenarios)

	Total daily intake	Air	Drinking water	Fish	Root crops	Leaf crops	Meat	Milk
Production								
I-a [1]	**7.59E-05**	1.02E-05	6.01E-05	4.88E-06	2.39E-07	5.92E-07	4.18E-10	7.78E-09
I-b [1]	**7.96E-06**	9.55E-07	6.37E-06	5.17E-07	5.55E-08	5.57E-08	4.4E-11	8.21E-10
I-c	**0.292**	8.81E-03	0.237	1.37E-04	0.0453	6.68E-04	1.58E-06	2.95E-05
Processing								
II-a1	See scenario I-b							
II-a2	**2.62E-03**	5.45E-04	1.71E-03	9.95E-07	3.27E-04	3.28E-05	1.25E-08	2.33E-07

	Total daily intake	Air	Drinking water	Fish	Root crops	Leaf crops	Meat	Milk
II-a3	**0.0163**	3.81E-03	0.0103	3.86E-06	1.97E-03	2.28E-04	7.66E-08	1.43E-06
II-b1	**0.0163**	6.54E-04	0.0125	7.17E-04	2.39E-03	4.62E-05	8.38E-08	1.56E-06
II-b2	**See scenario I-c**							
II-b3	**3.26E-03**	1.32E-04	2.5E-03	1.44E-04	4.78E-04	9.28E-06	1.68E-08	3.12E-07
II-b4	**1.87E-03**	7.48E-06	1.56E-03	7.68E-06	2.97E-04	1.45E-06	1.03E-08	1.91E-07
II-b5	**7.49E-04**	3.68E-06	6.22E-04	3.5E-06	1.19E-04	6.21E-07	4.11E-09	7.65E-08
II-b6	**9.74E-06**	2.61E-06	6.37E-06	5.17E-07	8.7E-08	1.52E-07	4.78E-11	8.9E-10
II-b7	**1.01E-04**	7.71E-05	1.56E-05	5.44E-07	2.99E-06	4.49E-06	2.76E-10	5.15E-09
II-b8	**1.93E-04**	1.57E-06	1.53E-04	9.3E-06	2.92E-05	1.91E-07	1.01E-09	1.88E-08
II-b9	**1.83E-05**	3.13E-06	1.23E-05	9.98E-07	1.69E-06	1.88E-07	8.79E-11	1.64E-09
II-b10	**1.29E-04**	5.31E-06	1.03E-04	5.89E-07	1.97E-05	3.76E-07	6.91E-10	1.29E-08
II-b11	**1.38E-04**	6.4E-06	1.04E-04	6.49E-07	2E-05	4.4E-07	7.02E-10	1.31E-08
II-b12	**4.56E-05**	2.71E-06	3.38E-05	2.44E-06	6.46E-06	1.79E-07	2.29E-10	4.27E-09
II-b13	**9.45E-06**	1.03E-06	7.4E-06	6.01E-07	3.54E-07	6.12E-08	5.1E-11	9.5E-10
II-b14	**4.68E-05**	2.48E-06	3.53E-05	2.19E-06	6.73E-06	1.67E-07	2.38E-10	4.43E-09
II-b15	**3.28E-05**	3.13E-06	2.43E-05	5.87E-07	4.64E-06	1.98E-07	1.67E-10	3.11E-09
II-b16	**1.15E-05**	2.04E-06	7.39E-06	5.22E-07	1.41E-06	1.24E-07	5.32E-11	9.92E-10
II-b17	**1.63E-05**	6.37E-06	8.2E-06	6.66E-07	6.59E-07	3.73E-07	6.83E-11	1.27E-09
II-b18	**7.07E-05**	5.43E-05	1.07E-05	5.36E-07	2.04E-06	3.16E-06	1.92E-10	3.58E-09
II-b19	**2.58E-04**	2.05E-05	1.87E-04	1.3E-05	3.58E-05	1.32E-06	1.28E-09	2.39E-08
II-b20	**1.71E-05**	8.57E-06	6.61E-06	5.36E-07	9.34E-07	5.01E-07	6.27E-11	1.17E-09

Exposure via air

The contribution of air to the total daily intake is very variable for the different scenarios: it varies from ±0.5% in scenarios II-b4/b5 to 76-77% in scenarios II-b7/b18. In the scenarios with the highest total daily intakes (I-c, II-a3, II-b1 and II-b2) the air contribution is 3, 23, 4 and 3%, respectively.

Exposure via food and water

For most scenarios, drinking water and root crops contribute the most to the daily intake via the oral route. It is to be noted that the intake of TBHP via drinking water and food as calculated with EUSES represents the worst case as TBHP is rapidly metabolized in the aquatic compartment into several metabolites, TBA being the most prominent.

Regional exposure

On a regional scale, the total daily intake of TBHP is 8.07E-06 mg/kg bw/day. The contribution of air to this total daily intake (12%, based on a regional PEC in air (total) of 3.34E-03 $\mu g/m^3$ (EUSES)) is minor as compared to the contribution of food.

4.1.1.5   Combined exposure

As there is no consumer exposure, combined exposure concerns only the combination of worker exposure and exposure of man via the environment However, for workers a conclusion i or a conclusion iii is reached for all endpoints that are also of concern for man exposed via the environment. The additional exposure via the environment will not change these conclusions. Therefore, no combined exposure assessment was performed.

4.1.2   Effects assessment: Hazard identification and dose (concentration) - response (effect) assessment

4.1.2.1   Toxicokinetics, metabolism and distribution

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).

4.1.2.1.1   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 4.1.2.A**. The results indicate that SC exposure resulted in somewhat longer terminal half-lives and higher AUC values for total radioactivity.

Table 4.1.2.A. ^{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-$^{.}$CH3 radical adduct or POBN-$^{.}$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 4.1.2.7.2). 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 4.1.2.B**), 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 4.1.2.B. ^{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 [14C]-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). [14C]-TBHP (6.4 μl) was applied on top of the rat skin membranes (surface 0.64 cm², 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 4.1.2.C**). 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 4.1.2.D**). 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 4.1.2.C. In vitro penetration parameters of TBHP (as total radioactivity)

covering	charcoal			glass		
exposure time	8	8	4	8	8	4
dose (mg/cm²)	0.1	1.0	6.0	0.1	1.0	6.0
mean flux constant (slope)(µg/cm².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 4.1.2.D. 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/cm²	1.0 mg/cm²	6.0 mg/cm²	0.1 mg/cm²	1.0 mg/cm²	6.0 mg/cm²
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 4.1.1D in section 4.1.1.2).

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 4.1.2.D), 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.

4.1.2.1.2   Studies in humans

<u>In vivo studies</u>

None

4.1.2.1.3   Summary of toxicokinetics, metabolism and distribution

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.
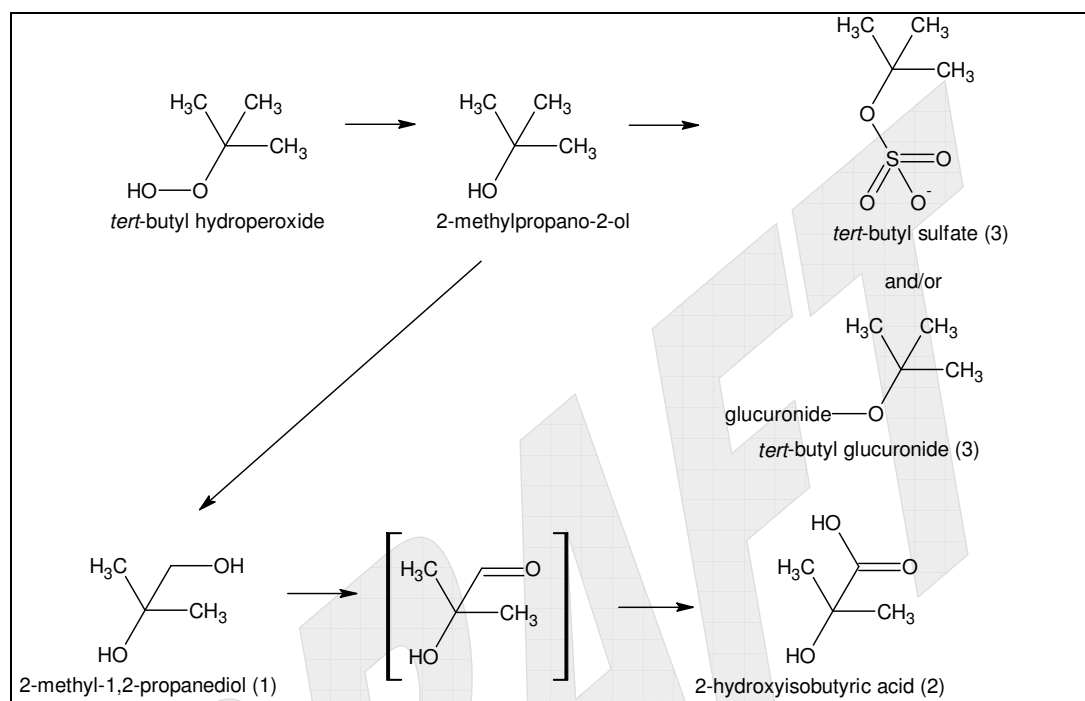
4.1.2.2   Acute toxicity

4.1.2.2.1   Studies in animals

<u>In vivo studies</u>

The results of the available acute toxicity studies are summarized in **Table 4.1.2.E**.

Table 4.1.2.E. A summary of acute toxicity studies

Route	Species	Endpoint LD$_{50}$/LC$_{50}$	Method	Reference
Oral	Rat ♂♀, adult Sprague-Dawley (n=5/sex/dose)	560 mg/kg bw 70% TBHP	EPA 40 CFR-63.81-1	Field and Field, 1981; SIDS, 1995
	Rat ♂, adult Wistar (n=5/dose)	406 mg/kg bw 70% TBHP	Other**	Floyd and Stokinger, 1958 Izmerov et al., 1982*
	Rat	800 mg/kg bw	Unknown	Izmerov et al., 1982*
	Mouse	800 mg/kg bw	Unknown	
Inhalation	Rat ♀♂, adult Sprague-Dawley (n=5/sex/dose; 4h)	1850 mg/m³ 100% TBHP (= 2643 mg/m³ 70% TBHP)	OECD-like	Thackara and Rinehart, 1980; SIDS, 1995
	Rat ♂, adult Wistar (n=6/dose; 4h)	1845 mg/m³ 100% TBHP (= 2636 mg/m³ 70% TBHP)	Other	Floyd and Stokinger, 1958
	Mouse ♂, adult Swiss (n=10/dose; 4h)	1292 mg/m³ 100 % TBHP (= 1846 mg/m³ 70% TBHP)	Other	Floyd and Stokinger, 1958
Dermal	Rabbit ♀♂, adult New Zealand White (n=5/sex/dose)	628 mg/kg bw 70% TBHP	OECD	Kingery and Valerio, 1982; SIDS, 1995
Intra-peritoneal	Rat ♂, adult Wistar (n=5/dose)	87 mg/kg bw 100% TBHP	Other	Floyd and Stokinger, 1958
	Mouse ♂, adult CFT–Swiss (n=6/dose)	270 mg/kg bw 100% TBHP	Other	RajeshKumar and Muralidhara, 1999
	Rat	200 mg/kg bw	Unknown	Izmerov et al., 1982*
	Mouse	246 mg/kg bw	Unknown	Izmerov et al., 1982*

* For this secondary study no study-details were available, only LD$_{50}$ values were reported; it is not clear whether the data refer to 70 or 100% TBHP.
** Method described but different from OECD method.

Inhalation

In the acute inhalation study of Thackara and Rinehart (1980) rats were exposed for four hours to aerosols of aqueous TBHP (17.2% TBHP by weight). The calculated nominal exposure concentrations were 0, 1090, 1570, 2180, or 2910 mg/m³ for pure TBHP. At all exposure levels signs of respiratory and ocular irritation were rapidly produced. Animals exposed to the lowest dose appeared to recover during the 14-day post-exposure period. In all cases where mortality was produced, the animals died within three days of exposure. Gross necropsy findings included lung discoloration. The calculated LC$_{50}$-value was 1850 mg/m³ for 100% TBHP. However, due to the fact that in this study no actual TBHP concentrations and no aerosol particle size were measured the exact exposure concentrations remain unclear.

Further, bearing in mind the high vapour pressure of TBHP, it is likely that at least part of the TBHP evaporated and was available as a vapour.

In the acute inhalation study of Floyd and Stokinger (1958) rats and mice were exposed for four hours to TBHP vapours. The different nominal concentrations used were not reported. In both rats and mice high dosage levels of TBHP vapour (doses not specified) caused porphyrin deposition in the nostrils and irregular respiration. Autopsy of mice and rats killed by the exposure to TBHP vapour showed hyperaemia of the lungs, with haemorrhages on the lung surface. For rats, the LC_{50}-value was 1845 mg/m³ 100% TBHP. For mice, the LC_{50} was 1292 mg/m³ 100% TBHP.

Dermal

When TBHP was administered dermally to the abraded skin of albino rabbits (dose levels 0, 480, 576, 720, 864, 1248, or 1997 mg/kg bw 70% TBHP; 24 hours under occlusive dressing) no clinical effects were observed in the lowest dose groups, although some animals died. At the highest dose levels the following clinical effects were observed: cyanosis, ataxia, lethargy, slow and laboured respiration, nasal discharge, congestion, prostration, nystagmus, red discharge from nares, and convulsions and vocalization immediately prior to death. Death occurred within 48 hours. Local skin reactions revealed area of necrosis surrounded by an inflammatory ring and blanching beyond that. For survivors atonia was present and became more pronounced with time. Cracking and exfoliation with bleeding also occurred. For those animals found dead the exposure site revealed massive haemorrhage and rupture of the small capillaries. Gross pathologic findings were dark coloured lungs, liver, spleen, and urinary bladder. The LD_{50}-value was 628 mg/kg bw 70% TBHP (Kingery and Valerio, 1982).

Oral

A single gavage administration of TBHP was given to six groups of ten Sprague-Dawley rats (five/sex) at doses of 420, 560, 700, 840, 980, or 1120 mg/kg bw 70% TBHP. The following toxicity signs were observed at most dose levels: depression, pallor, and loss of righting, lacrimation, hypothermia, and haematuria. Necropsy findings were gastric erosions, blood in GI tract, haematuria and greyish-tan lungs at most doses. At 840 mg/kg bw and higher necropsy findings included also pale kidneys and greyish-tan stomachs, duodenums, pancreas and adrenals. In the highest dose-group pale pancreas was also observed. Mortality occurred within 48 hours. The LD_{50} was 560 mg/kg bw 70% TBHP (Field and Field, 1981).

In the study by Floyd and Stokinger (1958) an LD_{50} of 406 mg/kg bw 100% TBHP was reported. Death occurred within five days and there was no weight loss in any of the surviving animals. No further details were given.

Other routes

In rats, intraperitoneal administration of single doses of TBHP (20% TBHP in propylene glycol) resulted in signs of weakness, some loss of equilibrium, a coarse pelage, prostration, and at higher doses porphyrin deposition in the nostrils. Death occurred within six days. The LD_{50}-value was 87 mg/kg bw 100% TBHP (Floyd and Stokinger, 1958).

In mice intraperitoneal administration of doses ranging from 500 – 3500 μmol/kg bw TBHP resulted in an LD_{50} of 3000 μmol/kg bw (equivalent to 270 mg/kg bw 100% TBHP). No toxicity details were given in this study (Kumar and Muralidhara, 1999).

4.1.2.2.2   Studies in humans

No data available.

4.1.2.2.3   Summary of acute toxicity

Although not all the studies are according to OECD-guidelines and some are rather dated the data are sufficient to fulfil the Annex VII requirements for acute toxicity. After acute exposure the oral LD_{50} was 406 mg/kg bw 70% TBHP and 560 mg/kg bw 100% TBHP for rats and 800 mg/kg bw (purity not specified) for mice. The dermal LD_{50} was 628 mg/kg bw 70% TBHP for rabbits. With respect to inhalation the LC_{50} was 1850 mg/m³ 100% TBHP for rats and 1292 mg/m³ 100% TBHP for mice.

It can be concluded that TBHP is harmful after acute oral and dermal exposure (Xn, R21/22). However, the two inhalatory studies differ with respect to the resulting classification. The study by Thackara and Rhinehart was performed with an aerosol and resulted in an LC_{50} of 1.850 mg/L. However, bearing in mind the vapour pressure of TBHP, it is likely that at least part of the TBHP evaporated and was available as a vapour. The study by Floyd and Stokinger was performed using vapours and resulted in an LC_{50} of 1.8 and 1.3 mg/L for rats and mice, respectively. This indicates classification of 100% TBHP with R23 because it is within the limits of 0.5 to 2 mg/L for a vapour. If the results are converted to concentration for 70% TBHP, than the LC_{50} values in rats are just above 2 mg/l indicating R20 for 70% TBHP but just below 2 mg/l in mice indicating R23 for 70% TBHP. As it is unknown whether humans resemble more to mice or rats in this respect, classification with R23 is proposed for 70% TBHP.

Classification with T; R23 and Xn; R21/22 was confirmed by the TC-C&L.

4.1.2.3   Irritation

The results of available irritation **studies are summarized in Table 4.1.2.F.**

Table 4.1.2.F. Summary of irritation studies

	Species	Grading	Result	Method	Reference
Skin	Rabbit ♂♀, adult New Zealand White	See **Table 4.1.2.G**	Corrosive	EPA 40 CFR 163.81-5	Field, 1981a
	Rabbit ♂, adult New Zealand White	*	Irritant/Corrosive	Other	Floyd and Stokinger, 1958

	Species	Grading	Result	Method	Reference
Eye	Rabbit ♀♂, adult New Zealand White	See **Table 4.1.2.H**	Irritant	EPA 40 CFR 163.81-4	Field, 1981b
	Rabbit ♂, adult New Zealand White	*	Irritant	Other	Floyd and Stokinger, 1958
	Rabbit (no details given)	*	Irritant	Other	Küchle, 1958
Respiratory tract	Mouse ♀♂, adult Swiss Webster		Irritant RD_{50}=77.1 mg/m³	Sensory irritation test	Arts and Zwart, 1992

* No scores given

4.1.2.3.1   Skin

Studies in animals

In the study of Field (1981a) 70% TBHP appeared to be a severe dermal irritant. In this study six New Zealand White rabbits (three/sex) were clipped free of hair over the back covering an area of approximately 10% of the body surface. The left side of each rabbit was abraded; the right side remained intact. On each of the rabbits, two areas on the abraded side and two areas on the intact side were exposed to a 0.5 ml aliquot of 70% TBHP for 24 hours, which was covered with four layers of gauze. The patches were covered with a rubber dental dam and both secured with tape. All rabbits became cyanotic and depressed within one hour. Three of six animals (two females, one male) died within 24 hours following test compound administration. At 24 hours (or at death) all rabbits showed moderate to severe oedema and four had mild to moderate erythema. Changes were the same for intact and abraded skin. At 72 hours a mild to moderate erythema was seen in two of three survivors, slight oedema was seen in one and severe oedema in the remaining two survivors. Fourteen days post-dosing two of the remaining rabbits had skin described as 'parchment-like'. The skin of these two animals appeared necrotic and was beginning to slough. For results see **Table 4.1.2.G**.

