ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Substance name: Di-tert-butyl peroxide
CAS number: 110-05-4
EC number: 203-733-6

General comments

Date	Submitted by Organisation/MSCA	Comment	Response	Rapporteurs' comments
2009/07/16	Hungary / National Institute of Chemical Safety	Firstly, in view of the precautionary principle the proposed classification and labelling can be supported but in our opinion there is a need for further information and/or testing to confirm the proposed classification. Secondly, di-tert-butyl peroxide contains tert-butyl hydroperoxide as an impurity in concentration lower to 0.1%. In general, hydroperoxides are much more toxic than diperoxides but tert-butyl hydroperoxide is classified „only" as Muta. Cat. 3; R68.	Classification proposal is based on available data. If further information and/or testing are required, testing proposal should be addressed to ECHA. Tert-butyl hydroperoxide classification as Muta. Cat. 3; R68 was agreed at the TC C&L of September 2007. It is present in di-tert-butyl peroxide at concentration lower than 0.1%. Due to its classification as Muta. Cat. 3; R68, it would trigger classification only if present at concentration ≥ 1%.	As far as classification & labelling is concerned the Precautionary Principle does not apply. Uncertainty is covered by the classification criteria. The proposed classification is justified by the available data.
2009/07/27	Ireland / Health & Safety Authority	The Irish CA is in agreement with the	The remark concerning IUCLID references in table 1	The rapporteurs agrees with the response from

		proposal of France to classify DTBP as Mut. Cat. 3 R68 (Mut. 2 H341). In addition, we have a few additional comments in relation to the Annex XV report for DTBP. *Physico - chemical properties:* Reference is made in Table 1 to IUCLID section 3.1 et seq, however these are now numbered 4.1 at seq in IUCLID 5. The information contained in the table is not included in the IUCLID file for the substance. *Mutagenicity: In vivo data Table 2:* The statistical test used to analyse the results has not been reported. Given that the mean MPE/1000PE for the vehicle treated females is outside the historical range for the test in that laboratory, it may be more appropriate to use the historical control data as the basis for the statistical analysis of the concurrent	has been taken into account and the background document has been changed accordingly. Concerning the remark that information of table 1 are not reported in IUCLID, sections 1 and 2 only are warranted in the technical dossier for Annex VI dossier of "hand-over" substance from ECB such as di-tert butyl peroxide. Statistical tests used for the studies reported in the dossier have been added in the background document. As mentioned by Irish CA, vehicle treated females group (5) was used as control although their mean MPE/1000PE is outside historical range. It was not discussed in the study neither in our proposal and we agree that use of historical controls could have been proposed. However, it is important to note that it will not change the conclusions: historical control mean MPE/1000PE is smaller than the mean	France.

		test data.	MPE/1000PE of the vehicle treated females of the study. Using vehicle treated females as control allowed to show an increase of MPE/1000PE in treated groups, statistically significant at low and high dose. Using historical controls would only have increased statistical power.	

Mutagenicity

Date	Submitted by Person/Organisation/MSCA	Comment	Response	Rapporteurs' comments
2009/07/16	Hungary / National Institute of Chemical Safety	Pg 9 "In vivo data" - How can the intraperitoneal administration to be considered as a relevant exposure route? Pg 10 "In vivo data" - Unfortunately acute and/or repeated dose toxicity studies are missing from the dossier however clinical signs (diarrhoea, lethargy) after dose administration may be occurred by toxic effects of the test substance.	Most of in vivo mutagenicity studies used in hazard assessment are dosed acutely by oral or intraperitoneal routes. Classification as a Category 2 mutagen would generally apply if intraperitoneal *in vivo* tests show mutagenicity. Guidance for the preparation of an Annex XV Dossier on Harmonised Classification and Labelling specifies that details of the reviewed relevant information need only be entered under relevant headings. As recommended, the note 'not evaluated for this	In vivo micronucleus tests in somatic cells with i.p. administration generally lead to classification for mutagenicity. The guidance document on the application of the CLP criteria clearly specifies that positive micronucleus tests, with i.p. administration justify classification as Germ Cell Mutagen Cat. 2 (i.e Mutagen Category 3, R68 according to

			dossier' was entered under the headings not used. Acute and/or repeated dose toxicity studies might confirm clinical signs observed in the studies presented but are not relevant information for mutagenicity endpoint. OECD guideline 474 specidies that dose levels should cover a range from the maximum to little or no toxicity. The reported information regarding clinical signs allows to show toxicity.	67/548/EEC).
2009/07/24	Germany	The following classification is proposed: based on Directive 67/548/EEC criteria: Muta. Cat. 3; R68 (Possible risks of irreversible effects); and based on GHS criteria: Muta. 2 – H341 (Suspected of causing genetic effects). The German CA supports the classification of the substance di-tert-butyl peroxide based on regulation (EC) No		Taken into account by both the dossier submitter and the rapporteurs.

		1272/2008 in category 2 as a substance which causes concern for humans owing to the possibility that it may induce heritable mutations in the germ cells of humans with the hazard statement H341. The in vivo mouse bone marrow micronucleus test (OECD 474) with intraperitoneal administration leads to a significant increase in micronucleated polychromatic erythrocytes (MPE) already at the lowest concentration tested (500 mg/kg). The data from oral administration are weakly positive, as a marked increase in MPE was observed in 1/5 male animals of the high dose group (5000 mg/kg) and 1/5 female animals in the mid dose group (2500 mg/kg). The available in vivo mutagenicity test in germ		

		cells (OECD 483) shows, that if the substance is administered intraperitoneal in concentrations of 200, 1000, and 2000 mg/kg, neither the mean mitotic index [%] nor the structural chromosome aberrations of spermatogonial cells are altered. The existing data from the in vivo somatic cell mutagenicity test are clearly positive, thus constituting in the classification regarding germ cell mutagenicity (see 3.5.2.1 CLP regulation). Category 2 is appropriate as there are no supporting data that the substance has potential to cause mutations to germ cells. These supporting data would be required for classification in category 1B (see table 3.5.1 CLP regulation). In the case of di-tert-butyl peroxide both	Taken into account	

		the in vitro mutagenicity test and the in vivo mammalian germ cell cytogenetic assay yield the information that there is no potential to cause mutations to germ cells. Because the supporting evidence of having the potential to cause mutations to germ cells is missing, the substance has to be classified in category 2. Due to the clearly positive results of the in vivo somatic cell mutagenicity test with intraperitoneal administration the classification concerning mutagenicity may not be waived. Concerning the test descriptions the German CA has some minor remarks: Page 10 (paragraph 1) The last sentence ('The only deviation…') is to be	Taken into account. Taken into account	

		deleted because the use of only one sampling time is correct. In accordance with the OECD Guideline 474 sample 'should be collected once between 18 and 24 hours following the final treatment for the bone marrow' if two or more daily treatments are used (see paragraph 3 of 'Treatment schedule'). Page 10 The reference for the second in vivo micronucleus assay is missing after the first sentence of the test description. Page 13 (paragraph 2) The last sentence is to be deleted because the use of only one sampling time is correct. Following a repeat treatment schedule in accordance with the OECD Guideline 483 'animals should then be sacrificed 24 hours (1.5 cell cycle length) after the last		

		treatment. Additional sampling times may be used where appropriate.’ (see paragraph 4 of ‘Treatment schedule’)		