SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

2-methylpropan-2-ol
(tertiary butyl alcohol)

EC No 200-889-7

CAS No 75-65-0

Evaluating Member State(s): United Kingdom

Dated: October 2019

Template Version 2.1
March 2015

Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2013

Before concluding the substance evaluation a Decision to request further information was issued on: 15 May 2015

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

2-Methylpropan-2-ol (tertiary butyl alcohol or TBA) was originally selected for substance evaluation in order to clarify concerns about:

- Suspected CMR (carcinogenicity and mutagenicity)
- Wide dispersive use
- Consumer use

During the evaluation the following additional concerns were identified:

Human Exposure

- the scope of the exposure assessments
- the practicality of recommendations for RPE to be used by professionals working in sectors that traditionally have little or no experience with this RMM
- the approach taken to calculate consumer exposures.

Environment

- Concerns about the PNEC value derived from the fish study using *Clarias gariepinus* (African catfish)
- Concerns about assumptions made in the environmental exposure modelling for specific risk management measures and biodegradability of the substance

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

TBA was not identified as a priority substance under the Existing Substances Regulation and no other regulatory processes have been initiated for this substance.

The eMSCA is aware of the following previous assessments of the human heath effects of TBA:

- WHO: International Programme on Chemical Safety Environmental Health Criteria 65 (1987) (Butanols; four isomers) (WHO, 1987) http://www.inchem.org/documents/ehc/ehc/ehc65.htm

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in table 1 below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION	
Conclusions	**Tick box**
Need for follow-up regulatory action at EU level	
Harmonised Classification and Labelling	

Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	✓

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

4.1.1. Harmonised Classification and Labelling

Not applicable

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

Not applicable

4.1.3. Restriction

Not applicable

4.1.4. Other EU-wide regulatory risk management measures

Not applicable

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

Table 2

REASON FOR REMOVED CONCERN The concern could be removed because	Tick box
Clarification of hazard properties/exposure	✓
Actions by the registrants to ensure safety, as reflected in the registration dossiers(e.g. change in supported uses, applied risk management measures, etc.)	

Human health – hazard

TBA was originally selected for substance evaluation in order to clarify concerns about carcinogenicity and mutagenicity.

Mutaganicity

Following the initial assessment period it was concluded that TBA has been well investigated for mutagenicity, *in vitro* and *in vivo*, and there was no positive evidence for mutagenicity. Overall, this evaluation concluded that any concerns for mutagenicity were unfounded.

Carcinogenicity

The carcinogenic potential of TBA has been well investigated in two standard studies, one in rats and one in mice.

Oral TBA administration resulted in increased incidences of two tumour types in laboratory animals: renal tumours in male rats, which the eMS concludes were male-rat specific and thus not relevant to humans; and benign thyroid tumours in female mice, which occurred only at excessively high doses and via a non-genotoxic mode of action, and which the eMS therefore concludes are of low relevance to humans. Overall, the concern for carcinogenicity has been clarified and no further information is requested.

Human health – exposure

TBA is not considered to present an unacceptable risk to workers or consumers from any identified use. However, the eMSCA has identified several areas where registrants can usefully improve the information provided in their registrations to increase the accuracy and transparency of their chemical safety assessments.

To ensure that exposure scenarios and suitable exposure assessments are available for each of the uses identified by registrants, each registrant should:

- check which of the uses that they currently identify in their CSR are still relevant and that an exposure scenario is available for each identified use;
- for any use that takes place under SCC at registrants' own sites ensure that the SCC are described in the registration;
- for any use that takes place under SCC at downstream user sites, ensure that there is evidence that confirmation has been received from the downstream user that SCC are implemented at their site;
- ensure that sufficient information is provided on any PPE that is required;
- ensure that wherever analogous measured data is used in the exposure assessment, sufficient contextual data is available to allow the suitability of the data to be examined. This has been identified as an area to address in relation to worker exposure assessments, but if in future, analogous data is used for the consumer exposure assessment, it is equally important that supporting contextual data is provided for that analogous data; and,
- provide scientific justifications for the choice of parameters and exposure models used to estimate consumer exposure.

Environment and environmental exposure

In March 2016, the Registrant submitted an updated Registration dossier to address the requirements of the Decision. This included:

- a new long-term toxicity on aquatic invertebrates (*Daphnia magna*) study;
- site specific monitoring information; and

- an updated CSR including updated PNEC, environmental exposure information and RCRs.

The Registrant has updated the environmental risk assessment to consider TBA as inherently degradable not meeting criteria. The eMSCA agrees this is appropriate based on available degradation data.

The eMSCA has reviewed the *Daphnia magna* study and agrees with the presented NOEC and reliability rating of 1. The 21-d NOEC from the study (100 mg/L) is used to derive the aquatic PNEC using an assessment factor of 50 based on 2 chronic ecotoxicity endpoints. The eMSCA agrees this is appropriate for the available ecotoxicity data.

The eMSCA has reviewed the revised exposure assessment in the 2016 update and recalculated the RCRs using the revised PNECs. The eMSCA agrees there are no environmental risks based on the data supplied.

Overall, based on the available data and the updated environmental risk assessment, there are no remaining concerns for the environment and no further information is required at this time. Should the tonnage change or new information (such as a change in risk management measures) become available, the CSR should be updated to reflect this.

5.2. Other actions

Not applicable

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Not applicable

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

2-methylpropan-2-ol (tertiary butyl alcohol) was originally selected for substance evaluation in order to clarify concerns about carcinogenicity and mutagenicity. In this report this substance will be referred to as TBA. The initial grounds for concern as taken from the original CoRAP justification document were:

"Carcinogenicity

Two carcinogenicity studies are available: one in rats and one in mice.

In the rat study, kidney toxicity (nephropathy, linear papillary mineralization and focal renal tubule hyperplasia) and an increase incidence in renal tumours were observed. It is suggested the kidney tumours were due to alpha-2urinary-globulin nephropathy and hyaline droplet formation was confirmed. However, kidney toxicity was also observed in females (nephropathy in all groups and hyperplasia in the top group). Evaluation of the existing information would confirm whether or not the tumours observed in male rats were a species specific effect.

The incidence of thyroid adenomas was increased in female and, to a lesser extent, male B6C3F1 mice. The relevance of these tumours has been investigated in a study investigating hepatotoxicity and thyroid hormone levels. An evaluation is required to determine whether this study allays our concerns for these tumours.

Genotoxicity

TBA was negative in most *in vitro* and *in vivo* studies. However, a positive result was observed in the presence of metabolic activation for *S.typhimurium* strain TA 102 (in one study). A weight of evidence evaluation would determine whether this result was of concern and determine whether any further testing was required.

Exposure

The RCR values should be verified".

During the evaluation also other concerns were identified. The additional concerns were:

Human Exposure

- the scope of the exposure assessments
- the practicality of recommendations for RPE to be used by professionals working in sectors that traditionally have little or no experience with this RMM
- the approach taken to calculate consumer exposures.

Environment

- Concerns about the PNEC value derived from the fish study using *Clarias gariepinus* (African catfish)
- Concerns about assumptions made in the environmental exposure modelling for specific risk management measures and biodegradability of the substance

The evaluation was targeted to the human health hazard concerns. Only a brief review of the environmental risk characterisation values was conducted. Further information given in Table 3 below.

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Human Health Hazard All information was evaluated paying particular attention to CMR endpoints. Relevant NTP studies were downloaded from the NTP website and evaluated. Information on the proposed mode of actions was requested from the registrants and also obtained through literature searches. For mutagenicity, the relevant publications/study reports were requested (where possible) and evaluated. In addition, information referred to in the IUCLID on reproductive/developmental toxicity, repeated dose toxicity and toxicokinetics was obtained and evaluated to inform on the toxicological profile of the substance and identify appropriate values for DNEL derivation.	Mutagenicity concerns not substantiated – no further action. Carcinogenicity TBA is non-genotoxic. Evaluation of the existing information confirmed that the tumours observed in male rats were a species-specific effect and not relevant to humans - no further information is needed. The tumours in the thyroid gland of female mice occurred only at excessively high doses and so are of low relevance for humans – no further information is needed. No additional concerns identified
Human exposure assessment All exposure scenarios were assessed from registrations that were active during the initial assessment period (March 2013-14) and all additional information provided by the registrants during the decision making period and subsequent follow-up assessment was taken into account.	During the course of the evaluation, the eMSCA identified areas of the registrants' exposure assessment that needed further work. These additional actions are described in Part A section 5.
Physico-chemical properties Analytical information provided in the dossiers was assessed to confirm substance identity and composition. The physico-chemical data was screened paying particular attention to those endpoints important to other parts of the evaluation, specifically water solubility, partition coefficient and vapour pressure.	No issues identified.
Environment A brief review of all of the relevant environmental fate, behaviour and toxicity data was performed. Where data did not indicate a significant issue and fitted the general pattern of the substance, these were not reviewed in depth. Two studies were targeted for a more in-depth evaluation. The first was the fish study used to provide the aquatic PNEC in the 2013 CSR, and the second was the biodegradation test most relevant for the exposure modelling. A brief review of the environmental exposure modelling and risk characterisation was also conducted. The focus of this was the generic	The review confirmed the general low environmental hazard profile of the substance. Several issues were found for the areas targeted for in-depth evaluation. As a result further information was required to consider concerns for the aquatic environment including: - Justification for the level of degradation used in environmental exposure modelling. - Site specific monitoring of TBA in effluent before and after wastewater treatment.

risk management measures, generic exposure modelling input assumptions and the values of the RCRs.	- Long-term toxicity testing on aquatic invertebrates. The information was provided and the environmental concerns were resolved. No further action is required.

7.2. Procedure

An initial meeting was held with registrants in March 2013 to discuss the process. As a result of this discussion, and requests made during the evaluation, additional exposure information was provided and the exposure assessment in the CSR was revised. The initial evaluation was based on the information contained in registrations in March 2013 and the updated exposure assessments provided by the registrant to the UK REACH CA in August and October 2013.

The draft evaluation report (SEv report) was circulated to the registrants in January 2014 and a meeting to discuss the conclusions was held in February 2014.

Questions remained at the end of the initial evaluation period. The decision making process was therefore initiated and a decision was issued on 29 May 2015 covering requests for more information on worker exposure, site specific monitoring data of TBA in effluent before and after wastewater treatment and a long term toxicity test on aquatic invertebrates.

The deadline for submitting the information was 5 September 2016.

An updated dossier was submitted on 11 March 2016 initiating the one year follow up period.

The new information resolved the environmental concerns held by the eMSCA but did not resolve all of the concerns the eMSCA identified with the human exposure assessment. The eMSCA therefore held a teleconference with the reigistrants on 30 March 2017 and agreed a way forward to resolve the remaining concerns. An additional dossier update was submitted in August 2018 containing information that was considered in this assessment.

7.3. Identity of the substance

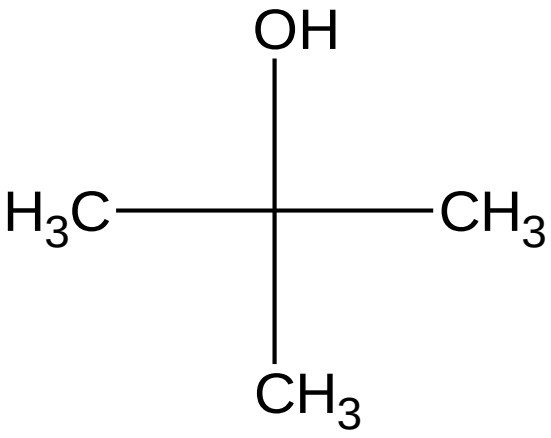
Information from the ECHA dissemination site is given in table 4.

Table 4

SUBSTANCE IDENTITY	
Public name:	2-methylpropan-2-ol
EC number:	200-889-7
CAS number:	75-65-0
Index number in Annex VI of the CLP Regulation:	603-005-00-1
Molecular formula:	$C_4H_{10}O$
Molecular weight range:	74.1216
Synonyms:	Tertiary butyl alcohol, t-butanol, tert-butyl alcohol, TBA

Type of substance ☒ Mono-constituent ☐ Multi-constituent ☐ UVCB

Structural formula:



Multiconstituent/UVCB substance/others

Generally the information provided by the registrants was sufficient to confirm the identity of the registered substance. However, it is recommended that registrants consider the requirements of Annex VI 2.3.5 to ensure that they are compliant and have data specific to their registration.

Each registrant provided some analytical information to support the composition reported in section 1.2 of their dossiers, but registrants are reminded that they should include sufficient information for the analysis to be reproduced.

Table 5

Constituent			
Constituents	**Typical concentration**	**Concentration range**	**Remarks**
2-methylpropan-2-ol (EC 200-889-7)	>80% (w/w)		Confidential

No validation information such as recovery rates, limit of detection or quantitation were given for any method although one report included chromatograms of standards for some of the known impurities. Some of the analytical reports identified small amounts of impurities (<1%) which were not reported in section 1.2 and for some of the impurities the typical concentration reported was outside the range given. Registrants are reminded to check their dossiers to ensure compositional information reported in IUCLID (Section 1.2) is correct and supported by the analytical information provided (IUCLID section 1.4).

7.4. Physico-chemical properties

The physico-chemical properties reported in the registration dossiers are summarised in Table 6.

Most of the values are taken from secondary reference sources such as the Merck Index and the CRC Handbook of Chemistry and Physics or other published material. The only tests conducted on the substance as registered are; partition coefficient, surface tension, flash point and viscosity.

Three physico-chemical properties are used in other areas of this evaluation and are described in more detail below;

Water Solubility;

The two solubility results were taken from secondary literature respectively giving values of 1000 g/l at 25°C and the other just describes TBA as miscible with water at 30°C. Neither specifies purity or method of measurement.

Octanol-water Partition Co-efficient;

One value is given from a recent (Unpublished, 2009a) study conducted on the registered substance using an appropriate method (shake flask). The value of 0.32 is consistent with values found in the literature.

Vapour Pressure;

Two values are given in the dossier, both taken from secondary literature sources. The values reported at 25°C range from 5413-6132 Pa. Neither specifies purity or the method used.

Table 6

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Off-white solid block Odour: camphor-like Substance melts to become a clear colourless liquid at ca. 25°C and is close to solid liquid transition at STP
Melting/freezing point	25.7°C (published data, secondary source)
Boiling point	82.4°C (published data, secondary source)
Relative density	0.786 at 20°C (published data, secondary source)
Vapour Pressure	5134 Pa at 25°C (published data, secondary source)
Surface tension	69.8 mN/m at 20°C, 1.09 g/L aqueous solution EC Method A5 (Ring method) Also measured neat at 25°C - result 22.3 mN/m TBA is not considered surface active in aqueous solution. According to the registrant no Harkins-Jordan correction was required for the ring assembly used as the smaller dimension ring gives a direct reading of surface tension.

Water solubility	1000 g/L at 25°C (published data secondary source) TBA is considered to be miscible with water
Partition coefficient n-octanol/water (Log Kow)	0.32 at 22.5°C (pH 6.8-7.3) EC Method A5 (Flask method) Registered substance tested using an appropriate study
Flash point	15°C EC Method A9 (ISO 13736:1997 (Petroleum products and other liquids - Determination of flash point - Abel closed cup method)) A preliminary test indicated the result to be <23°C *TBA meets the criteria for classification as flammable.*
Flammability	Waiver for EC methods A10, A12 & A13 Due to the nature of the substance EC Method A9 was performed in preference to Method A10. According to the registrant experience in use and testing material indicates no concern for pyrophoricity or reactivity in contact with water.
Explosive properties	Waiver According to the registrant there are no chemical groups associated with explosivity
Auto ignition temperature	470°C Method given as DIN 51794 (published data, secondary source)
Oxidising properties	Waiver According to the registrant TBA does not contain chemical groups likely to react exothermically with combustible materials
Granulometry	Waiver According to the registrant the substance forms a ductile mass at room temperature and smaller particles are not formed on handling
Stability in organic solvents and identity of relevant degradation products	Waiver According to the registrant the stability of TBA in organic solvents is not expected to be critical
Dissociation constant	Waiver According to the registrant no dissociation is expected under naturally relevant pH conditions
Viscosity	5.72 mm²/s (static) at 25°C 2.23 mm²/s (static) at 45°C OECD guideline 114 (Capillary Viscometer Method)

7.5. Manufacture and uses

7.5.1. Quantities

The tonnage given on the ECHA dissemination site (checked April 2019) is given in the table below.

Table 7

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☐ 10 – 100 t	☐ 100 – 1000 t	☐ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1,000,000 t	☒ > 1,000,000 t	☐ Confidential

7.5.2. Overview of uses

The most common technical function of TBA is as an intermediate. Over 95% of the manufactured tonnage is used under strictly controlled conditions (SCC), mainly to produce methyl tertiary butyl ether (MTBE). TBA is also used in the preparation of block polymers and as an alkylating agent for aromatics and phenols in the production of t-butyl aromatics. It is used as an ethanol denaturant. Owing to its amphiphilic properties a small part of the annual production is used as a process solvent in the manufacture of pharmaceuticals and as a solubiliser in water based preparations such as cleaning agents and coatings. TBA is not supplied to consumers as the substance itself, but products such as cleaning agents and coatings containing TBA may be available to consumers.

Table 8

USES	
	Use(s)
Uses as intermediate	Industrial use as intermediate
Formulation	Formulation and packing of preparations and mixtures containing TBA Formulation of the substance and its mixtures in batch or continuous operations within closed or contained systems
Uses at industrial sites	Industrial use of coatings, paints, inks and surface agents Industrial cleaning and degreasing Use of TBA in waste water treatment (industrial)* Use of small quantities of TBA within laboratory settings Use of TBA in fuels (industrial)*
Uses by professional workers	Professional use of coatings, paints, inks and surface agents Professional cleaning and degreasing Use of TBA in waste water treatment (professional)* Use in a laboratory setting as an analytical reagent Use of TBA in fuels (professional)*
Consumer Uses	Consumer use in washing and cleaning products Consumer use of a formulated paint or coating for general repair and maintenance Consumer use in adhesives and sealants* Consumer use of TBA in fuels*
Article service life	Not applicable

* although these uses are listed in the information ECHA was disseminating from registrations in October 2018, the lead registrant reports that these use categories are not applicable for TBA and are no longer supported.

In order to better understand the types of products where TBA may be found, the registrants have performed a survey of safety data sheets listing TBA as a component. Cleaning products

containing TBA included cable cleaners, degreasers, stain removers, sterilisation reagents, contact cleaners, skin cleaners, acne treatment pads, mildew removers and flux cleaners. The maxium concentration in any product was 5%. Coating products containing TBA included stain protectors, concrete sealants, fire suppressant coatings, epoxy resins, primers, penetrants and paints. The maximum concentration in any product was 20% with the highest levels being found in fire suppressant coatings.

Most of the products that have been identified appear to be supplied for industrial or professional use, but the possibility that products containing TBA are supplied to the consumer market cannot be excluded.

TBA is one of the substances that are approved for use as an ethanol denaturant. It is used at concentrations up to 5% in ethanol. The denatured ethanol may then be used for a variety of products. It is therefore possible that TBA residues may be present in a wide range of products including cosmetics and may be detected in chemical analyses of these products. Given that TBA residues will be a minor constituent of products formulated with denatured ethanol this use does not give cause for concern and will not be considered further in this evaluation.

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

Table 9

HARMONISED CLASSIFICATION ACCORDING TO ANNEX VI OF CLP REGULATION (REGULATION (EC) 1272/2008)							
Index No	**International Chemical Identification**	**EC No**	**CAS No**	**Classification**		**Spec. Conc. Limits, M-factors**	**Notes**
				Hazard Class and Category Code(s)	**Hazard statement code(s)**		
603-005-00-1		200-889-7	75-65-0	Flam. Liq. 2	H225 (flammable aerosol)		
				Eye Irrit. 2	H319 (Causes serious eye irritation)		
				Acute Tox. 4 *	H332 (harmful if inhaled)		
				STOT SE 3	H335 (may cause respiratory irritation)		

7.6.2. Self-classification

- In the registration(s)*:*

In addition to the harmonised classification given above the registrants include;

STOT SE 3 H336 May cause drowsiness or dizziness

- The majority of the aggregated self-classifications in the C&L Inventory have notified the harmonised classification, some include the additional classification as used by the registrants, one only gives Acute Tox 4 and another notifies the substance as *not classified*.

7.7. Environmental fate properties

The substance was not nominated as an environmental priority. Due to this, only a brief review of all of the relevant environmental fate and behaviour data was performed. Where data did not indicate a significant issue and fitted the general pattern of the substance, these were not reviewed in depth. One study was targeted for a more in-depth evaluation, which was the biodegradation test most relevant for the exposure modelling.

All studies were included in the registration dossier, unpublished reports are indicated by year only.

7.7.1. Degradation

7.7.1.1 Abiotic degradation

7.7.1.1.1 Hydrolysis

OECD 111: <10% at pH 4, 7, 9 in 5-d screening test at 50 ºC, Unpublished (2009a).

7.7.1.1.2 Phototransformation/photolysis

No data available.

7.7.1.2 Biodegradation

7.7.1.2.1 Biodegradation in water

7.7.1.2.1.1 Estimated data

No data available.

7.7.1.2.1.2 Screening tests

Key study: ready biodegradation study: OECD 301B: 4% mineralisation after 29 days. Unpublished (2003a).

Supporting study: ready biodegradation study: OECD 301A: 99% mineralisation after 28 days. Failed ten-day window, Unpublished (1994).

The registrant considers the 2003 study as the key study. This is based on test item properties including vapour pressure (5413 Pa at 25°C) which is considered 'quite high'. In the Unpublished 1994 study test vessels were loosely closed and shaken and controls for moderately volatile chemicals were not included. The eMSCA notes that the 2003 OECD 301B test method is suitable for non-volatile test substances and it is unclear if controls to limit volatilisation were taken during the test.