Table 4.1.2.G Summary of test results for skin irritation (Field, 1981a)

Mean scores observed after	24 hours (n =6)		72 hours (n = 3)	
	intact skin	abraded skin	intact skin	abraded skin
Erythema	0.33	0.84	1.67	1.67
Oedema	3.5	3.5	3	3

Similar irritating effects were seen in the study of Floyd and Stokinger (1958) where TBHP on the skin of New Zealand White rabbits resulted in erythema, oedema, and vesication within two or three days. In this study the back of rabbits was sheared and to six spots one or two drops of TBHP were applied and spread to a circular area about two centimetres in diameter. Exposure duration was 24 hours (no occlusion). Readings were at 24, 48, and 72

hours, but no scores were given. The maximum non-irritating strength of TBHP was investigated using dilutions of TBHP (dilutions made with propylene glycol) and was determined to be 35% but no further details were provided.

In the acute dermal toxicity study of Kingery and Valerio (1982; as already described in section 4.1.2.2.1.) even the lowest doses of 0.5 – 0.75 ml/kg bw TBHP resulted in severely irritated necrotic skin. The exposure was 24 hours on abraded skin under occlusion.

In the in vitro percutaneous absorption study of Maas (2004), described in 4.1.2.1.1, skin surface damage was often found at the end of the exposure period especially with the higher dose levels and under occlusive conditions.

Studies in humans

No data available.

4.1.2.3.2   Eye

Studies in animals

In an eye-irritation study of Field (1981b) nine New Zealand White rabbits received a 0.1 ml aliquot of 70% TBHP in one of the eyes. The other (untreated) eye was used as a control. The treated eyes of six of the rabbits were not washed, whereas from the other three rabbits the treated eyes were washed after 30 seconds with water. In both groups severe irritation occurred as evidenced by iritis and corneal opacities, keratitis, corneal ulcers, and keratoconus. Iritis and corneal opacities persisted in both groups in most of the animals through 21 days.

Two earlier studies (Floyd and Stokinger, 1958; Küchle, 1958) also reported that concentrated solutions of 70% TBHP affected the cornea, iris, and conjunctiva of the eyes of rabbits, but in these studies no scores were given. Washing the eyes with water four seconds after application prevented reactions. Floyd and Stokinger (1958) determined the maximum non-irritating strength of TBHP in the eye to be 7% (dilutions made with polypropylene glycol). Application of 35% resulted in severe eye irritation that was not reversible within 7 days.

An additional eye irritation test (Beek, 1978), which is not available to the rapporteur, is summarised in the category justification/test plan submitted to the US EPA by the Organic Peroxide Producers Safety Division Toxicology Committee/HPV Testing Working Group of the Society of the Plastic Industry, Inc. In general, the results confirmed the severe irritating and corrosive effects on the eye found in the other studies.

Table 4.1.2.H Summary of test results for eye irritation (Field, 1981b: n=6 animals: 3m, 3f)

Mean scores observed after	24 hours		48 hours		72 hours		96 hours		7 days		10 days		13 days		16 days		19 days		21 days		48 days	
Effect	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m*	f	m*	f	m*	f	m	f
Unwashed (n=3/sex)																						
Cornea opacity	2	2	2	2	1.7	3	4	3	3	2.3	2.7	2.3	3.3	2	3.5	2	3.5	2	3.5	1.7	nd	nd
Cornea area	4	4	4	4	4	4	4	4	4	4	3.3	4	4	4	4	4	4	4	4	4	nd	nd
Iris	1	1	1	1	1	**	1	**	**	**	**	1	**	0.3	**	0.3	**	0.3	**	0.3	nd	nd
Conjunctiva redness	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.3	1	0.3	1	0	nd	nd
Conjunctiva chemosis	3	1.3	3	1	2	1	1.3	0.7	0.7	0	1	0	1	0	.5	0	0	0	0	0	nd	nd
Conjunctiva discharge	2	2	1.7	1.3	1.7	1.3	1.3	1.3	1.3	1	1.3	0.7	1	1	1	0.3	1	0.3	0	0	nd	nd
Washed (2 females, 1 male)																						
Cornea opacity	2	1.5	2	3	2	3	2	3	2	2	3	1.5	3	1.5	3	1.5	3	1.5	nd	nd	3	1.5
Cornea area	4	4	4	4	4	4	4	4	4	4	3	4	4	3	4	3	4	3	nd	nd	4	2.5
Iris	1	1	1	**	1	**	1	**	1	1	1	1	**	0.5	**	0.5	**	0.5	nd	nd	**	0.5
Conjunctiva redness	1	1	1	1	1	1	1	1	0	0.5	1	1	1	0.5	0	0.5	0	0.5	nd	nd	0	0
Conjunctiva chemosis	2	2	2	2.5	1	2	1	1.5	0	0.5	0	0.5	0	0.5	0	0.5	0	0	nd	nd	0	0
Conjunctiva discharge	2	2	2	1.5	1	2	1	1.5	1	0.5	1	1	1	1	0	0.5	1	1	nd	nd	0	0.5

* One male rabbit died after 17 days; ** Could not be visualized due to opacity of the cornea; nd = not determine

Signs of ocular irritation were reported in the acute inhalatory toxicity study by Thackara and Rinehart (1980) described in 4.1.2.2.1.

Studies in humans

No data available.

4.1.2.3.3 Respiratory tract

Studies in animals

A sensory irritation test (Arts and Zwart, 1992) was performed with male Swiss Webster mice (four/dose) who were exposed to 13, 21, 45, or 82 ppm TBHP (48, 77, 166, or 303 mg/m³ 100% TBHP, respectively). After a single period exposure of 30 minutes, animals were kept for a 7-day observation. No abnormalities in behaviour and no significant weight loss were seen during the 7-day observation period. Almost all mice (15/16) had a decreased breathing rate and a post-inspiratory apnoea. The concentration that produced a 50% decrease in breathing frequency (i.e. RD_{50}) for mice was determined at 20.9 ppm (= 77.1 mg/m³) 100% TBHP. One male mouse exposed to the highest concentration (82 ppm) showed spungy-like, foamy lungs at autopsy. This was considered to be treatment related by the study authors.

In a second study of Arts and Zwart (1992) male Wistar [Crl: WI (WU)BR] rats (five/dose) were exposed to 21, 45, 82, 152, or 269 ppm TBHP (77, 166, 303, 561, or 993 mg/m³ 100% TBHP, respectively). After a single period exposure of 30 minutes, animals were kept for a 7-day observation. During exposure, clear restlessness was observed in most rats exposed to the highest concentration levels. Some rats showed a considerable decrease in breathing frequency, and great fluctuations in lung mechanical properties, but no relation with the exposure concentration was observed. The study-authors suggest that these effects might be stress-related. No gross changes were found at the autopsy at 7 days after exposure. No general conclusions could be obtained regarding potential changes in lung mechanical properties after TBHP exposure in rats.

In a cytogenicity study of Ben-Dyke and Hogan (1981; see section 4.1.2.7) ten male and ten female Sprague-Dawley rats were exposed to TBHP vapours for six hours/day for five consecutive days at concentrations of 0, 9, 30, 74 or 94 ppm (0, 33, 111, 273, or 347 mg/m³ 100% TBHP, respectively). (Slightly) increased incidences of lacrimation, red nasal discharge, mucoid nasal discharge and dry rales were observed in the exposed animals (no details regarding statistical significance were reported). No exposure related macroscopic changes were observed at the necropsy. It is noted that no clear concentration relationship was observed for the effects (exposure concentrations 9-94 ppm). This is, however, in agreement with the study with mice of Arts and Zwart (1992) in which 15/16 animals at concentrations between 13 and 82 ppm showed a reduction in breathing frequency.

The acute inhalation studies showed discoloration, hyperaemia and haemorrhages of the lung after necropsy. Also, signs of respiratory irritation were reported in the acute inhalatory toxicity study by Thackara and Rinehart (1980) described in 4.1.2.2.1.

Studies in humans

No data available.

4.1.2.3.4 Summary of irritation

The available data are acceptable to fulfil the Annex VII requirements for irritation testing to the eyes and skin, although it is to be noticed that the skin was exposed for 24 hours. TBHP is corrosive to the skin and causes serious damage to the eyes. Classification with C, R34 (which covers both effects) is proposed. TBHP also induces respiratory tract irritation, which indicates a classification with Xn, R37. However, R37 is also covered by R34 but is needed at concentrations below 10%. No specific concentration limits are proposed for skin and eye irritation because of the shortcomings and limited details on the studies with lower concentrations. Classification with C; R34 and with Xn; R37 between 5 and 10% was confirmed by the TC-C&L.

For a quantitative risk characterisation for respiratory tract irritation, the concentration of 33 mg/m³ from the study of Ben-Dyke and Hogan (1981) with rats will be taken forward to the risk characterisation as a LOAEC. Though no clear concentration relationship was observed for the effects which occurred, the effects which occured at the concentration of 33 mg/m^3 (the lowest tested concentration) are regarded as adverse treatment related effects indicating irritation.

4.1.2.4 Corrosivity

See irritation.

4.1.2.5 Sensitisation

4.1.2.5.1 Studies in animals

The results of available sensitisation studies are summarized in **Table 4.1.2.I**.

Table 4.1.2.I. Summary of skin sensitisation studies

Species	Method	Concentrations	Result	Method	Reference
Guinea-pig	GPMT	Intradermal induction: 1% TBHP (70%) Dermal induction: 30% Dermal challenge: 3%	Sensitiser (positive 6/10)	OECD 406	Prinsen, 2001
Guinea-pig	Buehler	Dermal induction: 2.5% TBHP (89.2%) Dermal challenge: 1%	Not acceptable	OECD 406	Morris, 1996

Skin

In vivo studies

In a GMPT according to OECD 406 (Prinsen, 2001), 10 male guinea-pigs (Dunkin Hartley) were induced on day 1 with 3 pairs of intradermal injection of 0.1 mL of FCA in saline (1:1), 1% TBHP (70%) in saline and a mixture (1:1) of 1% TBHP in saline and FCA. Skin readings were done 24 hours after treatment. On day 8, topical induction was performed on 8 cm^2

clipped skin with 30% TBHP for 48 hours. Skin readings were done directly after removal of the patches. Five control animals were treated similarly but without TBHP. The induction and challenge concentrations were based on range finding experiments. Dermal challenge was performed on day 22 on an unknown area of clipped skin using 3% TBHP for 24 hours. Skin readings were done at 24 and 48 hours after patch removal using the Magnusson and Kligman scale.

One control animal died on day 10. Slight erythema was found in the range finding study after intradermal injection with 1% in saline but no erythema was seen in the main study. Moderate and confluent erythema was found in the range finding study after dermal induction with 30% but only discrete or patchy erythema in the main study in 5 out of 10 animals. No erythema was seen in the range finding study after dermal treatment with 3%. Moderate to severe erythema was seen at 24 and 48 hours after challenge in 6 out of 10 treated animals. No skin reaction was found in the 4 remaining control animals.

TBHP is considered to be a sensitizer in this test.

In a Buehler test (Morris, 1996), 20 guinea pigs (Hartley) were induced on day 1, 8 and 15 with dermal exposure to 0.3 mL 2.5% TBHP (89.2%) in distilled water in Hill Top chambers for 6 hours on clipped skin. Ten control animals were treated in the same way with water. The treated site was scored after 24 hours. Fourteen days later, all animals were challenged in the same way using 1% TBHP. The next day, the treated site was chemically depilated and scored at 24 and 48 hours after patch removal using a modification of the Magnusson and Kligman scale. The scale contained an additional score ± for slight, patchy erythema. The induction and challenge concentrations were based on range finding experiments.

Exposure to 2.5% TBHP induced scores ± (3/4) to 1 (1/4) in the range finding and ± (12/20) to 1 (8/20) after the first induction, ± (8/20) through 1 (11/20) to 2 (1/20) after the second induction and ± (5/20) through 1 (11/20) to 2 (4/20) after the third induction. This is below the requirements for mild to moderate irritation during induction according to OECD 406. Exposure to distilled water induced scores 0 to ± in the induction phase of the controls. After challenge of the treated animals, the scores were 0 (1/20) and ± (19/20) at 24 hours and 0 (7/20) and ± (13/20) at 48 hours. In the control animals, the scores were 0 (2/10) and ± (8/10) at 24 and 48 hours.

This test is not acceptable because of the low irritation during the induction phase.

Respiratory tract

No data are available on respiratory sensitisation.

4.1.2.5.2   Studies in humans

No data available.

4.1.2.5.3   Summary of sensitisation

No information is available on the respiratory tract sensitising potential of TBHP. TBHP is a skin sensitizer in the GPMT. The provided Buehler test is not acceptable due to the low

irritation in the induction phase. TBHP is considered a strong sensitizer because 60% of the animals reacted after induction with 0.7% TBHP. This is based on the proposals of the sensitisation expert group (ECBI/81/02 Rev.2). Therefore, classification with R43 and a specific concentration limit of 0.1% is proposed. Classification with R43 and the specific concentration limit of 0.1% were confirmed by the TC-C&L.

4.1.2.6   Repeated dose toxicity

4.1.2.6.1   Studies in animals

In vivo studies

The results of the available repeated-dose toxicity study are summarized in **Table 4.1.2.J**.

Table 4.1.2.J. Summary of repeated dose toxicity studies

Exposure period	Route	Species	Doses	NOAEL/ LOAEL	Critical effects	Method	Reference
45 days	oral	rat ♀♂ adult Wistar	0, 3, 10, 30 mg/kg bw/day 70% TBHP	NOAEL: 30 mg/kg bw/day 70% TBHP (the highest dose tested)	None (effects on the stomach in the range-finding at 50 mg/kg bw/day)	OECD 422	Jonker *et al.*, 1993; SIDS, 1995
12 times over 17 days	oral, gavage	rat ♀♂ Fischer 344	0, 22, 44, 88, 175, 350 mg/kg TBHP	NOAEL: 22 mg/kg	Forestomach and stomach lesions	Other	Ritchie *et al.*, 2005a
13 times over 18 days	oral, gavage	mice ♀♂ B6C3F1	0, 22, 44, 88, 176, 350 mg/kg TBHP	NOAEL: 22 mg/kg in males and 44 mg/kg in females	Forestomach hyperplasia and body weight reduction (males)	Other	Ritchie *et al.*, 2005b
12 times over 17 days	dermal	rat ♀♂ Fischer 344	0, 22, 44, 88, 175 mg/kg TBHP	NOAEL: 88 mg/kg in males and 44 mg/kg in females	local skin effects	Other	Ritchie *et al.,* 2005c
13 times over 18 days	dermal	mice ♀♂ B6C3F1	0, 22, 44, 88, 176, 350 mg/kg TBHP	NOAEL: 44 mg/kg in males and 88 mg/kg in females	local skin effects	Other	Ritchie *et al.*, 2005d
5 consecutive days for 6 hours/day	inhalation	rat ♀♂ Sprague -Dawley	33, 111, 273, 347 mg/m³ 100% TBHP	NOAEL: 111 mg/m³ in males and 273 mg/m³ in females (study of limited value)	body weight	OECD like bone marrow aberration assay	Ben-Dyke and Hogan, 1981

Inhalation

In a bone marrow cytogenicity study of Ben-Dyke and Hogan (1981; see section 4.1.2.7) ten male and ten female rats (Sprague-Dawley) were exposed for five consecutive days to the vapour of TBHP (0, 33, 111, 273, 347 mg/m³ 100% TBHP) for six hours/day. No mortality occurred. The body weights of male animals exposed to the two highest doses and the body weights of female animals exposed to the highest dose of TBHP were lowered after three exposures. At necropsy, no macroscopical changes were observed. As no more parameters for general toxicity were studied, and the duration of exposure was only five days this study is of limited value for the evaluation of repeated dose toxicity after inhalation exposure.

Dermal

The abstracts of two dermal range finding studies were provided by the NTP.
TBHP was dermally administered in 50 percent aqueous acetone to male and female Fischer 344 rats at dosages of 0, 22, 44, 88, or 175 mg/kg bw (Ritchie *et al*., 2005c). Concentrations ranged between 4.4 and 35% TBHP. Core study animals were dermally administered TBHP 12 times on weekdays only during the 17-day study period. There were five rats/sex in each dosage group and they received 0.5 ml/kg bw. Body weight determinations were recorded weekly, and clinical observations were recorded daily for all animals. Rats were necropsied, with selected organs weighed and selected tissues processed for microscopic examination at study termination. Tissues other than skin and muscle underlying the site of application were only examined if gross lesions were observed. Haemotology and clinical chemistry was not performed. A special study group, consisting at termination of eight dosed (175 mg/kg TBHP) and eight control (0 mg/kg) male rats, was treated similarly to the core study group. On the last study day, each special study 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 special study were not provided in the abstract but some results were available in a review of the studies (NIEHS, 2006).
There were no early deaths in this study, no statistically significant changes in group mean body weights (treated groups versus controls), and no significant changes in mean gross organ weights or mean organ weight-to-body weight percentages. The only clinical sign observed was dermal irritation in 5/5 male and 5/5 female animals administered 175 mg/kg bw TBHP. Histopathology lesions in these groups included minimal to mild hyperkeratosis, hyperplasia and/or inflammation of the epidermis in 5/5 male and 5/5 female rats, and inflammation of the dermis in 5/5 male and 5/5 female rats. Additionally, one female rat assigned to the 88 mg/kg bw dosage group exhibited minimal epidermal hyperplasia and dermal inflammation. The NOAEL for 12 days of dermal delivery of TBHP in 50 percent aqueous acetone is 88 mg/kg bw for male Fischer 344 rats and 44 mg/kg bw for females. This corresponds with NOAEC of 17.6% for males and 8.8% for females.
There were no increases in the free radical formation measured in the lipid extracts of liver, kidney, blood, lung and heart from the high dose male animals.
These values can be used as a NOAEL or NOAEC for local effects but not for systemic effects due to the low number of studied parameters.

