Annex XV report

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: Di-tert-butyl peroxide

EC Number: 203-733-6

CAS Number: 110-05-4

Submitted by: France

Date: May 2009

Version : 2

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TABLES

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance name:

IUPAC name: Di-tert-butyl peroxide

CAS name: Peroxide, bis(1,1-dimethylethyl)

EC name: Di-tert-butyl peroxide

EC number: 203-733-6

CAS number: 110-05-4

Registration number(s): -

Molecular formula: $C_8H_{18}O_2$

Purity: 99.9 %

Impurities: Tert-butyl hydroperoxide : < 0.1 %

Proposed classification based on Directive 67/548/EEC:

O ; R7

F ; R11

Muta Cat 3 ; R68

Proposed classification based on CLP:

Org. Perox. Type E – H242

Flamm. Liq. 2 – H225

Muta. 2 – H341

Proposed labelling:

Symbol(s) : O, F, Xn
R-phrases: R7 - R11 – R68
S-phrases : S3/7 - S14 - S16 – S33 - S36/37/39

Proposed specific concentration limits (if any): -

Proposed notes (if any): -

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Chemical Name:	Di-tert-butyl peroxide
EC Name:	Di-tert-butyl peroxide
CAS Number:	110-05-4
IUPAC Name:	Di-tert-butyl peroxide

1.2 Composition of the substance

Chemical Name:	Di-tert-butyl peroxide
EC Number:	203-733-6
CAS Number:	110-05-4
IUPAC Name:	Di-tert-butyl peroxide
Molecular Formula:	$C_8H_{18}O_2$
Structural Formula:	$(CH_3)_3$-C-O-O-C-$(CH_3)_3$
Molecular Weight:	146.23 g/mole
Typical concentration (% w/w):	99.9 %
Concentration range (% w/w):	99-100 %

Chemical Name:	Tert-butyl hydroperoxide
EC Number:	200-915-7
CAS Number:	75-91-2
IUPAC Name:	Tert-butyl hydroperoxide
Molecular Formula:	$C_4H_{10}O_2$
Structural Formula:	$(CH_3)_3$-C-O-OH
Molecular Weight:	90.23 g/mole
Typical concentration (% w/w):	< 0.1 %
Concentration range (% w/w):	< 0.1 %
Classification	The following harmonised classification of tert-butyl hydroperoxide was agreed at TC C&L of September 2007:

According to 67/548/CEE	*According to CLP*
O; R7 R10 Muta. Cat. 3; R68 T; R23 Xn; R21/22 C; R34 R43 N; R51-53 with specific concentration limits: Xi; R37 when 5% ≤ C < 10% R43 when C ≥ 0.1%	Org. Perox. EF; H242 Flam. Liq. 3; H226 Muta. 2; H341 Acute Tox. 2; H330 Acute Tox. 3; H311 Acute Tox. 4; H302 Skin Corr. 1C; H314 Skin Sens. 1; H317 Eye dam. 1; H318 Aquatic Chronic 2; H411 with specific concentration limits: Skin Sens. 1; H317: C ≥ 0.1%

Considering that tert-butyl hydroperoxide (TBHP) is present in di-tert-butyl peroxide in concentration lower to 0.1%, no additional classification applies for di-tert-butyl peroxide due to its impurity.