Following the above ready biodegradation studies an extended inherent biodegradation study was undertaken and designed to limit volatilisation. This study (Unpublished (2009b)) was conducted according to the draft OECD 302D (CONCAWE method). This was to GLP and assessed as validity 1 by the registrant. The test was run for 56 days using a composite microbial inoculum derived from soil and activated sludge from a wastewater treatment facility (soil: mixed population of soil-micro-organisms, collected from a depth of up to 20cm below the soil surface

with stones/plant debris/invertebrates removed; activated sludge: mixed population of activated sewage sludge micro-organisms obtained from plant treating predominantly domestic sewage).

The test used 30.9 mg/L of TBA, which equated to 20 mg/L carbon. Biodegradation was followed by analysis of inorganic carbon, with samples taken every 3-4 days during the test. An inoculum blank was run, but the results are not included in the RSS. The registrant should consider adding these data as they are an important part of the study validation. The reference substance was n-hexadecane which reached 91% degradation by 28 days and 87% at 56 days. TBA achieved 66% degradation at 28 days and 66% at 56 days (note that each sample point uses a different bottle so there is some variation in the levels of degradation observed).

The registrant concluded that this level of degradation indicated the substance was inherently biodegradable based on a threshold of 60%. This is the level specified in the test guideline, although OECD 302D remains as a draft guideline and has not been agreed by OECD members (nor is it included in the REACH endpoint guidance R.7B). Other inherent tests use a threshold of 70% to demonstrate ultimate degradation, but these rely on DOC, rather than ThIC used here.

In the updated dossier in March 2016, the registrant considers the substance is 'inherently biodegradable not meeting criteria'. The eMSCA agrees this is appropriate.

7.7.1.2.1.3 Simulation tests (water and sediments)

Waived: In accordance with column 2 of REACH annex IX, further degradation testing does not need to be conducted as the chemical safety assessment does not indicate a need for further investigation.

7.7.1.2.1.4 Summary and discussion of biodegradation in water and sediment

The registrant concludes that the substance is 'inherently biodegradable not meeting criteria' in water.

7.7.1.2.2 Biodegradation in soil

Waived: In accordance with column 2 of REACH annex IX, further degradation testing does not need to be conducted as the chemical safety assessment does not indicate a need for further investigation.

7.7.1.3 Summary and discussion on degradation

The registrant considers the substance is 'inherently biodegradable not meeting criteria'. The eMSCA agrees this is appropriate.

7.7.2. Environmental distribution

7.7.2.1 Adsorption/desorption

Waived.

7.7.2.2 Volatilisation

No data available.

7.7.2.3 Distribution modelling

No data available.

7.7.2.4 Summary and discussion of environmental distribution

Not assessed.

7.7.3. Bioaccumulation

7.7.3.1 Aquatic bioaccumulation

Waived.

7.7.3.2 Terrestrial bioaccumulation

No data available.

7.7.3.3 Summary and discussion of bioaccumulation

TBA has a low measured logKow of 0.317. No experimental bioaccumulation data are available. Based on the logKow, TBA is considered to have low bioaccumulation potential.

7.8. Environmental hazard assessment

The substance was not nominated as an environmental priority. Due to this, only a brief review of all of the relevant ecotoxicity data was performed. Where data did not indicate a significant issue and fitted the general pattern of the substance, these were not reviewed in depth. In 2013, the fish study used to derive the aquatic PNEC was targeted for a more in-depth evaluation.

The registration update in March 2016 included a new chronic toxicity to *Daphnia magna* study which has been reviewed and included below as this forms the basis of the updated aquatic PNEC.

All studies were included in the registration dossier, unpublished reports are indicated by year only.

7.8.1. Aquatic compartment (including sediment)

7.8.1.1 Fish

Short-term toxicity to fish

OECD 203 using *Pimephales promelas*: 96-h LC_{50} >961 mg/l, NOEC = 961 mg/l Unpublished (2003b)

Long-term toxicity to fish

A five-day fish toxicity study using *Clarias gariepinus* (African catfish) is provided in the registration dossier to fulfil the long-term fish toxicity endpoint (Moreels, 2006). The test was not performed to a standard test guideline, although the method was similar to the OECD 212 (Fish, short-term toxicity test on embryo & sac fry stages). The study was not to GLP, but assessed as validity two by the registrant due to being published in a peer reviewed journal, and having analytical support and a clear method. Nominal concentrations were: 0, 500, 1000, 1500, 2000, 3000 and 4000 mg/l. Measured concentrations were significantly lower, being around 33-48% of the nominal, although the reasons for the difference are not explained in

the RSS, nor is it clear when the analysis was conducted. Two replicates at each concentration were run and flow-through conditions (5 l/day) were used in the test. The study endpoint was body deformation only, with the 120-h NOEC being 332 mg/l and the LOEC 560 mg/l based on measured concentrations.

According to the REACH endpoint guidance (R.7B), the embryo & sac fry test is less sensitive than the FELS study (OECD 210) but can be used to provide a long-term fish NOEC for substances with log Kow <4. In principle this would include TBA which has a log Kow of 0.32. African catfish are not listed as a recommended species in the OECD 212 test guideline. In addition, the guideline states that the test should run until the end of the sac-fry stage, defined as when the yolk sac is absorbed. This study was terminated 96 hours after hatching and it is not clear if this was arbitrarily chosen or did cover the entire sac-fry stage. Some species recommended in the OECD guideline only need four days post hatch, although their embryo stage durations are longer. The evaluating Member State notes that the duration of this study is only one day longer than a standard acute fish toxicity study.

In the OECD 212 guideline durations for the embryo and for the larval (sac-fry) stages vary depend on the fish species tested, and each may be up to 30 days long. Catfish are not listed as a test species in the OECD 212 guideline and so it is not known whether such rapid embryo and sac fry stages mean that it is a less sensitive species.

In the REACH guidance R7.8.4.1 and the OECD test guideline that the 212 test is less sensitive than the OECD 210 Fish Early Life Stage (FELS) test. Therefore if the test using Catfish is less sensitive than the standard species for the 212, potentially the results will be even less sensitive than would be obtained from a FELS test.

Overall the short duration of the egg and sac-fry stages for Catfish could mean lower sensitivity compared to OECD 212 fish species. Therefore a PNEC derived from the test may not be adequately protective.

The OECD 212 test guideline lists the following endpoints for use in statistical analysis: mortality, number of healthy larvae at test completion, time for start/completion of hatching, number of larvae hatching each day, length and weight of surviving animals, larvae exhibiting unusual behaviour, number of deformed/larvae. By comparison, the study reported non-viable eggs, dead larvae, deformation and percentage of healthy larvae at test completion. This means a significant number of the more subtle and potentially sensitive endpoints required by the OECD 212 test guideline were not reported.

Overall the eMSCA is not satisfied that the test provides a true measure of long-term toxicity for this substance given the points made above. However, given a chronic toxicity to invertebrates study is available and a relevant PNEC does not result in aquatic risks, the endpoint is not considered further at this time.

7.8.1.2 Aquatic invertebrates

Short-term toxicity to aquatic invertebrates

Key study: OECD 202 using *Daphnia magna* 48-h EC_{50} > 933 mg/l Unpublished (1994b)

Supporting study DIN 38412, Part II using *Daphnia magna* 48-h EC_{50} = 5504 mg/l (Kuhn et al, 1989)

Long-term toxicity to aquatic invertebrates

Key study: OECD 211 using *Daphnia magna* 21-d NOEC 100 mg/l Unpublished (2015)

The March 2016 Registration update includes a semi-static chronic toxicity to *Daphnia magna* study following GLP and OECD Test Guideline 211.

Following a range finding study, the definitive study was a limit test with one exposure concentration at nominally 100 mg/l and sealed vessels with 1 Daphnid per 100 ml test vessel. To prepare the treatment, the test item was warmed to 26°C to ensure it was liquid before direct addition to test media. Solutions were clear and colourless and renewed 3 times per week. Analytical measurement by GC-FID (Gas Chromatography with Flame Ionization Detection) were 83.5 to 98.4% of nominal for fresh solutions and 86.9 to 98.2% of nominal for expired solutions. On this basis, the results were based on nominal. Study conditions were acceptable, validity criteria were met and the study is considered valid.

There were no statistically significant differences between the 100 mg/l treatment and controls for the following endpoints:
- number of live young per adult;
- body length;
- time to first brood: and
- brood size.

In addition, no morphological or behavioral abnormalities were observed.

On this basis the 21-d NOEC for immobilisation and reproduction was considered to be ≥100 mg/l for all endpoints.

7.8.1.3. Algae and aquatic plants

Key study: OECD 201 using *Pseudokirchnerella subcapitata* 96-h E_rC_{50} > 976 mg/l; NOEC = 976 mg/l Unpublished (2003c)

Supporting study: OECD 201 using *Desmodesmus subspicatus* 72-h E_rC_{50} > 1000 mg/l; NOEC = 1000 mg/l Unpublished (1994c)

7.8.1.4. Sediment organisms

Waived

7.8.1.5. Other aquatic organisms

No data available.

7.8.2. Terrestrial compartment

7.8.2.1. Toxicity to soil macro organisms

OECD 207 using *Eisenia fetida*: 14-d LC_{50} > 1000 mg/l; NOEC = 1000 mg/l Unpublished (1994d)

7.8.2.2. Toxicity to terrestrial plants

Waived

7.8.2.3. Toxicity to soil micro-organisms

Waived

7.8.2.4. Toxicity to other terrestrial organisms

No data available.

7.8.3. Microbiological activity in sewage treatment systems

DIN 38412, part 8 (*Pseudomonas* Zellvermehrungshemm-Test) using *Pseudomonas putida*: 16-h EC_{50} >10 g/l; NOEC = 6.9 g/l Unpublished (1994e)

7.8.4. PNEC derivation and other hazard conclusions

7.8.4.1 PNEC water

In the 2013 dossier the registrant derived the aquatic PNEC from the 5-day NOEC in the (non-standard) long-term fish test using an assessment factor of 50 based on long term data for two trophic levels (fish and algae). This resulted in a freshwater aquatic PNEC of 6.64 mg/l.

Given the long-term fish test's short duration, use of a non-standard fish species and limited number of endpoints compared to the standard test guideline, the eMSCA was unconvinced that the Catfish study was adequate to fulfil the long-term fish endpoint.

On this basis, the eMSCA considered the aquatic PNEC should be revised and it was noted that using the available acute *Daphnia* toxicity data (EC_{50} = 993 mg/l) and an assessment factor of 1000, the freshwater aquatic PNEC would be 0.993 mg/l. Based on the environmental risk assessment, a chronic toxicity to invertebrates study was requested.

As described in section 7.8.1.2. above, a valid 21-day NOEC of 100 mg/l is available for invertebrates. In the March 2016 CSR the updated freshwater aquatic PNEC is 2 mg/l based on this NOEC and an assessment factor of 50 based on valid long term data for trophic levels (invertebrates and algae).

Considering an additional factor of 10, the marine aquatic PNEC is 0.2 mg/l.

7.8.4.2. PNEC sediment

The registrant has calculated the following PNEC sediment values based on equilibrium partitioning:

PNEC sediment (freshwater) 8.04 mg/kg dry weight
PNEC sediment (marine) 0.804 mg/kg dry weight

7.8.4.3. PNEC soil

The registrant has calculated a terrestrial PNEC of 1 mg/kg dry weight.

7.8.4.4. PNEC sewage treatment plant

The registrant has calculated a STP PNEC of 690 mg/l.

7.8.4.5. PNEC oral (secondary poisoning)

The registrant has calculated a PNECoral of 88,700 mg/kg food which applied an assessment factor of 30 to the oral DNEL. As the logKow is <3, the eMSCA has not reviewed the PNECoral.

Table 10 PNECs

PNEC DERIVATION AND OTHER HAZARD CONCLUSIONS		
Hazard assessment conclusion for the environment compartment	**Hazard conclusion**	**Remarks/Justification**
Freshwater	PNEC aquatic: 2 mg/l	Assessment Factor: 5 *Daphnia magna* 21-d NOEC 100 mg/l
Marine water	PNEC aquatic: 0.2 mg/l	Assessment factor: 500 *Daphnia magna* 21-d NOEC 100 mg/l
Intermittent releases to water	PNEC intermittent: 9.33 mg/l	Assessment factor: 100 *Daphnia magna* 48-h EC_{50} 933 mg/l
Sediments (freshwater)	PNEC sediment: 8.04 mg/kg dry weight	Equilibrium partitioning method
Sediments (marine water)	PNEC sediment: 0.804 mg/kg dry weight	Equilibrium partitioning method
Sewage treatment plant	PNEC STP: 690 mg/l	Assessment factor: 10 16-h NOEC 6.9 g/l
Soil	PNEC soil: 1 mg/kg dry weight	Assessment factor: 1000 *Eisenia fetida*
Air	A PNEC is not available	
Secondary poisoning	PNEC oral: 88,700 mg/kg food	Assessment factor: 30 Oral DNEL. As the logKw is <3, the eMSCA has not reviewed the PNEC oral.

7.8.5. Conclusions for classification and labelling

TBA has a harmonised classification (603-005-00-1) which does not include an environmental classification. In addition it is not self-classified for the environment.

On the basis of available data, the eMSCA agrees that TBA should not be classified for the environment.

7.9. Human Health hazard assessment

TBA was prioritised for Substance Evaluation due to concerns for carcinogenicity and genotoxicity; as such all information relating to these endpoints was evaluated (including repeated dose information).

The DNEL values and the absorption values used by the registrants were also evaluated to ensure that TBA is adequately controlled.

A detailed evaluation of the reproductive and developmental toxicity sections was carried out, as was a screen of all the other endpoints to ensure there were no additional potential concerns.

Unpublished studies available in the registration dossier are not referenced in this document.

7.9.1. Toxicokinetics

Toxicokinetic information on TBA is available from four studies available in the registration dossier and one study presented in the developmental section. These studies have been included to inform on the extent of absorption and fate of TBA.

7.9.1.1 Non-human information

Table 11: Overview of experimental studies on absorption, metabolism, distribution and elimination

Method	Results	Remarks	Reference
Toxicokinetic study Dermal route OECD guideline 417 Rat (Sprague-Dawley) Males 4/group Exposure regime: single 6-hour exposure Semi-occlusive Doses/conc.: approximately 2 MBq/kg, 7.5 mg/cm^2 TBA Immediately after application, each rat was housed in a metabolism cage. Each group of rats was sacrificed at the following time points and the amount of radioactivity determined, 6 h, 24 hours, 72 hours Values expressed as Mean total recovery of radioactivity (as percentage of radiochemical dose)	TERMINAL SACRIFICE AT 6 HOURS Total recovery: 97.8 % Absorption: 6.2 % (0.25 % urine, 0.01% faeces, 0.03 % cage wash, 0.9 % tissues, 4.98 % volatile organic material) Concentration of radioactivity in blood 6 hours after application: 3.25 µg eq/g TERMINAL SACRIFICE AT 24 HOURS Total recovery of radioactivity: 95.3% Absorption: 9.3 % (0.84 % urine, 0.01 % faeces, 0.11 % cage wash, 0.47 % tissues, 7.89 % as volatile organic material) Concentration of radioactivity in blood 24 hours after application: 2.80 µg eq/g TERMINAL SACRIFICE AT 72 HOURS Total recovery of radioactivity: 97.3% Absorption: 10.7 % (1.14% urine, 0.03% faeces, 0.1 % cage wash, 0.12 % tissues, 9.28 % volatile organic material) Concentration of radioactivity in the blood 72 hours after application: 0.7 µg eq/g	Key study Test substance: TBA	Unpublished (1998)
Pharmacokinetics Oral Gavage C57BL/6J mice	Absorption and distribution Complete within 1.5 hours Peak concentrations of 13 mM and average concentrations 8 mM	Supporting study Test substance: TBA	Faulkner, Weichart, Hartman and Hussain (1989)

6 females/group – 10.5 mmoles/kg (10 ml/kg of a 10 % solution in tap water) TBA or water for every 12 hours for 5 doses 24 hours after last dose, both groups were given another dose of TBA and blood samples taken at set timepoints up to 12 h.	Elimination Complete after 12 hours and occurred at a rate of 1.19 ± 0.18 mmoles/L/hr in water treated animals and 1.28 ± 0.29 mmoles/L/hr		
PBPK modelling A model structure, based on previous models, was used to predict the metabolism of TBA. The results of the model were compared with the blood, liver and kidney concentrations in female and male F344 rats exposed by inhalation to 250, 450 or 1750 ppm for 6 h/day for either 1 day or 8 days. These rats were killed at 2, 4, 6 and 8 hour after exposure and blood, liver, kidney were collected for analysis of TBA	In the model the extent of absorption via the inhalation route was set at 60 % (based on considerations for volatile substances outlined in Medinsky *et al* (1993)). The model compared well with the blood, liver, and kidney concentrations measured in female rats following both single and repeated exposure to TBA.	key study Test substance: TBA	Leavens TL and Borghoff SJ (2009)
Metabolism study Rats: Fischer 344 Oral gavage 250 mg/kg bw of [^{12}C]-or [^{13}C]-TBA administered to 3 male rats Urine samples were collected in 24 h intervals for 48 h. All	^{13}C NMR identified the following urinary metabolites (these were confirmed by mass spectra): 2-methyl-1,2-propanediol, 2-hydroxyisobutyrate and tertiary butyl alcohol sulphate (presumed), TBA and its glucuronide Although no quantification was attempted, a comparison of intensities of the signal strength revealed that the presumed tertiary	Key study Test substance: TBA	Bernauer U, Amberg, A, Scheutzow D, Dekant W (1998)

animals were individually housed and kept in metabolic cages for 72 h	butyl alcohol sulphate was the major metabolite.		
Pharmacokinetic investigation Intravenous administration Similar to EPA OPPTS 870.7485 Rats: F344 Doses/conc.: 37.5, 75, 150 or 300 mg/kg bw TBA Single administration Blood was drawn at 5, 10, 30, 40 and 60 min and 4,8,12, 16 and 24 h after TBA administration.	Absorption: not relevant as *i.v.* Distribution: Concentration versus time data suggested a two compartment model fit the available data best. The distribution phase was rapid - $T_{1/2}$ – 3 minutes. Values for the volume of the central compartment (Vc) were small and similar to the volume of the extracellular fluid (~0.25 L/kg) Volume of distribution at steady state (Vss) was also small (~1L/kg) suggesting significant tissue distribution. In male rats, Vss was significantly decreased at the highest dose (25 %); however, in females, Vss remained constant across tertiary butyl alcohol doses. Metabolism: no information on metabolism was presented in this study. Elimination: $t_{1/2}$ – 3.8 hours , apart from the 300 mg/kg bw where the t ½ was 4.3-5 hours This is consistent with the disproportionate increase in Area under the Curve (AUC) with increasing dose, particularly in male rats.	Supporting study Test substance: TBA	Poet et al (1997)

Absorption

Oral

Since TBA is a small, hydrophilic, molecule, absorption is predicted to be extensive. The results of one limited study in mice supports this prediction (Faulkner et al (1989)). On this basis, a default basis of 100 % will be taken forward to risk characterisation.

Dermal

Absorption via the dermal route has been investigated in a standard guideline toxicokinetic study (Unpublished, 1998). In this study, 4 male Sprague Dawley rats per group were exposed to TBA under a semi-occlusive dressing for 6 hours. Following application, each rat was placed

in a metabolism cage in order to collect urine, faeces and volatile materials. After 6 hours, the application site was washed to remove any unabsorbed dose. At time of termination, a blood sample was taken from the tail vein. Radioactive volatile material trapped in the meta-bowl apparatus, represented 4.4–8.4 % and 0.5-0.6 % of the dose at 1 and 6 hours respectively. The nature of the radioactivity in one of the traps at 1 h was TBA and may potentially be the result of evaporation from the application site rather than being expired by the rats. However, as this was not proven this radioactivity was included in the absorption calculations. Overall the dermal absorption of TBA was measured to be between 6-11 %. On this basis, an absorption value of 11 % (obtained at 72 h post exposure) will be taken forward to risk characterisation.

Inhalation

No experimental data are available informing on the extent of absorption via the inhalation route. A value of 60 % absorption was used in a PBPK model to model the pharmacokinetics of TBA (Leavens and Borghoff, 2009). This value takes into account the volatile nature of TBA, which can limit the extent of absorption. This value was used in the model, along with literature values for other parameters and other modifiers (e.g. to take account of the induction TBA metabolism). The model compared well with the blood, liver and kidney concentrations of TBA following single and repeated inhalation exposure (8 day) of TBA (250-1750 ppm per 6 h/day) to female rats (the model for male rats is complicated due to the binding of TBA in the kidneys). On this basis, a value of 60 % will be taken forward to risk characterisation.

Distribution

The distribution of TBA has been investigated in F344 rats (Poet et al, (1997)). In this study, rats were administered 37.5, 75, 150 or 300 mg/kg TBA via intravenous injection and blood samples collected at 5, 10, 20, 30 , 40, 60 min and 4, 8, 12, 16 and 24 hours and analysed. The pharmacokinetic parameters best described a two compartment model. The distribution phase was rapid with a t1/2 of 3 min, whereas the elimination phase t1/2, on the other hand, was 3.8 h for doses < 300 mg/kg and 5 h for a dose of 300 mg/kg.

Comparison of the value for the volume of the central compartment (Vc) and volume of distribution at steady state (Vss) suggested widespread distribution of TBA. The Vss values for males decreased with dose, which may be due to changes in partitioning as a result of the binding of TBA in tissues. A similar effect was not observed in females.

