Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the 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

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON TERT-BUTYL HYDROPEROXIDE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.
All attachments including confidential documents received during the public consultation have been provided in full to the dossier submitter, to RAC members and to the Commission (after adoption of the RAC opinion). Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website.

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Substance name: tert-butyl hydroperoxide
CAS number: 75-91-2
EC number: 200-915-7
Dossier submitter: The Netherlands

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
21.03.2014	Netherlands	LyondellBasell	Company-Manufacturer	1
Comment received				
The classification as proposed in the draft document is considered acceptable albeit severely conservative. However the read across to DTBP is considered inappropriate. Please see attached document. **(*ECHA note: The following attachment was provided [Attachment 1])*** Comments on the Draft CLH report for tert-butyl hydroperoxide				
Dossier Submitter's Response				
Thank you for your support for the proposed classification. We agree that the justification of the read-across of the mutagenic properties from DTBP to TBHP is limited and does not follow the ECHA guidance on read-across. We consider this only as supportive information and for that reason did not include this in the comparison with the criteria. The provided information on the mutagenicity of both substances show differences in the results in comparable tests indicating that there are differences in the mutagenic profile. Classification of TBHP based on read-across from DTBP only is not justified. However, the fact that DTBP is also positive in *in vivo* mutagencity tests is considered supportive seen the structural similarity. As both substance 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 is not shown for DTBP. Overall, we consider the observed mutagenicity of DTBP in vivo as supportive for the classification of TBHP for mutagenicity. It should be noted that our conclusion (classification with Muta 2:H341) would be the same without this supportive information.				
RAC's response				
Your support for classification and your reservation on the read-across with DTBP are noted. The substance DTBP share with TBHP the formation of some 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 remain speculative. Although not contradictory, data on DBTP are considered of limited use to conclude on the mutagenic classification of TBHP that is fully justified by the TBHP database on its own.

Date	Country	Organisation	Type of Organisation	Comment number
21.03.2014	Germany		Company-Manufacturer	2
Comment received				
We disagree with the read across (harmonization) between TBHP and DTBP as it is done in the dossier by the Dutch authorities, as neither the data set at hand nor the physico-chemical properties of these two peroxides justify such a read across/harmonization taken into account ECHA's guidance/rules for a scientifically sound read across. The justification by the Dutch Authorities for the harmonization was that, "The substance di-tert-butyl-peroxide (DTBP) was shown to be mutagenic to the bone marrow in an in vivo assay. As DTBP forms only radicals also formed by TBHP, it is likely that TBHP is also mutagenic." Neither literature data nor effects noted in vitro and in vivo by both substances support this conclusion. Experiments with DTBP did not result in positive in vitro mutagenicity results, whereas TBHP did. In vivo, DTBP showed some effects in an oral micronucleus assay at dose levels above the limit dose. However a micronucleus assay, conducted in conjunction with a 90-day inhalation study was negative. While a micronucleus study following IP administration was positive in bone marrow, the spermatogonial assay was negative. Those data does not fit to the data at hand for TBHP. In addition, whereas TBHP is water soluble, DTBP is almost insoluble in water. Taken these data together, we consider the read across/harmonization approach between DTBP and TBHP as not scientifically justified and not in line with ECHA's own guidelines.				
Dossier Submitter's Response				
See our response to comment 1.				
RAC's response				
See response to comment 1.				

Date	Country	Organisation	Type of Organisation	Comment number
21.03.2014	Sweden		MemberState	3
Comment received				
The SE CA supports classification of tert-butyl hydroperoxide (CAS No. 75-91-2) as specified in the proposal. SE agrees with the rationale for the classification into the proposed hazard class and differentiation.				
Dossier Submitter's Response				
Thank you for your support				
RAC's response				
Your support is noted.				