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

REACH ref Annex, §	Property	IUCLID section	Value	Reference]
VII, 7.1	Physical state at 20 C and 101.3 KPa	3.1	*Liquid*	
VII, 7.2	Melting / freezing point	3.2	*-30 to –40 °C*	*Ref. (11)*
VII, 7.3	Boiling point	3.3	*111 °C*	*Ref. (11)*
VII, 7.4	Relative density	3.4 density	*0.791 at 25°C*	*Ref. (13)*
VII, 7.5	Vapour pressure	3.6	*2600 to 2700 Pa at 20°C* *12000 Pa at 50°C*	*Ref. (13) and (15)*
VII, 7.6	Surface tension	3.10	-	
VII, 7.7	Water solubility	3.8	*0.1 g/l at 20°C*	*Ref. (15) and (16)*
VII, 7.8	Partition coefficient n-octanol/water (log value)	3.7 partition coefficient	*Calculated : 3.45 at 25°C*	*Ref. (17)*
VII, 7.9	Flash point	3.11	6 to 6.1 °C	*Ref. (13) and (15)*
VII, 7.10	Flammability	3.13	*Highly Flammable (EU classified R11)*	*Ref. (15)*
VII, 7.11	Explosive properties	3.14	-	
VII, 7.12	Self-ignition temperature		*170 °C*	92/69/A15 *Ref. (15)*
VII, 7.13	Oxidising properties	3.15;	*Yes (EU classified R7)*	*Ref. (15)*
VII, 7.14	Granulometry	3.5	-	
IX, 7.15	Stability in organic solvents and identity of relevant degradation products	3.17	*Half-life (0.2 mole/l, benzene, 126-149-193°C) = 10h-1h-1min* *Half-life (0.2 mole/l, n-decane, 129-149-189°C) = 10h-1h-1min* *Deg. Products : methane, ethane, propanone, tertiobutanol, tertiobutoxymethane, 1,1-dimethyloxiranne*	*Ref. (15)*
IX, 7.16	Dissociation constant	3.21	-	
IX, 7.17	Viscosity	3.22	-	
	Auto flammability	3.12	-	
	Reactivity towards container material	3.18	-	
	Thermal stability	3.19	*SADT = 80°C (BAM)*	*Ref. (15)*
			Vapor density / air = 5.03 Refractive index (20°C) = 1.389 Active Oxygen content = 10.9 %	*Ref. (15)* *Ref. (15)* *(calculated)*

2 MANUFACTURE AND USES

Not relevant for this type of dossier.

3 CLASSIFICATION AND LABELLING

3.1 Classification in Annex I of Directive 67/548/EEC

Index Number: 617-001-00-2 (inserted in the 19° ATP)

According to 67/548/CEE	*According to CLP*
O ; R7 F ; R11	Org. Perox. E - H242 Flam. Liq. 2 - H225

3.2 Self classification(s)

Not relevant

4 ENVIRONMENTAL FATE PROPERTIES

Not evaluated in this dossier.

5 HUMAN HEALTH HAZARD ASSESSMENT

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

No data.

5.2 Acute toxicity

Not evaluated in this dossier

5.3 Irritation

Not evaluated in this dossier

5.4 Sensitisation

Not evaluated in this dossier.

5.5 Repeated dose toxicity

Not evaluated in this dossier.

5.6 Mutagenicity

5.6.1 In vitro data

The genotoxic potential of di-tert-butyl peroxide was evaluated in 2 independent gene mutation assays with *Salmonella typhimurium* TA97, TA98, TA100, TA1535, TA 15/37 and/or TA1538. Di-tert-butyl peroxide did not induce a significant increase in the number of revertant colonies over that shown in the solvent control plates for any of the *Salmonella typhimurium* strains with and without S9 activation, at doses up to and including 10,000 µg/plate. Positive controls produced the expected response in all experiments (3,10).

The genotoxic potential of Di-tert-butyl peroxide (purity 98%) was also evaluated in a SOS chromotest using *Escherichia Coli* PQ37, PM21, GC4798. There is no data available regarding the product concentration or the use of a metabolic activation system. The SOS Inducing According to the Potency (SOSIP) values was calculated with the method of Quillardet and Hofnung, di-tert-butyl peroxide did not show a clear SOS inducing activity. Only in strain PQ37, a borderline activity was found: an induction factor of 1.49 is to be considered as genotoxic according to Quillardet's criteria. However, the SOSIP value of $4.3x10^{-5}$ was extremely low. Absolutely no SOS inducing activity could be found for di-tert-butyl peroxide when using strain PM21 or GC4798 (20).

5.6.2 In vivo data

A micronucleus assay was performed by intraperitoneal (IP) route to assess the genotoxic potential of di-tert-butyl peroxide *in vivo* (5). Four groups of five male and five female OF1 mice received two intraperitoneal treatments of di-tert-butyl peroxide (purity 98.9%, no information on impurity profile) at dose-levels of 0, 500, 1000 or 2000 mg/kg/day, at a 24-hour interval. One group of five males and five females received the positive control test item (cyclophosphamide - CPA) once by oral route at the dose-level of 50 mg/kg.
The animals of the treated and vehicle control groups were killed 24 hours after the last treatment and the animals of the positive control group were killed 24 hours after the single treatment. Bone marrow smears were then prepared and evaluated in random and blinded manner.
For each animal, the number of the micronucleated polychromatic erythrocytes (MPE) was counted in 2000 polychromatic erythrocytes. The polychromatic (PE) and normochromatic (NE) erythrocyte ratio was established by scoring a total of 1000 erythrocytes (PE + NE).
All animals treated with 1000 or 2000 mg/kg/d exhibited piloerection further to the second treatment. No mortality and no other clinical signs were reported. For males from the mid and high-dose treated groups and for the female from the low and high dose treated groups, a statistically significant increase in the frequency of MPE was noted. Main results are presented in Table 2 and historical control data in Table 3.