Metabolism

The metabolism of TBA has been investigated in male Fisher 344 rats (3 sex) following oral administration of 250 mg/kg bw TBA (Bernuer et al, 1998). The results suggested a sulphate conjugate of TBA was the major urinary metabolite. Other major metabolites present in the urine were 2-methyl-1,2-propanediol, 2-hydroxyisobutyrate and TBA glucuronide. In this paper, it was postulated that the likely pathway for metabolism of TBA involved oxidation of TBA by Cytochrome P450 to give 2-methyl-1,2-propanediol and then further oxidation to 2-hydroxyisobutyrate.

Following 8 days repeated exposure via the inhalation route, TBA tissue concentrations were found to be lower as compared to a single administration, suggesting TBA induces its own metabolism (Leavens and Borghoff (2009)). This assertion is supported by information presented in toxicokinetic summary of the NTP report on TBA (NTP (1997)) and the increased rate of excretion observed following pre-treatment with TBA (Faulkner et al, (1989)).

Excretion

TBA and/or its metabolites have been shown to be excreted via the urine following oral administration. No information on whether TBA is excreted via the faeces or in expired air is available.

In the study by Poet et al (1997), a two compartment model best described the pharmacokinetics of TBA. Elimination half-lives (t1/2) of 3.8 h for doses < 300 mg/kg and 5 h for doses of 300 mg/kg were derived. The results suggest that elimination of TBA is saturated at higher doses (≥ 300 mg/kg).

7.9.1.2 Human information

Table 12: Summary of the human volunteer study using TBA

Method	Results	Remarks	Reference
Metabolism study One human volunteer TBA: one 5 mg/kg bw gel capsule of [13C]-TBA Urine collected in 12-h intervals for 48 h and analysed by 13C NMR	^{13}C NMR identified the following urinary metabolites (these were confirmed by mass spectra): The urine showed the presence of TBA, TBA glucuronide, 2-hydroxyisobutyrate and 2-methyl-1,2- propanediol, plus another metabolite that could not be elucidated. TBA sulphate was only present in trace amounts. 2-hydroxyisobutyrate was the major metabolite based on signal intensities.	Key study Test substance: TBA	Bernauer U, Amberg, A, Scheutzow D, Dekant W (1998)

Toxicokinetic information in humans is limited to a single study, where one human individual received an oral dose of 5 mg/kg bw TBA. Analysis of the urine suggests the same metabolites were present in humans as in rats.

7.9.1.3 Summary and discussion on toxicokinetics

Animals

Limited information on oral absorption is available. However, the structure and physico-chemicals properties suggest absorption will be extensive. Therefore, 100 % absorption is assumed. A guideline dermal toxicokinetic study is available and indicates uptake of TBA is 11%. An absorption value of 60 % has been derived for the inhalation route.

TBA is expected to be well and rapidly distributed (particularly to well perfused organs). Studies have shown that TBA is metabolised to 2-methyl-1,2-propanediol, 2-hydroxyisobutyrate, TBA sulfate and TBA glucuronide.

Excretion of TBA and/or its metabolites has been shown via the urine (no other route has been investigated). Elimination of TBA appears to saturate at doses ≥ 300 mg/kg.

Humans

Information in humans is limited to a metabolism study in one human volunteer. In this study, 13C NMR analysis of the urine confirmed the same metabolites were present in humans as in rats.

7.9.2. Acute toxicity and Corrosion/Irritation

These endpoints were not an original concern and have only been included for information.

7.9.2.1 Acute toxicity

Non-human information

Oral

Three studies are available; one study is reported as being similar/equivalent to guideline, whereas the other two are non-guideline. The results of all studies suggest the LD50 is > 2000 mg/kg. Clinical signs (including piloerection, ataxia, and decreased muscle tone at the lowest dose level) were reported at all dose levels. A LOAEL of 1950 mg/kg was derived. No detailed evaluation has been carried out and no additional concerns noted.

Inhalation

TBA has a harmonised classification as Acute tox. 4 (H332).

One acute toxicity inhalation study is available in the registration dossier (reported as similar/equivalent to guideline). This reports an LC50 > 10000 ppm/4h (single dose level; approximately 30 mg/L). Clinical signs (ocular discharge, prostration, ataxia and generalised weakness) were observed as were focal areas of redness on the lungs.

Dermal

The results of the one acute dermal toxicity study (reported as equivalent/similar to guideline) suggest the LD50 is > 2000 mg/kg (limit test). Effects included clinical signs (ataxia, prostration and ocular discharge) and moderate skin irritation. No detailed evaluation has been conducted and no additional concerns noted.

Other routes

Information is available from a toxicokinetic study (Poet et al, 1997; see section 5.1.1 for more details) on the acute toxicity of TBA via the intravenous route. No deaths were observed following intravenous administration of 300 mg/kg bw TBA suggesting the LD50 is > 300 mg/kg bw.

Human information

No information available

Summary and discussion of acute toxicity

TBA does not meet the criteria for classification via the oral or dermal routes.

TBA has a harmonised classification as acutely toxic via the inhalation route: Acute tox. 4 (H332).

TBA has also been classified as STOT-SE 3. According to the registrants' dossier this classification was based on the presence of central nervous clinical signs during and immediately after exposure by all three routes of exposure. However, according to the Annex VI entry to the CLP this classification was imposed due to the possibility of respiratory irritation not narcotic effects.

7.9.2.2 Irritation

This endpoint was not an original concern and has only been included for information.

Skin

The results of a non-guideline skin irritation study (that appears to have been conducted to generally accepted methods) suggest TBA does not meet the classification as a skin irritant. No detailed evaluation has been carried out and no additional concerns noted.

Eye

One non-guideline study is available. This was a modified-draize study making evaluation of the results difficult due to the non-standard scoring system employed. However, since TBA has a harmonised classification for eye irritancy (Eye Irrit. 2 H319) this is not considered of concern.

Respiratory tract

No specific studies have been conducted. TBA has a harmonised classification for respiratory irritation (STOT-SE3 (H335)); however, the basis for this classification is unclear from the information available.

Summary and discussion of irritation

TBA does not meet the classification criteria as irritating to skin.

TBA has a harmonised classification as an eye irritant: Eye Irrit. 2 (H319)

TBA has a harmonised classification for respiratory irritation: STOT-SE3 (H335)

7.9.2.3 Corrosivity

The available information suggests TBA is not corrosive. No additional concerns noted.

7.9.3. Sensitisation

Skin

The skin sensitisation potential of TBA has been investigated in a guideline study (OECD 406; guinea pig maximisation test). The results of the study suggest TBA is not a skin sensitiser. No detailed evaluation was conducted. No additional concerns noted.

Respiratory system

No information available.

Summary and discussion on sensitisation

TBA does not meet the classification for sensitising to the skin. No information is available on its potential to cause respiratory sensitisation.

7.9.4. Repeated dose toxicity

This endpoint is not the primary focus of the evaluation and has only been evaluated to provide information on the toxicological profile of the substance and to decide which values should be taken forward for DNEL derivation.

7.9.4.1 Non-human information

Information on the repeated dose toxicity of TBA is available from a suite of NTP studies: 13-week drinking water studies in both rat and mouse; 18-day inhalation studies in both rat and mouse and 90-day inhalation studies in both rat and mouse. The study reports were downloaded from the NTP website and evaluated. In addition, repeated dose toxicity information is available from the carcinogenicity studies and reproductive toxicity studies. The information is summarised in the table below.

Table 13: Summary of repeated dose studies

Method	**Results**
13-week (drinking water) F344/N rats 10/sex/dose 0, 2.5, 5, 10, 20 and 40 mg/ml equivalent to 230, 490, 840, 1520 or 3610 mg/kg bw/day in males and 290, 590, 850, 1560, 3620 mg/kg bw/day in females NTP (1995)	**40 mg/ml** *Mortality:* All males, 4 females *Bodyweight:* 21 % ↓ bodyweight (females), 44 % ↓ bodyweight gain (females) *Clinical chemistry and haematology:* 40 % ↑ alanine aminotransferase, 79/80 %↓ urine volume (males/females) compared to controls *Organ weights:* 36/81 % ↑ abs/relative kidney weight (females), 9/41 % ↑ abs/relative liver weight (females) Kidney nephropathy (males/females) and hyaline droplets (males), transitional epithelial hyperplasia and inflammation of urinary bladder (males/females), grossly visible calculi (males) **20 mg/ml** *Bodyweight:* 17 % ↓ bodyweight (males), 39 % ↓ bodyweight gain (males) *Clinical chemistry:* ↑ sorbital dehydrogenase 75/75 % ↓ urine volume (males/females) *Organ weights: :* 39/40 % ↑ abs/relative kidney weight (females), 26/54 % ↑ abs/relative kidney weight (males), 15/16 % ↑ abs/relative liver weight (females), 31 % ↑ relative liver weight (males)

	↑ mineralisation of the kidney (males), kidney nephropathy (males/females) and hyaline droplets (males), transitional epithelial hyperplasia and inflammation of urinary bladder and grossly visible calculi (males) **10 mg/ml** *Bodyweight:* 12 % ↓ bodyweight (males), 17 % ↓ bodyweight gain (males) *Clinical chemistry:* 75/80 % ↓ urine volume (males/females) *Organ weights:* 30/28% ↑ abs/relative kidney weight (females), 16/32 % ↑ abs/relative kidney weight (males), 12/11 % ↑ abs/relative liver weight (females), 20 % ↑ relative liver weight (males) ↑ mineralisation of the kidney (males), kidney nephropathy (males/females) and hyaline droplets (males) **5 mg/ml** *Organ weights:* 16/15 % ↑ abs/relative kidney weight (females), 17/26 % ↑ abs/relative kidney weight (males), 10/9 % ↑ abs/relative liver weight (females), 8 % ↑ relative liver weight (males) ↑ mineralisation of the kidney (males), Kidney nephropathy and hyaline droplets (males) **2.5 mg/ml** 19/17 % ↑ abs/relative kidney weight (females),12/19 % ↑ abs/relative kidney weight (males), 11/9 % ↑ abs/relative liver weight (females) Kidney nephropathy and hyaline droplets (males) A LOAEL of 2.5 mg/ml (230 mg/kg bw) was derived
2-year study Rat (F334/N) 60/dose/sex 10 animals from each dose group were sacrificed after 15 months 0, 1.25, 2.5 and 5 mg/l in males equivalent to 0, 90, 200 and 400 mg/kg bw/day in males	**Non-neoplastic lesions – *pathological findings in kidney summarised in the table below*** **Interim Evaluation** ***10 mg/L (females only)*** ↑ hyperactivity 13 % ↓ bodyweight 22/42 % ↑ absolute/ relative kidney weight 56 % ↓ urine volume, 3 % ↑ urine specific gravity ***5 mg/L*** 11 % ↓ bodyweight (males)

0, 2.5, 5 or 10 mg/L equivalent to 0, 180, 330 and 650 mg/kg bw/day in females NTP (1995)	18/21 % ↑ absolute/ relative kidney weight (females), 20 % ↑ relative kidney weights (males), 37 % ↓ urine volume (females), 1 % ↑ urine specific gravity (females) ***2.5 mg/L*** 8/14 % ↑ absolute/ relative kidney weight (females), 15 % ↑ relative kidney weights (males), ***1.25 mg/L*** No adverse effects **<u>Terminal</u>** ***10 mg/L (females only)*** 21 % ↓ bodyweight ***5 mg/L*** 24 % ↓ bodyweight (males) ***2.5 mg/L*** 17 % ↓ bodyweight (males) ***1.25 mg/L (males only)*** 15 % ↓ bodyweight A LOAEL of 1.25 mg/ml (90 mg/kg bw/day) was derived for general toxicity
13 week study Oral (drinking water) B6C3F mice 0, 2.5, 5, 10, 20 and 40 mg/ml equivalent to 350, 640, 1590, 3940 or 8210 mg/kg bw/day in males and 500, 820, 1660, 6430, 11620 mg/kg bw/day in females NTP (1995)	**40 mg/ml** *Mortality:* 2 males and one female *Clinical signs:* emaciation, ataxia and hypoactivity in males and emaciation in females *Bodyweight and water consumption*: 24/51 % ↓ bodyweight (males/females), 53/50 % ↓ bodyweight gain (males/females), ↓ water consumption for first half of study in males and throughout study in females *Organ weights:* 12/35 % ↑ absolute and relative kidneys (females) *Haematology:* 10.5, 9.8 and 9.6 % ↑ in haemocrit, haemoglobin, erythrocytes in males *Pathology:* hyperplasia of urinary bladder transitional epithelium in all males and 3 females. Chronic inflammation of the bladder in all males and 6 females. Reproductive parameters: Estrous cycle length significantly increased (5 days compared to 3.9 in controls). **20 mg/ml**

	Bodyweight: 14 % ↓ bodyweight (males), 30 % ↓ bodyweight gain (males) *Pathology:* hyperplasia and chronic inflammation of urinary bladder transitional epithelium in six males **10 mg/ml, 5 mg/ml, 2.5 mg/ml** No treatment related adverse effects A NOAEL of 10 mg/ml (1590 mg/kg bw/day) was derived
2-year study Mouse (B6C3F1) 60/dose/sex 0, 5, 10 or 20 mg/ml equivalent to 0, 540, 1040 and 2070 mg/kg bw/day in males and 0, 510, 1020 and 2110 mg/kg bw/day in females NTP (1995)	**20 mg/ml** 12 % ↓ terminal female bodyweight Urinary bladder: chronic inflammation: 37 males (severity 2.4) and 4 females (severity 2) Transitional epithelium hyperplasia: 17 males (severity 1.8) and 3 females (1.0 severity) Thyroid: Follicular cell hyperplasia: 47 males (severity 2.2) and 18 females (severity 2.1) Liver: 29/59 males with fatty livers **10 mg/ml** Urinary bladder: chronic inflammation: 1 male (severity 1.0) and 0 females Transitional epithelium hyperplasia: 1 male (severity 1.0) and 0 females Thyroid: Follicular cell hyperplasia: 33 (severity 1.7) males and 15 females (severity 1.4) Liver: 8/59 males with fatty livers **5 mg/ml** Urinary bladder: chronic inflammation: 3 males (severity 1.7) and 0 females Transitional epithelium hyperplasia: 3 males (severity 1.7) and 0 females Thyroid: Follicular cell hyperplasia: 28 (severity 1.9) males and 18 females (severity 1.6) Liver: 5/59 males with fatty livers **Controls** Urinary bladder: chronic inflammation: 0 males and 0 females Transitional epithelium hyperplasia: 1 males (severity 2) and 0 females

	Thyroid: Follicular cell hyperplasia: 19 (severity 1.8) males and 5 females (severity 1.2) Liver: 12/59 males with fatty livers A LOAEL of 5 mg/ml (510 mg/kg bw/day) is derived for general toxicity
18-day (inhalation) Vapour (whole body) F344/N rats 5/sex/dose 0, 450, 900, 1750, 3500 and 7000 ppm equivalent to 0, 1385, 2759,5305, 10680 and 21188 mg/m^3 NTP (1997)	**7000 ppm** All animals died on day 2 of the study **3500 ppm** *Bodyweight:* 14/13 % ↓ in terminal bodyweight (males/females), 26 %/34 % ↓ bodyweight gain (males/females) Clinical signs including ataxia, hyperactivity and hypoactivity were observed at ≥ 900 ppm A NOAEL of 450 ppm (1.4 mg/L; equivalent to 1385 mg/m3) was derived
90-day (inhalation) Vapour (whole body) F344/N rats 10 sex/concentration 0, 135, 270, 540, 1080 and 2100 ppm, equivalent to 0, 406, 825, 1643, 3274 and 6369 mg/m^3 NTP (1997)	**2100 ppm** *Clinical signs:* emaciation and hypoactivity at one observation period only *Organ weights*: 10 % ↑ kidney weight (males), 9 % ↑ relative kidney weight (both sexes), 9 % ↑ relative liver weight (females) *Necropsy:* Mild nephropathy observed in all animals from both sexes *Haematology and clinical chemistry*: 5 % ↓ haemocrit, haemoglobin and erythrocytes (males), 9 % ↓ alkaline phosphatase in males, slight ↓ urine pH in both sexes. **1080 ppm** *Organ weight:* 11 % ↑ kidney weight (males), 8 % ↑ relative kidney weight (males), 9 % ↑ relative liver weight (females) *Necropsy:* Mild nephropathy observed in both sexes *Haematology and clinical chemistry:*4-6 % ↓ haemocrit, haemoglobin and erythrocytes (males), 11 % ↓ alkaline phosphatase in males **540 ppm , 270 , 135 ppm** Minimal – mild nephropathy observed in kidneys of animals from both sexes compared to minimal effects in the control. No other toxicological adverse effects observed

	A LOAEL of 135 ppm (0.4 mg/L; equivalent to 406 mg/m3) was derived
18-day (inhalation) Vapour (whole body) B6C3F$_1$ mice 5/sex/concentration 0, 450, 900, 1750, 3500 and 7000 ppm equivalent to 0, 1385, 2759, 5305, 10683 and 21294 mg/m^3 NTP (1997)	**7000 ppm** All animals died on day 2 **3500 ppm** One male died on day 3 *Clinical signs of toxicity:* prostrate from first exposure to day 3. Other effects included hypoactivity, ataxia and rapid respiration. *Organ weights*: 28 % ↑ liver weight (females), 15/24 % ↑ relative liver weight (males/females), 26/29 % ↓ in absolute/relative thymus weight (females) **1750 ppm** *Clinical signs of toxicity:* hypoactivity, hyperactivity, ataxia and urogenital wetness **900, 450 ppm** No treatment related effects observed A NOAEC of 900 ppm (2.8 mg/L; equivalent to 2759 mg/m^3) was derived
90-day (inhalation) Vapour (whole body) B6C3F$_1$ mice 10 sex/concentration 0, 135, 270, 540, 1080 and 2100 ppm, equivalent to 0, 406, 825, 1643, 3274 and 6369 mg/m^3 NTP (1997)	**2100 ppm** 1 male died (week 7) *Bodyweight:* 19 % ↓ bodyweight (females), 24 % ↓ bodyweight gain (females) *Organ weights:* 20 % ↑ relative liver weight (females) **1080 ppm** 5 males died (feed/water problem) *Bodyweight:* 19 % ↓ bodyweight gain (females) *Organ weights:* 9 % ↑ relative liver weight (females) **540, 270, 135 ppm** No treatment related effects A NOAEC of 540 ppm (1.7 mg/L; equivalent to 1643 mg/m3) was derived.

Repeated dose toxicity: oral

Ninety-day studies and information from carcinogenicity studies in rat and mice are available.

Rats

In a 90-day NTP study, groups of 10 male and 10 females F344/N rats were administered 0, 2.5, 5, 10, 20 and 40 mg/ml TBA (equivalent to 0, 230, 490, 840, 1520 or 3610 mg/kg bw/day in males and 290, 590, 850, 1560, 3620 mg/kg bw/day in females) via the drinking water. All males and six of the females in the top dose died during the study. Bodyweight was significantly lower in top dose females (21 %), and in males receiving 20 mg/ml (17 %) and 10 mg/ml (12 %). Water consumption in both sexes dosed ≥ 10 mg/ml was less than control animals, correlating with the decrease in urine volume. A dose-related increase in clinical signs (ataxia, hypoactivity (males), hyperactivity (females and emaciation) were reported (specific details of the effects at each dose were not provided). The kidney, bladder and liver were identified as target organs. An increase in absolute and relative kidney weight was observed in both sexes at all doses (> 12 % absolute weight at 2.5 mg/ml). A dose related increase in the severity of nephropathy (also referred to as chronic progressive nephropathy; CPN) was observed in males from 2.5 mg/ml (see table below – note at the top dose level all males died). This lesion was described in the NTP report as spontaneous background lesion in F344 rats, which was exacerbated by treatment. Exacerbation of this lesion is regarded as relevant to human health (see section 7.9.6.1 for a more detailed discussion). A statistically significant increase in the incidence of mineralisation was observed in males ≥ 10 mg/ml. In females, a dose related increase in the incidence of nephropathy but not of severity was observed from 10 mg/ml[2]. Staining of kidneys (Mallory Heidenhaim and Lee's methylene blue basic fuschin stains) revealed an increase in the presence of hyaline droplets and crystalline structures associated with the hyaline droplets within the renal tubule epithelium and tubule lumina of male kidneys (but not females) of all dose groups. The presence of the hyaline droplets and mineralisation suggests TBA induces α2u-globulin nephropathy in male kidneys; a rat-specific effect not considered relevant to humans. Both types of nephropathy are known to increase kidney weights. As exacerbation of CPN is considered relevant to human health and it is not known whether the increased kidney weights were caused by CPN or α2u-globulin nephropathy, the kidney weight findings cannot be dismissed and are considered relevant for human health.

Effects in the kidney of male/female rats following 90-day administration of TBA

Sex	Concentration/effect	0 mg/ml	2.5 mg/ml	5 mg/ml	10 mg/ml	20 mg/ml	40 mg/ml
Male	Mineralisation	0	0	2 (1.5)	8** (1.4)	4* (1.0)	4* (1.0)
	Nephropathy	7 (1.0)	10 (1.6)*	10 (2.6)**	10 (2.7)**	10 (2.6) **	7 (1.1)
	Hyaline Droplet Accumulation	0	+	++	++	++	0
Female	Mineralisation	10 (1.7)	10 (2.0)	10 (2.0)	10 (2.0)	10 (2.0)	6 (1.2)

[2] According to the NTP publication, nephropathy is a spontaneous lesion in F344/N rats and in a 90-day study usually consists of scattered renal tubules lined by basophilic regenerating tubule epithelium. The increase in severity observed in males was characterised by increased number and size of foci of regeneration. Occasionally a diluted tubule with a protein cast was present in animals with moderate severity.

	Nephropathy	2 (1.0)	3 (1.0)	5 (1.0)	7 (1.0)*	8 (1.0)*	7 (1.0)*

* Statistically significant (P ≤ 0.05), **statistically significant (P≤ 0.01), ++ or + indicates increased accumulation compared to controls, (brackets) average severity of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate – Note these values reflect effects in this study and are not comparable to the extent of the effects observed in the 2-year study.