Date	Country	Organisation	Type of Organisation	Comment number

21.03.2014	Netherlands		Individual	4

Comment received

While in vitro mutagenicity and genotoxicity data are positive, TBHP does not meet the criteria for classification as a Cat 2 mutagen based on the following:
☐ The positive results of germ cell tests, following IP administration, are considered evidence for a local mutagenic effect. No local mutagenicity tests, with TBHP, are available. There is a data gap for in vivo mutagenicity tests which is necessary to meet the criteria for classification in accordance with ECHA's guidance.
☐ 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.
☐ 2-Methylpropan-2-ol was tested for mutagenicity by the NTP in 1995 and all in vitro and in vivo results were negative. Therefore, by relevant routes of exposure, TBHP will not be mutagenic due to its rapid conversion to the non-mutagenic 2-methylpropan-2-ol.
☐ While the in vitro data demonstrates genotoxicity, TBHP was negative in several in vivo bone marrow micronucleus tests and in two Comet assays, one in rat liver after subcutaneous exposure and one in lung following inhalation exposure for 28 days.
☐ Harmonization with the current DTBP classification is not appropriate based on the dataset for that substance.
In conclusion, based on the above, with the current dataset, TBHP is not classifiable as a Muta 2; H341 and does not meet the criteria for classification of Muta 1A; H340 or Muta 1B; H340.

See below and attachment for full details.

(ECHA note: The following attachment was provided [Attachment 2]. Comment number 10 is related)

Tert-butyl hydroperoxide CL Position Paper, by AkzoNobel Functional Chemicals

Dossier Submitter's Response

We agree that most in vivo mutagenicity tests with TBHP, except the dominant lethal tests, are negative. However, these negative results can be explained by the kinetic data showing that TBHP is quickly transformed into the non-mutagenic metabolite 2-methylpropan-2-ol. As TBHP is unlikely to reach the target organ for the mutagenicity testing (bone marrow), the negative results do not show that TBHP does not have mutagenic properties. The negative results in the Comet assay in the liver after subcutaneous injection can also be explained by the kinetics. The negative results of the Comet assay in the lung after inhalation exposure could be explained by the limited amount of TBHP which reached the alveolar region of the lung as no histopathological effects were observed. Clear histopthological effects were observed in the upper respiratory tract. However, performance of a Comet assay on this tissue was considered not possible due to the limited recovery of viable cells. The kinetic data also show that TBHP is unlikely to reach the germ cells after exposure via relevant routes.The postive results in the dominant lethal tests after ip exposure can be explained as a local effect, which require classification as Muta Cat 2.

In the position paper, ANFC states that the guidance (version 4, page 379, final paragraph) does not appear to be applied properly because the guidance states that a local in vivo, somatic cell test is required to support the positive in vitro test. Such a test is indeed not available. However, in our opinion the positive dominant lethal test should be regarded as a local test. The fact that germ cells have been tested instead of somatic cells should not be regarded as not following the guidance because when considering local mutagencity there is no difference between somatic cells and germ cells. Therefore classification in category 2 is warranted.

We do not agree that an ip study is not suitable for clasification and labelling for germ cell

mutagenicity. According to the CLP guidance (volume 4 page 384):
"*If there are positive results in at least one valid in vivo mutagenicity test using intraperitoneal application, or from at least one valid in vivo genotoxicity test using intraperitoneal application plus supportive in vitro data, classification is warranted. In cases where there are additional data from further in vivo tests with oral, dermal or inhalative substance application, a weight of evidence approach using expert judgement has to be applied in order to to come to a decision. For instance, it may be difficult to reach a decision on whether or not to classify in the case where there are positive in vivo data from at least one in vivo test using intraperitoneal application but (only) negative test data from (an) in vivo test(s) using oral, dermal, or inhalative application. In such a case, it could be argued that mutagenicity/genotoxicity can only be shown at internal body substance concentrations which cannot be achieved using application routes other than intraperitoneal. However, it also has to be taken into account that there is generally no threshold for mutagenicity unless there is specific proof for the existence of such a threshold as may be the case for aneugens. Thus, if mutagenicity/genotoxicity can only be demonstrated for the intraperitoneal route exclusively, then this may mean that the effect in the in vivo tests using application routes other than intraperitoneal may have been present, but it may not have been detected because it was below the detection limit of the oral, dermal, or inhalative test assays*." For TBHP, the ip study on germ cells is the main local study and positive whereas the other in vivo studies are mainly targetting tissues that require systemic availability. As the systemic availability is unlikely, this explains the negative results of these studies. The only other local in vivo test (Comet assay in the lung after inhalation) was negative but it can be questioned whether sufficient TBHP reached the lungs as no histopathological changes were observed in the lungs. Such changes were only observed in the upper respiratory tract where a Comet assay was not feasible. Therefore, the positive ip study on germ cells warrant classification. As it is considered a local study, it warrants classification in category 2.