TBHP was administered dermally in 50 percent aqueous acetone to male and female B6C3F1 mice at dosages of 0, 22, 44, 88, 176, or 352 mg/kg bw (Ritchie *et al*., 2005d). Concentrations ranged between 1.1 and 17.6% and were applied at 2 mL/kg bw. Animals were dermally administered 13 times (on weekdays) during the 18-day study period. There were five

mice/sex for each dosage group. Body weight determinations were recorded weekly, and clinical observations were recorded daily for all animals. Mice were necropsied with selected organs weighed and selected tissues processed for microscopic examination at study termination. Tissues other than skin and muscle underlying the site of application were only examined if gross lesions were observed. Haemotology and clinical chemistry was not performed.

There were no early deaths in this study, no statistically significant changes in group mean body weights (treated groups versus controls), and no significant changes in organ weights or organ-to-body weight ratios. The only clinical sign observed was dermal irritation at the site of application in 100 percent of male (5/5) and female (5/5) animals administered 352 mg/kg bw TBHP. There was dermal histopathology observed in the 88 to 352 mg/kg bw male dosage groups and in the 176 to 352 mg/kg bw female dosage groups. This histopathology involved the epidermis and dermis in the skin area of application, and also included the underlying skeletal muscle in 2/5 male and 1/5 female mice administered 352 mg/kg bw. Two female (2/5) mice in the 0 mg/kg bw dosage group also exhibited histopathology, involving the epidermis (1/5) and/or dermis (2/5) at the site of application, attributed to effects of the acetone vehicle (Table 4.1.2.K). Based on the histopathology results, the NOAEL for TBHP administered dermally to B6C3F1 mice was 44 mg/kg bw for males and 88 mg/kg bw for females. This corresponds with a NOAEC of 2.2% for males and 4.4% for females. These values can be used as a NOAEL or NOAEC for local effects but not for systemic effects due to the low number of studied parameters.

Table 4.1.2.K Pathological observations on the skin of treated mice

Dose	Males			Females		
	Epidermis	Dermis	Skeletal muscle (SOA)	Epidermis	Dermis	Skeletal muscle (SOA)
0 mg/kg bw/day	-	-	-	Hyperkeratosis 1/5 [1.0] Hyperplasia 1/5 [1.0]	Inflammation 2/5 [1.0] Fibrosis 1/5 [1.0]	-
22 mg/kg bw/day	ND	ND	ND	ND	ND	ND
44 mg/kg bw/day	-	-	-	ND	ND	ND
88 mg/kg bw/day	Hyperkeratosis 1/5 [1.0] Hyperplasia 3/5 [1.3]	Inflammation 2/5 [1.5]	-	-	-	-
176 mg/kg bw/day	Hyperkeratosis 2/5 [1.5] Hyperplasia 5/5 [2.0] Necrosis 1/5 [1.0] Ulceration 1/5 [1.0] Atrophy-follicles 1/5 [1.0]	Inflammation 5/5 [2.2]	-	Hyperkeratosis 1/5 [2.0] Hyperplasia 3/5 [1.7] Necrosis 1/5 [3.0] Atrophy-follicles 1/5 [1.0]	Inflammation 4/5 [2.0] Fibrosis 1/5 [1.0] Inflammation subcutis 1/5 [2.0]	-
352 mg/kg bw/day	Hyperkeratosis 5/5 [3.0] Hyperplasia 5/5 [2.8] Necrosis 4/5 [1.5] Ulceration 5/5 [2.4] Atrophy-follicles 5/5 [1.6]	Inflammation 5/5 [2.6] Fibrosis 2/5 [1.5] Inflammation subcutis 4/5 [1.8]	Degeneration 2/5 [1.5]	Hyperkeratosis 5/5 [2.8] Hyperplasia 5/5 [3.0] Necrosis 3/5 [2.3] Ulceration 1/5 [2.0]	Inflammation 5/5 [3.0] Fibrosis 4/5 [1.0] Inflammation subcutis 4/5 [1.5]	Degeneration 1/5 [1.0]

ND: not determined, -: no effects, SOA: site of application

Oral

In the study of Jonker et al. (1993) two range-finding studies and a Combined Repeated Dose and Reproductive/Developmental Toxicity Screening Test (OECD 422) were described. In all three studies 70% TBHP was given orally by gavage (10 mL/kg bw) to male and female rats

(Crl:WI(WU)BR) at different concentrations in water, at a volume-to-body weight ratio of 10 ml/kg bw:

a. In the first range-finding experiment rats were given 0, 107, 179, 250, or 357 mg/kg bw/day 70% TBHP for five days (four/dose/sex). Postgavage lethargy, reduced food intake and body weight gain were seen at 179 mg/kg bw and above in one or both sexes. At autopsy, all treated rats showed moderate (at 107 mg/kg bw) or severe (at 179 mg/kg bw and above) submucosal oedema in the stomach wall.

b. In the second range finding study rats were given 0, 12.5, 25, or 50 mg/kg bw/day 70% TBHP for five days (two/dose/sex). There were no treatment-related clinical signs. At autopsy limited to slight oedema of the stomach was seen in one male of the 50 mg/kg bw group.

c. In the main study rats were given 0, 3, 10 or 30 mg/kg bw/day 70% TBHP for maximally 45 consecutive days (twelve/dose/sex). The general toxicity results of this study are given in **Table 4.1.2.L** (the reproductive toxicity results are described in section 4.1.2.8). The observations for general toxicity generally comply with those for a 28-day toxicity study. In male rats a dose-related increase in the incidence of renal lesions (multifocal increased accumulation of tubular proteinaceous material and multifocal tubular nephrosis) was observed at all doses. In male rats, multifocal increased tubular proteinaceous material deposits of the 'very slight/slight' severity category increased with dose (statistically significantly only in the 30 mg/kg bw/day group). In addition, multifocal tubular nephrosis of the 'very slight/slight' severity category increased with dose with nearly the same incidences in male rats (also statistically significantly only in the 30 mg/kg bw/day group) (see Table 4.1.2.L). In contrast, no renal lesions were observed in female rats of the 0 mg/kg bw or 30 mg/kg bw groups, the only ones examined. The lesions were characterized by rounded intracytoplasmatic hyaline inclusions and pyknotic nuclei in the proximal tubular cells, and by degeneration and desquamation of tubular cells. The study authors state that these renal lesions resemble those reported to occur in α2u-globulin nephropathy, a toxicological syndrome which to date appears to be limited to male rats, and which is believed to have no significant implications for man. In a mechanistic follow-up study (see study by Wijnands (2002) in section on 'Mechanistic studies' below), the increased accumulation of proteinaceous material was stated to have shown a positive reaction to the immunohistochemical staining for α2u-globulin. A dose-related increase (2-fold) in plasma concentration of bilirubin was observed in males given 10 and 30 mg/kg bw/day, while a dose-related decrease (halving) was seen in females. The changes in bilirubin concentrations were not accompanied by hepatotoxic signs, nor by evidence of changes in red blood cells. Further, the effects in males and females were in the opposite direction.
Jonker et al. (1993) and Wijnands (2002) concluded that the NOAEL which is considered relevant for human risk characterisation was 30 mg/kg bw/day as the kidney effects in male rats were concluded to resemble α2u-globulin nephropathy.

Table 4.1.2.L Summary of general toxicity test results for the oral Combined Repeated Dose and Reproductive/ Developmental Toxicity Screening study (Jonker *et al.*, 1993)

Dose (mg/kgbw)	0		3		10		30		dr
Parents	m	f	m	f	m	f	m	f	
Mortality	0/12	0/12	0/12	1/12	0/12	1/12	0/12	0/12	
Clinical findings	No toxicological relevant effects								
Body weight	No effects								

Dose (mg/kgbw)	0		3	10	30		dr
Food consumption	No effects						
Haematology							
- monocytes						is	
- reticulocytes					ds		
- packed cell volume				ds			
Clinical biochemistry							
- albumin				ds			
- bilirubin (total)			is	ds	is	ds	dr
Organ weights							
- relative kidney weight				is			
Macroscopic observations	No toxicological relevant effects						
Microscopic observations#							
- renal lesions*			i	i	is		dr
a)	4/12	0/12	6/12	9/12	10/12	0/12	
b)	4/12	0/12	5/12	9/12	10/12	0/12	
Fertility	No toxicological relevant effects						

i = increased, d = decreased, is/ds = increased/decreased significantly, dr = dose-related
# including ovaries, uterus, testes, epididymes and seminal vesicles
* a) = multifocal increased tubular proteinaceous material
  b) = multifocal tubular nephrosis

The kidney slides of the study by Jonker *et al.* (1993) were reviewed by a kidney specialist (Hard, 2007). In this review it is stated that:
- no changes indicative of tubule cell cytotoxicity were observed in the high- and mid-dose male and the high-dose female kidney compared to controls;
- no increase in hyaline droplet accumulation was observed in the H&E stained kidney tissue using either conventional microscopy or fluorescence microscopy for enhanced visualisation of hyaline droplets;
- there was no difference in the incidence and severity of chronic progressive nephropathy (CPN) between the high dose males and females compared to the controls.

Hard (2007) concluded that TBHP did not cause any nephropathic alteration in this study. Additionally, the kidney slides of the study by Wijnands (2002) were reviewed by Hard (2007), and it was concluded that also in that study TBHP did not cause any nephropathic alteration.

Apparently, the nature and degree of the renal effects described in the studies by Jonker *et al.* (1993) and Wijnands (2002) were deemed too marginal by Hard (2007) to be biologically or toxicologically relevant and the histological picture was considered to be within normal variation.

In conclusion, the Jonker et al. (1993) and Wijnands (2002) studies revealed very mild renal alterations (which according to them resembe male rat specific α2u-globulin nephropathy) that were reviewed by a kidney specialist and were considered to be insufficient to indicate a clear toxicologically relevant, treatment-related effect. Therefore, from the studies of Jonker *et al.* (1993) and Wijnands (2002) the NOAEL is considered to be 30 mg/kg bw/day 70% TBHP, the highest dose tested.

The abstracts of two oral range finding studies were provided by the NTP.

TBHP in 0.5 percent aqueous methylcellulose was delivered by gavage to male and female Fischer 344 rats at dosages of 0, 22, 44, 88, 175, or 350 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 five Core Study rats per sex or eight Special Study male rats in each treatment group. Body weight determinations were recorded weekly, and clinical observations were recorded daily for all Core Study animals. Core Study rats were subjected to a complete necropsy at study termination; selected organs were weighed, and protocol specified organs and other organs exhibiting gross lesions were processed for microscopic examination (to the NOEL). A Special Study group, consisting at termination of eight treated (175 mg/kg TBHP) and eight control (0 mg/kg) male rats, was dosed similarly to the Core Study groups. On the last study day, each Special Study 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 the special study were not provided in the abstract but some results were available in a review of the studies (NIEHS, 2006).

There were 40 percent early deaths among males (2/5) in the 350 mg/kg bw TBHP group which were considered to be treatment related. All male (5/5) and female (5/5) rats in the 350 mg/kg treatment groups exhibited post-treatment lethargy, thinness, abnormal breathing, ruffled fur, and/or ataxia. Treatment related and statistically significant reductions in group mean terminal body weights were found for both males (43.8 percent of control mean body weights) and females (68.2 percent of control mean body weights) in the 350 mg/kg bw TBHP groups.

Microscopic findings occurred in the esophagus (hyperplasia, inflammation, necrosis, and/or ulceration) of 3/5 males and 2/5 females treated with 350 mg/kg bw TBHP. Histopathologic changes were also seen in the forestomachs (hyperplasia, inflammation, ulceration and/or serosa fibrosa) and/or glandular stomachs (inflammation and/or ulceration) of 5/5 males and 5/5 females treated with 350 mg/kg bw TBHP. Similarly, microscopic findings were identified in the forestomach and/or glandular stomach of at least one male or female animal in every TBHP group except for those treated with 22 mg/kg bw. These esophagus and stomach lesions were thought to be directly related to the effects of repeated gavage treatment with TBHP.

Statistically significant decreases in group mean organ weights were identified for the liver, heart, lung (females only), kidney, and thymus of animals treated with 350 mg/kg bw TBHP. However, there was either no statistically significant change from controls or a significant increase in group mean percent organ-to-body weight values for the liver, heart, lung and kidney, suggesting that these variances from control levels were at least partially related to the reductions in mean body weights also seen in these groups. Microscopic findings of lung inflammation in 2/5 females treated with 88 mg/kg, 1/5 males and 1/5 females treated with 175 mg/kg bw, and 3/5 males and 2/5 females treated with 350 mg/kg bw TBHP. This suggests that the gavaged test article, thought to be directly responsible for tissue damage in the esophagus, may have affected pulmonary system tissue after evaporation from the stomach and inhalation. The presence of food material in the bronchiole in 2/5 males treated with 350 mg/kg bw TBHP supports this position. Microscopic findings of liver inflammation (average severities = 1.0 to 2.5) in 11/20 males and females treated with 175 or 350 mg/kg bw TBHP present an undetermined etiology, as liver inflammation was also observed in 6/10 male and female controls (average severity = 1.0).

The thymus of both males and females in the 350 mg/kg bw treatment groups exhibited statistically significant decreases in group mean organ weights (reductions up to 84.7% of controls) and in group mean percent organ-to-body weights, consistent with microscopic findings of lymphoid necrosis in 6/10 male and female animals in these groups. Although thought to be partially attributable to body weight reductions and general stress, this severe effect on the thymus was nevertheless thought to be a direct toxicological effect of repeated gavage administration of TBHP. Based on the histopathology results, the NOAEL for 12 days of gavage treatment with TBHP in 0.5% aqueous methylcellulose is 22 mg/kg bw.

A two to five fold increase in the 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 the free radical formation. This study is of limited value due to the low number of studied parameters.

TBHP was administered in 0.5 percent aqueous methylcellulose by the gavage route to male and female B6C3F1 mice at dosages of 0, 22, 44, 88, 176 or 350 mg/kg at a volume of 10 mL/kg bw (Ritchie *et al*., 2005b). Animals were dosed 13 times on weekdays only during the 18-day study period. There were five mice/sex in each dosage group. Body weight determinations were recorded weekly, and clinical observations were recorded daily for all animals. Mice were necropsied with selected organs weighed and selected tissues processed for microscopic examination at study termination.

There was one early death occurring in the male 22 mg/kg bw TBHP dosage group that appeared to be unrelated to the chemical treatment, as there were no deaths in higher dosage groups. The only clinical sign observed throughout the study was ruffled fur in two male mice that were administered 350 mg/kg bw TBHP. Group mean body weights were statistically significantly reduced, relative to controls, in male mouse groups treated with TBHP at dosages of 44, 88, 176 or 350 mg/kg bw. TBHP exposure resulted in significant changes in organ weight and/or organ-to-body weight ratio in the livers of female mice. Treatment related histopathology included hyperplasia and/or inflammation in the forestomach of both male ($\geq$ 44 mg/kg bw) and female ($\geq$ 88 mg/kg bw) mice, esophageal hyperplasia in male mice ($\geq$ 88 mg/kg bw), and hepatocellular hypertrophy in females at 350 mg/kg bw. The no observed effect level for 13 days of gavage delivery of TBHP in 0.5 percent aqueous methylcellulose to B6C3F1 mice was 22 mg/kg bw in males and 44 mg/kg bw in females, based on changes in mean body weight (males) and histopathology. This study is of limited value due to the low number of studied parameters.

Mechanistic studies

The provided toxicokinetic study (de Bie and Grossouw, 2004, see 4.1.2.1) showed that TBHP is rapidly converted to 2-methylpropan-2-ol. In 13 week and 2 year studies of NTP (1995) 2-methylpropan-2-ol was given in drinking water (0, 2.5, 5, 10, 20 and 40 mg/ml) to F344/N rats and B6C3F$_1$ mice. No nephropathy was observed in male and female mice. In the 13 week study with male rats a significant increase in kidney nephropathy and hyaline droplet accumulation was found at the lowest tested dose of 2.5 mg/ml (corresponding to 230 mg/kg bw/day) and above. In females, no hyaline droplet accumulation was found but an increase in kidney nephropathy at 10 mg/ml (corresponding to 850 mg/kg bw/day) and above. In the chronic study, an increase in kidney nephropathy was observed in males at 5 mg/ml and in females at 2.5 mg/ml and above.

The mechanism of 2-methylpropan-2-ol induced kidney effects in the male rat has been studied (Williams and Borghoff, 2001, Borghoff *et al*., 2001). The concentration of α2u-

globulin in kidney cytosol of male F344 rats was significantly increased at 12 hours following a single gavage treatment with 2-methylpropan-2-ol at 500 mg/kg bw compared to control. Binding of [^{14}C]-2-methylpropan-2-ol to α2u-globulin was found after *in vivo* exposure as shown using co-elution between 2-methylpropan-2-ol and α2u-globulin with several methods. The reversibility was shown by displacement with d-limonene oxide. After 10 day inhalatory exposure of male and female F344 rats to 2-methylpropan-2-ol at 0, 250, 450 or 1750 ppm (770, 1385 or 5387 mg/m^3) for 6 hours/day, a dose related increase in protein droplet accumulation within the proximal tubules was seen in the males (significant at 1750 ppm). The protein droplets in control and exposed males stained positive for α2u-globulin. However, an exposure related increase in staining was not found. No staining was seen in females. The amount of α2u-globulin in the male kidney was only statistically significantly increased at 1750 ppm. Cell proliferation was dose-dependently increased in males but not in females. The increase was already significant at the lowest dose of 250 ppm. A positive correlation was found between α2u-globulin levels and cell proliferation but this was not statistically significant.

In order to investigate the mechanism behind the renal effects observed in the 45-day study with TBHP (see study by Jonker et al. (1993) in section on 'Oral studies' above), groups of 8 male rats (Crl:WI(WU)BR) were treated by oral gavage with aqueous TBHP-70 at 0 or 30 mg/kg bw/day for 42 days (Wijnands, 2002). Observations included clinical signs, body weight, food consumption, gross necropsy, kidney weights and histopathological examination of the kidney including immunohistochemical staining for α2u-globulin.