Absolute and relative liver weights of females at all dose levels and relative liver weights of males at ≥ 5 mg/ml were increased. Apart from increased alanine transaminase activity in top dose females, these increases were not accompanied by any other effects. In the bladder, grossly visible calculi, microscopic inflammation[3] of the lamina propria and hyperplasia of the transitional epithelium was observed in males in the 20 mg/ml group. Inflammation and hyperplasia were also observed in females dosed 40 mg/ml. Due to the effects in the kidney in males, a LOAEL of 2.5 mg/ml (equivalent to 230 mg/kg bw) is derived from this study. This is consistent with the value derived by the registrants.

In a 2-year NTP carcinogenicity study, groups of 60 male and 60 females F344/N rats were administered 0, 1.25 (males only), 2.5, 5 or 10 (females only) mg/ml TBA (equivalent to 0, 90, 200 and 400 mg/kg bw/day in males and 0, 180, 330 and 650 mg/kg bw/day in females) via the drinking water. After 15 months, 10 animals/sex/group were sacrificed and urinalysis, haematological and organ weight (brain, kidney and liver) investigations conducted. At 15 months, females in the top two doses had reduced urine specific gravities and volume consistent with their decreased water intake.

Terminal survival rates were reduced in a dose dependent manner in males (12, 10, 4 and 2 animals from the control to high dose group), whereas survival in females was lower in top dose females only (29, 28, 26 and 17 animals from the control to the high dose group). In males, terminal bodyweight was adversely reduced from the lowest dose (15 %), whereas female bodyweight was only reduced at the top dose (20 %). There was a dose related increase in water consumption in males and a dose-related decrease in females.

In this study, the kidney was identified as the target organ.

At the 15 month interim, relative kidney weights of 2.5 and 5 mg/ml treated males and absolute and relative kidney weights of all treated females were statistically significantly increased compared to controls. Nephropathy was present in all animals, with the severity slightly increased in all exposed groups of males (non-statistically significantly) (see table below). Increased incidence and severity of mineralisation of the kidney was observed in 2.5 and 5 mg/ml treated males, although this increase was only statistically significant at 5 mg/ml.

Effects in the kidney of male/female rats following 15 month and 2-year administration of TBA

Sex	Sampling time and evaluation type	Effect/ concentration	0 mg/ml	1.25 mg/ml	2.5 mg/ml	5 mg/ml	10 mg/ml
Male	15-month - standard	Mineralisation	1 (1.0)	2 (1.0)	5 (1.8)	9**(2.3)	
		Nephropathy	10 (2.4)	10 (2.7)	10 (2.8)	10 (2.6)	
	15-month – extended	Renal tubule hyperplasia	0	0	2	0	

[3] According to the NTP publication, chronic inflammation consisted of increased numbers of macrophages, lymphocytes and plasma cells in the lamina propria. The hyperplasia varied in severity and morphology. In some rats, it was a diffuse lesion consisting of an increased number of layers of mucosal epithelium. In other rats, the lesions were papillary, consisting of simple unbranched fibrovacular cores covered with thickened layers or branches of transitional epithelium projecting into the lumen of the urinary bladder.

Females	15-month - standard	Mineralisation	10 (2.8)		10 (2.9)	10 (2.9)	10 (2.8)
		Nephropathy	10 (1.5)		10 (1.4)	10 (2.0)	10 (1.8)
Males	Terminal – standard	Nephropathy	49 (3.0)	49 (3.1)	50 (3.1)	50 (3.3)*	
		Transitional cell hyperplasia	25 (1.7)	32 (1.7)	36**(2.0)	40**(2.1)	
		Mineralisation	26 (1.0)	28 (1.1)	35 (1.3)	48** (2.2)	
		Mineralisation, linear	0	5* (1.0)	24**(1.2)	46** (1.7)	
		Renal tubule hyperplasia	3 (1.7)	7 (1.7)	6 (2.0)	6 (1.7)	
	Terminal – extended	Renal tubule hyperplasia	12 (2.3)	16 (2.3)	14 (2.2)	23* (2.8)	
	Combined	Renal tubule hyperplasia	14 (2.1)	20 (2.3)	17 (2.2)	25 ** (2.7)	
Females	Terminal – standard	Inflammation, supparative	2 (1.0)		3 (1.3)	13**(1.0)	17**(1.1)
		Mineralisation	49 (2.6)		50 (2.6)	50 (2.7)	50 (2.9)
		Nephropathy	48 (1.6)		47 (1.9)*	48 (2.3)**	50 (2.9)**
		Renal tubule hyperplasia	0		0	0	1 (1.0)
		Transitional epithelium, hyperplasia	0		0	3 (1.0)	17** (1.4)

* Statistically significant (P ≤ 0.05), **statistically significant (P≤ 0.01),, (brackets) average severity of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4= marked.

At termination, a dose related increase in incidence and severity of foci of linear mineralisation was observed in males. This type of mineralisation has been specifically associated with accumulation of α2u-globulin in male rats[4] . The severity of the kidney nephropathy[5] was increased in all female dose groups and in 5 mg/ml treated males when compared to controls. Signs associated with nephropathy (inflammation, mineralisation and transitional epithelial hyperplasia) were also increased from 2.5 mg/ml in males and 5 mg/ml in females. There was no progression of transitional cell hyperplasia to neoplasms.

The incidence of focal renal tubule hyperplasia[6] was increased in all treated male groups (not dose dependently). Renal tubule hyperplasia was also observed in one 10 mg/mL female. Additional male rats with hyperplasia (11, 13, 11 and 19 animals in control through to high dose groups) were identified following examination of further sections of the kidney in male

[4] According to the NTP study, a component of this mineralisation included linear foci in the renal papilla of exposed males (0/50, 5/50, 24/50, 46/50 – control to high dose). These foci consisted of distinctive linear deposits along radiating medullary collecting ducts. The more common pattern of mineral deposits at the junction of inner and outer stripes at the corticomedullary junction was observed in females and control males.

[5] According to the NTP study, nephropathy was characterised as thickened tubule and glomerular basement membranes, basophilic foci or regenerating renal tubule epithelium, intratubule protein casts, focal mononuclear inflammatory cell aggregates within areas of interstitial fibrosis and scarring and glomerular sclerosis.

[6] According to the NTP study, lesions were regarded as renal tubule hyperplasia rather than foci of regeneration observed with nephropathy if there was no thickening of the basement membranes and more disorganisation and crowding, sometimes with stratification of tubule epithelial cells with hyperplastic lesions.

rats. The extent of the hyperplasia was statistically significant increased in males at 5 mg/ml. For further information on these effects and the step sections see Section 5.8.

A LOAEL for toxicity of 1.25 mg/L (equivalent to 90 mg/kg bw/day) TBA was derived, since adverse effects were observed at all doses; in the low-dose group, these comprised effects on bodyweight in males. This value is consistent with those derived by the registrant.

Mice

In a 13-week NTP study, groups of 10 male and 10 female B6C3F1 mice were administered TBA via the drinking water at doses of 0, 2.5, 5, 10, 20 and 40 mg/ml (equivalent to 0, 350, 640, 1590, 3940 or 8210 mg/kg bw/day in males and 500, 820, 1660, 6430, 11620 mg/kg bw/day in females). At 40 mg/ml, 2 males and 1 female died. At this dose, terminal bodyweight was lower in both males (25 %) and females (15%) and was also lower in males of the 20 mg/ml dose group (14 %). Clinical findings included emaciation, ataxia and hypoactivity in males and emaciation in females. The increased estrous cycle length observed in females of this dose was considered a consequence of the toxicity observed and not a specific effect of TBA.

Water consumption was reduced in the top dose, although not throughout the study in males. Slight increases in haemoglobin and haemocrit values at the top two doses are consistent with slight dehydration. The kidney and bladder were identified as target organs. Absolute (12 %) and relative (35 %) kidney weights of 40 mg/L females were increased compared to controls. No nephropathy was noted. In the bladder, hyperplasia of the transitional epithelium was present in all males and three females dosed with 40 mg/ml and in six male mice dosed with 20 mg/ml[7] . Chronic inflammation of the bladder (primarily macrophages, lymphocytes and plasma cells) was observed in six males and six females in the 40 mg/ml and six males in the 20 mg/ml group.

A NOAEL of 10 mg/ml (equivalent to 1590 mg/kg bw/day) can be derived from this study based on the effects on bodyweight and bladder at 20 mg/ml. This value is consistent with the registrant.

In a 2-year NTP carcinogenicity study, groups of 60 male and 60 female B6C3F1 mice were administered 0, 5, 10 or 20 mg/ml TBA (0, 540, 1040 and 2070 mg/kg bw/day in males and 0, 510, 1020 and 2110 mg/kg bw/day in females) via the drinking water. Survival was reduced in high dose males (27, 36, 34, 17 survivors from control to high dose) and as a consequence the interim kill was not conducted in either sex. Survival was similar in females from all treated groups (36, 35, 41, 42 from control to high dose). In the 20 mg/ml group, female bodyweight was 10-15 % lower than control from week 13 and was 12 % lower than controls at the end of the study. Male bodyweight gain was 5 – 10 % lower at various stages during the treatment period. There was no difference at termination. Mean bodyweight of 10 mg/ml treated females was about 6 % lower than controls throughout the study and bodyweight was slightly lower than controls in 5 mg/ml females as well. Water consumption in both sexes was similar to the controls.

In this study the thyroid, urinary bladder and liver were identified as target organs.

[7] According to the NTP summary, the transitional epithelium hyperplasia consisted of an increase in the thickness of the mucosal epithelium and included both diffuse and focal proliferative lesions. The diffuse lesions were generalised with 2-3 fold increase in numbers of layers of epithelial cells in the mucosa. Focal hyperplastic lesions had more epithelial layers and often had a papillary appearance with finger-like projections of epithelium in to the lumen.

In the thyroid a dose-related increase in the incidence of follicular cell hyperplasia was observed in both sexes. The extent of this increase reached statistical significance in all dose groups in males and in the top two dose groups in females.

In the urinary bladder, the incidence of chronic inflammation and transitional cell epithelium hyperplasia was increased at the top dose in both sexes. Similarly, an increase in the number of males with fatty liver was also observed at the top dose.

A LOAEL of 5 mg/ml (510 mg/kg bw/day) was derived for toxicity, since adverse effects were reported at all doses. At the LOAEL, a statistically significant increase in the incidence of follicular cell hyperplasia in both sexes occurred. This value is consistent with that of the registrant.

In addition to the NTP studies, information on repeated dose toxicity is available from a screening study conducted with TBA and showed similar effects as observed in other oral repeated dose studies (see section 7.9.7).

Repeated dose toxicity: inhalation

Information on the inhalation repeated dose toxicity of TBA is available from NTP studies conducted in both rats and mice and for durations of 18 and 90 days.

Rats

In an 18-day NTP study, groups of 5 male and 5 female F344/N rats were exposed to TBA by inhalation at concentrations of 0, 450, 900, 1750, 3500 and 7000 ppm (equivalent to 0, 1385, 2759, 5305, 10680, 21188 mg/m3) for 6 hours a day, 5 days per week. Investigations in this study were similar to the 90-day studies, apart from no haematology, clinical chemistry, urinalysis or sperm morphology/vaginal cytology evaluation was conducted. All animals in the top concentration group died on day 2. No deaths were observed in any other concentration group. Clinical signs including hypoactivity, hyperactivity and ataxia were observed at all concentration levels ≥ 900 ppm (no details on incidence, etc provided). At 3500 ppm, terminal bodyweights were significantly lower than controls in both sexes (> 10 %); concomitantly bodyweight gain was also lower than the controls at this concentration level. A NOAEC of 450 ppm (1385 mg/m3) was determined for this study based on clinical signs observed at 900 ppm. This is consistent with the NOAEC derived by the registrants.

In a 90-day NTP study, groups of 10 male and 10 female F344/N rats were exposed to TBA by inhalation at concentrations of 0, 135, 270, 540, 1080 and 2100 ppm (equivalent to 0, 406, 825, 1643, 3274 and 6369 mg/m^3 [8]) for 6 hours a day, 5 days per week. At 2100 ppm, emaciation and hypoactivity were observed at one observation time point only. No effects on bodyweight were observed. The minor changes in clinical chemistry and haematology were not sufficiently severe to be considered adverse. At the top concentration, absolute kidney weight was increased in males (10%), and was accompanied by signs of mild chronic nephropathy in all males (see table below). In top concentration females, relative liver weight was increased (9 %).

Effects in the kidney of male rats following 90-day administration of TBA

[8] In the repeated dose inhalation studies the mg/m3 equivalents were calculated using the equation on page 26 of http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECD-GD39.pdf using a M.wt of 74.1216 and assuming the studies were conducted at 25 ºC (chamber temperature given as 24-28 ºC for 18-days and 21-27 ºC for the 90-day studies) and 101 KPa atmospheric pressure.

Sex	Concentration/effect	0 ppm	135 ppm	270 ppm	540 ppm	1080 ppm	2010 ppm
Male	Nephropathy	9 (1.0)	8 (1.4)	9 (1.4)	10 (1.6)	10 (1.9)	10 (2.0)

(Brackets) average severity of lesions in affected animals: 1 = minimal, 2 = mild

At the next concentration (1080 ppm), similar effects were noted in the male kidney (11 % ↑ weight and nephropathy) and female liver (9 % ↑ relative weight). At the lower concentration levels, nephropathy was less severe than at the higher concentrations, but still more severe than in the control (nephropathy considered mild in controls). Sections of kidney from male rats in the 0, 1080 ppm and 2100 ppm concentration group were stained by Mallory-Heidernhain method for the presence of tubular hyaline droplets. There were no differences between the controls and treated groups in the number, shape or size of the droplets.

In this study no NOAEC could be identified due to the increase in severity of the chronic nephropathy observed in males of all concentration groups. The LOAEC is the lowest concentration tested 135 ppm (406 mg/m3). This is consistent with the value derived by the registrants.

Mice

In an 18-day NTP study, groups of 5 male and 5 female B6C3F1 mice were exposed to TBA by inhalation at concentrations of 450, 900, 1750, 3500 and 7000 ppm (equivalent to equivalent to 0, 1385, 2759, 5305, 10683, 21294 mg/m3) for 6 hours a day, 5 days per week. Investigations in this study were similar to the 90-day studies, apart from no haematology, clinical chemistry, urinalysis or sperm morphology/vaginal cytology evaluation was conducted. All animals in the top concentration group died on day 2. One male died on day 3 in the 3500 ppm concentration group. In this concentration group, animals were prostrate following the first exposure through to day 3. Clinical signs after this time included hypoactivity, ataxia and rapid respiration. At this concentration, female liver weight was increased as was relative liver weight in both sexes. Thymus weight was lower in females of this concentration (26 % ↓ absolute weight). At the next concentration level (1750 ppm), effects were limited to clinical signs (hypoactivity, hyperactivity, ataxia and urogenital wetness). No toxicologically adverse effects were observed at any other concentration level. The NOAEC for this study was 900 ppm (2759 mg/m3) based on clinical signs at 1750 ppm. This is consistent with the value derived by the registrants.

In a 90-day NTP study, groups of 10 male and 10 female B6C3F1 mice were exposed to TBA by inhalation to 0, 135, 270, 540, 1080 and 2100 ppm (equivalent to 0, 406, 825, 1643, 3274 and 6369 mg/m3) for 6 hours a day, 5 days per week. In the top concentration group, 1 male died in week 7. Five males also died in the 1080 ppm concentration group; however, these deaths were attributed to problems with the water/food and not treatment with TBA. Bodyweight (↓19 %) and bodyweight gain (↓ 24%) were adversely affected in top concentration females, whereas only bodyweight gain (↓ 19%) was affected in the 1080 ppm concentration group. Relative female liver weights were also increased in these two groups (9 and 20 % in females of the 1080 and 2100 ppm groups, respectively); however, as there were no histopathological changes observed, these increases may be secondary to the reduced bodyweight, particularly at the top concentration. No adverse treatment related effects were noted at ≤ 540 ppm. The NOAEC for this study is 540 ppm (1643 mg/m3); at the next concentration level (1080 ppm) there were effects on female bodyweight gain. This NOAEC is consistent with the value derived by the registrants.

Repeated dose toxicity: dermal

No information available

Repeated dose toxicity: other routes

No information available

7.9.4.2 Human information

No information available

Summary and discussion of repeated dose toxicity

The effects of repeated exposure to TBA have been extensively investigated in standard subchronic and carcinogenicity studies via the oral route and subacute and subchronic studies via the inhalation route.

The study NOAELs and LOAELS are summarised below;

Route	Duration	Species	LOAEL/C	NOAEL/C	Reference
Oral, drinking water	90-day	Rat	230 mg/kg bw/day	Not derived	NTP (1995)
Oral, Drinking water	2-year	Rat	90 mg/kg/bw/day	Not derived	NTP (1995)
Oral, Drinking water	90-day	Mice	3940 mg/kg bw/day	1590 mg/kg bw/day	NTP (1995)
Oral, Drinking water	2-year	Mice	510 mg/kg/bw/day	Not derived	NTP (1995)
Inhalation	18-day	Rat	2759 mg/m^3	1385 mg/m^3	NTP (1997)
Inhalation	90-day	Rat	406 mg/m^3	Not derived	NTP (1997)
Inhalation	18-day	Mice	5305 mg/m^3	2759 mg/m^3	NTP (1997)
Inhalation	90-day	Mice	3274 mg/m^3	1643 mg/m^3	NTP (1997)

In rats, for the oral route, sub-chronic and carcinogenicity studies consistently showed the kidney to be the principal target organ for TBA toxicity. The effects in the kidney ranged from increased weight, increased incidence/severity of nephropathy and the presence of hyaline droplet (males only) indicative of α2u-nephropathy (see section 3.6 for a more detailed mode of action analysis). Other effects, seen at higher exposures (40 mg/ml) in the sub-chronic studies included death and bodyweight effects. In the carcinogenicity studies, bodyweight effects occurred in males of all dose levels (≥ 90 mg/kg bw/day) and may be a result of the increased kidney toxicity in these animals. A dose related increase in clinical signs (ataxia, hypoactivity, hyperactivity and emaciation) was observed in the oral sub-chronic study and similar effects observed in the top dose of the chronic study (10 mg/ml). LOAELs of 230 mg/kg bw/day for sub-chronic and 90 mg/kg bw/day for chronic exposure were derived. NOAELs were not derived, since adverse effects were observed at all doses.

Via the inhalation route, in the sub-chronic study similar effects were observed as via the oral route, with the kidney as the target organ. In the sub-acute study, the driving effect was clinical signs (hypo/hyperactivity and ataxia) observed at all doses ≥ 2759 mg/m3. At higher concentrations (≥ 10680 mg/m^3) other effects included deaths and bodyweight effects. A

NOAEC of 1385 mg/m^3 was derived for sub-acute exposure and a LOAEC of 406 mg/m^3 was derived for sub-chronic exposure.

Repeated oral exposure of mice showed them to be less sensitive to TBA than rats. In the sub-chronic study effects were only observed at doses ≥ 20 mg/ml. At this dose level, effects on bodyweight and in the bladder (transitional cell hyperplasia and chronic inflammation) were observed. Deaths, clinical signs, kidney and liver effects were observed at the highest dose (40 mg/ml). A NOAEL was derived for sub-chronic exposure of 10 mg/ml (3940 mg/kg bw/day).

In the carcinogenicity study, effects on the bladder and bodyweight were again observed at 20 mg/ml (highest dose tested). The target organ in this study was the thyroid with a dose-related increase in the incidence of thyroid follicular cell hyperplasia observed from the lowest dose. A LOAEL of 510 mg/kg bw/day was derived from this study.

Via the inhalation route in mice, effects were more varied. In the subacute study, clinical signs (hypoactivity, hyperactivity and urogenital wetness) were observed at doses ≥ 1750 ppm (5305 mg/m^3). Other effects were observed at the highest dose only (7000 ppm or 21194 mg/m^3) and included death, liver weight increase and decreased thymus weight. In the sub-chronic study, no clinical signs were observed, although it is noted that the top dose in this study (2100 ppm) is relatively close to the LOAEL for these effects in the 18-day study and may reflect experimental variation. In the sub-chronic study, reduced bodyweight was the lead effect and was reduced from 1050 ppm (3274 mg/m^3). A NOAEL of 900 ppm (2759 mg/m^3) was derived for sub-acute exposure and 540 ppm (1643 mg/m^3) for sub chronic exposure.

7.9.5. Mutagenicity

TBA was originally prioritised because of concerns for mutagenicity, since a positive result in an Ames test was reported. On this basis, the following original papers were requested and evaluated: Williams-Hill et al (1999), McGregor et al (2005) and McGregor et al (1988). In addition, a translation of the Tang et al (1997) paper was provided by the registrants. The NTP summaries (which include details on methodology and tables of results) of the following studies were used for the evaluation: Zeiger et al (1987) and Galloway et al (1987). It was not possible to gain access the results of an unpublished study (Unpublished (1979)). However, since the results of this study are consistent with those from other studies, this was not considered an issue.

