March 20, 2014

To: Whom It May Concern

From: AkzoNobel Functional Chemicals

Subject: Proposed harmonized classification and labeling of tert-butyl hydroperoxide (TBHP) as a category 2 mutagen in accordance with Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Executive Summary

In the August 2013 CLH 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.

AkzoNobel Functional Chemicals (ANFC) does not support this proposed classification. ANFC does not feel the data and its interpretation, provided in the CLH report, meet the criteria for classification as a Cat 2 mutagen (CLP).

Introduction

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

525 W. Van Buren St.
Chicago, IL 60607

T +1-312-544-7347
F +1-312-544-7408
www.akzonobel.com

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 t**his 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, t**here 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.

Conclusion

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.

Submitted by:
Glen Rosini
Global REACH Project Manager
AkzoNobel Functional Chemicals – OP/OMS
REACHPC@akzonobel.com

Prepared by:
Louette Rausch
Senior Toxicologist
Akzo Nobel BA Specialty Chemicals