Microscopic examination revealed very slight (3/8) to slight (2/8) multifocal tubular nephrosis in the cortex of the kidneys of treated animals. The tubular nephrosis was characterized by degeneration and desquamation of proximal tubular cells, some of which showed pyknotic nuclei. The lesions in the proximal tubules were associated with an increased accumulation of hyalin proteinaceous material, intraluminal as well as intracytoplasmatic. Very slight (3/8) to slight (2/8) basophilic tubules were found in treated animals compared to very slight basophilic tubules in three out of eight controls. The immunohistochemical staining for α2u-globulin showed a very slight positive reaction in all controls and a very slight (2/8) to slight (6/8) positive reaction in treated rats. The slight increase in staining in the treated rats was less than the staining seen in control rats of another strain. It was stated by the author that it was clear that the increased accumulation of proteinaceous material observed in close association with the degenerative changes of the proximal tubules in the cortex of the kidneys showed a positive reaction to the immunohistochemical staining for α2u-globulin.

The kidney slides of the study by Wijnands (2002) were reviewed by a kidney specialist (Hard, 2007). In this review it is stated that:
- no increase in hyaline droplet accumulation was observed in the H&E stained kidney tissue using either conventional microscopy or fluorescence microscopy for enhanced visualisation of hyaline droplets;
- there was no difference in the severity of CPN between the two groups;
- blind reading of the slides containing kidney tissue immunohistochemically-stained for α2u-globulin did not provide a distinct seperation between the control and treated groups of male rats and did not substantiate a treatment-related effect.
Hard (2007) concluded that TBHP did not cause any nephropathic alteration in this study.

With regard to the α$_{-2u}$globulin immunohistochemistry, the staining of the intracytoplasmic proteinaceous inclusions of all rats (controls and treated) in this study was less intense than that observed in the reference sample of a normal (untreated) rat provided by the University of

North Carolina where the immunochemistry had been performed. Obviously, the reaction was very mild and within narrow borders. Blind and random re-evaluation of the slides by Hard (2007) could not reproduce a dose-response relationship. Thus, in contrast to its metabolite 2-methylpropan-2-ol, for TBHP itself the involvement of α_{-2u}globulin accumulation with associated nephropathy was not confirmed. It is true that the doses tested for TBHP were not as high as for 2-methylpropan-2-ol. However, it is not likely that TBHP can be tested at a dose in the range of 200 mg/kg bw/d for a longer time, given that 179 mg TBHP/kg bw/day for 5 days already resulted in severe effects on the stomach (see range-finding part of the Jonker et al. (1993) study).

In vitro studies

No data available.

4.1.2.6.2   Studies in humans

No data available.

4.1.2.6.3   Summary of repeated dose toxicity

The data for oral repeated dose toxicity are sufficient to fulfil the Annex VII requirements. A NOAEL of 30 mg/kg bw/day 70% TBHP (calculated NAEL of 21 mg/kg bw/day for 100% TBHP) from a 45-days gavage study will be taken forward to the risk characterisation. This NOAEL, the highest tested dose for a sufficient period in rats, can be used for systemic and for local effects. Local effects on stomach and/or forestomach were observed at higher doses, i.e. from 50 mg/kg bw/day in range-finding studies to the 45-day study, and from 44 mg/kg bw/day in two limited gavage studies of shorter duration in rats and mice. In the latter study with mice, also reductions in body weight were observed at 44 mg/kg bw/day and higher.

After dermal exposure of rats and mice for approximately 14 days only local effects were found at doses up to 350 mg/kg bw/day. A NOAEL of 44 mg/kg bw/day for local effects was derived from these studies. This NOAEL can be taken forward to the risk characterisation as a NOAEL for local effects but not as a NOAEL for systemic effects due to the limitations of the studies such as the low number of parameters studied. This NOAEL corresponds with a NOAEC of 2.2%.

For inhalation repeated dose toxicity no (useful) data were available.

4.1.2.7   Mutagenicity

The results of available genotoxicity studies are summarized in **Table 4.1.2.M**.

Table 4.1.2.M Summary of in vitro and *in vivo* genotoxicity tests

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

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

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
DNA fragmentation	Rat hepatocytes	Fluorimetric analysis of alkaline DNA unwinding	0 – 0.5 mM TBHP		+	Latour *et al.,*1995

In vitro eukaryotic test systems						
Type of test	**Species**	**Method**	**Concentrations**	**Remarks**	**Results**	**Reference**
DNA strand breaks	Rat hepatocytes	Comet assay	250 µM during 20 minutes		+	Lee *et al.*, 2004

In vivo genotoxicity tests with insects						
Type of test	**Species**	**Method**	**Concentrations**	**Remarks**	**Results**	**Reference**
Dominant lethal mutation	Drosophila melanogaster, adults ♀	vapour	5 ml 50 Molar percent solution*	Sifter technique	_	Altenburg, 1954
	eggs	immersed or in vapour	various dilutions of 50 Molar percent solution*		+	
Sex linked recessive lethal mutation	Drosophila melanogaster, adults	oral	2000 and 2500 ppm 100% TBHP in feed (nominal)		_	Woodruff e*t al.*, 1985; SIDS 1995
		injection	0.2 - 0.3 ul of 1000 and 2000 ppm 100% TBHP in water		+	
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

In vivo genotoxicity tests with mammals						
Type of test	**Species**	**Method**	**Concentrations**	**Remarks**	**Results**	**Reference**

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

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

* purity of TBHP not specified

4.1.2.7.1   Studies in vitro

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, 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 to oxygen. 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).

4.1.2.7.2   Studies *in vivo*

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 were treated by gavage with a single dose of TBHP (70%) at 1 ml/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 were significantly increased compared to control rats (n=3). 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 compared to stomach (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 (4.1.2.10) indicated that the TBHP had reached the liver.

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 4.1.2.2.1). 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.

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.

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 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.

4.1.2.7.3   Summary 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 intrinsic genotoxic *in vitro*.

The data set on genotoxicity of TBHP *in vivo* towards somatic cells is limited. Therefore 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.

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

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 travel from the abdominal cavity through the inguinal channel to the testis.

The ADME study (de Bie and Grossouw, 2004, see 4.1.2.1) 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* and probably genotoxic *in vivo*. TBHP was negative in several mutagenicity tests on 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. TBHP is mutagenic in germ cells after *in vivo* exposure (changes in sperm morphology and an increase in dominant lethal mutations) but this was only seen after intraperitoneal exposure. 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 confined to somatic cells in the tissues of first contact and could possibly result in local carcinogenicity. The formal 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. 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.

Classification with Muta. Cat.2; R46 is not justified because TBHP does not reach the gonads after oral, inhalation and dermal exposure. However, classification with Muta. Cat. 3; R68 is proposed because it is assumed that TBHP will be mutagenic at the sites of first contact in somatic cells. Classification with Muta. Cat. 3; R68 was confirmed by the TC-C&L.

4.1.2.8   Carcinogenicity

4.1.2.8.1   Studies in animals

<u>In vivo studies</u>

Inhalation

No data available.

Dermal

In a carcinogenicity study of Van Duuren *et al.* (1967) 30 female Swiss mice were exposed to 100 mg of a 3% TBHP-solution (in benzene) onto the dorsal skin 3 times weekly for 522 days. The dose, therefore, was 3 mg/mouse three times per week. A vehicle control (n=30) and an untreated group (n=100) were included. Mice were observed regularly. Complete autopsy was performed. Histopathology was performed on all abnormal appearing tissues and organs. Haematology and clinical chemistry were not investigated. An effect on survival was not found and in none of the mice local papillomas or carcinomas were found.
However, the skin damage in mice treated with the vehicle alone (benzene) was more severe than reported in the 3% TBHP (in benzene) group. Furthermore, only one dose level was tested which is probably not sufficiently high to be the maximal tolerated dose (MTD) (see studies of Floyd and Stokinger, 1958 and Ritchie *et al.*, 2005c/d). Also, no attempts were made to reduce loss from the skin surface through evaporation. Therefore, the results of this study can not be used for risk assessment.

In the study of Hoshino *et al.* (1970) it was tested whether TBHP had a potentiating effect on tumour formation in mice induced by the potently carcinogenic 4-nitroquinoline 1-oxide. A total of 158 female mice of ddNN strain, about 40 days of age, were divided in four groups. Prior to the treatment hair was clipped off the back over the area of 1 cm in diameter. All chemicals to be tested were applied on the bare skin. After the treatment, mice were kept under observation of 450 days. During this period, mice found moribund were sacrificed and

pathological examination was performed routinely, including the tumours and grossly abnormal tissues. Experimental groups were as follows: one group (n=20) was given 270 applications of TBHP (0.02 ml of a 16.6% solution of TBHP in benzene; 6 days a week), a second group (n=50) was given 20 applications of a submanifestational dose of 4-nitroquinoline 1-oxide (0.02 ml of a 0.25% solution; 3 times a week), a third group (n=38) was treated with 20 applications of 4-nitroquinoline 1-oxide (0.02 ml of a 0.25% solution; 3 times a week), followed by a 10-day interval, followed by 270 applications of TBHP (0.02 ml of a 16.6% solution of TBHP in benzene; 6 days a week), and a fourth group (n=50) was treated with 20 applications of 4-nitroquinoline 1-oxide, followed by a 10 day interval, followed by 270 applications of 2-methylpropan-2-ol (0.02 ml of a 16.6% solution 2-methylpropan-2-ol in benzene; 6 days a week). Mice treated with TBHP alone showed ulcers, erosion, and hair-follicle hyperplasia soon after the start of the experiment but they all recovered completely from these damages later. Treatment with the solvent benzene was not tested but the absence of comparable irritation during the treatment with 2-methylpropan-2-ol in benzene shows that the irritation was caused by TBHP. No tumour was found. Treatment of mice with 4-nitroquinoline 1-oxide alone resulted in no acute skin damage and no malignant skin tumours. However, treatment of mice with 4-nitroquinoline 1-oxide, followed by TBHP, resulted in 9 malignant and 4 benign skin tumours (papillomas). Treatment of mice with 4-nitroquinoline 1-oxide, followed by 2-methylpropan-2-ol, resulted in 1 malignant tumour and no papillomas. The results with 2-methylpropan-2-ol can also be used as a negative control for benzene as the effect of benzene on tumor formation after initiation was not tested.

The results of the carcinogenicity studies are given in **Table 4.1.2.N**.

Table 4.1.2.N Summary of carcinogenicity studies

Route	Species	Doses	No. of tumour bearing animals		Remarks	Reference
			malignant	benign		
Dermal	Mice ICR/Ha Swiss female, 8 weeks old	100 mg of 3% TBHP-solution (in benzene), 3 times weekly, 522 days	0/30	0/30	Inadequate study	Van Duuren *et al.*, 1967
Dermal	Mice ddNN strain female, 40 days old	0.02 ml of 16.6% TBHP in benzene, 6 times weekly, 315 days	0/20	0/20		Hoshino *et al.*, 1970
		0.02 ml of 0.25% 4 nitroquinoline 1-oxide, 3 times weekly, 46 days	0/50	1/50		
		pretreatment with 4-nitroquinoline 1-oxide, followed by 0.02 ml 16.6% TBHP*	9/38	4/38		
		pretreatment with 4-nitroquinoline 1-oxide, followed by 0.02 ml 16.6% 2-methylpropan-2-ol*	1/50	0/50		

* See text for treatment schedule

Oral

No oral data with TBHP are available. However, TBHP is rapidly converted into 2-methylpropan-2-ol after uptake. The carcinogenicity of 2-methylpropan-2-ol was tested by the NTP in drinking water studies (1995). There was no increase of tumours in female F344/N rats. In male rats, a small increase in renal tubular hyperplasia and adenoma was seen. This increase was significant at the middle dose of 200 mg/kg bw/day. In B6C3F1 mice a small increase in thyroid follicular cell hyperplasia was found. In males a non-significant increase in follicular adenomas was found and in females a significant increase at the highest dose of 2110 mg/kg bw/day. The NTP concluded in their report that there was some evidence of carcinogenic activity of 2-methylpropan-2-ol in male rats and female mice, no evidence in female rats and equivocal evidence in male mice.

The increase in renal tubular adenomas in the male rat might possibly be caused at least in part, by male rat specific α2u-globulin nephropathy (see also section 4.1.2.6).

In vitro studies

No data available.

4.1.2.8.2   Studies in humans

No data available.

4.1.2.8.3   Information on structurally related substances

Hanausek *et al*. (2004) evaluated nine organic peroxides for their ability to increase biomarkers of tumor promotion in mouse skin. This test was developed as a screening model for the assesment of carcinogenic properties of organic peroxides towards the skin model. All positive controls used in this study were known to induce c-Ha-ras oncogene mutations and it is stated that all complete carcinogens and tumor initiators studied in the mouse skin model have been shown to produce such mutations.
Female SENCAR mice (n=5) were shaved and dermally treated with the organic peroxide or controls in 200 ul aceton (except for MEKP in dimethyl phthalte) 2 times a week for 4 weeks. The dose levels (none-irritating) for each organic peroxide were benzoyl peroxide (BZP): 62.5 and 125 umol, di-t-butyl peroxide (DTBP): 100 and 200 umol, t-butyl-peroxybenzoate (TBPB): 50 and 100 umol, p-t-butyl usipropylbenzene hydroperoxide (TBIBHP): 100 and 200 umol, cumene hydroperoxide (CHP): 25 and 50 umol, dicetyl peroxydicarbonate (DPD): 2.0 and 4.0 umol, dicumyl peroxide (DCP): 100 and 200 umol, methyl ethyl ketone peroxide (MEKP): 100 and 200 umol, O,O-t-butyl-O-(2-ethylhexyl) monoperoxycarbonate (TBEC): 100 and 200 umol, 7,12-dimethylbenz[a]anthracene (DMBA, positive control): 100 nmol and 12-O-tetradecanoylphorbol-13-acetate (TPA positive control): 3.2 nmol. Dosed skin was harvested at 2 and/or 4 days after final dosing for determination of the epidermal thickness (hyperplasia), dermal cellularity (inflammation) and 8-hydroxy-2'deoxyguanosine (oxidative DNA damage). Only BZP, TBPB and TBIBHP and the positive control DMBA (complete carcinogen) exhibited significant increases in all three biomarkers. CHP and the positive control TPA (tumor promotor) produced increases in epidermal hyperplasia and dermal inflammation, MEKP and DCP only epidermal hyperplasia and TBEC only dermal inflammation. DTBP and DPD had no effect on the three parameters.

The ability of TBPB and TBIBHP to produce mutations in codons 12, 13 and 61 of the C-Ha-*ras* protooncogene was tested with a 8 or 12 week comparable exposure to the high dose (n=5) and compared with the positive controls N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), benzo[a]pyrene (B[a]P), urethane and DMBA. Increases in specific mutations were found with the positive controls but not with TBPB and TBIHP. Treatment with DMBA also induced tumors in this system.

TBPB and TBIHP were also tested as initiators in a initiation/promotor study with TPA as promotor (n=5) and compared with the positive controls MNNG, B[a]P, urethane and DMBA in a comparable 16 week test with 12 weeks of exposure. All positive controls induced skin tumors with Ha-*ras* mutations. However, no increases in skin tumors and mutations were found with the two organic peroxides.

It was concluded by Hanausek *et al.* that TBPB and TBIBHP could have tumor-promoting activity, but are not likely to possess tumor initiating or complete carcinogenic activity in the mouse skin model.

The provided study is a screening assay with only limited validation. The exposure period in the assay is shorter than in the standard OECD test for carcinogenicity. A conclusion on the carcinogenic potential towards the skin of organic peroxides could not be based on this study alone. Several references within the study by Hanausek *et al.* show that more extensive dermal studies are available for some organic peroxides. Furthermore, a justification of the read-across using data on other organic peroxides for TBHP is lacking.

4.1.2.8.4   Summary of carcinogenicity

No inhalatory and oral carcinogenicity studies with TBHP are available. TBHP is, however, rapidly converted to 2-methylpropan-2-ol and for this compound oral carcinogenicity studies are available. These studies show very small increases in systemic tumours. However, these dose levels of 2-methylpropan-2-ol can not be reached by treatment with TBHP because these levels are above the LD50 of TBHP for mice, and above the dose level of TBHP inducing local effects to the stomach in rats. From these observations it is concluded that chronic exposure to TBHP will most probably not result in 2-methylpropan-2-ol levels that can induce systemic tumours.

It is assumed that TBHP will be mutagenic at the sites of first contact in somatic cells. However, based on the rapid conversion of TBHP, it is unlikely that TBHP can reach the systemic circulation through normal routes of exposure. Consequently, carcinogenicity limited to tissues that are exposed to the parent TBHP (i.e. tissues of first contact) cannot be excluded.

Useful data on the potential local carcinogenic effects of TBHP are not available, unfortunately. In a single very limited dermal study one clearly toxic concentration of TBHP was capable of promoting the development of dermal tumors after induction by 4-nitro-quinoline 1-oxide. Therefore information on the local carcinogenicity is needed. This information could be derived either by read-across from other substances in case the read-across is sufficiently validated and based on sufficient data or based on tests. This should ideally be through the oral, dermal and inhalatory route because it is unknown whether and how local carcinogenicity can be extrapolated from one route to the other. However, from a practical point of view it is proposed to start with one route and the need for additional routes will depend on the results. Information on the carcinogenicity by the inhalatory route is

preferred because this route is relevant for both workers and humans exposed via the environment.

The NTP had nominated TBHP for carcinogenicity testing, apparently for the dermal route. Fourteen-day oral and dermal range finding studies have meanwhile been performed (described under 4.1.6.2). The NIEHS recommended in 2006 after the range-finding studies that no further studies should be done with TBHP because of the minimal exposure to TBHP, the negative result in the study by van Duuren et al (1967) and the results of a number of other organic peroxides in a short-term initiation and/or promotion protocol (Hanausek *et al.*, 2004). The argument for not testing, that exposure is minimal, is not consistent with the current exposure estimates as specified in section 4.1. In addition, the study by Van Duuren is assessed as inadequate in the RAR. The results of the study by Hanausek *et al.* indicate that several organic peroxides are negative in this screening model. However, seen the limitations of this model it is difficult to extrapolate this result. References in this study show the presence of more extensive carcinogenicity studies with some organic peroxides. However, a full study on all available carcinogenicity data on organic peroxides and the justification of the read-across approach is not available.

In conclusion, the available data are insufficient to determine whether TBHP may be carcinogenic at the sites of first contact. Besides, it is to be noted that no information is available on whether dermal carcinogenicity data can be extrapolated to the sites of first contact after inhalation and oral exposure.

4.1.2.9 Toxicity for reproduction

The results of the available reproductive toxicity studies are summarized in **Table 4.1.2.O**.

