

REVISED TEST PROPOSAL FOR CONCLUSION ON TERTIARY BUTYL HYDROPEROXIDE (CAS NO. 75-91-2)

The Netherlands, January 2008

Introduction

A conclusion i for carcinogenicity was concluded at TCNES IV '07 meaning that further information on the carcinogenic potential of TBHP is required. However, no conclusion was reached on the required test. Several member states indicated that additional details on the proposed 28-day inhalation test with additional mutagenicity determinations in the respiratory tract are needed before a definitive decision can be made.

Justification of the conclusion i

No inhalatory or oral carcinogenicity studies with TBHP are available. TBHP is, however, rapidly converted to 2-methylpropan-2-ol and for this compound oral carcinogenicity studies in mice and rats are available. These studies show very small increases in systemic tumours at dose levels of 2-methylpropan-2-ol that can not be reached by treatment with TBHP because these levels are above the LD50 of TBHP for mice, and above the dose level of TBHP inducing local effects to the stomach in rats. From these observations it is concluded that chronic exposure to TBHP will most probably not result in 2-methylpropan-2-ol levels that can induce systemic tumours.

TBHP is considered to be mutagenic at the sites of first contact in somatic cells. However, based on the rapid conversion of TBHP, it is unlikely that TBHP can reach the systemic circulation through normal routes of exposure. Consequently, carcinogenicity limited to tissues that are exposed to the parent TBHP (i.e. tissues of first contact) cannot be excluded.

Useful data on the potential local carcinogenic effects of TBHP are not available, unfortunately. In a single very limited dermal study one clearly toxic concentration of TBHP was capable of promoting the development of dermal tumours after induction by 4-nitroquinoline 1-oxide. Therefore information on the local carcinogenicity is needed (**conclusion i**). This information could be derived either by:

- read-across from other substances in case the read-across is sufficiently validated and based on sufficient data, or
- carcinogenicity tests.

This should ideally be through the oral, dermal and inhalatory route because it is unknown whether and how local carcinogenicity can be extrapolated from one route to the other. However, from a practical point of view it is proposed to start with one route and the need for additional routes will depend on the results. Information on the carcinogenicity by the inhalatory route is preferred because this route is relevant for both workers and humans exposed via the environment.

Considering that:

- the local mutagenic potency of TBHP is probably low because genotoxic effects were mainly seen at very high dose levels;
- the risk assessment of comparable substances which also have corrosive and mutagenic properties like e.g. formaldehyde is mainly based on the assumption that the mutagenicity and carcinogenicity is restricted to irritating concentrations;

- only very limited data are available on the local irritating potential of TBHP after inhalation exposure, it is not appropriate to ask for an inhalatory carcinogenicity study straight away.

Instead, we propose an inhalatory repeated dose toxicity study to investigate which level could be derived as a NOAEC for local irritation. In addition, investigation of the relationship between irritation in the respiratory tract and genotoxicity of TBHP towards specific parts of the respiratory tract, including nose epithelium, upper respiratory tract and lower respiratory tract, is proposed. The results of the proposed studies will not allow a conclusion on the carcinogenic properties directly. The only test to provide an answer to that question would be a full carcinogenicity study. However, it is useful to study if the local genotoxicity and carcinogenicity is restricted to irritating concentrations and therefore the studies may provide a first indication of this relationship. Dependent on the outcome, additional studies might need to be considered to confirm this. In analogy with comparable substances where carcinogenicity is restricted to irritating concentrations, then a threshold approach can be considered for risk characterisation. This could be sufficient for a well substantiated risk characterisation with a minimal use of animals.

Test proposal

Part I –Rodent 28 Day Repeated Dose Inhalation Toxicity Study of TBHP vapour

GLP and OECD Guideline 412 Compliant Study

Objectives:

- 1) Determine inhalation toxicity using 28 day repeated exposure to TBHP vapor
- 2) Define respiratory tract histopathology
- 3) Define respiratory tract cellular proliferation

Test Animals:

Young adult, male and female rats

Sets of animals for probe study, main study, respiratory tract histopathology and cellular proliferation, post exposure/recovery (for respiratory tract histopathology and cellular proliferation)

Test Material:

Commercial Tertiary Butyl Hydroperoxide (T-Hydro® Solution:
70% TBHP, 30% Water, ≤ 0.003% hydroquinone) provided by Lyondell

Route:

To be determined (whole-body or snout-only inhalation exposure to vapour)

Exposure levels:

To be determined (air control and 3 concentrations of TBHP (determined from a 3 day exposure probe study: i.e., an irritating, a slightly irritating and a non-irritating concentration)

Exposure duration:

Minimum of 28 exposures, (~6 weeks); 6 hours/day; 5 days/week

Study Endpoints:

Main study

- 1) Clinical observations

- 2) Feed consumption
- 3) Body weight and body weight gain
- 4) Hematology
- 5) Clinical Chemistry
- 6) Gross Necropsy
- 7) Organ Weights
- 8) Histopathology of gross lesions and routine organs/tissues

Respiratory tract histopathology

Histopathology of nasal cavity (nasopharyngeal tissues: at least 4 levels; 1 level to include the nasopharyngeal duct and including Nasal Associated Lymphoid Tissue (NALT)), larynx, trachea (at least 2 levels including 1 longitudinal section through the bifurcation and 1 transverse section) and lung (all lobes at one level, including main bronchi)

Respiratory tract cellular proliferation

- 1) BrdU mini-pumps implanted 3 days prior to final exposure
- 2) Tissues for analysis: nasal epithelium, liver, lung, gastrointestinal tract (for BrdU control)
- 3) Analyze nasal epithelium, lung, liver for ULLI & ND & LI (Unit Length Labelling Index; numeric density; labelling index)

Part II – COMET assay on respiratory tissues after inhalation exposure to TBHP

The 28-day repeated dose inhalation study should be followed by a study to determine DNA damage (strand breaks) in the same rat strain exposed for 6 hours/day during three consecutive days to TBHP at 5 or 6 concentrations to get a good impression of the dose – effect curve for DNA damage for comparison with the dose-response curve and NOAEC for local irritation. This should at least include the NOAEC and the LOAEC for local irritation derived from the 3-days range-finding study and from the 28-day study plus a negative control group. It is proposed to perform the COMET assay in accordance with most recent methodology available (recommendations of IWGTP workshop and current literature). The assay should be performed in the different parts of the respiratory tract, i.e., nasal epithelium, upper airways and lower respiratory tract using alkaline conditions.

This assay is not a standard OECD test (OECD tests for mutation induction testing in the respiratory tract are not available) but intended to determine the mode of action of TBHP. Non standard OECD tests have been requested and used in the past by the TC-NES. COMET assays in the lung after inhalation exposure have been done before for regulatory purposes. In the COMET assay for TBHP an extension (compared to COMET assays with lung tissue) with nasal epithelium and upper respiratory tract tissue is proposed which may require the development of special techniques.

The exact details of the studies will be determined by consultation between industry and the rapporteur.

Interpretation of the results

Part I – 28-day repeated dose inhalation

The NOAEC of the repeated dose inhalation study shall be used for a quantitative risk characterisation for local effects after repeated inhalatory exposure.

Part II – COMET assay on respiratory tissues after inhalation exposure to TBHP

Based on the results of the 28-day inhalation study (including the results from the 3-days range-finding study) and the COMET assay the relationship between irritation and local genotoxicity could be established. Interpretation of the possible changes in % DNA in tail

(tail intensity) at cytotoxic concentrations will be difficult because both cytotoxicity and genotoxicity can result in an increase in % DNA in tail (tail intensity). However, at non-cytotoxic concentrations, an increase in % DNA in tail (tail intensity) can be attributed to genotoxicity. If the genotoxicity results show that TBHP induces increases in % DNA in tail (tail intensity) only at concentrations also inducing local irritation then this is a first indication that the possible carcinogenicity of TBHP is restricted to concentrations above a certain threshold and would reduce the necessity for a carcinogenicity study. Of course this will depend on the quality of the COMET assay data, the adequacy of the dose-response for cell proliferation and the robustness of the dose-response for irritation. The presence of a threshold may be confirmed using additional mechanistic studies. On the basis of the total data base, the NOAEC for local irritation may possibly be used as a starting point (with some extrapolation factors) in the risk characterisation for possible local carcinogenicity (using a threshold approach). This results in a conclusion i on hold for carcinogenicity because the conclusions drawn for local genotoxicity and local irritation will result in risk reduction measurements when necessary that are assumed also to be protective against possible local carcinogenicity.

If the results show genotoxicity (increase in % DNA in tail (tail intensity)) below the concentrations inducing local irritation then no safe level for local genotoxicity and possible local carcinogenicity can be determined. Then for risk assessment purposes the LOAEC for local genotoxicity may still be used as a starting point for risk characterisation using the same approach as for non-threshold carcinogens. If this characterisation still indicates a concern then further information is required.

Conclusion

The described 28-day inhalation study with an additional study for the determination of strand breaks in respiratory tissues is a feasible starting point of a tiered approach which could provide an answer on the possible risk for carcinogenic effects of TBHP without directly requesting a 2-year carcinogenicity study.