For a response regarding the read-across from DTBP see the response to comment 1. The absence of the formation of spin-trappable radicals after incubation of DTBP with rat liver mitochondria confirms the differences in mutagenic profile between TBHP and DTBP but does not show that DTBP does never form radicals. ANFC also refers to a number of screening studies with other peroxides DTBP that could be used if read-across between TBHP and DTBP is considered acceptable. These studies focus on the initiating and promoting capacity for local tumours. However, carcinogenicity is outside the scope of this proposal.

Based on the comments in the position paper (attachment 2) there seem to be some misunderstanding regarding the CLH process. Information regarding the CLH process is available on the ECHA website (http://echa.europa.eu/addressing-chemicals-of-concern/harmonised-classification-and-labelling) including the working procedure.

RAC's response

RAC notes the following elements in response:

- RAC agrees with the DS that positive germ cell tests by IP route provide evidence of local mutagenicity of TBHP.
- RAC recognises that there is no evidence that TBHP reaches the gonads by a physiological route of exposure and in consequence does not support a classification as Muta 1B. Toxicokinetic data provides evidence that it is likely that TBHP does not reach systemic circulation, and in particular the gonads, after single exposure.
- Although the metabolite 2-methylpropan- 2-ol is not mutagenic, TBHP may form reactive radicals at the site of contact and local mutageniticity is not excluded by this element.

- The negative Comet assay in the lung (inhalation exposure) may be explained by an insufficient exposure in the lower parts of the respiratory tract compared to nasal and tracheal tissues.
- It is also noted that no toxicokinetic data are available after repeated exposure, to demonstrate absence of systemic exposure to TBHP when metabolism may be saturated and antioxidant defenses depleted.
- See response to comment 1 regarding read-across with DTBP

Date	Country	Organisation	Type of Organisation	Comment number
21.03.2014	Germany		MemberState	5
Comment received				
The CLH proposal is supported.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Your support is noted.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
21.03.2014	Netherlands		Individual	6
Comment received				
No further action proposed until the question of mutagenicity is definitively addressed.				
Dossier Submitter's Response				
Carcinogenicity is outside the scope of our proposal.				
RAC's response				
Carcinogenicity is outside the scope of the proposal.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
21.03.2014	Netherlands	LyondellBasell	Company-Manufacturer	7
Comment received				
The dominant lethal assay in mice does indicate mutagenic potential. We agree with the Dutch approach that only site of contact mutation is relevant due to the rapid metabolism of this product.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Your support is noted.				

Date	Country	Organisation	Type of Organisation	Comment number
21.03.2014	Germany		Company-Manufacturer	8
Comment received				
In the August 2013 CHL report, on tert-butyl hydroperoxide (TBHP), the TC-C&L concluded				

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

We would like to comment on the above document as follows:

Background

The TC-C&L in September 2007 agreed to the provisional classification for Muta. Cat. 3; R68 (Muta. 2 H341). As DSD will be replaced by CLP, this recommendation was not included in an ATP and a new proposal, in accordance with CLP, was therefore required. The Committee for Risk Assessment (RAC) requested a re-evaluation due to the differences between the DSD and CLP criteria. The information used in the current evaluation was based on the RAR of TBHP plus additional information available in the transitional report, mainly a Comet assay in the lung.

Justification of C&L proposal by the Dutch Authorities

Interpretation of the data set available for TBHP
We agree with the Dutch evaluation that there is convincing evidence that the substance is mutagenic/ genotoxic in vitro. As highlighted by the Dutch authorities, there is limited data available to conclude that the substance will be mutagenic in vivo at the sites of first contact in somatic cells as there are contradictory findings for this scenario. Nevertheless, the data (mainly the findings in a dominant lethal test after i.p. administration) are considered by the Dutch authorities sufficient to propose the above given classification, which should be noted represents a severely conservative interpretation of the data.

Additional justification based on a read across (harmonization) between d-t-butyl peroxide (DTBP) and TBHP

We disagree with the read across (harmonization) between TBHP and DTBP as it is done in the dossier by the Dutch authorities, as neither the data set at hand nor the physico-chemical properties of these two peroxides justify such a read across/harmonization taken into account ECHA's guidance/rules for a scientifically sound read across.

