Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

tert-butyl hydroperoxide

EC number: 200-915-7
CAS number: 75-91-2

CLH-O-0000001412-86-27/F

Adopted

04 December 2014

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4 December 2014

CLH-O-0000001412-86-27/F

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

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

Chemical name: tert-butyl hydroperoxide

EC number: 200-915-7

CAS number: 75-91-2

The proposal was submitted by **the Netherlands** and received by RAC on **19 August 2013.**

In this opinion, all classifications are given in the form of CLP hazard classes and/or categories.

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **4 February 2014**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **21 March 2014**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by RAC: **Elodie Pasquier**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation. The comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was reached on **4 December 2014.** The RAC opinion was adopted by **consensus**.

OPINION OF RAC

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

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	tert-butyl hydroperoxide	200-915-7	75-91-2	Muta. 2	H341	GHS08 Wng	H341			
RAC opinion	TBD	tert-butyl hydroperoxide	200-915-7	75-91-2	Muta. 2	H341	GHS08 Wng	H341			
Resulting Annex VI entry if agreed by COM	TBD	tert-butyl hydroperoxide	200-915-7	75-91-2	Muta. 2	H341	GHS08 Wng	H341			

SCIENTIFIC GROUNDS FOR THE OPINION

RAC general comment

Tert-butyl hydroperoxide (TBHP) has currently no entry in Annex VI to the CLP Regulation. The dossier submitter proposed only the hazard class mutagenicity for harmonisation

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

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

Additionally, the dossier submitter (DS) noted that a similar substance, di-tert-butyl peroxide (DTBP), was shown to be mutagenic to the bone marrow in an *in vivo* assay. As DTBP forms only radicals which are also formed by TBHP, it is likely that TBHP is also mutagenic.

Comments received during public consultation

During public consultation, two MSCAs and one industry organisation agreed with the proposed classification.

One MSCA suggested that the statement in the proposal that TBHP is very likely not to be systemically available could be more appropriately replaced with a statement that there is no data demonstrating that TBHP is systemically available.

In response, the DS argued against this suggestion as there is extensive kinetic data available in the CLH proposal which allow an assessment of the systemic availability.

Three industries raised the issue that using the DTBP data as a basis for harmonisation is not appropriate, and is not in line with ECHA's own guidance.

In response, the DS agreed that the justification for the read-across of the mutagenic properties from DTBP to TBHP is limited and does not follow the ECHA guidance on read-across. However, the DS considered this only as supportive information and for that reason did not include this in the comparison with the criteria. The DS also claimed that the information provided on the mutagenicity of both substances shows differences in the results in comparable tests indicating that there are differences in the mutagenic profile. According to the DS, classification of TBHP, solely based on read-across from DTBP is not justified. However, the fact that DTBP is also positive in *in vivo* mutagencity tests is considered supportive given the structural similarity. As both substances are used for the generation of radicals and the main metabolite of both substances, 2-methylpropan-2-ol, is not mutagenic, it is considered likely that both substances induce mutagenicity via the formation of radicals, although this has not not shown for DTBP.

Overall, the DS considers the observed mutagenicity of DTBP *in vivo* as supportive for the classification of TBHP for mutagenicity.

Assessment and comparison with the classification criteria

No human data are available and classification as Muta 1A is therefore not appropriate.

Positive results from *in vivo* heritable germ cell mutagenicity tests in mammals are available for TBHP from studies of good quality. These positive results were obtained by the IP route. In the absence of evidence of mutagenicity of TBHP on germ cells by a physiological route of exposure, these data are therefore considered to be insufficient to warrant classification as Muta. 1B.

Negative results are obtained in *in vivo* mutagenicity tests on somatic cells located at a distance from the site of exposure after single exposure. In some of these studies, signs of effects in the tested organs were noted at the highest doses but toxicokinetic data after single exposure shows that TBHP is rapidly metabolized to the non-genotoxic 2-methylpropan-2-ol whereas DNA-reactive radicals are expected to be formed from the parent TBHP. Therefore, the reactive TBHP may not reach the tested systemic organs. The negative results for systemic genotoxicity in somatic cell after single exposure therefore does not exclude a potential for mutagenicity of TBHP after repeated exposure when metabolism is saturated and antioxidant defenses are depleted, as well as for local mutagenicity as supported by the largely positive *in vitro* database in absence of metabolic activation.