7.9.5.1 Non-human information

In vitro data

Table 14: Summary of *in vitro* data

Method	Results	Remarks	Reference
Ames Equivalent to OECD 471 (Deviations: Only four strains tested) *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 0-10,000 *µg/plate*	+S9: Negative - S9: Negative Pre-incubation method Positive controls included	Key Study Test Substance: TBA	Zeiger *et al* (1987)

Ames OECD 471 *S. typhimurium* TA102 Dose range 0-3.75 mg/plate	+S9:Positive Pre-incubation method Vehicle (2,2,-trifluoroethanol) At 2.75 mg/plate there was the greatest number of revertants. At higher doses the number of revertants decreased in a dose dependent manner. No information on cytotoxicity and no negative control information included	Supporting study Test Substance: TBA	Williams-Hill *et al* (1999)
Ames OECD 471 *S. typhimurium* TA102 Dose range 0-5000 µg/plate	+S9: Negative - S9: Negative Tested in two laboratories, using two different methods (pre-incubation and plate incorporation) and two different solvents (DMSO or water)	Key Study Test Substance: TBA	McGregor *et al* (2005)
Ames Method of Ames *et al* (1975) *S. typhimurium* TA 1535, TA 1537, TA 98, TA 1538 and TA 100 Dose range : 10-1000 µg/plate	+S9: Negative - S9: Negative Pre-incubation and plate incorporation assay No information on negative or positive control incidences provided, although apparently included	Reliability 4 Supporting study Test substance: TBA	Unpublished (1979)
Mammalian Cell gene mutation assay (mouse lymphoma assay) Equivalent to OECD 476 0-5000 *µ*g/ml	+S9:Negative - S9: Negative* *Slight increase (1.6-fold) in mutation frequency at top dose (5000 *µ*g/ml). Positive controls included and expected responses observed	Key study Test substance: TBA	McGregor *et al* (1988)
Chromosome Aberration study CHO cells Similar to OECD 473 (Deviations include use of single cultures, 9 – 9.5 hr exposure to test substance in the absence of S9 and 2 hr exposure in the	+S9:Negative - S9: Negative A statistically significant increase was observed at the top dose of one trial without S9 (6 % of cells had aberrations compared to 0% in the controls). This increase is not considered biologically relevant. No increase was observed in the second trial; however, due to severe toxicity,	Key study Test Substance: TBA	Galloway *et al*, (1987)

presence, no repeat of the without S9 for a longer exposure period, only 100 metaphases scored) 0-5000 µg/ml	only 13 metaphases were scored at the top dose in this trial. Positive controls included and behaved as expected		
Sister Chromatid exchange Equivalent to OECD 479 (deviations: only one culture per dose) 0-5000 *µ*g/ml	+S9: Negative -S9: Negative A weakly positive result was observed in one trial without S9; however, the result of the second trial was negative Positive controls included	Supporting study Test Substance: TBA	Galloway *et al*, (1987)
DNA damage/repair (comet assay) No guideline followed 1-30 mMol/L Human Leukemia (HL 60) cells	-S9: Positive A dose dependent increase in the percentage of DNA damage was observed. However, there are a number of methodological and reporting issues that cast doubt on the reliability of this result.	Supporting study Test substance: TBA	Tang *et al* (1997)

The *in vitro* genotoxicity of TBA has been investigated in four Ames tests, a mammalian cell gene mutation assay (mouse lymphoma assay), a chromosome aberration study, a sister chromatid exchange study and a comet assay. The majority of studies were non-guideline, but were conducted using methods considered equivalent to guideline.

The majority of the Ames tests were negative. A positive result was reported in strain TA 102 in the presence of metabolic activation in the published Williams-Hill et al (1999) study. In this study, the number of revertants increased in a concentration-dependent manner up to approximately 2.75 mg/plate. At this concentration, the number of revertants had almost doubled. At higher concentrations the number of revertants decreased concentration-dependently. Although the authors concluded TBA was mutagenic, no criteria as to what constituted a positive result were included in the paper and no information on toxicity was provided, which is imperative given the very high concentration level employed. In follow up, the mutagenic potential of TBA in strain TA102 was investigated in a study conducted at two independent laboratories (McGregor et al (2005)). The first laboratory used a pre-incubation method to restrict evaporation, whereas the second used the plate incorporation procedure (as was used in the Williams-Hill study). Both studies were conducted in accordance with OECD 471 in GLP-accredited laboratories. No significant increase in the numbers of mutations per plate was observed in either laboratory. Given the increase reported in the Williams-Hill study was less than 2-fold and the results of the follow-up studies showed no significant increase in mutations, TBA is not considered mutagenic in bacteria. The mutagenicity of TBA in bacteria is considered adequately investigated.

The mutagenic potential of TBA has been investigated in mammalian cells in a mouse lymphoma assay (McGregor et al (1988)). In this study, a small concentration-related increase was observed in one study conducted in the absence of S9; however, the increase was only 1.6-fold at the top concentration (5000 ug/plate) and, as such, is not considered to constitute a positive result. The repeat study did not meet the quality control standards for the assay and

as such has not been included in the evaluation. However, as the result of the first trial was negative, this deficiency is not considered sufficient to warrant a repeat of the study. The results of both trials conducted in the presence of metabolic activation were clearly negative. Overall, TBA does not appear to be mutagenic in mammalian cells *in vitro*.

The results of a chromosome aberration study (non-guideline) and a sister chromatid exchange study have been included in an NTP summary of TBA. These summaries have been used as the basis of this evaluation. A weak positive result was observed in one test of the SCE assay; however, as this result was not reproducible, the overall result of the study is considered negative (in accordance with the criteria outlined in OECD guideline 476). There were a number of deficiencies in the *in vitro* chromosome aberration study; however, a statistically significant increase in the number of cells with aberrations was observed at the top dose in one trial of the chromosome aberration study (6 % of cells had aberrations compared to none in the controls) in the presence of S9. The increase is small compared to the positive control, was not concentration-related, and may have been exacerbated by the absence of aberrations in control cells. On this basis the biological significance of this increase is doubtful. This conclusion is supported by the negative response observed in the *in vivo* micronucleus study (see the table below). Severe toxicity in the repeat trial meant that only 13 metaphases could be scored at the top dose.

A concentration-related positive response was reported in an *in vitro* comet assay following incubation of TBA with HL-60 cells for one hour (Tang et al, 1997). No OECD guideline is available for the *in vitro* comet assay and at the time the methodology is unlikely to have been well developed. There are a number of issues with the reporting and interpretation of the data that reduce confidence in the result. Firstly, the results are presented as % DNA damage; however, it is not clear what this relates to. No information on the % DNA present in the tail or tail length or the number of cells undergoing apoptosis has been reported (a minimum requirement for acceptance of an *in vivo* comet assay by EFSA: http://www.efsa.europa.eu/en/efsajournal/doc/2977.pdf). Furthermore, the results of three separate experiments have been grouped together and no information on the variability of the data is available to determine whether this approach was justified. Finally, there are concerns with the assessment of cytotoxicity (estimated by measuring % LDH released). The authors concluded that TBA was not cytotoxic based on a similar % of LDH released in treated cells (3.62 – 4.49 %) and controls (3.29 %). However, similar levels of LDH release were also reported for other substances in the same paper, but in these cases (due to a comparison to the internal control) the extent (2.79 - 4.15 %) was considered indicative of cytotoxicity, raising doubts over the use of LDH as a measure of cytotoxicity. Overall, the results of this study are not considered reliable. Since a robust *in vivo* micronucleus study is already available, there is insufficient residual concern to warrant a repeat of this study or further *in vitro* investigations.

In vivo data

Table 15: Summary of *in vivo* data

Method	**Results**	**Remarks**	**Reference**
Mouse micronucleus assay B6C3F1 mice, 10 sex/dose Oral (drinking water) 13 week administration Peripheral blood samples	Negative Deaths (3 males and 5 females) were observed in the top dose No effect on NCE/PCE ratio Positive controls included	Key Study Test Substance: TBA	NTP (1995)

0, 10 , 20, 40 mg/L equivalent to 0, 1590, 3940 and 8210 mg/kg bw/day in males and 0, 1660, 6430 and 11620 mg/kg bw/day in females			

One study investigating the potential for TBA to cause cytogenetic damage to peripheral blood cells of mice following 13 week exposure is available. No increase in micronucleus formation was observed following oral administration of very high doses of TBA. No change in the P/N ratio was observed; however, detection of TBA in the blood (see section 7.9.1) suggests the bone marrow will have been exposed. In addition, deaths in the high dose group suggest the maximum tolerated dose was exceeded.

7.9.5.2 Human information

No information available.

Summary and discussion of mutagenicity

There are a number of studies available investigating the mutagenic potential of TBA *in vitro* and *in vivo*. Although a positive response was reported in the Ames test for strain TA102, this result was not replicated in two tests conducted in two independent GLP-accredited laboratories and, therefore, TBA is not considered mutagenic in bacteria. The results of the other *in vitro* studies did not provide any convincing evidence that TBA was mutagenic.

The results of an *in vivo* micronucleus study indicate TBA is not clastogenic or aneugenic.

The genotoxic potential of TBA is considered to have been adequately investigated. Overall, TBA is considered not genotoxic.

7.9.6. Carcinogenicity

TBA was prioritised for Substance Evaluation as treatment related tumours were observed in the rat and mouse carcinogenicity studies.

7.9.6.1 Non-human information

The carcinogenic potential of TBA has been investigated in carcinogenicity studies in both rats and mice (NTP, 1995). As both were NTP studies, the summaries were downloaded from the NTP website and used as the basis of the evaluation.

Carcinogenicity: oral

Rat

In a 2-year NTP study, groups of 60 male and 60 female F344/N rats were administered 0, 1.25 (males only), 2.5, 5 or 10 (females only) mg/ml TBA (equivalent to 0, 90, 200 and 400 mg/kg bw/day in males and 0, 180, 330 and 650 mg/kg bw/day in females) via the drinking water. The non-neoplastic effects are reported in the repeated dose section.

At the 15 month interim termination, a renal tubule adenoma was detected in one male of the 5 mg/L group.

At termination, the incidences of focal renal tubule hyperplasia[9] and adenoma were increased in exposed males and a carcinoma was observed in one 5 mg/ml male. Renal tubule hyperplasia was also observed in one 10 mg/mL female. Due to the effects observed in male kidneys, further sections from all groups were examined. This identified additional male rats with hyperplasia (11, 13, 11 and 19 animals in control through to high dose groups) and renal tubule adenomas (7, 8, 15, 10 from control through to high dose groups). Renal tubule carcinomas were also identified in two 1.25 mg/L males and one 2.5 mg/L male. When the standard and extended evaluations were combined, there was a statistically significant increase in hyperplasia in males at 5 mg/L and of adenoma incidence in 2.5 mg/L males. Inclusion of the adenoma observed in the high dose males at the interim kill leads to a statistically significant increase in adenoma incidence in the 5 mg/L males.

Incidence of neoplastic lesions in male kidneys from a 2-year rat study

Dose (mg/ml)	0	1.25	2.5	5
Interim kill				
Animal no	10	10	10	10
Adenomas	0	0	0	1
Terminal kill – standard and extended investigations combined				
Animal no	50	50	50	50
Renal tubule hyperplasia	14 (2.3)[a]	20 (2.3)	17 (2.2)	25** (2.7)
Renal tubule adenoma	7	7	10	10
Renal tubule adenoma, multiple	1	4	9**	3
Renal tubule carcinoma	0	2	1	1
Renal tubule adenoma and carcinoma	8	13	19	13

a: Average severity of lesions in affected animals: 1=minimal; 2=mild; 3= moderate; 4 = marked.

Although an increase in benign tumour incidence was observed in treated groups, the increase was only marginal. Furthermore, no increase in the incidence of carcinomas was observed. However, a literature search revealed that the tumours had been re-evaluated by a Pathology Working Group (PWG) (Hard *et al*, 2011). This group considered the tumours were treatment related, raising concern for these findings. The results of this re-evaluation of the tumour findings are presented below. The use of contemporary diagnostic criteria by the PWG resulted in the reclassification of two control renal tumours as an amphophilic-vacuolar (A-V) adenoma and an oncocytoma; neither of which is considered to be relevant to test article administration. A lower incidence of tubule adenomas in the control group meant the combined incidence of renal tumours was now statistically significantly higher in all treated groups. One carcinoma was also downgraded to an adenoma in both the low and mid dose group.

Incidence of renal tubule tumour types in standard and step sections of kidney in male rats of the 2-year study as evaluated by the PWG.

Dose (mg/mL)	0	1.25	2.5	5

[9] According to the NTP study, lesions were regarded as renal tubule hyperplasia rather than foci of regeneration observed with nephropathy if there was no thickening of the basement membranes and more disorganisation and crowding, sometimes with stratification of tubule epithelial cells with hyperplastic lesions.

Oncocytoma	2	1	0	0
Amphophilic-vacuolar (A-V) tumour	1	0	1	1
Adenoma	3	9	9	9
Multiple	1	3	9	3
Carcinoma	0	1	0	1
Adenomas and Carcinomas combined	4	13*	18**	12*
All tumour variants	4	14	18**	13

The registrants have argued that the renal tubule tumours are not relevant to humans. This argument is based upon an expert review of the tumours (Unpublished (2001)). This review was not available in the registration dossier during the initial evaluation period so was requested. In summary, this expert review examined the findings from the oral 90-day and chronic studies in both rats and mice and identified two possible causes of the adenomas: α2u-globulin nephropathy or an exacerbation of chronic progressive nephropathy (CPN). The review by the PWG group also concluded that both modes of action were contributing to the tumours observed in the male kidneys of rats treated with TBA. This review is outlined in more detail in the section on CPN below.

α2u-globulin nephropathy

Background

Alpha2urinary-globulin nephropathy is a male-rat-specific effect and, as such, tumours that occur via this mechanism are not considered relevant to human health. Substances that induce α2u-globulin nephropathy do so by reversibly binding to the α2u protein in the renal proximal tubule. This binding prevents the α2u-globulin from being degraded by proteolytic enzymes within phagolysosomes. The α2u-globulin accumulates, resulting in proximal tubule necrosis and compensatory cell proliferation, which increases the likelihood of mutation and potentially tumourgenesis (Borghoff et al, 2001).

Criteria to determine whether a substance causes renal tumours by this mode of action have been developed by the US EPA (1991) and IARC (1998). At the time of the initial evaluation the registrants had not demonstrated in their dossier that these criteria have been met[10], although one mode of action study was summarised in the registration dossier (Williams and Borghoff (2001)). To determine whether this mode of action was feasible, the eMSCA considered the information in the Williams and Borghoff (2001) study, plus that of a related publication (Borghoff, 2001 – referenced in the Williams and Borghoff study), and information from the NTP studies were evaluated and compared to the IARC criteria.

According to IARC, the main criteria that need to be satisfied for this mode of action are:

Criteria	***Comparison with the criteria***
Essential evidence	
Renal tumours occur only in male rats	Satisfied. Renal tumours were not observed in female F344 rats or $B6C3F_1$ mice following chronic exposure

[10] It is noted that this information has been included in the updated dossier

Acute exposure exacerbates hyaline drop formation	Satisfied. An increase in protein droplets was observed in male F344 rats following 10 days of inhalation exposure to 1750 ppm TBA. (Borghoff *et al* (2001))
α2u-Globulin accumulates in hyaline droplets	Satisfied. It was confirmed by Enzyme-linked immunosorbent assay (ELISA) that the accumulating protein observed in the above study was α2u-globulin protein (Borghoff *et al* (2001))
Sub-chronic histopathological changes including granular cast formation and linear papillary mineralization	Satisfied. No specific signs were observed in the 90-day study in rats. However, upon re-evaluation by the PWG, sporadic basophilic tubules containing cellular debris were detected in 5/10 males of the 20 mg/L group, which were considered the precursor of granular casts (Hard, *et al* (2011)). Linear papillary mineralisation was observed in all treated males and increased in incidence and severity with dose in the chronic study. A similar observation was not noted in female rats or mice (NTP 1997 and Hard, *et al* (2013))
Absence of hyaline droplets and characteristic histopathological changes in female rats and in mice	Satisfied. No evidence of hyaline droplet accumulation in mice of either sex or females rats.
Negative for genotoxicity in a battery of tests	Satisfied (see section 7.9.5)
Supportive evidence	
Reversible binding of chemical to α2u-globulin	Satisfied. Application of several biochemical techniques demonstrated that TBA reversibly binds to α2u-globulin (Williams and Borghoff (2001))
Increased and sustained proliferation in P2 segment of proximal tubules in male rat kidneys	Satisfied. BRDU immunohistochemistry-stained kidney sections revealed a statistically significant increase in the labelling index in the renal cortex (where the proximal convoluted tubules are situated) in TBA-exposed male rats, but not female rats (Borghoff et al (2001))
Dose-response relationship between hyaline droplet severity and renal tumour incidence	In the 90-day study, an increased accumulation of hyaline droplet was observed with dose. In addition, via the inhalation route, a statistically significant increase in α2u-globulin concentration in male rat kidney exposed to 1750 ppm (Borghoff et al (2001)). Renal tumour incidence, however, was not as clearly dose related, with the highest incidence observed in the 2.5 mg/ml group.

TBA meets all the essential criteria and the majority of the supporting IARC criteria for α2u–globulin nephropathy. It is possible that failure to see a dose response relationship between hyaline droplet severity and renal tumour incidence may be due to the influence of the CPN (see below). Overall, the available information demonstrates that TBA causes α2u–globulin nephropathy. No further information is required.

Chronic progressive nephropathy

In addition to the above, the registrants have proposed that the kidney tumours may also be caused by CPN. No detailed analysis of the data in support of this mode of action was included in the registration dossier at the time of the initial evaluation and reference was limited to an expert review of the tumours (Unpublished (2001)). Following a literature search, it became apparent more information was available. On this basis, several papers were requested by the eMSCA, evaluated and an assessment of this proposed mode of action conducted.

Background

Chronic progressive nephropathy is a spontaneous renal disease in rats, which can progress to end-stage kidney disease; a prelude to death from chronic renal failure (Hard *et al* (2013)). Males are more predisposed to CPN than females with respect to onset, incidence and severity of progression (Hard *et al* (2013)). When CPN reaches the most advanced stages there is an increased risk for the development of atypical tubule hyperplasia and renal tubule tumours. Some chemicals are thought to exacerbate the progression of the disease. Chemicals exacerbating the disease to advanced grades can be associated with a marginal increase in atypical tubule hyperplasia and renal tubule tumours (Hard, *et al* (2013)).

In 2011, a PWG re-examined information available from the repeat dose studies and chronic studies available for TBA in both sexes.

In their review, the PWG re-examined the following:

- Ninety-day rat NTP drinking water study: all hematoxylin and eosin (H&E)-stained kidney sections from all control and high dose group from the 90-day study, as well as a representative sample of Mallory Heidenhan (MH) stained slides from each of these groups.
- Two-year NTP study: all original H&E-stained slides from control and high dose groups (standard sections); plus:
- All animals with renal tumours in other dose groups; and
- A selection of animals with hyperplasia; and
- All control and high dose females that survived longer than 700-days (29 control and 16 high dose animals); and.
- All renal tumours diagnosed by NTP in the step sections of the kidney were re-examined.

The PWG concluded that CPN was observed in both the 90-day study and the 2-year carcinogenicity study. The severity of CPN was graded on a scale of 1 to 4 (minimal, mild, moderate, and marked) based on percentage of kidney involved, similar to the convention used by the NTP.

In the 90-day study, the PWG review group considered the incidence and severity of CPN was higher in males of the 20 mg/L than the control.

In the 2-year study, the PWG agreed the incidence and severity of CPN was again higher in the high dose group (5 mg/L) than the controls, with a mean grade of 2.8 in the controls (compared to 2.9 reported in the NTP study) and 3.2 in the high dose group (compared to 3.3. reported in the NTP study). Linear mineralisation of the renal papilla was present at levels of severity ranging from mild to moderate in many 5 mg/mL males.

Hyperplasia of the epithelial lining of the renal papilla (transitional cell hyperplasia) was considered to be moderately more prevalent in high dose males than controls and, due to its morphology, was a component of CPN and not a change directly induced by TBA.

The PWG went on to compare the severity of the CPN in rats with renal tumours. The majority of rats with tumours (excluding A-V and oncocytic tumours) had high grades of CPN; the mean grade of high-dose rats with renal tumours was 3.5 compared to 2.9 for rats with no tumours.

Relationship of renal tubule adenomas/carcinomas to chronic progressive nephropathy (CPN) in male rats, demonstrated by NTP and PWG data.

Dose (mg/mL)	0		1.25		2.5		5	
	NTP	PWG	NTP	PWG	NTP	PWG	NTP	PWG
Rats with renal tumours and 100 % grade 3 or 4 CPN	8/8 100%	6/7 86 %	13/14 93%	-	191 100 %	-	12/13 93 %	11/13 85 %
Mean CPN for rats with renal tumours	3.5	3.3	3.6	-	3.7	-	3.4	3.5
Mean CPN for rats without renal tumours	2.9	2.7	2.8	-	2.8	-	3.2	2.9

In slides examined from females, the PWG concluded the severity of CPN (3.2 in the high dose group compared to 1.6 in the controls) was increased in the high dose group compared to controls. An increased incidence of hyperplasia of the lining epithelium of the renal papilla in high dose females was also observed. The hyperplasia was attributed to advanced-stage CPN and not considered a direct proliferative change of TBA. No increase in the number of renal tumours was observed. No linear mineralisation of the papilla was observed in females.

Overall, the PWG group supported the NTP findings that renal tubule adenoma incidence was increased in male rats in the 2-year study. The adenoma incidence was increased in all treated groups, but not dose dependently with the highest incidence in the 2.5 mg/ml group. The PWG also confirmed the presence of hyaline droplets accumulation in the 90-day study supporting the presence of α2u-globulin nephropathy. Over 93% of male rats with renal tumours also had moderate to severe CPN, suggesting a relationship between CPN exacerbation and renal tumour incidence. A relationship between advanced CPN and tumours was considered particularly likely at mid and low dose as no clear evidence of α2u-globulin nephropathy at these doses was noted (and may explain the dose response); whereas at higher doses both modes of action appeared to play a role. Failure to observe tumours in females, even though CPN was also increased, may indicate the importance of the α2u-globulin nephropathy in tumour formation in males.