The justification by the Dutch Authorities for the harmonization was that, "The substance di-tert-butyl-peroxide (DTBP) was shown to be mutagenic to the bone marrow in an in vivo assay. As DTBP forms only radicals also formed by TBHP, it is likely that TBHP is also mutagenic." Neither literature data nor effects noted in vitro and in vivo by both substances support this conclusion. Experiments with DTBP did not result in positive in vitro mutagenicity results, whereas TBHP did. In vivo, DTBP showed some effects in an oral micronucleus assay at dose levels above the limit dose. However a micronucleus assay, conducted in conjunction with a 90-day inhalation study was negative. While a micronucleus study following IP administration was positive in bone marrow, the spermatogonial assay was negative. Those data does not fit to the data at hand for TBHP. In addition, whereas TBHP is water soluble, DTBP is almost insoluble in water.

Taken these data together, we consider the read across/harmonization approach between DTBP and TBHP as not scientifically justified and not in line with ECHAs own guidelines.

Dossier Submitter's Response
See our response to comment 1.
RAC's response
See response to comment 1.

Date	Country	Organisation	Type of Organisation	Comment number
21.03.2014	Sweden		MemberState	9
Comment received				
p.9. As commented above we agree with the proposed classification of TBHP. However, we would have preferred to present the arguments for the proposed classification as phrased in the paragraph below, which differs slightly from how they are presented in section 2.2 Short summary of the scientific justification for the CLH proposal. TBHP was positive in several in vitro studies. The in vivo dataset is limited and most studies were negative. It is noted that these negative studies used test methods where systemic exposure of the target cells is required for the detection of a possible genotoxic effect. However, the available data does not support that TBHP is systemically available. TBHP was positive in two in vivo studies using the dominant lethal assay in mice exposed by intraperitoneal injection. Since TBHP can migrate from the abdominal cavity through the inguinal channel to the testis, this mutagenic effect is considered not to have resulted from systemic exposure but from interaction of the test substance with the DNA of germ cells following site of contact exposure. With that, TBHP fulfills the requirements for classification in Muta. 2 (CLP). This involves that the potential for TBHP to affect germ cells following other routes of administration should be considered to ascertain if the substance should be classified in Muta. 1B (CLP). Since TBHP has been shown to be unstable in blood in in vivo ADME studies and no data is available demonstrating that TBHP has the ability to interact with the DNA of germ cells following systemic exposure, sufficient information to conclude that TBHP poses a mutagenic hazard to germ cells is not available. Therefore, TBHP does not fulfill the requirements for classification in Muta. 1B (CLP). p.47. We would have preferred not to make any judgment about the likelihood for TBHP to reach the bone marrow as made in the sentence "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." Instead, we would have suggested the following wording: "TBHP is rapidly converted to the non-mutagenic compound 2-methylpropan 2-ol and there is no data available demonstrating that TBHP reaches the bone marrow." p.47. We would have preferred not to make any judgment about the likelihood for TBHP to reach the gonads as made in the sentence "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." Instead, we would have suggested the following wording: "TBHP is rapidly converted to 2-methylpropan 2-ol and there is no data available demonstrating that TBHP reaches the gonads." p.47. We would have preferred not to make any judgment about the likelihood for TBHP to reach the germ cells as made in the sentence "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." Instead, we would have suggested the following wording: "There is no data available demonstrating that TBHP reaches the germ cells after oral, inhalation and dermal exposure, meaning that there is no support for concluding that exposure to TBHP would result in inheritable genetic damage."				

p.48. We would have preferred not to make any judgment about the likelihood for TBHP to reach the germ cells as made in the sentences "However, this test was positive after intraperitoneal exposure whereas the kinetic data show that TBHP does not reach the systemic circulation, and thus does not reach germ cells, after oral, inhalation and dermal exposure. Classification with Muta 1B; H340 is not justified because TBHP will not induce germ cell mutagenicity via normal routes of exposure." Instead, we would have suggested the following wording: "However, this test was positive after intraperitoneal exposure whereas the kinetic data does not support that TBHP reaches the systemic circulation and, accordingly, there is no data supporting that TBHP reaches germ cells after oral, inhalation and dermal exposure. Classification with Muta 1B; H340 is not justified because there is no data supporting that TBHP will induce germ cell mutagenicity via normal routes of exposure."