Table 2 Summary of study results – *in vivo* IP micronucleus assay

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

*: $p<0.05$; **: $p<0.01$

Table 3 Historical control data from 8 micronucleus studies on bone marrow in mouse performed between March 2002 and July 2004.

Group	MPE/1000PE		PE/NE ratio	
	mean	range	mean	range
Males				
Vehicle	0.66	0.3-1.7	0.44	0.3-0.6
CPA	23.9	17.0-33.4	0.61	0.4-0.8
Females				
Vehicle	0.59	0.0-1.1	0.64	0.4-1.0
CPA	19.24	12.7-25.6	0.8	0.6-1.1

Under these experimental conditions, it was concluded that di-tert-butyl peroxide induce damage to the chromosomes or the mitotic apparatus of mice bone marrow cells after two intraperitoneal administrations, with a 24-hour interval, at the dose-levels of 500, 1000 or 2000 mg/kg/day. The study was performed according to GLP and the OECD 474 guideline. The only deviation to guideline was use of a single sampling time. (5).

In an other independent micronucleus assay, four groups of five male and five female ICR mice were exposed to di-tert-butyl peroxide (purity 99.5%, no information on impurity profile) by gavage at dose-levels of 0, 1250, 2500 or 5000 mg/kg. One group of five males and five females received the positive control test item (CPA) at the dose-level of 60 mg/kg.
Animals of the positive control group were sacrificed 24 hours after dosing. Bone marrow cells of the treated group were collected and prepared 24, 48 and 72 hours after dose administration. For each animal, the number of micronucleated polychromatic erythrocytes (MPE) was counted in 1000 polychromatic erythrocytes.
Clinical signs after dose administration included diarrhea in males at all test article dose levels and in females at 5000 mg/kg and lethargy in females at 2500 and 5000 mg/kg. Reductions of up to

15% in the ratio of PE to total erythrocytes were observed in some of treated groups. The number of MPE per 1000 cells in test-article treated groups was statistically increased relative to the vehicle control in females at 1250 and 5000 mg/kg at 24 hours only; no statistically significant increases were observed in males.
The test was repeated with a 24 hrs-harvest only. In this repeated test, clinical signs after dose administration included diarrhea in males at 2500 and 5000 mg/kg and in females at 5000 mg/kg. There were no reductions in the ratio PE/total erythrocytes, and the number of MPE per 1000 cells in test-article treated groups was statistically increased relative to the vehicle control in females at 2500 and 5000 mg/kg, and in males at 5000 mg/kg at 24 hrs.
Main results are presented in Table 4 and historical control data in Table 5.

Table 4 Summary of study results – *in vivo* gavage micronucleus assay

Group	Doses	**MPE/1000PE**			**PE/NE ratio**		Time of sacrifice
	(mg/kg)	mean	(sd)		mean	(sd)	
Initial study							
Males							
Vehicle	-	1.4	(1.14)		0.54	(0.07)	24h
Test item	1250	1.6	(1.34)		0.55	(0.06)	
	2500	2.6	(1.52)		0.51	(0.13)	
	5000	3.6	(2.07)		0.52	(0.12)	
CPA	60	32.8	(8.58)	*	0.47	(0.10)	
Females							
Vehicle	-	0.6	(0.55)		0.55	(0.03)	24h
Test item	1250	3.6	(3.71)	*	0.56	(0.08)	
	2500	1.8	(2.05)		0.59	(0.04)	
	5000	3.4	(3.36)	*	0.55	(0.06)	
CPA	60	29.6	(7.37)	*	0.52	(0.07)	
Males							
Vehicle	-	1.4	(2.61)		0.55	(0.04)	48h
Test item	1250	1.2	(1.30)		0.55	(0.08)	
	2500	1.4	(0.55)		0.59	(0.05)	
	5000	2.6	(2.30)		0.47	(0.11)	
Females							
Vehicle	-	1.4	(0.89)		0.51	(0.06)	48h
Test item	1250	0.8	(0.45)		0.57	(0.04)	
	2500	1.2	(0.84)		0.58	(0.04)	
	5000	2.8	(2.17)		0.45	(0.17)	
Males							
Vehicle	-	1.0	(1.41)		0.53	(0.11)	72h
Test item	1250	0.2	(0.45)		0.52	(0.08)	
	2500	1.2	(1.10)		0.55	(0.13)	

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

*: $p<0.05$

Table 5 Historical control data from micronucleus studies on bone marrow in mouse by all route performed between 1993 and 1995.