Human relevance

The authors of the Hard *et al* (2011) paper and the registrants have argued that there is no known human kidney disease that shows the combination or pattern of histopathological

changes that characterise CPN. On the basis of differences in biology and pathology, they have argued there appears to be no counterpart to CPN in humans. Therefore, the registrants consider that an increase in the renal tubule tumours in rats associated with chemical-induced exacerbation of CPN is not relevant to humans.

Discussion

Although CPN appears to be rat-specific, the mechanism by which TBA exacerbates the CPN is unknown. Therefore, it is possible an equivalent mechanism exists in humans and that chemicals that act via this mechanism may result in a different form of renal toxicity. As such, the CPN effects in kidney are considered by the eMSCA to be relevant to humans with regards repeated dose toxicity. With regards carcinogenicity, it is less clear whether chemicals that act via this mechanism (if it exists in humans) would result in renal tumours in humans.

It is noted that the incidence of renal tubule tumours was only increased in male rats, although CPN was exacerbated by TBA in both sexes. On this basis, the fact that TBA also causes α2u-globulin nephropathy would appear pivotal to tumour formation. Moreover, this means that even if the mechanism whereby chemicals exacerbate CPN were to exist in humans, tumour formation would appear unlikely as α2u-globulin nephropathy is not relevant to humans. On this basis the eMSCA agrees with the registrant that the kidney tumours seen in male rats are unlikely to be relevant to humans and considers no further information is needed.

Therefore, the NOAEL for carcinogenicity is the top dose (males) of 5 mg/ml (equivalent to 400 mg/kg bw /day). This value is consistent with the registrant.

Mice

In a 2-year NTP study, groups of 60 male and 60 female B6C3F mice were administered 0, 5, 10 or 20 mg/ml TBA (0, 540, 1040 and 2070 mg/kg bw/day in males and 0, 510, 1020 and 2110 mg/kg bw/day in females) via the drinking water. Survival was reduced in high dose males (43 natural or moribund deaths compared to 33 in the controls). As a consequence the interim kill was not conducted. Water consumption in both sexes was similar to the controls. In the high-dose group, female bodyweight was 10-15 % lower than control from week 13 and was 12 % lower than controls at the end of the study. Male bodyweight gain was 5 – 10 % lower at various stages during the treatment period. There was no difference at termination. No other signs of toxicity were reported.

In this study the thyroid and liver were identified as target organs.

Thyroid gland: The incidence of follicular cell hyperplasia was significantly increased in all treated male groups and in the two top dose female groups. An increased incidence of follicular cell adenoma was observed in top dose females (15 % overall rate compared to 3 % in controls and 0-5% in historical controls). An increased adenoma incidence was also observed in mid, but not top dose males. The NTP study authors postulated that failure to see an increase in the top group males may be due to the reduced survival in this group. However, reduced survival does not appear to fully explain failure to see an increase and therefore, the increase in the mid-dose appears to be due to chance. A follicular cell carcinoma was also observed in top dose males. Overall, there is evidence of an increased incidence of adenomas in the thyroids of females.

Thyroid Histopathological Findings in the 2-year study in mice

Dose (mg/ml)	0	5	10	20
Females				
Follicular cell hyperplasia	19 (1.8)[a]	28 (1.9)	33* (1.7)	47**(2.2)

Follicular cell adenoma	2/58 (3 %)	3/60 (5%)	2/59 (3 %)	9/59 (15 %[11])
First incidence	729T	729T	729T	646
Historical control	3.4 % (0-5 %)			
Males				
Follicular cell hyperplasia	5 (1.2)	18** (1.6)	15*(1.4)	18** (2.1)
Follicular cell adenoma	1/60 (2 %)	0/59 (0 %)	4/59 (7 %)	1/57 (2 %)
First incidence	727	-	616	728T
Historical control	1.7 % (1-2 %)			
Follicular cell, carcinoma	0/60	0/59	0/59	1/57 (2 %)
First incidence				580
Historical control range	0 %			

a: Average severity of lesions in affected animals: 1=minimal; 2=mild; 3= moderate; 4 = marked. T= terminal sacrifice

* Statistically significant (P ≤ 0.05), **statistically significant (P≤ 0.01),

The NTP study authors postulated that although the proliferative lesions may be due to a direct action of TBA, they may also be due to altered hepatic microsomal enzymes resulting in perturbation of hypothalamus, pituitary and thyroid (HPT) axis. However, there is no evidence in the available repeated dose studies consistent with liver enzyme induction, such as hepatocyte hypertrophy.

A common cause of thyroid tumours in rodents is perturbation of the HPT axis. In rodents, perturbation of the HPT axis is caused by an increase in UDP-glucuronyltransferase (UGT) activity. Increased UGT activity results in increased excretion of T4, lowering serum T4 levels (and sometimes T3 levels). To counter this decrease, the pituitary releases more thyroid-stimulating hormone (TSH). Chronic TSH stimulation of the thyroid gland leads to thyroid hypertrophy, hyperplasia and adenoma of the thyroid gland. In 1999 the EU Specialised Experts on carcinogenicity (EU Specialised Experts, 1999) concluded that "certain rodent thyroid tumours mediated by UDP glucuronyl transferase (UDT) induction are not relevant for human health".

Epidemiological data on substances that act via this mode of action (e.g. phenobarbitone) do not show any increased risk of thyroid cancer in humans. Although these substances produce hypothyroidism by decreasing T4 levels, there are quantitative differences in thyroid homeostasis between humans and rats that mean that the decrease in T4 does not result in elevated TSH levels in humans - a key requirement for this mode of action; hence, tumours induced in rodents through this mode of action are of reduced concern for the induction of tumours in humans.

To support this mode of action, several key events should be observed. These are increased UGT activity, changes in thyroid hormone levels, increased thyroid growth and thyroid lesions.

An expert review (Unpublished, 2001b) commissioned by the registrants concluded the adenomas were likely due to perturbation of the HPT axis. However, the report was based on the existing findings from the NTP data and not specific mode-of-action studies. Therefore, the

[11] This was not flagged as being statistically significant in the table of results but was in the text of the discussion and conclusion of the study report.

information reviewed was not sufficient for concluding that the tumours were induced via this mode of action.

In 2009, the registrants commissioned a 3 and 14-day mechanistic study to investigate this potential mode of action (Unpublished, 2009c). The results of this study are summarised below.

Table 16: Summary of the mechanistic study

Method	***Results***	***Reference***
14-day study Mouse (B6C3F1) 15 female mice 0, 2, 20 mg/ml for 3 or 14 days Positive control: 80 mg/kg bw/day phenobarbitone Investigations include: Total P450 content determined. Enzyme activities of EROD, PROD, BROD and Lauric Acid hydroxylation. qPCR of Cytochrome P450 (Cyp1a1, Cyp2b9, Cyp2b10, Cyp3a11), Sulfortransferase (Sulta2, Sultn), UDP-glucuronoyltransferase (Ugt1a1, Ugt2b1, Ugt2b5), Beta-2-microglobulin (control)	*3 days* No clinical signs 20 mg/ml: Mean water consumption ↓ 78 % between day 1-4 20 mg/ml: 18 % ↓ TSH compared to control (non-statistically significantly) No change in mean T3 and mean T4 levels 20 and 2 mg/ml: No change in transcription levels UGPAse isoenzymes *14 days* No treatment-related clinical signs observed 20 mg/ml: water consumption decreased 65 % over entire study period 2 mg/ml: mean water consumption reduced by 8 % between day 1-8 and 12 % between days 8-15. No change in TSH observed at either dose level Mean T3 concentrations ↓ 12% and 13 % at 2 and 20 mg/ml Mean T4 concentrations ↓ 15 and 22 % at 2 and 20 mg/ml Minimal to slight hepatoceullar hypertrophy at 20 mg/ml 20 and 2 mg/ml: no change in transcription levels of UGPase	Unpublished, (2009c)

The results of the 3 and 14-day study indicate that T3 and T4 levels are decreased (by 13 and 22% respectively at the top dose after 14-days) after administration of TBA, which is consistent with the proposed mechanism. However, the transcript levels (measured as a surrogate for enzyme activity) of the three isoforms for UGP-glucuronoyltransferase were not increased at either timepoint and no changes in TSH levels were observed.

It is noted that the positive control, phenobarbitone, increased UDP-glucuronyltransferase transcript levels (by 29-83%) and caused clear decreases in T3 (21%) and T4 (48%); however, no changes in TSH were observed.

It is unclear why no increases in TSH were detected, particularly with the positive control substance. However, as noted in OECD TG 407[12], thyroid histopathological examination can be a more reliable indicator of HPT-axis perturbation than hormone measurements:

"Definitive identification of thyroid-active chemicals is more reliable by histopathological analysis rather than hormone levels"

[12] https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecdtg407-2008.pdf

It is also possible the study duration was too short, given that tumours were only observed after approximately 18-months in the 2 year studies. Therefore, this study is not considered to support or refute the postulated MoA.

Although the mode of action for thyroid tumour induction has not been established, the available evidence indicates that TBA is a non genotoxic mouse thyroid carcinogen, only causing thyroid adenomas at doses in excess of the modern limit dose of 1000 mg/kg/day. Histopathological investigations clearly demonstrate that TBA induces thyroid follicular cell hyperplasia at doses below that causing tumours. Thyroid follicular hyperplasia is consistent with TBA-mediated chronic perturbation of the HPT-axis. As only benign tumours occurred, the increase was only observed in one sex of one species and at the maximally tolerated dose (12 % reduction in bodyweight of females, increased deaths of males), at a very high dose (double the test-guideline limit value), the concern for these tumours in relation to a human hazard is low.

Liver: In males, the overall incidence of hepatocellular adenoma was slightly higher in the mid dose group as compared to control (40/59, 41/60, 44/59, 26/59: control to high dose). A similar pattern was also observed with carcinomas (25/59, 29/60, 35/59, 19/59: control to high dose). Given the lack of dose response and the prevalence of these types of tumours in control B6C3F1 mice, the increased incidence of adenomas and carcinomas is considered to be within normal variability and not treatment related. As noted, liver tumours occur with a very high spontaneous incidence in this mouse strain and the decreased survival rate at the top dose is not considered to have compromised the results.

A NOAEL for carcinogenicity of 10 mg/ml (equivalent to 1020 mg/kg bw/day) is derived based on the increase in thyroid tumours observed in top dose females. This value is consistent with the value proposed by the registrants in their dossier update.

Carcinogenicity: inhalation

No information available

Carcinogenicity: dermal

No information available

7.9.6.2 Human information

No information available

Summary and discussion of carcinogenicity

The carcinogenic potential of TBA has been well investigated in two standard studies: one in rats and one in mice. Two dose-related tumour types were reported in these studies: renal tumours only in male rats, and benign thyroid tumours only in female mice.

In the well-conducted rat study, kidney toxicity (CPN, linear papillary mineralization and focal renal tubule hyperplasia) and an increase in the incidence of renal tumours were observed in males. The increased tumour incidence occurred against a background of α2urinary-globulin nephropathy, hyaline droplet formation and CPN. It is suggested the kidney tumours were due to alpha-2u-globulin nephropathy. However, kidney toxicity was also observed in females (nephropathy in all groups with hyperplasia and CPN observed at the top dose), but without progression to renal tumours. Therefore it is very likely that the male kidney tumours are α2u-globulin-related. Overall, the tumours observed in male rats were likely to be a species-specific effect and not relevant to humans.

In mice, the incidence of thyroid follicular cell hyperplasia was significantly increased in males administered 540 mg/kg/day and above and in the two female groups at doses of 1,020

mg/kg/day and above. An increased incidence of follicular cell adenoma was only observed in top dose females (15 % overall rate compared to 3 % in controls and 0-5% in historical controls) administered an estimated dose of dose of 2,110 mg/kg/day via the drinking water, a dose that is well in excess of the currently accepted limit of 1000 mg/kg/day and that approached the maximum tolerated dose (12% reduction in bodyweight of females, increased deaths in males). At the next dose of 1020 mg/kg bw/d (close to the limit dose of the test guideline), there was not an increase in thyroid tumours in either sex. Given the occurrence of tumours in the thyroid that were benign and only occurred in one sex of one species, at very high doses that approached the maximum tolerated dose, the eMS concludes that the concern for human health is low and that no further information need be requested to clarify it.

In conclusion, information on the carcinogenic potential of TBA in laboratory animals is available from two well-conducted studies, one in rats and one in mice. Oral TBA administration resulted in increased incidences of two tumour types: renal tumours in male rats, which the eMS concludes were male-rat specific and thus not relevant to humans; and benign thyroid tumours in female mice, which occurred only at excessively high doses and via a non-genotoxic mode of action, and which the eMS therefore concludes are of low relevance to humans. Overall, the concern for carcinogenicity has been clarified and no further information is requested.

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

Effects on fertility

Although not identified as an initial concern, reproductive toxicity was evaluated as information on reproductive toxicity is available from a screening study conducted on TBA.

Non-human information

Table 17: Summary of available animal data

Method	Results	Remarks	Reference
Reproductive/developmental toxicity screening study Modified OECD 421 Oral (gavage) Sprague-Dawley Rats 12/sex/dose 0, 64, 160, 400 and 1000 mg/kg bw/day F0 males: dosed 4 weeks prior to mating until termination (9 weeks overall) F0 females: dosed 4 weeks prior to mating until termination (PND 21)	F0 toxicity 1000 mg/kg bw/day *Clinical signs:* CNS toxicity (unresponsiveness/lethargy and ataxia), vocalisation and rapid breathing. Reported as of moderate intensity in males and mild in females *Bodyweight and food consumption:* *Males:* 13 % ↓ bodyweight gain day 0-69*. *Females:* No effect on bodyweight. No effect on pre-mating bodyweight gain; 16 % ↓ in gestation bodyweight gain; 200 % ↑ lactation bodyweight gain. Slightly lower food consumption during lactation	Key Study Test material: TBA	Unpublished (2004)

Offspring: Dosed PND 21 until termination (PND 27) Modifications included: An increased males dosing period to allow for one full cycle of spermatogenesis to be assessed. Sacrifice of pups on Day 21 not PND 4. Administration of TBA to selected pups for one week postweaning Purity 99.6 %	*Organ weight and histopathology:* 30 % ↑ relative kidney weight in males, 15 % ↑ relative male liver weight 400 mg/kg bw/day *Clinical signs:* lower incidence than high dose of findings among females (only) from week 2 to week 4. *Bodyweight and food consumption:* No notable effects on bodyweight or food consumption in either sex *Organ weights:* 15 % ↑ relative kidney weight in males 160 mg/kg bw/day 12 % ↑ relative kidney weight in males 64 mg/kg bw/day 8 % ↑ relative kidney weight in males* Mating and performance No effect on mating performance or number of pregnancies was observed in any treatment group. Slight increase in gestation length to 23 days in 6/12 top dose and 5/12 mid dose dams (< 2 in other groups). F1 *Viability* 1000 mg/kg bw/day 153 pups were born. 6 pups were born still born (2 in controls) a further 32 pups died between days 1-4 (4 in controls). 1 further pup died during the period 1-21 days. Mean litter size was 10 on PND 1 (compared to 15 in control), 74.1 % viability index for pups surviving 4 days (96.4 % in controls) 400, 160 and 64 mg/kg bw/day – No effect on pup viability		

	Bodyweight and food consumption 1000 mg/kg bw/day 10 % ↓ pup weight PND1*, 16 % ↓ pup weight PND7, 8 % ↓ pup weight PND21 Direct treatment to pups had no further effect on bodyweight NOAEL for parental effects was 64 mg/kg bw/day. The NOAEL for reproductive toxicity was 1000 mg/kg. NOAEL for this study for offspring effects is 400 mg/kg bw/day.		

* Non-statistically significant increase

The reproductive toxicity of TBA was investigated in an enhanced guideline reproductive/developmental screening study. In this study, Sprague-Dawley rats (12/dose/sex) were administered, via oral gavage, 0, 64, 160, 400 or 1000 mg/kg bw/day TBA for 4 weeks pre-mating and then until termination: week 9 (males) and PND 21 (females). Selected pups were then administered TBA directly for a period of seven days before termination on PND 27.

At 1000 mg/kg bw/day, F0 toxicity manifested itself as clinical signs (unresponsiveness/ lethargy, ataxia, increased vocalisation and rapid breathing). Bodyweight was mildly affected in males (non significant decreases in male bodyweight from week one) and during the later stages of gestation in females. The only effect on food consumption was a 15 % reduction in females over the first two weeks of lactation. During lactation a large increase in female bodyweight gain was observed. The significance of this finding is unknown. Increases in both liver and kidney weight were observed in males. At 400 mg/kg bw/day, lower incidences of clinical signs (as compared to the high dose) were observed in females, although only transiently (weeks 2-4). Kidney weight was increased in males of this dose level and was the only effect observed at 160 and 64 mg/kg bw/day. This increase was not statistically significant at the lowest dose and, therefore, is not considered adverse.

There was no effect on mating performance or number of pregnancies observed in any treatment group. A slight effect on gestation length was observed; with half the females from the top dose group and almost half the females in the mid dose with a gestation length greater than 22 (all but one was a shift to 23 days). The shift was reported to be within the normal range (21-23 days, although with a distinct node of 22) and is therefore considered a chance finding.

In the F1 generation, there was no effect on the number of implantations per pregnancy; however, significant pup mortality was observed at 1000 mg/kg bw/day. Six out of 153 pups were stillborn with a further 32 pups dying between days 1-4 (the majority were found dead on day 1). The deaths include one total litter loss. The incidence of still born deaths is likely to be within the normal variation for this type of effect and is therefore not considered treatment related. The deaths observed post-parturition were only observed in the presence of maternal toxicity (unresponsiveness/lethargy and ataxia) and are considered likely to be a secondary consequence of this toxicity and not a direct effect of TBA.

In addition to the reduction in pup survival, pup bodyweight gain was also affected in pups treated with 1000 mg/kg bw/day. Direct treatment of the pups for 7 days from PND 21 had no further effect on the pups.

No treatment related effects were noted at any other dose.

The parental NOAEL was 64 mg/kg bw/day based on increased kidney weight in males. The NOAEL for this study for reproductive toxicity is 1000 mg/kg bw/day, the highest dose tested. The NOAEL for this study for offspring effects is 400 mg/kg bw/day based on increased pup mortality (mostly post-natal) at the top dose. This is consistent with the values derived by the registrant for parental and offspring toxicity. A NOAEL for reproductive toxicity was not derived by the registrants.

No multigeneration study is available for TBA. Instead the registrants have provided information on the results of a 2-generation study conducted on **Methyl tertiary butyl ether (MTBE).** This study was conducted via the inhalation route. The following justification for this read-across was provided in the CSR.

"Based *on metabolism studies which demonstrate that methyl tertiary butyl ether (MTBE) is rapidly metabolized to tertiary butyl alcohol in vivo, data from a two-generation study conducted with MTBE are also relevant for evaluation of the developmental toxicity of tertiary butyl alcohol.*"

Information from two studies summarised in the registrant's dossier, support the registrant's argument that TBA is a metabolite of MTBE (Bernauer *et al* (1998)), although the conversion of MTBE would not appear to be complete (Miller *et al*, 1997).

The eMSCA considers that read-across to MTBE has not been sufficiently justified. In particular, no comparison of the toxicological profile of the two substances has been conducted for all endpoints to show the two substances are similar. In addition, no consideration has been given to the potential contribution of the other metabolites of MTBE (e.g. formaldehyde, acetone) to the toxicological profile. As TBA was not prioritised for Substance Evaluation on the basis of reproductive toxicity and since no effects on reproductive function were observed in the enhanced reproductive toxicity screening study, the eMSCA has not evaluated this area further, but acknowledges there is a potential data gap.

Human information

No information available.

Developmental toxicity

No standard guideline developmental toxicity study is available. The registrants have addressed developmental toxicity using information from the reproductive screening study and data from the published literature; an inhalation study in rats and two oral studies in mice.