Dossier Submitter's Response

The Swedish comments concern the language used regarding the systemic availability of TBHP and its potential to reach the germ cells after relevant routes of exposure. The main suggestion is to replace the statements that TBHP is very likely not systemically available with statements that it is unknown whether TBHP is systemically available. We do not agree with this suggestion as there is extensive kinetic data available in the CLH proposal which allow an assessment of the systemic availability. In an in vivo study it was shown that after intravenous injection of 5 mg TBHP/kg bw, no TBHP could be detected in the blood after 15 minutes (first measurement) but only metabolites. This indicates that TBHP is transformed very quickly into its metabolites. For other routes of exposure it is expected that the TBHP concentration in the blood will be lower than after IV injection. Therefore, TBHP in the blood after exposures via other routes will also be transformed very quickly. Therefore, it was concluded that "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.". We could accept to weaken the statements regarding the systemic bioavailability of TBHP from "TBHP does not reach the systemic circulation" and "very likely that TBHP did not reach the bone marrow" into "systemic availability of TBHP is not expected". This would be in line with the conclusion on the kinetic data. However, we do not support the suggestion to state that there is no data available demonstrating that TBHP reaches the bone marrow or the gonads.

RAC's response

Your support for classification is noted.
RAC agrees that toxicokinetic data provides evidence that it is likely that TBHP does not reach systemic circulation and in particular the gonads after single exposure. It is noted that no data are available after repeated exposure when metabolism may be saturated and antioxidant defenses depleted.

Date	Country	Organisation	Type of Organisation	Comment number
21.03.2014	Netherlands		Individual	10

Comment received

In their 2006 discussions, the TC-C&L member states did not unanimously support classification of tert-butyl hydroperoxide (TBHP) as a Cat 3 mutagen in accordance with DSD. However, in September 2007 the TC-C&L agreed to the provisional classification for Muta. Cat. 3; R68 (Muta. 2 H341). As DSD will be replaced by CLP, this recommendation was not included in an ATP and a new proposal, in accordance with CLP, is required. The Committee for Risk Assessment (RAC) requested a re-evaluation because of the differences

between the DSD and CLP criteria. The re-evaluation and recommendation was limited to mutagenic properties only. The information used in the current evaluation was based on the RAR of TBHP plus additional information available in the transitional report, mainly a Comet assay in the lung.
These comments are submitted in the framework of the public consultation initiated by the ECHA Risk Assessment Committee (RAC) the basis of Article 37.4 of the CLP Regulation 1272/2008.
AkzoNobel Functional Chemicals hereby submits that it wishes to participate to the RAC meeting during which the TBHP classification will be discussed, so that it can address any questions directly.
Data Summary
TBHP is considered mutagenic and genotoxic in vitro based on 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. Moreover, the fact that TBHP induces DNA base damage and DNA fragmentation indicates that TBHP is genotoxic in vitro.
The data set on genotoxicity of TBHP in vivo towards somatic cells is limited. Consequently, the TC-C&L felt that it is difficult to reach a conclusion on the genotoxicity in vivo of TBHP. The data set includes an oral study with exposure to a dose exceeding the oral LD50. As lower dose levels were not tested, the TC-C&L made the worst case assumption that mutagenicity will occur at all dose levels including the levels to which humans are exposed. Other in vivo data show that TBHP does not induce chromosomal aberrations in bone marrow and was negative in several other tests on the bone marrow as well. A limited Comet assay in rat liver after subcutaneous exposure was negative. A 2009 Comet assay, conducted as part of a 28-day inhalation study, with rat lung was also negative. In this study, no information was provided regarding DNA damage in the upper respiratory tract where the most severe toxicological effects were observed. Determination of DNA damage in nasal and bronchial epithelial cells was not possible in the COMET assay using the techniques described in the feasibility study. If additional long-term repeat dose studies become available, the Committee should take the new results into consideration prior to making a final decision.
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. It was the opinion of the TC-C&L that this could be a local effect of TBHP on the testis because substances can migrate from the abdominal cavity through the inguinal channel to the testis. According to the TC-C&L, there are no local mutagenicity tests with TBHP available.
While TBHP is genotoxic and mutagenic in vitro, TBHP was negative in several mutagenicity tests in the bone marrow. TBHP has been shown to be unstable in blood in in vivo ADME studies and therefore it is very likely that TBHP did not reach the bone marrow due to its rapid conversion to 2-methylpropan-2-ol following parenteral administration. No detectable levels of TBHP would be expected after oral, dermal and inhalatory exposure due to the slower absorption and the first pass effect in the liver after oral exposure. It should be noted that 2-methylpropan-2-ol was tested for mutagenicity by the NTP in 1995 and all in vitro and in vivo results were negative.
TBHP is mutagenic in germ cells after in vivo exposure (changes in sperm morphology and an increase in dominant lethal mutations) after intraperitoneal exposure. This positive rodent dominant lethal mutation test would normally fulfill the criteria for classification in category 1B. However as noted by the TC-C&L, it is unlikely that TBHP will reach the gonads through relevant routes of exposure in view of the rapid conversion to 2-methylpropan- 2-ol. Therefore, the positive results of these germ cell tests are considered evidence for a local