The *in vivo* evidence for local mutagenicity is further provided by the induction of dominant lethal mutations in germ cells by the IP route, where TBHP can migrate from the abdominal cavity through the inguinal canal to the testis. The four conclusive studies available were all performed in the same research laboratory and appear to have been designed with no major deficiencies. The results of induction of inheritable mutations were repeated and supported by the concurrent observation of oxidative stress and DNA damage in sperm cells. The relevance of the findings on germ cells for local effects in somatic cells is supported by the positive results of *in vitro* tests that were performed on somatic mammalian cells.

Local genotoxicity was directly investigated in a Comet assay with 5-day inhalation exposure in lung tissues and gave a negative result. However, the significance of this result was questioned by the lower exposure that is expected in the lower parts of the respiratory tract. The investigation of nasal and tracheal epithelium that receive a higher exposure, as evidenced by the histopathological effects observed in these tissues, was not possible due to methodological difficulties. This negative Comet result in the lung is therefore not considered sufficient to rule out the local mutagenicity of TBHP as evidenced by mutagenicity on germ cell by IP exposure that justifies a classification as Muta. 2.

It is also noted that most of the negative tests performed on somatic cells were performed with a single exposure whereas positive results on germ cells were obtained with repeated exposure. Only a bone marrow chromosomal aberration assay was performed with a 5-day exposure by inhalation (Ben-Dyke, 1981) but toxicokinetics data via this route are lacking, and it is hence not possible to conclude on exposure of the bone marrow to the reactive TBHP under these exposure conditions.

Toxicokinetic data indicates that TBHP is not likely to be systemically available and in particular not to reach germ cells after single exposure via a physiological route of exposure. However, the toxicokinetics of TBHP under repeated conditions of exposure, when metabolism is saturated and antioxidant defenses are depleted, has not been investigated. In addition, the formation of free radicals has been observed in the liver, kidney and blood after repeated exposure to TBHP by gavage (Ritchie, 2005a). Although insufficient to conclude on genotoxicity, it gives an indication that TBHP may induce oxidative damage at distant sites after repeated exposure by a physiological route.

Therefore, the potential for systemic genotoxicity of TBHP after repeated exposure cannot be fully excluded. Similarly, the absence of mutagenicity of TBHP in germ cells by a physiological route in particular under repeated exposure has not been demonstrated and the concern raised by the positive dominant lethal assays by IP route cannot be ruled out with certainty. This is considered as a supportive element to warrant a classification as Muta. 2.

It is also noted that the substance DTBP shares with TBHP the formation of the same reactive radicals. DTBP is genotoxic in somatic cells at distant sites, which brings some support to the mutagenic effect of TBHP. Some differences between the two substances are noted: difference in water solubility, expected higher stability of DTBP, possibility to form additional radicals (more reactive) from TBHP. These differences may explain the difference in the *in vitro* results (negative for the more stable DTBP) as well in the *in vivo* results (negative at distant sites for unstable TBHP). However, the explanations of the differences in the mutagenic profile between the two substances remains speculative and, although not contradictory, data on DBTP are considered of limited use to conclude on the mutagenic classification of TBHP.

Overall, RAC concludes that a classification of TBHP as Muta. 2; H341 is warranted based on the evidence of mutagenic potential provided by the available positive *in vitro* studies and the reproducible positive results reported in dominant lethal mutation assays performed by the intra-peritoneal route.

These studies show evidence for local, site of initial contact mutagenicity of TBHP, and as such are not contradicted entirely by the available negative results in studies for genetic toxicity in the liver and bone marrow. Besides, there remains a potential that repeated high dose exposure to TBHP could lead to a saturation of its primary metabolism and the depletion of anti-oxidant defences systemically in target tissues. This has not been tested experimentally. As such, the possibility of a mutagenic potential on germ cells and somatic cells at distance sites cannot be fully excluded, in particular after repeated exposure.

ANNEXES:

Annex 1 Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in RAC boxes.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and rapporteurs' comments (excl. confidential information).