Table 4.1.2.O Summary of reproductive toxicity studies

Exposure period	Route	Species	Doses	NOAEL	Critical effects	Method	Reference
45 days	Oral	rat ♀♂ adult Wistar	0, 3, 10, 30 mg/kg bw/day 70% TBHP	30 mg/kg bw/day 70% TBHP	none (dams, pups)	OECD 422	Jonker *et al.*, 1993; SIDS, 1995
10 days	Oral	rat ♀ adult Wistar	0, 5, 15, 50 mg/kg bw/day 70% TBHP	50 mg/kg bw/day 70% TBHP	none (dams, pups)	OECD 414	Smits-van Prooije, 1993

4.1.2.9.1 Effects on fertility

Studies in animals

In an oral Combined Repeated Dose and Reproductive/Developmental Toxicity Screening Test (according to OECD guideline 422) of Jonker *et al.* (1993), the effect of TBHP on the reproduction and development of pups of rats were studied. Male and female rats (Crl:WI(WU)BR; twelve/sex/dose) were exposed daily by oral gavage (10 mL/kg bw) to 0, 3, 10, or 30 mg/kg bw 70% TBHP for maximally 45 consecutive days (14 days premating, up to

7 days mating, 21-22 days gestation, 4 days lactation). Parent animals and their offspring were killed on day 4 of lactation. Observations included clinical signs, body weight, food and water consumption, haematology, clinical chemistry and pathology (see section 4.1.2.5), fertility and reproductive performance, litter parameters, and abnormalities of the pups. There were no toxicological relevant effects on fertility after TBHP exposure. Unfortunately, the female fecundity index (females pregnant / females mated) was low in several groups (Table 4.1.2.P). No significant toxicological effects were observed on reproductive indices and litter data, except for pup mortality between day 1 and 4 *post partum* in the 3 and 30 mg/kg bw groups (control: 0/87 (0%), 3 mg/kg: 11/43 (26%) with one total litter loss of 10 pups, 10 mg/kg: 1/111 (1%) and 30 mg/kg: 5/81 (6%). The study authors stated that the loss of pups in the 3 mg/kg bw group was probably not toxicological relevant because these pups were largely from one litter, and because no increased mortality in the next higher dose group was observed. The higher pup mortality at 30 mg/kg could be treatment related according to the study authors. However, the incidence of postnatal death was considered to be very low and therefore of minor toxicological significance. No information on historic control data were provided to show that a 6% postnatal mortality is within the normal range. No toxicological relevant effects on body weight, clinical observations and macroscopical observations in the pups were observed. From these results it can be concluded that the NOAEL for reproductive/developmental toxicity in this study is 30 mg/kg bw 70% TBHP, the highest dose tested.

Table 4.1.2.P Delivery and litter data from Jonker et al., 1993

Parameter	Control	3 mg 70% TBHP/kg bw/day	10 mg 70% TBHP/kg bw/day	30 mg 70% TBHP/kg bw/day
Females placed with males	12	12	11	12
Females mated	11	11	11	11
Females pregnant	8	5	10	7
Females with liveborn pups	8	4	10	7
Liveborn pups	87	43	111	81
Liveborn pups per litter	10.9	10.8	11.1	11.6
Pup mortality day 4	0	11 (26%) [ds]	1 (0.9%)	5 (6.2%) [ds]

ds: decreased significantly

It is unclear from the available parts of the studies by Richie et al. (2005a and b) on the 14-day oral toxicity of TBHP in rats and mice whether histopathological examination of the reproductive organs was performed.

TBHP is rapidly converted to 2-methylpropan-2-ol. Limited information on the effects of 2-methylpropan-2-ol on the fertility is available from one study.

According to an abstract (Nelson, 1989), groups of 18 male rats were exposed by inhalation exposure of to 6000 and 12000 mg/m^3 2-methylpropan-2-ol for 7 hours a day for 6 weeks. The males were mated with untreated females. The litters were culled and fostered by untreated controls. The offspring was tested for behavioural effects. There were few differences from controls in the behavioural measurements.

In the 13-week oral repeated dose study on 2-methylpropan-2-ol in rats and mice (NTP, 1995) the reproductive evaluation included cauda, epididymis and testis weights, epididymal spermatozoal measurements and oestrous cycle characterization. Some effects on cyclicity

were found at a near lethal level of 20 mg/mL in rats (not significant) and at 40 mg/mL (significant) in mice. In male mice, the absolute testis weight was decreased at 40 mg/mL and the relative testis weight was increased at 20 and 40 mg/mL. These changes in testis weight were probably secondary to the significant reductions in body weight at both dose levels. The histopathological examination at the highest dose levels and controls included ovary, uterus, testis and prostate. However, no tables with results were provided. Furthermore, no results were provided in the text.

The data from the 15-month interim evaluation (n=10, rats only) and of the final evaluation after 2 years (approximately 50 animals for rats and 60 for mice) consist of tables with the percentage of animals showing a particular effect without information on the severity and on the statistical significance of the effect. Therefore, these results can only be used as a rough indication on the presence or absence of effects on the reproductive organs. Therefore effects were only considered as significant if an effect was seen at both time points (rats) and showed a dose effect relation. No significant effects were seen on the reproductive organs of the male and female rat and the female mice. In male mice an increase in degeneration of the germinal epithelium from 3% in the controls to 10% at the highest dose of 20 mg/mL was found.

Overall, the results indicate a NOAEL for 2-methylpropan-2-ol of 10 mg/mL in mice, corresponding with approximately 1000 mg/kg bw/day, based on an increase of the degeneration of the germinal epithelium at 20 mg/mL after 2-years of exposure. In rats, a NOAEL for 2-methylpropan-2-ol of 5 mg/mL for males and 10 mg/mL for females (highest dose tested, corresponding with 420 and 650 mg/kg bw/day, respectively) was found.

In some genotoxicity tests, in which TBHP was given to male animals after i.p. injection, reduced fertility and effects on fertility parameters (caudal sperm counts) were observed (Kumar and Muralidhara, 1999 and 2007; Kaur *et al.*, 2006). These studies have been discussed in section 4.1.2.7 into more detail. This reduction in fertility is not taken into account, as i.p. injection is considered an irrelevant route of exposure for fertility. The reduction of fertility is related to direct contact of the testis with TBHP, which would never occur under normal conditions of exposure.

Studies in humans

No data available.

4.1.2.9.2   Developmental toxicity

Studies in animals

In an oral embryotoxicity/teratogenicity study with TBHP (Smits-van Prooije, 1993), according to OECD guideline 414, mated female albino Wistar (Crl:Wi(WU)BR) rats were given TBHP by gavage, at levels of 0, 5, 15, or 50 mg/kg bw 70% TBHP (n=24/dose) from day 6 up to and including day 15 of gestation. On day 21 of gestation the females were killed. Observations were clinical signs, body weight, food consumption; numbers of corpora lutea, implantation sites, resorptions, live and dead foetuses, malformations of the foetus; weight of ovaries, uterus, foetuses and placentas; length and sex of foetuses. During the study no mortality occurred. In the 50 mg/kg bw group maternal body weight gain and food intake during the administration period were slightly (5-10%) but not statistically significantly affected. No differences were observed in the maternal performance and reproduction

parameters between any of the groups. Upon macroscopic foetal examination no compound-related defects were observed. Upon microscopic foetal examination no visceral and skeletal malformations, anomalies or variants were observed that could be related to the treatment with TBHP. It can be concluded that TBHP is not embryo/feototoxic and does not induce teratogenic effects at dose levels up to the highest dose tested of 50 mg 70%-TBHP/kg bw/day. In this study the NOAEL for both maternal toxicity and developmental toxicity is 50 mg/kg bw/day 70% TBHP, the highest dose tested.

Remark: As no maternal toxicity was seen in this study, it is questionable whether the tested doses were high enough. However, the dose levels in the study were based on the results of a five-day range finding study in which a narrow range was observed between the no-effect level (50 mg/kg bw) and the levels causing submucosal oedema in the stomach wall (107 mg/kg bw 70% TBHP) and reduced food intake (statistically significant from 179 mg/kg bw 70% TBHP) (Jonker et al., 1993). For this ten-day study, the study authors judged a dose of 50 mg/kg bw 70% TBHP to be sufficiently close to the level shown to induce maternal effects.

TBHP is rapidly converted to 2-methylpropan-2-ol. Information on the effects of 2-methylpropan-2-ol on the development is available from several studies.

According to an abstract (Nelson et al., 1989a), groups of 15 pregnant Sprague-Dawley rats were exposed by inhalation exposure to 6000 and 12000 mg/m^3 2-methylpropan-2-ol for 7 hours a day from gestation day 1 to 19. The litters were culled and fostered by untreated controls. The offspring was tested for behavioural effects and the level of neurotransmitters in some parts of the brain was determined. The highest dose was maternally toxic, thereby reducing feed intake and maternal weight gain. There were few differences from controls in the behavioural measurements. The high concentration produced elevations in a number of neurotransmitters. No additional information was available.

In an inhalatory developmental study, female Sprague-Dawley rats (n=15) were treated with 0, 2000, 3500 or 5000 ppm (6156, 10773 and 15390 mg/m^3) 2-methylpropan-2-ol for 7 hours a day from gestation day 1 to 19 (Nelson et al., 1989b). Maternal toxicity was seen at the highest dose as narcosis, unsteady gait, reduced body weight gain and reduced food uptake. At the two lower doses only unsteady gait was seen. Foetal effects were restricted to a dose dependent reduction in body weight which was significant at the lowest dose and a dose-dependent increase in skeletal variations which was significant from 3500 ppm. There was no increase in malformations.

According to an abstract (Abel and Bilitzke, 1992), pregnant rats consumed liquid diet containing 10.9%, 1.3% or 0.65% 2-methylpropan-2-ol from gestation day 8 until parturition. Each group had its pair-fed controls. 2-methylpropan-2-ol reduced maternal weight gain, litter sizes (from 11 to 3 pups per litter), birth weights, and weights at weaning and increased perinatal mortality (from 2% to 14%) and postnatal mortality (from 6% to 100%). No additional information (abstract) was available.

Groups of 15 pregnant Swiss-Webster mice were exposed to liquid diet containing 0, 0.5, 0.75 or 1.0% 2-methylpropan-2-ol from gestation day 6 to 20 (Daniel, 1982). The controls and other treatment groups were pair-fed based on the 1% 2-methylpropan-2-ol animals. Based on an average daily uptake of 23.5 g of liquid diet and the average weight of 37 g on day 15 of pregnancy, this roughly corresponds with 3000, 4500 and 6000 mg/kg bw/day. Fifty percent of the offspring were fostered by untreated animals. Behavioural test were performed on the

offspring on several days after birth. The pups were not examined for variations or malformations. 2-methylpropan-2-ol exposure resulted in a dose dependent decrease in litters, neonates per litter and pup body weight and an increase in stillborn pups (Table 4.1.2.Q) but no information on the statistical significance was provided. Postnatal weight gain was reduced in nonfostered pups at 0.75 and 1.0% 2-methylpropan-2-ol. Developmental delay in post-parturitional behaviour by 2-methylpropan-2-ol was found with 0.75 and 1.0%. A NOAEL of 3000 mg/kg bw/day for 2-methylpropan-2-ol was derived.

Table 4.1.2.Q. Postparturation data of mice treated with 2-methylpropan-2-ol on day 6 to 20.

	Control	0.5%	0.75%	1.0%
Number of litters	11/15	12/15	8/15	7/15
Neonates/litter	10.4±4.0	10.3±4.4	7.4±2.3	5.3±2.8
Total stillborn	3	6	14	20
Foetal weight day 2	1.78±0.21	1.66±0.24	1.45±0.14	1.10±0.10

Groups of pregnant CBA/J or C57BL/6J mice (n=5 -12) were treated by gavage with 0 or 10.5 mmol 2-methylpropan-2-ol per kg bw every 12 hours from day 6 through day 18 (corresponding with approximately 1500 mg/kg bw/day). Both type of mice showed a significant increase in resorptions per litter and a decrease in live foetuses per litter. No increase in soft tissue or skeletal malformations was found. A LOAEL of 1500 mg/kg bw/day is derived (Faulkner *et al*., 1989).

Studies in humans

No data available.

4.1.2.9.3   Summary of toxicity for reproduction

In an oral screening study no toxicological relevant effects on fertility, reproductive performance, and development were seen up to 30 mg/kg bw/day 70% TBHP, the highest dose tested. TBHP is rapidly converted to 2-methylpropan-2-ol. Therefore only 2-methylpropan-2-ol will be available to the reproductive organs. Chronic exposure to 2-methylpropan-2-ol up to levels of 420 mg/kg bw/day or higher in rats and mice did not induce effects on the reproductive organs. This confirms the absence of effects on fertility in the oral screening study.

In a developmental toxicity study a dose of 50 mg/kg bw/day 70% TBHP, the highest dose tested, did not result in developmental toxicity. It is recognised that these negative results do not exclude the potential for reproductive and developmental effects at higher doses than tested. However, considering these negative test results on relevant parameters and given the effects seen in the range-finding studies (especially submucosal oedema in the stomach wall, in males at 50 mg/kg bw and higher and in females at 107 mg/kg bw and higher) and in the repeated dose toxicity study (effects in males at much lower levels in organs other than reproductive organs), further testing at higher dose levels is not expected to provide relevant additional information.

With regard to the significant increase in post-natal pup mortality at 30 mg/kg bw/day in the oral screening study, this observation is not considered a treatment related effect (see section 4.1.2.9.1: study by Jonker *et al.*, 1993). This is supported by the studies with 2-methylpropan-2-ol where increased post-natal mortality was only seen at much higher dose levels (lower doses not tested) in rats (Abel and Bilitzke, 1992) and not in mice at much higher dose levels (Daniel and Evans, 1982).

The available data on 2-methylpropan-2-ol indicate reproductive effects at much higher dose levels than can achieved by exposure to TBHP. Therefore, these effects are unlikely to be found after exposure to TBHP.

4.1.2.10   Other data

TBHP is widely used as a model compound to study the effects of oxidative stress in vivo and in vitro. Not all available studies are included in the text below.

A few studies describe the effects *in vivo* after intraperitoneal exposure to TBHP, in which TBHP is used as an inducer of oxidative stress. Younes and Wess (1990) report a study in which male Wistar rats were injected intraperitoneally with 100 mg/kg bw TBHP (purity not specified) in 2 ml olive oil. TBHP led to an enhanced ethane exhalation as a marker of *in vivo* lipid peroxidation, as well as a moderate hepatotoxicity as evidenced by a rise in plasma activities of liver-specific enzymes (glutamate-pyruvate transaminase and sorbitol dehydrogenase) and an increase in hepatic calcium content. Furthermore, a depletion of hepatic glutathione by 17% was observed. All effects apart from the reduction of glutathione were antagonized by pretreatment with the iron chelator deferrioxamine and potentiated by pretreatment with iron sulphate.

Di Meo *et al.* (1997) studied the effects of a ten day intraperitoneal treatment with 1 mmol/kg bw (= 91.12 mg/ kg bw/day 100%) TBHP on the antioxidant capacity of blood, liver and heart in male Wistar rats. TBHP easily diffuses across biological membranes and only a short time is required for equilibration of peroxide with blood and its presence in heart and liver. A significant decrease in antioxidant potential as measured by a luminescence technique was determined in the blood and heart, but not in the liver. Treatment with TBHP lowered the body weight, but no effect was observed on heart weight/body weight ratio. Besides producing a decrease in antioxidant defences of cardiac cells, in TBHP-treated rats an increased heart rate and modifications in the electrophysiological properties of the myocardium were observed.

Trotta *et al.* (1981) reported that incubation of red blood cells with 1 mM TBHP resulted in lipid peroxidation. Using red cells containing either oxy-, met- or carboxyhaemoglobin and incubating them with TBHP indicated that the formation of methaemoglobin and non-intact haemoglobin might act as scavengers of either TBHP or reactive intermediates necessary for propagation of chain reactions involved in lipid peroxidation. The authors proposed that depending on the availability of glucose and the liganded state of haemoglobin, lipid peroxidation and haemoglobin alterations represent extremes of a spectrum of oxidative damage.

Maples et al. (1990) studied the radical formation in rat erythrocytes after treatment per os. Treatment with 700 mg/kg bw TBHP resulted in detectable amounts of haemoglobin thiyl radical adduct at 2 hours after treatment as detected by ESR in the presence of the spin trap DMPO. The minimal dose required for the detection was 50 mg/kg. Pretreatment with

diethylmaleate, an indiscriminate thiol blocking agent, resulted in lower levels of nonprotein sulfhydryl and a twelve fold increase in haemoglobin thiyl radical adduct. Pretreatment with l-buthionine-(S,R)-sulfoxime, an inhibitor of GSH synthesis, resulted in lower levels of nonprotein sulfhydryl and an eight fold increase in haemoglobin thiyl radical adduct. After treatment with 175 mg/kg TBHP per os, a 50% decrease in nonprotein sulfhydryl levels in the erythrocytes was found after 20 minutes (first measurement) which fully recovered within 80 minutes. Only a partial recovery was seen after treatment with TBHP in rats pre-treated with a glutathione reductase inhibitor.

Rush and Alberis (1986) studied the metabolism of TBHP in isolated rat hepatocytes. The metabolism of TBHP by the glutathione peroxidase/reductase system results in a rapid depletion of reduced glutathione and NADPH. Regeneration of NADPH can occur through the pentose phosphate pathway, but only when the pathway is stimulated, for example, by $NADP^+$ and possibly oxidized glutathione, both of which can be elevated in hepatocytes exposed to TBHP. Isolated rat hepatocytes exposed to 0.5 mM TBHP for 30 minutes metabolized more $[1\text{-}^{14}C]$-glucose to $^{14}CO_2$ than control cells whereas $^{14}CO_2$ evolution from $[6\text{-}^{14}C]$-glucose was unchanged, indicating that TBHP increases the activity of the pentose phosphate pathway and not glycolyses. Inhibition of the pentose phosphate pathway with 6-aminonicotinamide potentiated the toxicity of TBHP, indicating that the regeneration of NADPH by the pentose phosphate pathway may play a significant role in protecting hepatocytes from TBHP-induced damage.

Jones *et al.* (1989) reports a study in which the toxicity of TBHP was determined in rat hepatocytes during hypoxia and anoxia. It appeared that the cytotoxicity of 0.6 mM TBHP *in vitro* is mainly due to the reduced GSH-levels rather than to lipid peroxidation.

In vitro incubation of human erythrocytes with TBHP resulted in the formation of methaemoglobin and glutathionyl haemoglobin (Murakami and Mawatari, 2003).