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

Discussion
As stated previously, a number of the member states did not agree with this proposal in previous discussions (see Appendix A and B of the CLH report). While the CLH notes that there were disagreements with the proposal, the actual argumentation was not provided in the report.
The TC-C&L's proposal to classify TBHP as a Cat 2 mutagen seems to hinge on a study demonstrating mutagenic activity in germ cells after in vivo intraperitoneal exposure (changes in sperm morphology and an increase in dominant lethal mutations). The Committee postulated that the positive effect was possibly due to be a local effect of TBHP on the testis because substances can migrate from the abdominal cavity through the inguinal channel to the testis. This was based on negative results by relevant routes of exposure and ADME studies which demonstrate the TBHP is rapidly converted to a non-genotoxic metabolite, 2-methylpropan-2-ol. The Committee states that its conclusion for classification is supported by chapter 3.5.1 of the Guidance on the application of Regulation (EC) No 1272/2008. However, the criteria do not appear to have been applied appropriately.
According to ECHA's Guidance on the application of the CLP criteria (v3 Nov. 2012, pg. 288), "It is also warranted that where there is evidence of only somatic cell genotoxicity, substances are classified as suspected germ cell mutagens. Classification as a suspected germ cell mutagen may also have implications for potential carcinogenicity classification. This holds true especially for those genotoxicants which are incapable of causing heritable mutations because they cannot reach the germ cells (e.g. genotoxicants only acting locally, "site of contact" genotoxicants).

This means that if positive results in vitro are supported by at least
one positive local in vivo, somatic cell test, such an effect should be considered as enough evidence to lead to classification in Category 2. If there is also negative or equivocal data, a weight of evidence approach using expert judgment has to be applied."
Since TBHP does not reach the gonads through relevant routes of exposure in view of the rapid conversion to 2-methylpropan- 2-ol, the positive results of germ cell tests, following IP administration, are considered evidence for a local mutagenic effect. While the data cited in the CLH report appears to support the claim that TBHP is mutagenic in vitro, by the Member States' own admission there is a data gap for in vivo mutagenicity tests. According to the report, "there are no local mutagenicity tests with TBHP available. The data set on genotoxicity of TBHP in vivo towards somatic cells is limited. Consequently it is difficult to reach a conclusion on the genotoxicity in vivo of TBHP." Therefore, in the opinion of ANFC, with no valid positive local in vivo, somatic cell test to support the positive in vitro test,