Group	MPE/1000PE		PE/NE ratio	
	mean	range	mean	range
Males				
Vehicle	0.59	0-8	0.56	0.04-0.87
CPA	17.45	0-109	0.51	0.06-0.85
Females				
Vehicle	0.61	0-8	0.58	0.09-0.89
CPA	16.79	1-70	0.53	0.04-0.83

In conclusion, the initial and repeat assays demonstrated 1/5 females at 1250 and 5000 mg/kg (initial), and 1/5 males at 5000 mg/kg (repeat) with statistically significant increases in the number of MPE per 1000 cells. The test material was concluded to be weakly positive in the mouse micronucleus test. The study was performed according to GLP and the OECD 474 guideline (6).

A mammalian germ cell cytogenetic assay was carried out and consisted of five groups, each containing 5 male ICR mice (4). Animals in these groups were intraperitoneally exposed to either with the controls (negative or positive) or with di-tert-butyl peroxide (purity 98.6%, no information on impurity profile) at a dose of 200, 1000 or 2000 mg/kg/day. Corn oil was used as vehicle and negative control. Mitomycin C, at a dose of 4 mg/kg, was used as the positive control article. The test and negative control article were administered on two consecutive days, separated by

approximately 24 hours. Colchicine was given 4-5 hours prior to sacrifice to arrest cells in metaphase. Twenty-four hours after the last dose, animals were sacrificed and testes were removed from animal body cavity. Spermatogonial cells were isolated from the tubules and smeared onto the microscope slide. The smears were stained with Giemsa stain. One hundred metaphase cells per each animal were scored for structural chromosome aberration. Mitotic index (MI) was calculated for each animal as the ratio of spermatogonial cells in mitosis per 1000 cells observed.
No mortality or clinical signs were observed in any of the mice during the course of the study. No statistically significant increase in the percentage of aberrant cells and no dose-related decrease of the mitotic index were observed in the test article-treated groups relative to the vehicle control ($p > 0.05$ Fisher's exact test). Main results are presented in Table 6.

Table 6 Summary of study results – *in vivo* IP spermatogonial chromosome aberration assay

Group	Doses (mg/kg/d)	# of cells	Mean mitotic index (%)	Cells with struct. aberr.	Struct. Aberr. (%)	Aberr. per cells		Sample time
						mean	(sd)	
Vehicle	-	500	8.5	0	0.0	0.000	(0.000)	24h
Test item	200	500	4.8	0	0.0	0.000	(0.000)	
	1000	500	4.8	0	0.0	0.000	(0.000)	
	2000	500	8.0	0	0.0	0.000	(0.000)	
Mitomycin	4	500	2.6	39	7.8*	0.088	(0.019)	

*: $p<0.01$

The results of the study indicate that, di-tert-butyl peroxide, when intraperitoneally administered on two consecutive days (at doses up to 2000 mg/kg/day), did not induce a significant increase in the percentage of spermatogonial cells with structural chromosome aberrations. The study was performed according to GLP and the OECD 483 guideline. However, only one harvest time was used in the study whereas two sample times are required by the guideline at the highest dose. (4).

5.6.3 Human data

No data.

5.6.4 Other relevant information

No data.

5.6.5 Summary and discussion of mutagenicity

Di-tert-butyl peroxide was clearly positive in the mouse bone marrow micronucleus test after an intraperitoneal administration and weakly positive after an oral administration. Di-tert-butyl peroxide was negative in the mammalian spermatogonial chromosome aberration test in male mice after an intraperitoneal administration.

Di-tert-butyl peroxide must be classified in category 3 of the mutagenic substances and labelled Xn; R68.

5.7 Carcinogenicity

Not evaluated in this dossier.

5.8 Toxicity for reproduction

Not evaluated in this dossier.

5.9 Other effects

Not evaluated in this dossier.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this dossier

7 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in this dossier.

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

New data shows that the substance has CMR properties that justify to revise its harmonised classification and labelling for this endpoint.

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