Buc-Calderon *et al.* (1991) studied the cytotoxicity of TBHP in isolated rat hepatocytes and reported that the most relevant intracellular events to explain cytotoxicity are both the GSH depletion and an increase in the phosphorylase *a* activity, which is an indicator of fluctuation in cytosolic Ca^{2+}. The study authors concluded that lipid peroxidation represents an important but secondary mechanism in the cytotoxicity of TBHP.

Several studies (Adams *et al.,* 1993, 1994; Chang and Adams, 1997) report that intracerebroventricularly administered TBHP (109.7 mg/kg bw) to male mice easily penetrates membranes and distributes throughout the brain. TBHP in the brain damaged many types of brain cells, and it increased cellular GSSG, decreased cellular GSH, and decreased the protein sulfhydryl content. In another study TBHP was administered intracerebroventricularly in six different brain regions in 2- and 8-month old male mice (Chang *et al.*, 1995). TBHP induced dose-dependent oxidative stress and damaged many types of brain cells.

Müehlematter *et al.* (1989) studied the mechanism of the promotional activity of TBHP in carcinogenicity. In mouse epidermal cells JB6 TBHP was reported to induce DNA strand breakage, poly ADP-ribosylation of chromosomal proteins and the expression of proto-oncogenes c-fos and c-myc. The effects were much more pronounced in the non-promotable clone 30, in comparison to the promotable clone 41. The latter has a higher amount of the antioxidant defense, i.e. a 2 – 3 times higher constitutive amount of catalase and CuZn-superoxide dismutase. The authors report the induction of a cellular prooxidant state by TBHP.

Greenley and Davies (1994) studied the radical generation in isolated rat liver nuclei after treatment with TBHP. The radicals detected are similar in type to those detected in the rat liver microsomal fraction, although the extent of radical production in the latter *in vitro* system is much higher.

Several studies are available on the inhibitory effect of antioxidants on intraperitoneal injection of TBHP. In one of the studies (Lee *et al*., 2004), male Sprague-Dawley rats received a single intraperitoneal injection with 20 mg/kg bw TBHP and sacrified 24 hours later. Strong increases were found in plasma alanine and aspartate aminotransferase indicating liver damage. The liver malondialdehyde concentration was significantly increased indicating lipid peroxidation in the liver. A significant decrease in liver glutathione concentration was found. Upon microscopic examination severe hepatic lesions were found including degeneration in hepatocytes and hepatic chords, focal necrosis, congestion in central vein and sinusoids and infiltration of lymphocytes.

A single subcutaneous injection of 117 mg/kg bw TBHP to male Fisher rats did not affect plasma triglyceride and cholesterol levels measured at 2 hours after treatment (Farombi *et al*., 2004). However, increases in liver and plasma 2 amino adipic semialdehyde and liver malondialdehyde were found indicating protein oxidation and lipid peroxidation. A decrease in erythrocyte catalase and glutathione peroxidase was found.

Conclusion

The other data indicate that the cytotoxicity of TBHP can be caused by radicals formed from TBHP or is secondary to the depletion of GSH levels due to the metabolism of TBHP or by a combination of both.

4.1.3   Risk characterisation [5]

4.1.3.1   General aspects

In the data set only animal studies are available. Most of the studies were not performed according to current standards and were in some cases not suitable to be used in risk assessment.

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.

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[5] Conclusion (i)   There is a need for further information and/or testing.
 Conclusion (ii)   There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
 Conclusion (iii)   There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

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.

Although not all the studies are according to OECD-guidelines and some are rather dated the data are sufficient to fulfil the Annex VII requirements for acute toxicity. After acute exposure the oral LD_{50} was 406 mg/kg bw 70% TBHP and 560 mg/kg bw 100% TBHP for rats and 800 mg/kg bw (purity not specified) for mice. The dermal LD_{50} was 628 mg/kg bw 70% TBHP for rabbits. With respect to inhalation the LC_{50} was 1850 mg/m³ 100% TBHP for rats and 1292 mg/m³ 100% TBHP for mice.

It can be concluded that TBHP is harmful after acute oral and dermal exposure (Xn, R21/22). However, the two inhalatory studies differ with respect to the resulting classification. The study by Thackara and Rhinehart was performed with an aerosol and resulted in an LC_{50} of 1.850 mg/L. However, bearing in mind the vapour pressure of TBHP, it is likely that at least part of the TBHP evaporated and was available as a vapour. The study by Floyd and Stokinger was performed using vapours and resulted in an LC_{50} of 1.8 and 1.3 mg/L for rats and mice, respectively. This indicates classification of 100% TBHP with R23 because it is within the limits of 0.5 to 2 mg/L for a vapour. If the results are converted to concentration for 70% TBHP, than the LC_{50} values in rats are just above 2 mg/l indicating R20 for 70% TBHP but just below 2 mg/l in mice indicating R23 for 70% TBHP. As it is unknown whether

humans resemble more to mice or rats in this respect, classification with R23 is proposed for 70% TBHP. Classification with T; R23 and Xn; R21/22 was confirmed by the TC-C&L.

The available data are acceptable to fulfil the Annex VII requirements for irritation testing to the eyes and skin, although it is to be noticed that the skin was exposed for 24 hours. TBHP is corrosive to the skin and causes serious damage to the eyes. Classification with C, R34 (which covers both effects) is proposed. TBHP also induces respiratory tract irritation, which indicates a classification with Xn, R37. However, R37 is also covered by R34 but is needed at concentrations below 10%. No specific concentration limits are proposed for skin and eye irritation because of the shortcomings and limited details on the studies with lower concentrations. Classification with C; R34 and with Xn; R37 between 5 and 10% was confirmed by the TC-C&L. A concentration of 33 mg/m³ will be taken forward to the risk characterisation for respiratory tract irritation as a LOAEC. (Slightly) increased incidences of lacrimation, red nasal discharge, mucoid nasal discharge and dry rales were observed in the exposed rats.

No information is available on the respiratory tract sensitising potential of TBHP. TBHP is a skin sensitizer in the GPMT. The provided Buehler test is not acceptable due to the low irritation in the induction phase. TBHP is considered a strong sensitizer because 60% of the animals reacted after induction with 0.7% TBHP. This is based on the proposals of the sensitisation expert group (ECBI/81/02 Rev.2). Therefore, classification with R43 and a specific concentration limit of 0.1% is proposed. Classification with R43 and the specific concentration limit of 0.1% were confirmed by the TC-C&L.

The data for oral repeated dose toxicity are sufficient to fulfil the Annex VII requirements. A NOAEL of 30 mg/kg bw/day 70% TBHP (calculated NAEL of 21 mg/kg bw/day for 100% TBHP) from a 45-days gavage study will be taken forward to the risk characterisation. This NOAEL, the highest tested dose for a sufficient period in rats, can be used for systemic and for local effects. Local effects on stomach and/or forestomach were observed at higher doses, i.e. from 50 mg/kg bw/day in range-finding studies to the 45-day study, and from 44 mg/kg bw/day in two limited gavage studies of shorter duration in rats and mice. In the latter study with mice, also reductions in body weight were observed at 44 mg/kg bw/day and higher.

After dermal exposure of rats and mice for approximately 14 days only local effects were found at doses up to 350 mg/kg bw/day. A NOAEL of 44 mg/kg bw/day for local effects was derived from these studies. This NOAEL can be taken forward to the risk characterisation as a NOAEL for local effects but not as a NOAEL for systemic effects due to the limitations of the studies such as the low number of parameters studied. This NOAEL corresponds with a NOAEC of 2.2%.

For inhalation repeated dose toxicity no (useful) data were available.

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 intrinsic genotoxic *in vitro*.

The data set on genotoxicity of TBHP *in vivo* towards somatic cells is limited. Therefore 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.

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

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 travel from the abdominal cavity through the inguinal channel to the testis.

The ADME study (de Bie and Grossouw, 2004, see 4.1.2.1) 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* and probably genotoxic *in vivo*. TBHP was negative in several mutagenicity tests on 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. TBHP is mutagenic in germ cells after *in vivo* exposure (changes in sperm morphology and an increase in dominant lethal mutations) but this was only seen after intraperitoneal exposure. 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 confined to somatic cells in the tissues of first contact and could possibly result in local carcinogenicity. The formal 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. 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.

Classification with Muta. Cat.2; R46 is not justified because TBHP does not reach the gonads after oral, inhalation and dermal exposure. However, classification with Muta. Cat. 3; R68 is proposed because it is assumed that TBHP will be mutagenic at the sites of first contact in somatic cells. Classification with Muta. Cat. 3; R68 was confirmed by the TC-C&L.

No inhalatory and oral carcinogenicity studies with TBHP are available. TBHP is, however, rapidly converted to 2-methylpropan-2-ol and for this compound oral carcinogenicity studies are available. These studies show very small increases in systemic tumours. However, these dose levels of 2-methylpropan-2-ol can not be reached by treatment with TBHP because these levels are above the LD50 of TBHP for mice, and above the dose level of TBHP inducing local effects to the stomach in rats. From these observations it is concluded that chronic exposure to TBHP will most probably not result in 2-methylpropan-2-ol levels that can induce systemic tumours.

It is assumed that TBHP will be mutagenic at the sites of first contact in somatic cells. However, based on the rapid conversion of TBHP, it is unlikely that TBHP can reach the systemic circulation through normal routes of exposure. Consequently, carcinogenicity limited to tissues that are exposed to the parent TBHP (i.e. tissues of first contact) cannot be excluded.

Useful data on the potential local carcinogenic effects of TBHP are not available, unfortunately. In a single very limited dermal study one clearly toxic concentration of TBHP was capable of promoting the development of dermal tumors after induction by 4-nitro-quinoline 1-oxide. Therefore information on the local carcinogenicity is needed. This information could be derived either by read-across from other substances in case the read-across is sufficiently validated and based on sufficient data or based on tests. This should ideally be through the oral, dermal and inhalatory route because it is unknown whether and how local carcinogenicity can be extrapolated from one route to the other. However, from a practical point of view it is proposed to start with one route and the need for additional routes will depend on the results. Information on the carcinogenicity by the inhalatory route is preferred because this route is relevant for both workers and humans exposed via the environment.

The NTP had nominated TBHP for carcinogenicity testing, apparently for the dermal route. Fourteen-day oral and dermal range finding studies have meanwhile been performed (described under 4.1.6.2). The NIEHS recommended in 2006 after the range-finding studies that no further studies should be done with TBHP because of the minimal exposure to TBHP, the negative result in the study by van Duuren et al (1967) and the results of a number of other organic peroxides in a short-term initiation and/or promotion protocol (Hanausek *et al.*, 2004). The argument for not testing, that exposure is minimal, is not consistent with the current exposure estimates as specified in section 4.1. In addition, the study by Van Duuren is

assessed as inadequate in the RAR. The results of the study by Hanausek *et al.* indicate that several organic peroxides are negative in this screening model. However, seen the limitations of this model it is difficult to extrapolate this result. References in this study show the presence of more extensive carcinogenicity studies with some organic peroxides. However, a full study on all available carcinogenicity data on organic peroxides and the justification of the read-across approach is not available.

In conclusion, the available data are insufficient to determine whether TBHP may be carcinogenic at the sites of first contact. Besides, it is to be noted that no information is available on whether dermal carcinogenicity data can be extrapolated to the sites of first contact after inhalation and oral exposure.

In an oral screening study no toxicological relevant effects on fertility, reproductive performance, and development were seen up to 30 mg/kg bw/day 70% TBHP, the highest dose tested. TBHP is rapidly converted to 2-methylpropan-2-ol. Therefore only 2-methylpropan-2-ol will be available to the reproductive organs. Chronic exposure to 2-methylpropan-2-ol up to levels of 420 mg/kg bw/day or higher in rats and mice did not induce effects on the reproductive organs. This confirms the absence of effects on fertility in the oral screening study.

In a developmental toxicity study a dose of 50 mg/kg bw/day 70% TBHP, the highest dose tested, did not result in developmental toxicity. It is recognised that these negative results do not exclude the potential for reproductive and developmental effects at higher doses than tested. However, considering these negative test results on relevant parameters and given the effects seen in the range-finding studies (especially submucosal oedema in the stomach wall, in males at 50 mg/kg bw and higher and in females at 107 mg/kg bw and higher) and in the repeated dose toxicity study (effects in males at much lower levels in organs other than reproductive organs), further testing at higher dose levels is not expected to provide relevant additional information.

With regard to the significant increase in post-natal pup mortality at 30 mg/kg bw/day in the oral screening study, this observation is not considered a treatment related effect (see section 4.1.2.9.1: study by Jonker *et al.*, 1993). This is supported by the studies with 2-methylpropan-2-ol where increased post-natal mortality was only seen at much higher dose levels (lower doses not tested) in rats (Abel and Bilitzke, 1992) and not in mice at much higher dose levels (Daniel and Evans, 1982).

The available data on 2-methylpropan-2-ol indicate reproductive effects at much higher dose levels (6000 mg 2-methylpropan-2-ol/m^3) than can achieved by exposure to TBHP (LC$_{50}$: 1850 mg/m^3). Therefore, these effects are unlikely to be found after exposure to TBHP.

4.1.3.2   Workers

Assuming that oral exposure is prevented by personal hygienic measures, the risk characterisation for workers is limited to the dermal and inhalation routes of exposure.

In the scope of the assessment of existing substances, dermal exposure to corrosive concentrations is not assessed. For the handling of corrosive substances and formulations, it is assumed that daily dermal exposure can be neglected because workers are protected from dermal exposure and immediate dermal contacts occur only accidentally. Techniques and equipment (including PPE) are used that provide a high level of protection from direct dermal contact. Eye protection is obligatory for activities where direct handling of TBHP occurs.

However, dermal exposure to dilutions of TBHP, that result in a substance or formulation which has no corrosive labelling (dilutions containing <10% TBHP, according to EU classification and labelling commission), also occurs. Dermal exposure to such non-corrosive dilutions of TBHP (in scenario 3 (Production of products containing <1% TBHP – general mixing and packaging of products') and scenario 4 (Use of products containing <1% THBP)) cannot be neglected and will be taken into account.

Furthermore, acute and repeated inhalation exposure to TBHP will be considered.

It is noted that the animal studies which are used as starting point for risk characterisation of the different toxicological endpoints are performed with dilutions of TBHP. However, the inhalation and dermal exposure values for the various worker scenarios are recalculated and expressed as '100%' TBHP (see **Table 4.1.1.1A**). Therefore, the (no) effect levels from the animal studies (in which dilutions of TBHP were used) are also recalculated to 100% TBHP.

4.1.3.2.1   Acute toxicity

Only acute inhalation animal studies are available for TBHP. The mouse and rat LC_{50} values, 1292 mg/m^3 and 1845 mg/m^3 (100% TBHP), respectively, from the studies of Thackara and Rinehart (1980) and Floyd and Stokinger (1958) show that mice are more sensitive to TBHP. No information on systemic non-lethal effects (which would be preferred for risk characterisation (see draft TGD, 2005)) was reported for the acute inhalation studies. Therefore, it should be noted that the data available for evaluation of acute inhalation exposure are limited. Starting-point for the risk assessment of acute inhalation toxicity is the 4-hour LC_{50} value of 1292 mg/m^3 for mice. Adapting this starting point by a factor of 6.7/10 for activity-driven differences of respiratory volumes in workers conducting light activity compared to being in rest, results in an adapted level of 866 mg/m^3. Comparison of this LC_{50} value with the estimated short-term exposure levels is presented in **Table 4.1.3.2A** as MOS values. The MOS values are evaluated by comparison with the minimal MOS (>>12.5). In Appendix A, **Table A-1** the assessment factors used to establish the minimal MOS are given. There is concern when the MOS is lower than the minimal MOS. As an LC_{50} is used as starting point for the risk characterisation, a factor of <10 as margin between the MOS and the established part of the minimal MOS is not considered acceptable.

Comparing the MOS value of scenario 1 with the established part of the minimal MOS (>>12.5), it is reasonably not expected that this will result in a concern for workers with regard to the occurrence of adverse effects after acute inhalation exposure to TBHP (an additional uncertainty factor of >10 is possible) (**conclusion ii**).

Comparing the MOS values of scenarios 2, 3 and 4 with the established part of the minimal MOS of >>12.5, it is concluded that the margin between the MOS and the established part of the minimal MOS is lower than 10. It should be noted that MOS for acute inhalation toxicity is based on a 4-hour LC_{50} while the actual human exposure concerns only 1-2 hr in some scenarios. By applying Haber's Law[6] the additonal 'margin of safety' based on these exposure duration differences can be assessed quantitatively. This approach results in MOS values of 109, 137.5 and 108 for scenarios 2, 3 and 4, respectively. As an additional uncertainty factor of >10 is possible next to the already established part of the minimal MOS with regard to

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[6] Nowadays a modification of Haber's Law ($C^n.t = k$) is used as for many substances it has been shown that n is not equal to 1. In case extrapolation of exposure duration is required, the n value should be considered. If this n value is not available from literature, a default value may be used. It is recommended to set n = 3 for extrapolation to shorter duration than the duration for which the LC_{50} was observed (ACUTEX TGD, 2006).

scenario 3, there is no concern for this scenario (**conclusion ii**). However, acute toxic effects due to acute inhalation exposure cannot be excluded for scenarios 2 and 4 (**conclusion iii**).

Table 4.1.3.2.A   Risk assessment for TBHP for acute toxicity after inhalation exposure

Occupational exposure scenario	Short-term exposure estimate in mg/m³ (duration)	MOS[A]	Conclusion[B]
1. Production and use of TBHP in the chemical industry Drumming, sampling, cleaning and maintenance, repair work	5 (1-2 hr)	>173	ii
2. Production and use of TBHP containing hardeners of plastics Transfer activities, emptying drums, sampling, packaging, cleaning and maintenance, repair work	10 (1-2 hr)	109	iii
3. Production of products containing <1% TBHP Emptying drum and cleaning and maintenance	10 (0-1 hr)	137.5	ii
4. Use of products containing <1% TBHP Manual application and cleaning of equipment	8 (2-4 hr)	108	iii

[A] based on a starting point of 866 mg/m³ (exposure duration 4 h) which was corrected for the actual human exposure duration in each specific scenario and the short-term inhalation exposure levels; [B] Based on comparison of the MOS with a minimal MOS of >>12.5.