Non-human information

Table 18: Summary of the available data

Method	Results	Remarks	Reference
Reproductive screening study Oral route	See summary in fertility section above.	Supporting Study Test material: TBA	Unpublished (2004)
Prenatal developmental toxicity	*Maternal toxicity* **5000 ppm**	Key Study	Nelson et al (1989)

Rats; Sprague-Dawley Inhalation (whole body) 15 pregnant control animals, 18 pregnant animals in the 2000 ppm group, 15 pregnant animals in the 3500 ppm and 13 pregnant animals in the 5000 ppm group Exposed 7h/day for days 1-19. Terminated on day 20. The uterus was removed and corpora lutea, resorptions and live foetuses counted. All foetuses were weighed and examined for external malformations. Half foetuses were examined for skeletal malformations and variations Purity ≥ 99 %	*Clinical signs*: unsteady gait (narcosis in preliminary study), impaired locomotor activity *Bodyweight gain and food consumption:* 56 %↓ bodyweight gain*. 22 % ↓ food consumption (statistically significant in first two weeks) **3500 ppm** *Clinical signs*: unsteady gait, impaired locomotor activity **2000 ppm** Unsteady gait. *Developmental toxicity* **5000 ppm** ~ 30 % ↓ foetal bodyweight 2 litters (4 foetuses) reported with "skeletal malformations" (majority rudimentary cervical ribs) [a]compared to 0 (0) in the control; 12 litters (76 foetuses) reported with skeletal variations compared with 10 litters (18 foetuses) in the control; 12 litters (27 foetuses) reported with visceral variations compared to 6 litters (6 foetuses) in the control **3500 ppm** ~ 12 % ↓ foetal bodyweight 2 litters (2 foetuses) reported with "skeletal malformations", 14 litters (53 foetuses) reported with skeletal variations **2000 ppm** 9 % ↓ foetal bodyweight A LOAEL of 2000 ppm is derived for both maternal and developmental toxicity	Test material: TBA	
Pre-natal developmental study Non-guideline Swiss Webster mice Oral (dietary)	**Dams** **1% and 0.75 % TBA** 10 % and 5% ↓ Bodyweight gain compared to controls on GD 20. Maternal sedation **Pups** **1%** ↓ litter number (7 compared to 11 in controls), ↓ pups/litter (5.3 compared to 10.4 in control), ↓	Supporting study Test substance: TBA	Daniel and Evans (1982)

15 animals per group. 45 animals for foster control group. 0, 0.5, 0.75 or 1 % TBA (all groups pair-fed according to the 1 %TBA group) from day 6 to day 20 Within 24 h of parturition, approx. half of maternal animals in each group were replaced with chow fed maternal animals, which had also delivered within 24 h of the treated or control group. A total of eight litters (4 fostered, 4 maintained)/group selected for behavioural studies (righting reflex, negative geotaxis, open field behaviour, cliff avoidance, roto rod performance). The litters each consisted of 7 pups apart from the top dose which only contained four pups	fetal weight (1.10g compared to 1.78 g in control), ↑ stillborn (20 compared to 3 in control) **0.75 %** ↓ litter number (8 compared to 11 in controls), ↓ pups/litter (7.4 compared to 10.4 in control), ↓ fetal weight (1.45g compared to 1.78 g in control), ↑ stillborn (14 compared to 3 in control) **0.5%** ↓ fetal weight (1.66g compared to 1.78 g in control), ↑ stillborn (6 compared to 3 in control) Behavioural effects 1 % and 0.75% Eye opening was delayed to day 16 in 1 % group (4-6 days behind other groups) and roto-rod performance ↓ on days 18-22. Both groups showed delays in cliff avoidance (days 6-10), field behavioural results and righting reflex (days 4-6) with cross-fostered pups fairing better suggesting effect is partly due to maternal exposure. A NOAEL has not been derived for this study		
Pre-natal Developmental toxicity Non-guideline Oral gavage (twice daily) Mice CBA/J or C57BL/6J CBA/J – 7 control animals, 12 treated animals C57BL/6J – 5 control animals, 9 treated animals	No information on maternal toxicity provided CBA/J 39 % resorptions compared to 15 % in the controls. 83 % litters had resorptions compared to 57 % in the controls. 25 % of litters had total resorptions compared to 0 in the control. 61 % live foetuses compared to 85 % in the control C57BL/6J 38 % resorptions compared to 9 % in the controls. 67 % litters had resorptions compared to	Test material: TBA	Faulkner, Wiechart, Hartman and Hussain (1989)

10.5 mmoles/kg (10 mg/kg of a 10 % solution in tap water) every 12 hours from day 6 to day 18 of gestation, equivalent to 778.2 mg/kg bw Uteruses examined for resorptions. Fetuses weighed and half fixed in Bouin's solution for hand razor sectioning; half fixed in Alizarin Red S for skeletal examination Purity: not stated	40 % in the control. 56 % of litters had total resorptions compared to 0 in the control. 44 % live foetuses compared to 91 % in the control A NOAEL has not been derived for this study		

- Estimated from graph, **Not statistically significant, ªAccording to the paper, the majority of reported "skeletal malformations" were rudimentary cervical ribs, which are not generally considered a malformation. *** calculated using default parameters (30 g bodyweight, 3.6 g food consumption).

Rats

Oral

No standard guideline developmental studies are available. Information on developmental toxicity is available from a reproductive screening study, which has been summarised above.

Inhalation

The developmental toxicity of TBA was investigated via the inhalation route (whole body) in a published study considered to be similar to guideline (Nelson et al, 1989). Sprague-Dawley rats (15 control and 18 treated/group) were exposed 7h/day between days 1-19 to 5000, 3500 or 2000 ppm TBA.

The equivalent mg/kg bw/day values have been estimated using the conversion equation in Appendix 1 of OECD guidance document 39 (temp was 24 ºC); a value of 0.34 m^3/kg bw calculated for 7 hour exposure based on the information in table 8.2 of Chapter 8 of ECHA's guidance; and an absorption value of 60 % for TBA. The resulting values were 1239, 2169 and 3098 mg/kg bw/day for the 2000, 3500 and 5000 ppm groups, respectively. These doses are higher than the maximum dose recommended by current OECD guidelines (1000 mg/kg bw/day).

At the top dose, in dams, bodyweight gain and food consumption was lower compared to the controls. Clinical signs included unsteady gait and impaired locomotor activity in the top two dose levels, whereas unsteady gait was observed at the lowest dose level. In foetuses, bodyweight gain was reduced in all dose levels (≥ 9 %) compared to controls. According to the paper there was a slight increase in the number of "skeletal malformations" at the top dose. The vast majority of these "malformations" were rudimentary cervical ribs. The eMSCA considers this type of effect to be a variation rather than a malformation, reducing concern. There were also an increased number of skeletal variations (top two doses) and visceral variations (top dose only) reported. Due to clinical signs in dams and reduced foetal weight at all dose levels, a LOAEL

of 1239 mg/kg bw/day is derived for both maternal and developmental toxicity. This is consistent with the values derived by the registrants.

Mice

Information is available on the developmental toxicity of TBA from two non-standard studies.

Oral

The effect of TBA on development and post-natal behaviour was investigated in a non-standard study in Swiss Webster mice (Daniel and Evans, 1982). These mice were administered with 0.5, 0.75 or 1 % TBA via the diet between days 6 to day 20. Only 4 litters/treatment groups were used in the behavioural investigations.

Equivalent mg/kg bw/day values were estimated using the food consumption and bodyweights of the mice provided in the paper on day 15 of gestation, the resulting doses were 3270, 4521 and 6250 mg/kg bw/day.

Maternal weight gain was adversely affected at 1 % (10 % lower than controls), the top dose. A lesser reduction in bodyweight was observed in the 0.75 % dose group (5 % lower than controls). A dose related reduction in litter size, number, weight and viability was observed. This is consistent with the effects observed in the screening study. At the top two doses (0.75 % and 1.0%) there were also behavioural effects observed in offspring (delayed righting reflex, open field performance differences, reduced roto-rod performance) suggesting that pups at these doses may be developmentally delayed, possibly as a result of the maternal toxicity observed. Given the non-standard nature of the study design, particularly the small group sizes employed, little confidence can be attributed to the findings of the study. No NOAEL has been derived for this study.

The pre-natal toxicity of TBA has been investigated in a limited reported, non-guideline study in two strains of mice (CBA/J or C57BL/6J) (Faulkner *et al*, 1989). The study reported that oral gavage administration of 10.5 mmoles/kg bw (780 mg/kg bw), twice daily between gestation days 6 to 18, led to an increased number of resorptions and a decrease in foetal viability. No information on the dams was provided so it is not possible to assess maternal toxicity. Although developmental effects are reported, the small group sizes, the very high dose levels (1560 mg/kg bw administered in total each day), the limited scope of the study and limited reporting mean little confidence can be attributed to the results. No NOAEL/LOAEL was derived.

Human information

No information available

Summary and discussion of reproductive toxicity

Reproductive toxicity

No effects on fertility were noted in a screening study conducted with TBA.

A multi-generation study is not available for TBA. The registrants have read-across data from MTBE to address this endpoint. As it stands, this read-across has not been adequately justified. However, no effects of concern were noted in the screening study that would warrant further investigation under Substance Evaluation.

Developmental toxicity

Information on developmental toxicity is available from an oral screening study in rats; a published developmental toxicity study (similar to guideline) conducted via the inhalation route and two non-standard published dietary studies in mice.

No malformations were observed in the only good quality study in rat suggesting that TBA is not developmentally toxic (Nelson *et al* (1989)).

At the top dose in the screening study, 6 pups were stillborn (compared to 2 in the control) and a further 32 pups died by day 4 (Unpublished (2004)). At this dose level, maternal toxicity was evident (ataxia, sedation and bodyweight reduction during gestation). It is likely that the deaths observed between days 1-4 may be due to neglect by the dams rather than a direct effect of TBA. Reduced foetal weights were also observed at all doses; the extent of the reduction was considered adverse in the top two doses.

The available studies in mice (Daniel and Evans (1982) and Faulkner *et al* (1989)) are not considered of sufficient quality to provide any useful information on the developmental toxicity of TBA.

Overall, TBA does not appear to be developmentally toxic, but there is insufficient information on developmental toxicity to make a definitive conclusion.

7.9.8. Other effects

Non-human information

Neurotoxicity

The effect of TBA on pup brain development has been investigated in a non-guideline study. The publication has not been requested and the summary is based on the information available within the dossier. No detailed evaluation has taken place.

Table 19: Summary of the animal data

Method	Results	Remarks	Reference
Neurotoxicity – postnatal brain development following exposure to TBA Rat (Long Evans): male/female Surgically implanted feeding cannula. Vehicle: Milk Doses: PND4: 1.44 g/kg bw PND5: 2.16 g/kg bw PND6: 0.6 g/kg bw PND 7: 2.69 g/kg bw Exposure:	Mortality Only 12 TBA pups and 8 control pups completed study. Most deaths caused by cannulation or gastric bloating. No information on number of pups in each exposure group. Blood collection (6 animals): Blood levels of TBA ranged from 33-33 mg TBA/100 ml blood. Average was 48.2 ± 13.1 mg TBA/100 ml. Clinical signs and developmental landmarks: During the period of test material administration, TBA exposed rats were visibly intoxicated and had difficulty performing reflex tests. Greatest impairment after PND 6 and 8, after highest alcohol administration.	Supporting Test material: TBA	Grant and Samson (1982)

PND4-7: animals were provided with test material in milk formula for 20 minutes every 2 hours. Method: Groups of females were mated and allowed to deliver. Twenty-four hours after parturition, litters were culled to 8 pups. Pups remained with dams until day 4 when they were cannulated. The neonatal rats were reared from PND 3-18 using artificial feeding technique. On PND 4-7, one half of the litters received TBA in the milk formula; the other half served as controls. From PND8: animals received formula until sacrifice on PND 18. Examinations: weighed daily and the amount of milk formula adjusted to provide weight gains comparable to dam-reared pups. After daily maintenance following tests conducted: righting reflex, cliff avoidance and negative geotaxis. Checked for teeth emergence, eye opening and ear development On PND 18, blood samples taken after last feed.	On PND8, no TBA-exposed pup completed the cliff avoidance assessment, whereas control animals did On PND8, All TBA-exposed pups took significantly longer in the righting reflex task compare to controls. There were no differences between groups in reflex testing from PND10 through euthanasia. TBA pups displayed signs of withdrawal (full body tremors, rigid extension of body extremities and vocalisations) following the alcohol exposure period. Withdrawal lasted well into PND10. No developmental delays in teeth emergence, eye opening and pinna detachment. Bodyweights: No significant difference compared to controls Organ weights: Statistically significant ↓ brain weight (16 %) and mean weight ratio (16 %) in TBA-exposed pups No effects on liver to BW or heart to BW ratios. Forebrain biochemical analysis: Absolute DNA and protein per DNA were slightly but not statistically significantly lower in the TBA-exposed group. No effect on absolute cholesterol, or cholesterol/tissue weight ratio, myelin density or myelination/arborisation (cholesterol/DNA) per cell Statistically significantly lower (15 %) absolute protein content Hindbrain biochemical analysis Absolute DNA levels were statistically significantly lower (16%) in the TBA-exposed group Cholesterol and total protein were decreased in TBA-exposed groups (not statistically significantly)		

Following sacrifice: Whole brain weighted than forebrain and hindbrain. DNA, protein and cholesterol extracted.	No significant differences in myelin or protein densities, cellular density, cellular size, myelination/ aborisation, or protein per DNA ratio.		

Information on developmental neurotoxicity is available from a non-guideline study in rats (Grant and Samson (1982)). In this study, pups were dosed directly with TBA on PND4-7 of lactation, which in contrast to the OECD guideline No 426 (developmental neurotoxicity study), where pups are exposed via lactation. Confidence in the findings of the study is reduced by the large number of deaths (possible due to dosing) and variable dosage given to the only dose group (between 600- 2690 mg/kg bw/day). Overall, this study provides no information on the potential developmental neurotoxicity of TBA.

Immunotoxicity

Apart from reduced thymus weight observed in one 18-day inhalation study in mice, no effects indicative of immunotoxicity were reported. Overall, TBA is not considered immunotoxic.

Specific investigations: other studies

Studies involving specific investigations (ie. mode of action studies) are summarised and discussed in the relevant sections.

7.9.9. Medical surveillance data

No information available.

7.9.10. Hazard assessment of physico-chemical properties

TBA is a very low melting point (ca 25 °C) substance so at room temperature can exist as an off-white solid block or a colourless liquid depending on the actual temperature.

It is classified as a flammable liquid category 2 based on a flash point study. No other hazardous properties were identified.

The neat substance should be labelled with the following precautionary statements;
P210 Keep away from heat/sparks/open flames/hot surfaces. – No smoking
P403 + P233 Store in a well-ventilated place. Keep container tightly closed.

The registrant recommends the following additional safe handling measures should be taken into consideration when handling the neat substance:

Ground/bond container and receiving equipment.
Use explosion-proof electrical/ventilating/lighting/.../ equipment.
Use only non-sparking tools.
In case of fire: Use dry chemicals, CO2, water spray or alcohol-resistant foam for extinction
Take precautionary measures against static discharge
Do not empty into drains.
Handle and open container with care in a well-ventilated area.

Consumers do not handle neat TBA. The hazardous properties of any consumer products containing TBA should be assessed separately according to the composition of the product supplied.

7.9.11. Derivation of DNEL(s) / DMEL(s)

Overview of typical dose descriptors for all endpoints

Table 20: Available dose-descriptor(s) per endpoint

Endpoint	Study	NOAEL/C	LOAEL/C	Associated effect and remarks
Acute toxicity	Acute oral		> 1950 mg/kg bw	Clinical signs. Not classified
	Acute Dermal		> 2000 mg/kg bw	Clinical signs (only one dose tested). Not classified
	Acute inhalation		> 10 000 ppm	Clinical signs (only one dose tested). Harmful
	Skin irritation	Not irritant		
	Eye irritation	Eye irritant		Information from standard study only
	Respiratory irritation	Respiratory irritant		Not sure what the basis of this classification was.
	Skin sensitisation	Not sensitising		
	Respiratory sensitisation	No information		
Repeated dose toxicity	18-day inhalation (rat)	1385 mg/m3		Clinical signs
	18-day inhalation (mice)	2759 mg/m3		Clinical signs
	90-day inhalation (rat)		406 mg/m^3	Chronic nephropathy in kidneys
	90-day inhalation (mice)	1643 mg/m^3		Reductions in bodyweight gain
	90-day Oral (rat)		230 mg/kg bw/day	Increased kidney weight and kidney nephropathy
	90-day Oral (mouse)	1590 mg/kg bw/day		Reduced bodyweight and effects in the bladder
	Chronic drinking water (rat)		90 mg/kg bw/day	Reductions in male bodyweight
	Chronic drinking water (mouse)		510 mg/kg bw/day	Follicular cell hyperplasia
Mutagenicity		N/A	N/A	Not mutagenic *in vitro* or *in vivo*
Carcinogenicity	Chronic drinking water (rat)	400 mg/kg bw/day		
	Chronic drinking water (mouse)	1020 mg/kg bw/day		increase in thyroid tumours observed in top dose females
Reproductive toxicity	Screening study (rat)	64 mg/kg bw/day		Increased kidney weights in parental animals
Developmental toxicity	Inhalation study (rat)	1239 mg/kg bw/day		Clinical signs in dams and reduced foetal weights

Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

Overall the profile shows that the rat is more sensitive to TBA than the mouse. The oral database is more extensive than the inhalation database. Therefore, it makes sense that the DNEL should be derived by route to route extrapolation using the results of the oral studies. However, it is noted that a relatively low LOAEL value was derived from the 90-day inhalation study in rats, so a DNEL will also be derived for this study to ensure the lowest DNEL is identified.

The following DNELs were derived for worker exposure:

- Acute inhalation systemic exposure (15 min)
- Long-term inhalation systemic exposure
- Long-term dermal systemic exposure

The following DNELS were derived for the general public

- Acute inhalation systemic exposure (15 min)
- Long-term inhalation systemic exposure
- Long-term dermal systemic exposure
- Long-term oral systemic exposure

Worker DNEL short-term inhalation

TBA is acutely toxic vial the inhalation route and peak exposures have been identified, therefore, it is necessary to derive an acute toxicity DNEL for the inhalation route.

The only acute inhalation study available in the registration dossier comes from a single dose (10 000 ppm) acute inhalation study. The lead effect at this dose level was clinical signs. Since this study only employed a single dose, it is of limited use to derive a DNEL. Therefore, information from an 18-day inhalation study conducted in rats was used to determine the acute inhalation DNEL. This study employed multiple doses and a clear NOAEC was derived.

The dose descriptor obtained from this 18-day study rat inhalation study (6 hour exposure) is the NOAEC of 1385 mg/m^3 and was based on clinical signs.

Since the short-term DNEL has a 15 minute reference period, it is necessary to convert the 18-day inhalation study NOAEC to an equivalent dose that would be inhaled over a 15 minute period. This is done using the modified Haber's rule $c^n t=k$ (TGD, Chapter R8, Appendix R8-8, page 108). Since it is not possible to determine an appropriate value for 'n' from the available data, a default values of 3 will be used to extrapolate from a longer to shorter exposure period.

$\sqrt[3]{(1385^3 \times 6 \times 4)} = 3995$ mg/m^3 (15 min)

It is also necessary to adjust this value by a factor of 0.67 to take account of the different doses that will be received due to differences in breathing rates between workers at rest and engaged in light activity.

3995 mg/m^3 x 0.67 = 2677 mg/m^3

The corrected starting point is 2677 mg/m^3 (15 minutes)

Assessment factors and DNEL calculation for worker DNEL short-term inhalation systemic effects		
Uncertainties	AF	Justification
Interspecies difference	2.5	Since the dose descriptor was derived from an inhalation study and is being used to derive an inhalation DNEL, no allometric scaling is necessary. There are no data for TBA to quantify other differences between animals and humans that could affect interspecies extrapolation. On this basis the default value of 2.5 to account for other species difference will be applied.
Intraspecies differences	5	There are no data to quantify the variability in susceptibility to the effects of long-term exposure to TBA in the human population. The default value of 5 for workers will therefore be used to take account of intraspecies variability
Differences in duration of exposure	1	The dose descriptor was obtained from an 18-day study. Although the DNEL is to account for short term acute exposure, there is evidence to suggest that the clinical signs occur relatively shortly after exposure to TBA and do not increase in severity with repeated exposure. Therefore, it is not considered necessary to apply a factor to take account of differences in duration of exposure.
Dose response and endpoint specific/severity issues	1	The starting point is a NOAEC. Although, the study report from which the NOAEC was derived has limited information, other studies have shown that the severity of the clinical signs increase with dose. A factor for uncertainties is not considered necessary
Quality of the data base	1	The quality of the database is adequate. The information comes from an NTP 18-day inhalation study. The results of which are supported by effects observed in other studies. This consistency provides confidence in the reliability of these studies. It is not necessary to apply a factor to take account of deficiencies in the quality of the database.
Overall assessment factor: 12.5		
Worker short-term inhalation DNEL 2677 /12.5 = **214 mg/m^3 (15 minute)**		

TBA is also classified for eye and respiratory irritation. There is insufficient information to calculate a DNEL for these effects. Therefore, a qualitative approach is warranted.

Worker long term inhalation systemic DNEL

Via the oral route, the lowest NOAEL was derived from a reproductive screening study in rats (64 mg/kg bw/day) and was based on an increase in kidney weight. However, a LOAEL of 90 mg/kg bw/day was derived from the oral chronic study in rats (based on

reduced bodyweight effects) and LOAEC of 406 mg/m^3 was derived from a 90-day inhalation study in rats (nephropathy). As these may result in a lower DNEL than that derived from the screening study, DNELs will be derived for all three studies.

To convert the oral values to inhalation, route to route extrapolation is required. In the absence of specific information the extent of absorption in humans is assumed to be the same as that in rat. Although an inhalation value of 60% has been estimated by PBPK modelling (see section 7.9.1), a worst-case assumption of 100% absorption will be used for extrapolation from the oral to the inhalation route, as recommended by ECHA's guidance Chapter R.8, section R.8.4.2. An oral absorption value of 100% will be used (see section 7.9.1).

The equations used were obtained from ECHA's Practical Guide 14; Table 2, page 38.

Corrected inhalation N(L)OAEC = oral N(L)OAEL*(1/0.38m^3/kg bw/day) *0.67* (Abs_{oral}/Abs_{inhal})).

Corrected inhalation NOAEC from the reproductive screening study:
64*****(1/0.38)*****0.67*****(100/100) = 112.8 mg/m^3 (NOAEC)

Corrected inhalation LOAEC from the chronic study in rats:
90*****(1/0.38)*****0.67*****(100/100) = 158.7 mg/m^3 (LOAEC)

The inhalation LOAEC from the 90-day inhalation study in rats has to be adjusted to take account of the different doses that will be received because of differences in breathing rates between experimental animals at rest and workers engaged in light activity. A factor of 0.75 will also be included to take account of differences in exposure duration, with workers assumed to be exposed to the substances for 8 h, whereas the exposure in the study was only 6 hours.