TBHP does not meet the criteria of a Cat.2 mutagen. The positive in vivo test was conducted by a route of administration that is not relevant in an occupational setting nor is it relevant to humans exposed via the environmental. Therefore the IP study alone is not suitable for risk assessment or for classification purposes. Relevant routes of administration did not demonstrate mutagenicity in vivo. Comet assays, conducted in lung and liver, did not demonstrate DNA damage and the metabolite formed following relevant routes of exposure was not mutagenic in vitro. As there is no relevant positive in vivo test to support the positive in vitro test, TBHP does not meet the criteria for classification as a germ cell mutagen even as a category 2.
The ECHA guidance also states that, "A Category 2 mutagen classification may also be based on positive results of a least one in vivo valid mammalian genotoxicity test, supported by positive in vitro mutagenicity results." While the in vitro data demonstrates mutagenicity, TBHP was negative in in vivo bone marrow micronucleus tests, in a Comet assay in rat liver after subcutaneous exposure and in a Comet assay in rat lung following inhalation. Therefore, based on a WOE, the data do not support classification as a Cat 2 mutagen and TBHP is not classifiable as a germ cell mutagen. This is in line with the ECHA guidance.
The TC-C&L also proposed, in the CLH report, that the TBHP classification should be harmonized with di-t-butyl peroxide (DTBP). The justification was that, "The substance di-tert-butyl-peroxide (DTBP) was shown to be mutagenic to the bone marrow in an in vivo assay. As DTBP forms only radicals also formed by TBHP, it is likely that TBHP is also mutagenic." However, according to a paper by Kennedy, C. et al, DTBP did not yield spin-trappable radicals in either non-respiring or respiring mitochondria and that there did not seem to be a correlation between tumor-promoting activity of peroxidic compounds and radical production in mitochondria (Biochem. and Biophys. Communications Vol. 160, No. 3, 1989).
The dataset of DTBP for other endpoints is also not comparable to the dataset of TBHP. TBHP and DTBP exhibit different physical-chemical properties in terms of vapor pressure, Log Pow and water solubility. DTBP is not mutagenic in vitro. In vivo, DTBP was inconclusive/weakly positive in an oral micronucleus assay at dose levels considered above the limit dose. A micronucleus assay, conducted in conjunction with a 90-day inhalation study was negative. Inhalation is considered the most relevant route of administration for DTBP. While a micronucleus study following IP administration was positive in bone marrow, the spermatogonial assay was negative. DTBP did not affect bio-markers of tumor promotion in mouse skin and was negative in a two-stage skin carcinogenicity and 81 week dermal carcinogenicity study. It should be noted that because there is no supportive data in vitro, the oral study is equivocal and the inhalation study and initiation/promotion studies are negative, ANFC is considering challenging the classification of DTBP, as a Cat 2 mutagen. Based on a WOE, it does not appear that DTBP should be classified as a germ cell mutagen. Since, in the opinion of ANFC, DTBP maybe inappropriately classified, the classification of TBHP should not be harmonized with the current classification of DTBP.
If the Committee does elect to use DTBP as a read-across substance it should do so consistently. A number of studies summarized in the transitional report (EU RISK ASSESSMENT - [TERTIARY BUTYL HYDROPEROXDE] CAS [75-91-2]), were disregarded due to lack of adequate read-across justification as well as being screening studies. The studies evaluated DTBP and other peroxides for their ability to increase bio-markers of tumor promotion in mouse skin as well as initiation promotion studies. DTBP did not affect the bio-markers. While these studies are screening studies, they can provide some information on a substance's potential as an initiator or promoter and if the Committee uses DTBP as a read-across substance, these studies should be taken into consideration as WOE.

Dossier Submitter's Response

See our response to comment 4.

RAC's response

See response to comment 4.

Date	Country	Organisation	Type of Organisation	Comment number
21.03.2014	Germany		MemberState	11
Comment received				
TBHP is genotoxic in vitro based on positive effects in numerous in vitro tests. The in vivo rodent dominant lethal mutagenicity test was positive after intraperitoneal exposure. Via routes of exposure relevant for humans, TBHP is not systemically available due to the rapid conversion of TBHP to 2-methylpropan-2-ol. We agree with the assessment that TBHP can induce genotoxic effects at a site of contact. Therefore, we support the proposed classification Muta 2, H341 due to local mutagenicity of TBHP.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Your support is noted.				

ATTACHMENTS RECEIVED

1. Comments on the Draft CLH report for tert-butyl hydroperoxide, Submitted by LyondellBasell on 21.03.2014. [Filename: LYB Comments on Draft CLH Proposal 21March2014]. [*Please refer to Comment number 1]*

2. Tert-butyl hydroperoxide CL Position Paper, by AkzoNobel Functional Chemicals. Submitted on 21.03.2014. [Filename: tert-butyl hydroperoxide CL Position Paper]. [*Please refer to Comment number 4*]