Dermal exposure

Only one acute dermal toxicity study with rabbits was available. Based on this study, TBHP (70%) is classified as harmful in contact with skin, because the LD_{50} in rabbits is 628 mg/kg bw (Kingery and Valerio, 1982). Doses levels of 480, 576, 720, 864, 1248 and 1997 mg/kg bw (70% TBHP) were tested in this study. Mortality of all tested animals was observed from dose levels of 720 mg/kg bw. One animal in the lowest dose group died (no explanation for this finding was given) and no animals exposed to 576 mg/kg bw died. The LD_{50} of 628 mg/kg bw is used as a starting point for acute dermal toxicity. Based on this value for a 70% TBHP dilution, a LD_{50} of 440 mg/kg bw is calculated for 100% TBHP. In Appendix A, **Table A-2** the assessment factors used to establish the minimal MOS are given. There is concern when the MOS is lower than the minimal MOS.

In scenario 1 and 2, dermal exposure is considered to occur only accidentally, so **conclusion ii** is justifiable.
In scenario 3 (Production of products containing <1% TBHP – general mixing and packaging of products') and scenario 4 (Use of products containing <1% THBP), the estimated external dermal exposures expressed as mg/kg bw/day are 0.06, 0.6 and 1.4, respectively, assuming a worker body weight of 70 kg. The MOS values between the LD_{50} value and the estimated external dermal doses are calculated to be 7333, 733 and 314, respectively. It should be noted that systemic effects may occur at lower dose levels than the LD_{50} dose level. However, comparing the MOS values with the minimal MOS of >>30, it is concluded that the MOS

values are clearly in excess of the minimal MOS even with regard to possible systemic effects (an additional uncertainty factor of 10 is possible).

Furthermore, the following aspects should be emphasised:

- at the dose levels tested in the acute dermal toxicity study local dermal effects were observed which may have resulted in a higher absorption of TBHP in the rabbits;
- the MOS for acute dermal toxicity is based on a 24-hour LD_{50} while the exposure duration for the various workers scenarios concern 1-6 hours, which may indicate an underestimation of the MOS value.

Therefore, comparing the MOS values with the established part of the minimal MOS and taking into account the above described aspects, it is reasonably not expected that this will result in a concern for workers with regard to the occurrence of adverse effects after short-term dermal exposure to TBHP (**conclusion ii**).

Table 4.1.3.2.B   Risk assessment for TBHP for acute toxicity after dermal exposure

Occupational exposure scenario	Exposure estimate in mg/day (mg/kg bw/day)[A]	MOS[B]	Conclusion[C]
3. Production of products containing <1% TBHP			
General: mixing and packaging of products	4 (0.06)	7333	ii
Emptying drum and cleaning and maintenance	42 (0.6)	733	ii
4. Use of products containing <1% TBHP			
Manual application and cleaning of equipment	27 (0.4)	1100	ii

[A] Between brackets: the external dermal dose in mg/kg bw/d, assuming a worker body weight of 70 kg; [B] Based on a calculated LD_{50} value of 440 mg/kg bw for 100% TBHP and dermal exposure levels; [C] Based on comparison of the MOS with a minimal MOS of >>30.

4.1.3.2.2   Irritation and corrosivity

Skin

Acute irritation

TBHP is considered to be a corrosive agent (concentrations ≥10%). Workers can be exposed to corrosive concentrations. However, dermal exposure to corrosive concentrations of TBHP is considered to occur only accidentally if the required protection is strictly adhered to. Therefore, **conclusion ii** is justifiable for scenarios in which corrosive concentrations of TBHP are handled.

Dermal exposure to irritating, but non-corrosive, dilutions of TBHP (concentrations <10%) also occurs. The data available do not permit a quantitative risk characterisation. However, it is assumed that existing controls (i.e., engineering controls and personal protective equipment based on classification and labelling with R38) are applied for these exposure situations. Therefore, in the case that engineering controls and personal protective equipment are effectively used, it is concluded that TBHP is of no concern for workers with regard to skin irritation for scenarios in which non-corrosive concentrations are handled (**conclusion ii**).

Eye

Given the results of the eye irritation studies, it is concluded that TBHP is of concern for workers with regard to local effects on the eye. However, ocular exposure can be excluded as effective use of personal protective equipment (for the eyes) is assumed. Therefore, in the case that engineering controls and personal protective equipment are effectively used, it is concluded that the substance is of no concern for workers with regard to eye irritation (**conclusion ii**).

Respiratory tract

TBHP is considered irritating to the respiratory tract and labelled with R34 (concentrations 10%) and R37 (concentrations 5% < C ≤ 10%).

For a quantitative risk characterisation the study of Ben-Dyke and Hogan (1981) is used. In this study, from a concentration of 33 mg/m³ (slightly) increased incidences of lacrimation, red nasal discharge, mucoid nasal discharge and dry rales were observed in the exposed rats compared to control animals. It is noted that no clear concentration relationship was observed for the effects (exposure concentrations 33 – 347 mg/m³). However, the effects which occurred at the lowest concentration were regarded as adverse treatment related effects indicating irritation. Therefore, the lowest tested concentration (33 mg/m³) is used as starting point for the quantitative risk characterisation.

Comparison of the concentration of 33 mg/m³ (LOAEC) with the estimated short-term exposure levels is presented in **Table 4.1.3.2C** as MOS values. The MOS values are evaluated by comparison with the minimal MOS (37.5). In Appendix A, **Table A-3** the assessment factors used to establish the minimal MOS are given. There is concern when the MOS is lower than the minimal MOS.

Table 4.1.3.2.C   Risk assessment for TBHP for acute (sensory) irritation after inhalation exposure

Occupational exposure scenario	Short-term exposure estimate in mg/m³ (duration 0-4 hr)	MOS[A]	Conclusion[B]
1. Production and use of TBHP in the chemical industry Drumming, sampling, cleaning and maintenance, repair work	5	6.6	iii
2. Production and use of TBHP containing hardeners of plastics Transfer activities, emptying drums, sampling, packaging, cleaning and maintenance, repair work	10	3.3	iii
3. Production of products containing <1% TBHP Emptying drum and cleaning and maintenance	10	3.3	iii
4. Use of products containing <1% TBHP Manual application and cleaning of equipment	8	4.1	iii

[A] Based on a concentration of 33 mg/m³ (LOAEC) and the short-term inhalation exposure levels; [B] Based on comparison of the MOS with a minimal MOS of 37.5.

Given the MOS values for respiratory sensory irritation as mentioned in **Table 4.1.3.2C** and the absence of labelling with S23 and S38, it is concluded that based upon the present information, local effects of the respiratory tract cannot be excluded for all scenarios.

4.1.3.2.3   Sensitisation

Skin

Based on the results of the GMPT study, it is concluded that TBHP is a skin sensitiser. The data are insufficient for a quantitative risk characterisation. However, as sensitisation is considered as a non-threshold effect and as dermal exposure may occur in different scenarios, it is concluded that TBHP is of concern for workers (**conclusion iii**).

Respiratory tract

No information is available on the respiratory tract sensitising potential of TBHP.

4.1.3.2.4   Repeated dose toxicity

Dermal exposure

Local effects
As indicated in section 4.1.3.2.2, TBHP is considered to be a corrosive agent (concentrations ≥10%). With regard to the available repeated dermal dose studies, a NOAEC of 2.2% TBHP (in 50 percent aqueous acetone) for local effects (histopathology lesions included minimal to mild hyperkeratosis, hyperplasia and/or inflammation of the epidermis and dermis) was derived in mice (Ritchie *et al.*, 2005c,d). It is noted that exposure was also to acetone, a substance which also may cause local dermal effects.

Dermal exposure to corrosive concentrations of TBHP is considered to occur only accidentally if the required protection is strictly adhered to. Therefore, **conclusion ii** is justifiable for scenarios in which corrosive concentrations of TBHP are handled (scenario 1 and 2).

In scenario 3 'Production of products containing <1% TBHP' and scenario 4 'Use of products containing <1% TBHP', workers may be exposed to TBHP concentrations lower than 1%. Comparing this TBHP exposure concentration with the NOAEC of 2.2%, it is concluded that TBHP is of no concern for workers with regard to local dermal effects (**conclusion ii**).

Systemic effects
Starting points for the risk characterisation for workers exposed by skin contact for systemic effects are (a) the NOAEL of 30 mg/kg bw/day from the 45 day oral gavage study performed by Jonker et al. (1993) in rats with 70% TBHP and (b) the estimated dermal exposure levels for the different occupational scenarios (see chapter 4.1.1.2 and **Table 4.1.1.2B**). Based on the NOAEL of 30 mg/kg bw/day for a 70% TBHP dilution, a NAEL of 21 mg/kg bw/day is calculated for 100% TBHP. Given the estimated frequency of exposure (10-50 d/year for all

other scenarios) semi-chronic exposure is assumed. Although TBHP is a corrosive substance route-to-route extrapolation is considered applicable.

In scenarios 1 and 2, dermal exposure is considered to occur only accidentally, so **conclusion ii** is justifiable. In scenarios 3 (Production of products containing <1% TBHP – general mixing and packaging of products') and 4 (Use of products containing <1% THBP), the systemic doses due to the dermal exposure are 0.002 and 0.0135 mg/kg bw/day, respectively, assuming a worker body weight of 70 kg and 3.5% dermal absorption. Comparison of the calculated NAEL for 100% TBHP with the estimated exposure level is presented in **Table 4.1.3.2.D** as MOS values. The MOS values are evaluated by comparison with the minimal MOS of 50. In Appendix A, **Table A-4** the assessment factors used to establish the minimal MOS are given. There is concern when the MOS is lower than the minimal MOS.

Based on a comparison of the MOS values with the minimal MOS value, it is concluded that there is no concern for workers with regard to systemic effects due to repeated dermal exposure to TBHP (**conclusion ii**).

Table 4.1.3.2.D   Risk assessment for TBHP for repeated-dose toxicity after dermal exposure (systemic effects)

Occupational exposure scenario	Dermal exposure estimate in mg/day (mg/kg bw/day)[A]	MOS[B]	Conclusion
3. Production of products containing <1% TBHP Full shift	4 (0.002)	10500	ii[c]
4. Use of products containing <1% TBHP Manual application and cleaning of equipment	27 (0.0135)	1560	ii[c]

[A] Between brackets: the internal dose due to dermal exposure in mg/kg bw/d, assuming a worker body weight of 70 kg and 3.5% dermal absorption; [B] Based on a calculated oral external NAEL of 21 mg/kg bw/day, an oral absorption of 100% and the internal dose due to dermal exposure in mg/kg bw/day; [C] Based on comparison of the MOS with a minimal MOS of 50.

Inhalation exposure

Local effects
There is no data available on respiratory irritation following repeated inhalation exposure.

Systemic effects
Starting points for the risk characterisation for workers exposed by inhalation for systemic effects are (a) the NOAEL of 30 mg/kg bw/day from the 45-day oral gavage study from Jonker et al. (1993) in rats with 70% TBHP, and (b) the estimated inhalation exposure levels for the different occupational scenarios (see chapter 4.1.1.2 and **Table 4.1.1.2B**). Based on the NOAEL of 30 mg/kg bw/day for a 70% TBHP dilution, a NAEL of 21 mg/kg bw/day is calculated for 100% TBHP. Given the estimated frequency of exposure (100-200 d/year for the chemical industry and 10-50 d/year for all other scenarios) chronic and semichronic exposure is assumed, respectively, for risk characterisation.

Comparison of the calculated NAEL for 100% TBHP with the estimated exposure level is presented in **Table 4.1.3.2.E** as MOS values. The MOS values are evaluated by comparison with the minimal MOS (100 for chronic exposure and 50 for semichronic exposure). In

Appendix A, **Table A-5** the assessment factors used to establish the minimal MOS are given. There is concern when the MOS is lower than the minimal MOS.

Given the MOS value for inhalation exposure for the scenario 'Production and use of TBHP in the chemical industry' as mentioned in **Table 4.1.3.2E**, there is no concern for this scenario (**conclusion ii**).

The MOS values for the scenarios 'Production and use of TBHP containing hardeners of plastics', 'Production of products containing <1% TBHP' and 'Use of products containing <1% TBHP – Full shift' are slightly lower than the minimal MOS (factor 1.1, factor 1.2 and factor 1.4, respectively) (see **Table 4.1.3.2.E**). Considering the relative small difference between the minimal MOS and the MOS and taking into account the uncertainties and worst case approaches taken in both exposure assessment and derivation of the minimal MOS, **conclusion ii** is considered acceptable for these scenarios. Furthermore, it is noted that due to concern for respiratory tract irritation risk reduction measures with regard to inhalation exposure are required.

The MOS value of 18 for the scenario 'Use of products containing <1% TBHP - Manual application and cleaning of equipment' is considered too low compared to the minimal MOS value of 50. Therefore, a **conclusion iii** is applicable for this scenario.

Table 4.1.3.2.E   Risk assessment for TBHP for repeated-dose toxicity after inhalation exposure (systemic effects)

Occupational exposure scenario	Inhalation exposure estimate in mg/m^3 (mg/kg bw/day)[A]	MOS[B]	Conclusion
1. Production and use of TBHP in the chemical industry			
Full shift	0.5 (0.07)	294	ii[C]
2. Production and use of TBHP containing hardeners of plastics			
Full shift	3.2 (0.46))	46	ii[D], see text
3. Production of products containing <1% TBHP			
Full shift	3.6 (0.51)	41	ii[D], see text
4. Use of products containing <1% TBHP			
Manual application and cleaning of equipment	8 (1.14)	18	iii[D], see text
Full shift	4 (0.57)	37	ii[D], see text

[A] Between brackets: estimated internal dose due to inhalation exposure in mg/kg bw/day assuming a worker body weight of 70 kg, a respiratory volume of 10 m^3 for a working day and a respiratory retention of 100%; [B] Based on a calculated external oral NAEL in rats of 21 mg/kg bw/day, an oral absorption of 100% and the internal dose due to inhalation exposure in mg/kg bw/day; [C] Based on comparison of the MOS with a minimal MOS of 100; [D] Based on comparison of the MOS with a minimal MOS of 50.

Combined exposure

The starting points for combined exposure are (a) the NOAEL of 30 mg/kg bw/day from the 45-day oral gavage study from Jonker et al. (1993) in rats with 70% TBHP, and (b) the combined exposure levels for the scenarios 'Production of products containing <1% TBHP' and 'Use of products containing <1% TBHP, Manual application and cleaning of equipment' (0.512 and 1.1535 mg/kg bw/day, respectively). Based on the NOAEL of 30 mg/kg bw/day for a 70% TBHP dilution, a NAEL of 21 mg/kg bw/day is calculated for 100% TBHP. Given the estimated frequency of exposure (10-50 d/year for the combined exposure scenarios) semichronic exposure is assumed for risk characterisation. The MOS values for the scenarios 'Production of products containing <1% TBHP' and 'Use of products containing <1% TBHP, Manual application and cleaning of equipment', are 41 and 18, respectively (the same values as for inhalation exposure alone (see Table 4.1.3.2.E)). Thus it can be concluded that the internal body burden due to inhalation and dermal exposure is mainly determined by inhalation exposure and therefore for combined exposure the same conclusions as for inhalation exposure are applicable: **conclusion ii** for the scenario 'Production of products containing <1% TBHP' and **conclusion iii** for the scenario 'Use of products containing <1% TBHP, Manual application and cleaning of equipment'.

4.1.3.2.5   Mutagenicity

Based on the available data, it is concluded that TBHP is considered genotoxic *in vivo* at sites of first contact. This conclusion is based on the worst case assumption that there is no threshold for this effect. Therefore, **conclusion iii** is reached.

4.1.3.2.6   Carcinogenicity

No inhalatory or oral carcinogenicity studies with TBHP are available. TBHP is, however, rapidly converted to 2-methylpropan-2-ol and for this compound oral carcinogenicity studies in mice and rats are available. These studies show very small increases in systemic tumours at dose levels of 2-methylpropan-2-ol that can not be reached by treatment with TBHP because these levels are above the LD50 of TBHP for mice, and above the dose level of TBHP inducing local effects to the stomach in rats. From these observations it is concluded that chronic exposure to TBHP will most probably not result in 2-methylpropan-2-ol levels that can induce systemic tumours.

TBHP is considered to be mutagenic at the sites of first contact in somatic cells. However, based on the rapid conversion of TBHP, it is unlikely that TBHP can reach the systemic circulation through normal routes of exposure. Consequently, carcinogenicity limited to tissues that are exposed to the parent TBHP (i.e. tissues of first contact) cannot be excluded.

Useful data on the potential local carcinogenic effects of TBHP are not available, unfortunately. In a single very limited dermal study one clearly toxic concentration of TBHP was capable of promoting the development of dermal tumours after induction by 4-nitro-quinoline 1-oxide. Therefore information on the local carcinogenicity is needed (**conclusion i**). This information could be derived either by:
-    read-across from other substances in case the read-across is sufficiently validated and based on sufficient data, or

- carcinogenicity tests.

This should ideally be through the oral, dermal and inhalatory route because it is unknown whether and how local carcinogenicity can be extrapolated from one route to the other. However, from a practical point of view it is proposed to start with one route and the need for additional routes will depend on the results. Information on the carcinogenicity by the inhalatory route is preferred because this route is relevant for both workers and humans exposed via the environment.

Considering that:
- the local mutagenic potency of TBHP is probably low because genotoxic effects were mainly seen at very high dose levels;
- the risk assessment of comparable substances which also have corrosive and mutagenic properties like e.g. formaldehyde is mainly based on the assumption that the mutagenicity and carcinogenicity is restricted to irritating concentrations;
- only very limited data are available on the local irritating potential of TBHP after inhalation exposure,
it is not appropriate to ask for an inhalatory carcinogenicity study straight away.

Therefore, we propose an inhalatory repeated dose toxicity study to investigate which level could be derived as a NOAEC for local irritation. In addition to a NOAEC for local irritation, information is needed with regard to the mutagenicity of TBHP towards specific parts of the respiratory tract, including nose epithelium, upper respiratory tract and lower respiratory tract at the same concentrations as tested in the inhalatory repeated dose toxicity study.

With regard to the inhalatory repeated dose toxicity study, 'an extended' 28-day inhalation study according to EU method B8 using at least three concentrations including an irritating, a slightly irritating and a non-irritating concentration could be performed. Histopathological examination should at least include all parts of the respiratory tract.
Regarding the information on mutagenicity of TBHP towards the respiratory tract, a local mutagenicity test could be conducted.

If the results show that TBHP induces mutations only at concentrations also inducing local irritation than the NOAEC for local irritation can also be used (with some extrapolation factors) as the NOAEC for local mutagenicity and possible local carcinogenicity. If the results show mutagenicity below the concentrations inducing local irritation then no safe level for local mutagenicity and possible local carcinogenicity can be determined and further information on the inhalatory carcinogenicity is required.

Furthermore, it is proposed to store the testis of the animals tested in the inhalatory repeated dose toxicity study to determine mutagenic effects on the germ cells and the liver to determine the systemic mutagenic effects, in case of positive results for mutagenicity in the respiratory tract. This is mainly to confirm the expected absence of systemic and germ cell effects.

4.1.3.2.7   Toxicity for reproduction

Fertility

No specific effects on fertility are shown in the oral Combined Repeated Dose and Reproductive/Developmental Toxicity Screening Study in rats (Jonker et al., 1993).

Therefore, no quantitative risk characterisation is performed and there is no reason for concern (**conclusion ii**).

Developmental toxicity

No specific teratogenic potential and/or impairment of embryo/fetal development are shown in the oral embryotoxicity/teratogenicity study in rats (Smits-van Prooije, 1993). Therefore, no quantitative risk characterisation is performed and there is no reason for concern (**conclusion ii**).

4.1.3.2.8   Summary of risk characterisation for workers

PM: will be added later.

4.1.3.3   Consumers

TBHP is mainly used in the chemical industry as starting material or as reactive ingredient. As TBHP will be totally converted the exposure of consumers to TBHP as impurities in other products is expected to be absent. The use of TBHP in some consumer products was indicated in several databases but individual consumer products containing TBHP could not be identified. A risk characterisation for consumers is therefore not possible. The total amount used in consumer products is low. Also, the two products that were identified but no longer on the market indicate that the concentration is low. Therefore, no risk characterisation is performed.

4.1.3.4   Humans exposed via the environment

Only oral and inhalatory exposure is taken into account for exposure via the environment as exposure is via air and via food and water. Further, the exposure duration is considered to be chronic as exposure via the environment is a continuous process.

4.1.3.4.1   Repeated dose toxicity

Starting points for the risk characterisation for humans exposed via the environment are (a) the NOAEL of 30 mg/kg bw/day from the 45 day oral gavage study performed by Jonker et al. (1993) in rats with 70% TBHP and (b) the total daily intake values as reported in table 4.1.1.4B. From the oral NOAEL, a NAEL of 21 mg/kg bw/day is calculated for 100% TBHP. This NAEL can be used for the risk characterisation for the exposure via food, assuming route to route extrapolation is allowed and 100% absorption for the oral and inhalation route.

A minimal MOS of 200 was determined based on assessment factors for interspecies (4 * 2.5) and intraspecies (10) differences and semichronic-to-chronic (2, the 45 days repeated dose study was assessed as a semichronic study).

Applying the minimal MOS of 200 to the NAEL of 21 mg/kg bw/day implies that only scenarios with a total daily intake above 0.105 mg/kg bw are of concern. This is only the case for the local sites I-c and II-b2, both with an estimated total daily intake of 0.292 mg/kg bw, mainly (97%) coming from food and water. Hence, for these two local sites a **conclusion iii*** is reached. For all other local sites (with 0.0163 mg/kg bw as the highest total daily intake), as well as for the regional scenario (total daily intake of 8.07E-06 mg/kg bw), a **conclusion ii** can be drawn.

* The exposure estimates for these two sites are only partly based on site-specific data. A conclusion i asking for more site-specific data in order to possibly refine the exposure estimates would be the preferred option. However, when dealing with the environmental aspects, Industry has not supported to provide additional exposure data (see environmental RAR).

4.1.3.4.2   Mutagenicity

Based on the available data, it is concluded that TBHP is considered genotoxic *in vivo* at sites of first contact. This conclusion is based on the worst case assumption that there is no

threshold for this effect. Therefore, **conclusion iii** is reached for all local sites and for the regional scenario.

4.1.3.4.3 Carcinogenicity

No inhalatory or oral carcinogenicity studies with TBHP are available. TBHP is, however, rapidly converted to 2-methylpropan-2-ol and for this compound oral carcinogenicity studies in mice and rats are available. These studies show very small increases in systemic tumours at dose levels of 2-methylpropan-2-ol that can not be reached by treatment with TBHP because these levels are above the LD50 of TBHP for mice, and above the dose level of TBHP inducing local effects to the stomach in rats. From these observations it is concluded that chronic exposure to TBHP will most probably not result in 2-methylpropan-2-ol levels that can induce systemic tumours.

TBHP is considered to be mutagenic at the sites of first contact in somatic cells. However, based on the rapid conversion of TBHP, it is unlikely that TBHP can reach the systemic circulation through normal routes of exposure. Consequently, carcinogenicity limited to tissues that are exposed to the parent TBHP (i.e. tissues of first contact) cannot be excluded.

Useful data on the potential local carcinogenic effects of TBHP are not available, unfortunately. In a single very limited dermal study one clearly toxic concentration of TBHP was capable of promoting the development of dermal tumours after induction by 4-nitro-quinoline 1-oxide. Therefore information on the local carcinogenicity is needed (**conclusion i**). This information could be derived either by:
- read-across from other substances in case the read-across is sufficiently validated and based on sufficient data, or
- carcinogenicity tests.

This should ideally be through the oral, dermal and inhalatory route because it is unknown whether and how local carcinogenicity can be extrapolated from one route to the other. However, from a practical point of view it is proposed to start with one route and the need for additional routes will depend on the results. Information on the carcinogenicity by the inhalatory route is preferred because this route is relevant for both workers and humans exposed via the environment.

Considering that:
- the local mutagenic potency of TBHP is probably low because genotoxic effects were mainly seen at very high dose levels;
- the risk assessment of comparable substances which also have corrosive and mutagenic properties like e.g. formaldehyde is mainly based on the assumption that the mutagenicity and carcinogenicity is restricted to irritating concentrations;
- only very limited data are available on the local irritating potential of TBHP after inhalation exposure,

it is not appropriate to ask for an inhalatory carcinogenicity study straight away.

Therefore, we propose an inhalatory repeated dose toxicity study to investigate which level could be derived as a NOAEC for local irritation. In addition to a NOAEC for local irritation, information is needed with regard to the mutagenicity of TBHP towards specific parts of the respiratory tract, including nose epithelium, upper respiratory tract and lower respiratory tract at the same concentrations as tested in the inhalatory repeated dose toxicity study.

With regard to the inhalatory repeated dose toxicity study, 'an extended' 28-day inhalation study according to EU method B8 using at least three concentrations including an irritating, a slightly irritating and a non-irritating concentration could be performed. Histopathological examination should at least include all parts of the respiratory tract.
Regarding the information on mutagenicity of TBHP towards the respiratory tract, a local mutagenicity test could be conducted.

If the results show that TBHP induces mutations only at concentrations also inducing local irritation than the NOAEC for local irritation can also be used (with some extrapolation factors) as the NOAEC for local mutagenicity and possible local carcinogenicity. If the results show mutagenicity below the concentrations inducing local irritation then no safe level for local mutagenicity and possible local carcinogenicity can be determined and further information on the inhalatory carcinogenicity is required.

Furthermore, it is proposed to store the testis of the animals tested in the inhalatory repeated dose toxicity study to determine mutagenic effects on the germ cells and the liver to determine the systemic mutagenic effects, in case of positive results for mutagenicity in the respiratory tract. This is mainly to confirm the expected absence of systemic and germ cell effects.

4.1.3.4.4   Reproductive toxicity

Fertility

No quantitative risk characterisation is performed as no adverse effects regarding this endpoint were observed up to dose levels causing other toxic effects (see repeated dose toxicity). Therefore, **conclusion ii** is applicable.

Developmental toxicity

No quantitative risk characterisation is performed as no adverse effects regarding this endpoint were observed up to dose levels causing other toxic effects (see repeated dose toxicity). Therefore, **conclusion ii** is applicable.

4.1.3.4.5 Summary of risk characterisation for exposure via the environment

The risk characterization for man indirectly exposed via the environment resulted for all local sites and for the regional scenario in a conclusion iii for the endpoint mutagenicity, a **conclusion i** for carcinogenicity and a conclusion ii for reproductive toxicity. For repeated dose toxicity the conclusions depend on the scenario. A conclusion iii was reached for the local sites I-c and II-b2, while for all other local sites and for the regional scenario a conclusion ii was reached.

4.1.3.5   Combined exposure

As there is no consumer exposure, combined exposure concerns only the combination of worker exposure and exposure of man via the environment. However, for workers a **conclusion i** or a conclusion iii is reached for all endpoints that are also of concern for man

exposed via the environment. The additional exposure via the environment will not change these conclusions. Therefore, no combined exposure assessment and risk characterization was performed.

4.2   HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.2.1   Effects assessment: Hazard identification

4.2.1.1 Explosivity

Pure TBHP is explosive. However, TBHP is commercially available and used mostly as TBHP-70 (T-Hydro), an aqueous solution of approximately 70 weight percent TBHP and 30 weight percent water. Detailed experimental data on explosive properties was not available on TBHP-70. However, general transport classification data (Peroxid-Chemie, 1993) indicate that TBHP-70 has no explosive properties in the sense of Directive 92/69/EEC. This is also in line with the current transport classification of TBHP-70.

4.2.1.2 Flammability

THBP-70 is flammable, but does not need to be classified as flammable according to the criteria. However, the flashpoint indicates that the substance may cause a combustible vapour or a vapour explosion. Therefore, TBHP should be labelled with R10.

4.2.1.3 Oxidizing potential

TBHP-70 should be classified as oxidizing (symbol O) and labelled with the R-sentence R7, because it is an organic peroxide.

4.2.2   Risk characterisation

TBHP is flammable and oxidizing and is labelled with respect to these physico-chemical properties. However, it is assumed that existing controls (i.e., engineering controls and personal protective equipment based on classification and labelling with R7 and R10) are applied for exposure situations. Therefore, in the case that engineering controls and personal protective equipment are effectively used, it is concluded that TBHP is of no concern with regard to physico-chemical properties (**conclusion ii**). There is no need for further information and/or testing.

5   RESULTS [7]

<mark>[Note: In the final report, chapters 0 and 5 should be as close as possible to the OJ]</mark>

5.1   INTRODUCTION

[click here to insert text]

5.2   ENVIRONMENT

<mark>[keep only appropriate conclusion(s)]</mark>

Conclusion (i)   There is a need for further information and/or testing.

Conclusion (ii)   There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (iii)   There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion () applies to [click here to insert text in accordance with conclusion(s)]

5.3   HUMAN HEALTH

5.3.1   Human health (toxicity)

5.3.1.1   Workers

Conclusion (i)   There is a need for further information and/or testing.

Conclusion (iii)   There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (i) is reached because:
local carcinogenicity can not be excluded as TBHP is considered mutagenic to the sites of first contact and useful data on the potential local carcinogenic effects of TBHP are not available. This conclusion applies to all scenarios.

Conclusion (iii) is reached because:
- systemic effects cannot be excluded after acute inhalation exposure in the scenarios 'Production and use of TBHP containing hardeners of plastics' and 'Use of products containing <1% TBHP';
- respiratory tract irritation cannot be excluded after inhalation exposure in all scenarios;

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[7] Conclusion (i)   There is a need for further informaon and/or testing.
   Conclusion (ii)   There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
   Conclusion (iii)   There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

- skin sensitisation cannot be excluded after dermal exposure in all scenarios;
- systemic effects cannot be excluded after repeated inhalation exposure in the scenario 'Use of products containing <1% TBHP - Manual application and cleaning of equipment'; and
- mutagenic effects after dermal and inhalation exposure cannot be excluded in all scenarios.

It might be possible that in some workplaces adequate worker protection measures are already being applied.

5.3.1.2   Consumers

Not applicable because there is no consumer exposure.

5.3.1.3   Humans exposed via the environment

Conclusion (i)   There is a need for further information and/or testing.

Conclusion (ii)   There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (iii)   There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (i) is reached because:
local carcinogenicity can not be excluded as TBHP is considered mutagenic to the sites of first contact and useful data on the potential local carcinogenic effects of TBHP are not available. This applies to all local sites and for the regional scenario.

Conclusion (ii) applies to the endpoint reproductive toxicity for all local sites and for the regional scenario, and to the endpoint repeated dose toxicity for the sites not mentioned below.

Conclusion (iii) applies to the endpoint mutagenicity for all local sites and for the regional scenario, and to the endpoint repeated dose toxicity for the local sites I-c and II-b2.

5.3.1.4   Combined exposure

A risk characterisation for combined exposure was not performed because the conclusions already made for each scenario will not be changed by adding the exposure via the environment.

5.3.2   Human health (risks from physico-chemical properties)

Conclusion (**ii**)   There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

TBHP is flammable and oxidizing and is labelled with respect to these physico-chemical properties. However, it is assumed that existing controls (i.e., engineering controls and personal protective equipment based on classification and labelling with R7 and R10) are applied for exposure situations. Therefore, in the case that engineering controls and personal protective equipment are effectively used, it is concluded that TBHP is of no concern with regard to physico-chemical properties (**conclusion ii**). There is no need for further information and/or testing.

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ABBREVIATIONS

[update the list to correspond to the substance RAR]

ADI	Acceptable Daily Intake
AF	Assessment Factor
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
bw	body weight / *Bw, b.w.*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT_{50}	Clearance Time, elimination or depuration expressed as half-life
d.wt	dry weight / dw
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 50 percent dissipation / degradation
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]

EbC50	Effect Concentration measured as 50% reduction in biomass growth in algae tests
EC	European Communities
EC10	Effect Concentration measured as 10% effect
EC50	median Effect Concentration
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EEC	European Economic Communities
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EN	European Norm
EPA	Environmental Protection Agency (USA)
ErC50	Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD	Emission Scenario Document
EU	European Union
EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
F(+)	(Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
GLP	Good Laboratory Practice
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission
HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 t/a)
IARC	International Agency for Research on Cancer
IC	Industrial Category
IC50	median Immobilisation Concentration or median Inhibitory Concentration
ILO	International Labour Organisation
IPCS	International Programme on Chemical Safety
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database (existing substances)
IUPAC	International Union for Pure and Applied Chemistry
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
Koc	organic carbon normalised distribution coefficient

Kow	octanol/water partition coefficient
Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidizing (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based PharmacoKinetic modelling
PBTK	Physiologically Based ToxicoKinetic modelling
PEC	Predicted Environmental Concentration

pH	logarithm (to the base 10) (of the hydrogen ion concentration $\{H^+\}$
pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst Case
S phrases	Safety phrases according to Annex III of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations
UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum
UVCB	Unknown or Variable composition, Complex reaction products of Biological material

vB	very Bioaccumulative
vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
v/v	volume per volume ratio
w/w	weight per weight ratio
WHO	World Health Organization
WWTP	Waste Water Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)

Appendix A

ESTABLISHMENT OF THE MINIMAL MOSS USED FOR THE WORKER RISK CHARACTERISATION

In the tables below calculations of the minimal MOS-values via assessment factors are given. The assessment factors are based upon the draft version of the TGD (2005).

Table A-1 Assessment factors applied for the calculation of the minimal MOS for acute toxicity after acute inhalation exposure (mouse and rat)

Aspect	Assessment factors
Interspecies differences[a]	2.5
Intraspecies differences	5
Differences between experimental conditions and exposure	assessed in a semiqualitative manner (see section 4.1.3.2.1)
Dose response / Type of critical effect [b]	>>1
Confidence of the database	1
Overall	>>12.5

[a] For inhalation studies only a factor 2.5 is used, and no correction is made for differences in body size, because extrapolation is based on toxicological equivalence of a concentration of a chemical in the air of experimental animals and humans; animal and humans breathe at a rate depending on their caloric requirements. Evenmore, for local effects this factor is not necessary.

[b] It is noted that the MOS values are calculated for a severe effect (lethality). It is expected that other toxic effects after acute exposure might occur at lower concentrations than the lethal concentrations.

Table A-2. Assessment factors applied for the calculation of the minimal MOS for acute toxicity after acute dermal exposure (rabbit)

Aspect	Assessment factors
Interspecies differences[a]	2.4 x 2.5
Intraspecies differences	5
Differences between experimental conditions and exposure	assessed in a semiqualitative manner (see section 4.1.3.2.1)
Dose response / Type of critical effect[b]	>>1
Confidence of the database	1
Overall	>>30

[a] Extrapolation based on differences in caloric demands, together with a factor 2.5 for remaining uncertainties.

[b] It is noted that the MOS values are calculated for a severe effect (lethality). It is expected that other toxic effects after acute exposure might occur at lower concentrations than the lethal concentrations.

Table A-3 Assessment factors applied for the calculation of the minimal MOS for acute irritation after acute inhalation exposure (rats)

Aspect	Assessment factors
Interspecies differences[a]	2.5
Intraspecies differences	5
Differences between experimental conditions and exposure	1
Dose response / Type of critical effect [b]	3
Confidence of the database	1
Overall	37.5

[a] For inhalation studies only a factor 2.5 is used, and no correction is made for differences in body size, because extrapolation is based on toxicological equivalence of a concentration of a chemical in the air of experimental animals and humans; animal and humans breathe at a rate depending on their caloric requirements. Evenmore, for local effects this factor is not necessary.

[b] For extrapolation of a LOAEC to a NAEC a factor 3 is considered sufficient.

Table A-4 Assessment factors applied for the calculation of the minimal MOS for systemic effects after semichronic dermal exposure based on a 45 days oral gavage study in rats with 70% TBHP

Aspect	Assessment factors for semichronic exposure
Interspecies differences[a]	4 x 2.5
Intraspecies differences	5
Differences between experimental conditions and exposure [b]	1
Dose response / Type of critical effect [c]	1
Confidence of the database	1
Overall	50

[a] Extrapolation based on differences in caloric demands, together with a factor 2.5 for remaining uncertainties.

[b] The 45 days oral repeated dose study was assessed as a semichronic study.

Table A-5 Assessment factors applied for the calculation of the minimal MOS for systemic effects after (semi)chronic inhalation exposure based on a 45 day oral gavage study in rats with 70% TBHP

Aspect	Assessment factors for chronic exposure	Assessment factors for semichronic exposure
Interspecies differences[a]	4 x 2.5	4 x 2.5
Intraspecies differences	5	5
Differences between experimental conditions and exposure[b]	2	1
Dose response / Type of critical effect [c]	1	1
Confidence of the database	1	1
Overall	100	50

[a] Extrapolation based on differences in caloric demands, together with a factor 2.5 for remaining uncertainties.

[b] The 45-days oral repeated dose study was assessed as a semichronic study. A factor 2 is used for extrapolation of a semichronic study to a chronic exposure situation.

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