Corrected inhalation LOAEC = 406 mg/m^3x 0.67 x 0.75 = 204 mg/m^3

Assessment factors and DNEL calculation for worker DNEL long-term inhalation systemic effects		
Uncertainties	AF	Justification
Interspecies difference	2.5	Since we are deriving an inhalation DNEL, no allometric scaling is necessary. There are no data for TBA to quantify other differences between animals and humans that could affect interspecies extrapolation. On this basis the default value of 2.5 to account for other species difference will be applied.
Intraspecies differences	5	There are no data to quantify the variability in susceptibility to the effects of long-term exposure to TBA in the human population. The default value of 5 for workers will therefore be used to take account of intraspecies variability
Differences in duration of exposure	1 (chronic study) 2 (90-day and reproductive	Chronic study. No factor is required as exposure is already long-term 90-day inhalation study: a default factor of 2 will be applied to extrapolate to long-term exposure. As the protocol for the reproductive screening study was enhanced, it is considered to be equivalent to a sub-

	screening study)	chronic study and therefore a default factor of 2 will also be applied.
Dose response and endpoint specific/severity issues	1 (reproductive screening study) 3 (other studies)	The starting point for the reproductive screening study is a NOAEL. A dose related increase in kidney weight was observed in this study. The starting point for the chronic oral study and the 90-day inhalation study is a LOAEC. For the chronic oral study, the LOAEL was based on bodyweight reduction. As the extent of this reduction was only 15 %, a factor of 3 is considered sufficient to account for the uncertainty of using a LOAEL as the starting point. Similarly, as the severity of the kidney nephropathy was estimated as minimal-mild (compared to minimal in controls), a factor of 3 is also considered appropriate to account for the associated uncertainty.
Quality of the data base	1	The quality of the database is adequate. The information comes from an NTP 90-day inhalation study an NTP chronic study and a well conducted reproductive screening study. The results of which are supported by the effects observed in other studies. This consistency provides confidence in the reliability of these studies. It is not necessary to apply a factor to take account of deficiencies in the quality of the database.
Overall assessment factor: 75 (90-day inhalation study) 37.5 (chronic oral study) 25 (reproductive screening study)		
Endpoint specific DNEL: 204/75 = 2.7 mg/m^3 (90-day inhalation study) 158.7/37.5 = 4.2mg/m^3 (chronic oral study) 112.8/25 = 4.5 mg/m^3(reproductive screening study) **Worker long term inhalation systemic DNEL= 2.7 mg/m^3**		

Worker Long term dermal systemic DNEL

The same studies were selected for worker long-term dermal DNEL derivation as for worker long-term inhalation DNEL derivation. The studies were conducted via the oral and inhalation route. To convert these values to dermal exposure, route-to-route extrapolation is required.

The equations used were obtained from ECHA's Practical Guide 14; Table 2, page 38.

Inhalation study

Dermal N(L)OAEL = Inhalation N(L)OAEC*sRVrat*0.67*(Abs_{inhal}/Abs_{dermal}))

Corrected dermal LOAEL from the 90-day inhalation study LOAEC:

406*0.38*0.67*****(100/11) = 940 mg/kg bw/day

Oral studies

Corrected dermal N(L)OAEL = oral N(L)OAEL*(Abs_{oral}/Abs_{dermal})).

Corrected dermal NOAEL from the reproductive screening study:

64*(100/11) = 582 mg/kg bw/day

Corrected dermal LOAEL from the chronic study in rats:

90*(100/11)= 818 mg/kg bw/day

Assessment factors and DNEL calculation for worker DNEL long-term dermal systemic effects		
Uncertainties	AF	Justification
Interspecies difference	10 (90-day inhalation study) 10 (oral studies)	Allometric scaling factor of 4 is applied for extrapolation from the inhalation to the dermal route.. For the two oral studies in rat, an allometric scaling factor of 4 is required to take account of differences in basal metabolic rates between rats and humans. There are no data for TBA to quantify other differences between animals and humans that could affect interspecies extrapolation. On this basis the default value of 2.5 to account for other species difference will be applied.
Intraspecies differences	5	There are no data to quantify the variability in susceptibility to the effects of long-term exposure to TBA in the human population. The default value of 5 for workers will therefore be used to take account of intraspecies variability
Differences in duration of exposure	1 (chronic study) 2 (90-day and reproductive screening study)	Chronic study. No factor is required as exposure is already long-term 90-day inhalation study: a default factor of 2 will be applied to extrapolate to long-term exposure. As the protocol for the reproductive screening study was enhanced, it is considered to be equivalent to a sub-chronic study and therefore a default factor of 2 will also be applied.
Dose response and endpoint specific/severity issues	1 (reproductive screening study) 3 (other studies)	The starting point for the reproductive screening study is a NOAEL. A dose related increase in kidney weight was observed in this study. The starting point for the chronic oral study and the 90-day inhalation study is a LOAEC. For the chronic oral study, the LOAEL was based on bodyweight reduction. As the extent of this reduction was only 15 %, a factor of 3 is considered sufficient to account for the uncertainty of using a LOAEL as the starting point. Similarly, as the severity of the

		kidney nephropathy was estimated as minimal-mild (compared to minimal in controls), a factor of 3 is also considered appropriate to account for the associated uncertainty.
Quality of the data base	1	The quality of the database is adequate. The information comes from an NTP 90-day inhalation study an NTP chronic study and a well conducted reproductive screening study. The results of which are supported by the effects observed in other studies. This consistency provides confidence in the reliability of these studies. It is not necessary to apply a factor to take account of deficiencies in the quality of the database.
Overall assessment factor: 300 (90-day inhalation study) 150 (chronic oral study) 100 (reproductive screening study)		
Endpoint specific DNEL: 940/300 = 3.1 mg/kg bw/day (90-day inhalation study) 818/150 = 5.5 mg/kg bw/day (chronic oral study) 582/100 = 5.8 mg/kg bw/day (reproductive screening study) **Worker Long term dermal systemic DNEL = 5.5 mg/kg bw/day** Whilst the DNEL calculated from the 90-day inhalation study is slightly lower, the eMSCA proposes to take forward the DNEL from the chronic study as this involves less extrapolation and it is unusual to calculate a dermal DNEL from an inhalation study when there are good quality oral data available.		

General population exposure

General population short-term inhalation systemic DNEL

TBA is acutely toxic vial the inhalation route and peak exposures have been identified, therefore, it is necessary to derive an acute toxicity DNEL for the inhalation route.

The only acute inhalation study available in the registration dossier comes from a single dose (10 000 ppm) acute inhalation study. The lead effect at this dose level was clinical signs. Since this study only employed a single dose, it is of limited use. Therefore, it was decided to use information from an 18-day inhalation study conducted in rats to determine the acute inhalation DNEL.

The dose descriptor is the NOAEC of 1385 mg/m3 obtained from an 18-day inhalation study (6 hour exposure) in rats and was based on clinical signs.

Since the short-term DNEL has a 15 minute reference period, it was necessary to convert the 18-day inhalation study NOAEC to an equivalent dose that would be inhaled over a 15 minute period. This was done using the modified Haber's rule $c^n t=k$ (TGD, Chapter R8, Appendix R8-8, page 108). Since it is not possible to determine an appropriate value for 'n' from the available data, a default value of 3 will be used to extrapolate from a longer to shorter exposure period.

$^{3}\sqrt{(1385^{3} \times 6 \times 4)} = 3995$ mg/m^{3} (15 min)

Assessment factors and DNEL calculation for general population DNEL short-term inhalation systemic effects		
Uncertainties	AF	Justification
Interspecies difference	2.5	Since the dose descriptor was derived from an inhalation study and is being used to derive an inhalation DNEL, no allometric scaling is necessary. There are no data for TBA to quantify other differences between animals and humans that could affect interspecies extrapolation. On this basis the default value of 2.5 to account for other species difference will be applied.
Intraspecies differences	10	It is necessary to apply a factor to take account of variability in the human population. There are no data to quantify variability in susceptibility to the effects of long-term exposure to TBA in the human population. The default factor of 10 for consumers will therefore be used.
Differences in duration of exposure	1	The dose descriptor was obtained from an 18-day study. Although the DNEL is to account for short term acute exposure, there is evidence to suggest that the clinical signs occur relatively shortly after exposure to TBA and do not increase in severity with repeated exposure. Therefore, it is not considered necessary to apply a factor to take account of differences in duration of exposure.
Dose response and endpoint specific/severity issues	1	The starting point is a NOAEC. Although, the study report from which the NOAEC was derived has limited information, other studies have shown that the severity of the clinical signs increases with dose. A factor for uncertainties is not considered necessary
Quality of the data base	1	The quality of the database is adequate. The information comes from an NTP 18-day inhalation study. The results of which are supported by the effects observed in other studies. This consistency provides confidence in the reliability of these studies. It is not necessary to apply a factor to take account of deficiencies in the quality of the database.
Overall assessment factor: 25		
General population short-term inhalation systemic DNEL 3995 /25 = **159.8 mg/m^{3} (15 minute)**		

TBA is also classified for eye and respiratory irritation. There is insufficient information to calculate a DNEL for these effects. Therefore, a qualitative approach is warranted.

General population long term inhalation systemic DNEL

The same studies were selected for general population long-term inhalation DNEL derivation as for worker long-term systemic inhalation DNEL derivation. The studies were conducted via the oral and inhalation route. To convert the oral values to inhalation, route to route extrapolation is required.

The equations used were obtained from ECHA's Practical Guide 14; Table 2, page 38.

Inhalation N(L)OAEC = oral N(L)OAEL*(1/1.15m^3/kg bw/day)* (Abs_{oral}/Abs_{inhal}).

Corrected NOAEC from the reproductive screening study: 64*(1/1.15)**100/100) = 55.7 mg/m^3

Corrected LOAEC from the chronic study in rats: 90*(1/1.15)*(100/100) = 78.3 mg/m^3

A LOAEC of 406 mg/m^3 was identified from the 90-day inhalation study in the rat. Since the animals were exposed for 6 hour per day, 5-days per week whereas consumers may be exposed for up to 24 hours per day/ 7 days per week it is necessary to adjust the starting point by a factor of 0.18 to take account of differences in the dose that will be obtained over the daily exposure period.

The corrected starting point is therefore:

406 mg/m^3 x (6/24) x (5/7) = 72.5 mg/m^3

Assessment factors and DNEL calculation for general population DNEL long-term inhalation systemic effects		
Uncertainties	AF	Justification
Interspecies difference	2.5	Since we are deriving an inhalation DNEL, no allometric scaling is necessary. There are no data for TBA to quantify other differences between animals and humans that could affect interspecies extrapolation. On this basis the default value of 2.5 to account for other species difference will be applied.
Intraspecies differences	10	It is necessary to apply a factor to take account of variability in the human population. There are no data to quantify variability in susceptibility to the effects of long-term exposure to TBA in the human population. The default factor of 10 for consumers will therefore be used.
Differences in duration of exposure	1 (chronic study) 2 (90-day and reproductive screening study)	Chronic study. No factor is required as exposure is already long-term 90-day inhalation study: a default factor of 2 will be applied to extrapolate to long-term exposure. As the protocol for the reproductive screening study was enhanced, it is considered to be a sub-chronic study and therefore a default factor of 2 will also be applied.
Dose response and endpoint	1 (reproductive	The starting point for the reproductive screening study is a NOAEL. A dose related increase in kidney weight was observed in this study.

specific/severity issues	screening study) 3 (other studies)	The starting point for the chronic oral study and the 90-day inhalation study is a LOAEC. For the chronic oral study, the LOAEL was based on bodyweight reduction. As the extent of this reduction was only 15 %, a factor of 3 is considered sufficient to account for the uncertainty of using a LOAEL as the starting point. Similarly, as the severity of the kidney nephropathy was estimated as minimal-mild (compared to minimal in controls), a factor of 3 is also considered appropriate to account for the associated uncertainty.
Quality of the data base	1	The quality of the database is adequate. The information comes from an NTP 90-day inhalation study an NTP chronic study and a well conducted reproductive screening study. The results of which are supported by the effects observed in other studies. This consistency provides confidence in the reliability of these studies. It is not necessary to apply a factor to take account of deficiencies in the quality of the database.
Overall assessment factor: 150 (90-day inhalation study) 75 (chronic oral study) 50 (reproductive screening study)		
Endpoint specific DNEL: 72.5/150 = 0.5 mg/m^3 (90-day inhalation study) 78.3/75 = 1.0 mg/m^3 (chronic oral study) 55.7/50 = 1.1 mg/m^3(reproductive screening study) **General population long term inhalation systemic DNEL =0.5 mg/m^3**		

General Population long-term dermal systemic DNEL

The same studies were selected for general population long-term dermal systemic DNEL derivation as for worker long term systemic inhalation. The studies were conducted via the oral and inhalation route. To convert these values to dermal, route-to-route extrapolation is required.

The equations used were obtained from ECHA's Practical Guide 14; Table 2, page 38.

Inhalation study

From the animal data a LOAEC of 406 mg/m^3 from a 90-day inhalation study in the rat was identified. Since animals were exposed for 5-days per week whereas consumers may be exposed for up to 7 days per week it is necessary to adjust the starting point by a factor of 5/7 (0.7) to take account of differences in the dose that will be obtained over the daily exposure period.

406 x 0.7 = 284.2 mg/m^3

Dermal N(L)OAEL = Inhalation N(L)OAEC*sRVrat*(Abs_{inhal} / $Abs_{dermal/}$)

Corrected dermal LOAEL from 90-day inhalation study:

284.2*1.15**(100/11) = 2971 mg/kg bw/day

Oral studies

Dermal N(L)OAEL = oral N(L)OAEL*(Abs_{oral}/Abs_{dermal}).

Corrected dermal NOAEL from reproductive screening study:

64*(100/11) = 582 mg/kg bw/day

Corrected dermal LOAEL from chronic study in rats:

90*(100/11) = 818 mg/kg bw/day

Assessment factors and DNEL calculation for general population DNEL long-term dermal systemic effects		
Uncertainties	AF	Justification
Interspecies difference	10 (90-day inhalation study) 10 (oral studies)	Allometric scaling is applied when extrapolating from an inhalation study to a value for dermal absorption. For the two oral studies in rat, an allometric scaling factor of 4 is required to take account of differences in basal metabolic rates between rats and humans. There are no data for TBA to quantify other differences between animals and humans that could affect interspecies extrapolation. On this basis the default value of 2.5 to account for other species difference will be applied.
Intraspecies differences	10	It is necessary to apply a factor to take account of variability in the human population. There are no data to quantify variability in susceptibility to the effects of long-term exposure to TBA in the human population. The default factor of 10 for consumers will therefore be used.
Differences in duration of exposure	1 (chronic study) 2 (90-day and reproductive screening study)	Chronic study. No factor is required as exposure is already long-term 90-day inhalation study: a default factor of 2 will be applied to extrapolate to long-term exposure. As the protocol for the reproductive screening study was enhanced, it is considered to be equivalent to a sub-chronic study and therefore a default factor of 2 will also be applied.
Dose response and endpoint specific/severity issues	1 (reproductive screening study) 3 (other studies)	The starting point for the reproductive screening study is a NOAEL. A dose related increase in kidney weight was observed in this study. The starting point for the chronic oral study and the 90-day inhalation study is a LOAEC. For the chronic oral study, the LOAEL was based on bodyweight reduction. As the extent of this reduction was only 15 %, a factor of 3 is considered sufficient to

		account for the uncertainty of using a LOAEL as the starting point. Similarly, as the severity of the kidney nephropathy was estimated as minimal-mild (compared to minimal in controls), a factor of 3 is also considered appropriate to account for the associated uncertainty.
Quality of the data base	1	The quality of the database is adequate. The information comes from an NTP 90-day inhalation study an NTP chronic study and a well conducted reproductive screening study. The results of which are supported by the effects observed in other studies. This consistency provides confidence in the reliability of these studies. It is not necessary to apply a factor to take account of deficiencies in the quality of the database.
Overall assessment factor: 600 (90-day inhalation study) 300 (chronic oral study) 200 (reproductive screening study)		
Endpoint specific DNEL: 2971 /600 = 4.9 mg/kg bw/day (90-day inhalation study) 818/300 = 2.7 mg/kg bw/day (chronic oral study) 582/200 = 2.9 mg/kg bw/day (reproductive screening study) **General Population long-term dermal systemic DNEL = 2.7 mg/kg bw/day**		

General population long term oral systemic DNEL

The same studies were selected for general population long-term oral systemic DNEL derivation as for long-term worker systemic inhalation DNEL derivation. The studies were conducted via the oral and inhalation route. To convert inhalation route to the oral route, route-to-route extrapolation is required.

Inhalation study

From the animal data a LOAEC of 406 mg/m^3 (90-day inhalation study in the rat, exposure 6 hours per day, 5 days per week) was identified. Since animals were exposed for 5-days per week whereas consumers may be exposed for up to 7 days per week it is necessary to adjust the starting point by a factor of 0.7 to take account of differences in the dose that will be obtained over the daily exposure period.

406*0.7 = 284.2 mg/m^3

The equations used were obtained from ECHA's Practical Guide 14; Table 2, page 38.

Oral NOAEL = Inhalation N(L)OAEC*1.15 m^3/kg bw/day*(ABS_{inhal}/ABS_{oral})

284.2*(1.15)*(100/100) = 326.8 mg/kg bw/day

Since the extent of oral absorption is assumed to be the same in rats and humans, no adjustment of the starting dose from the oral studies is required.

Assessment factors and DNEL calculation for general population DNEL long-term oral systemic effects		
Uncertainties	AF	Justification
Interspecies difference	10 (90-day inhalation study) 10 (oral studies)	Allometric scaling is applied when extrapolating from an inhalation study to a DNEL for dermal exposure. For the two oral studies in rat, an allometric scaling factor of 4 is required to take account of differences in basal metabolic rates between rats and humans. There are no data for TBA to quantify other differences between animals and humans that could affect interspecies extrapolation. On this basis the default value of 2.5 to account for other species difference will be applied.
Intraspecies differences	10	It is necessary to apply a factor to take account of variability in the human population. There are no data to quantify variability in susceptibility to the effects of long-term exposure to TBA in the human population. The default factor of 10 for consumers will therefore be used.
Differences in duration of exposure	1 (chronic study) 2 (90-day and reproductive screening study)	Chronic study. No factor is required as exposure is already long-term 90-day inhalation study: a default factor of 2 will be applied to extrapolate to long-term exposure. As the protocol for the reproductive screening study was enhanced, it is considered to be a sub-chronic study and therefore a default factor of 2 will also be applied.
Dose response and endpoint specific/severity issues	1 (reproductive screening study) 3 (other studies)	The starting point for the reproductive screening study is a NOAEL. A dose related increase in kidney weight was observed in this study. The starting point for the chronic oral study and the 90-day inhalation study is a LOAEC. For the chronic oral study, the LOAEL was based on bodyweight reduction. As the extent of this reduction was only 15 %, a factor of 3 is considered sufficient to account for the uncertainty of using a LOAEL as the starting point. Similarly, as the severity of the kidney nephropathy was estimated as minimal-mild (compared to minimal in controls), a factor of 3 is also considered appropriate to account for the associated uncertainty.
Quality of the data base	1	The quality of the database is adequate. The information comes from an NTP 90-day inhalation study an NTP chronic study and a well conducted reproductive screening study. The results of which are supported by the effects observed in other studies. This consistency provides confidence in the reliability of these studies. It is not necessary to

		apply a factor to take account of deficiencies in the quality of the database.
Overall assessment factor: 600 (90-day inhalation study) 300 (chronic oral study) 200 (reproductive screening study)		
Endpoint specific DNEL: 326.8/600 = 0.5 mg/kg bw/day (90-day inhalation study) 90/300 = 0.3 mg/kg bw/day (chronic oral study) 64/200 = 0.3 mg/kg bw/day (reproductive screening study) **General population long term oral systemic DNEL = 0.3 mg/kg bw/day**		

Summary of critical DNELs

	Worker	General population
DNEL short term local effects	Not quantifiable	Not quantifiable
DNEL short-term inhalation	214 mg/m^3 (15 minute)	159.8 mg/m^3 (15 minute)
DNEL long-term inhalation	2.7 mg/m^3	0.5 mg/m^3
DNEL long-term dermal	5.5 mg/kg bw/day	2.7 mg/kg bw/day
DNEL long-term oral	-	0.3 mg/kg bw/day

In the dossier updated in 2016 the Registrants used the DNELs derived by the eMSCA.

7.9.12. Conclusions of the human health hazard assessment and related classification and labelling

No toxicological information is available on the effects of exposure to TBA in humans, the only information available to address the potential human health risks of TBA comes from studies in animals.

TBA has a harmonised classification on Annex VI of Regulation (EC) 1272/2008 for acute inhalation toxicity category 4 (H332), STOT SE 3 (H335) and eye irritation category 2 (H319). The information in the dossier supports this classification.

A number of repeat dose toxicity studies conducted in animals are provided in the dossier. Information is available via the oral route from 90-day and carcinogenicity studies in both rats and mice. Via the inhalation route, information is available from 18-day and 90-day inhalation studies in both rats and mice. In rats, the kidney was identified as the principal target organ and clinical signs (including ataxia, hypoactivity, hyperactivity and emaciation) were commonly observed. Via the inhalation route, similar effects were observed in the sub-chronic study as via the oral route, with the kidney in males as the main target organ. In the sub-acute study, the driving effect was clinical signs (hypo/hyperactivity and ataxia) observed at all doses ≥ 2759 mg/m^3.

Repeated oral exposure of mice showed them to be less sensitive to TBA than rats. In the subchronic study, effects (effects on bodyweight and transitional hyperplasia and chronic inflammation of the bladder) were only observed at doses ≥ 20 mg/ml. In the chronic study, the target organ was the thyroid with a dose-related increase in the incidence of thyroid follicular cell hyperplasia observed in both sexes. Via the inhalation route, in a sub-acute study, clinical signs (hypoactivity, hyperactivity and urogenital wetness) were observed at doses ≥ 1750 ppm (5305 mg/m^3). In the sub-chronic study, a reduction in bodyweight was the lead effect from 1050 ppm (3274 mg/m^3). No classification required.

The mutagenic profile of TBA has been investigated *in vitro* and *in vivo*. Although a positive response was reported in the Ames test strain for TA102, the result was not replicated in tests conducted in two independent GLP accredited laboratories. TBA is not considered mutagenic in bacteria. The results of the other *in vitro* studies and the *in vivo* study did not provide any convincing evidence that TBA is genotoxic. Overall, the genotoxic potential of TBA has been adequately investigated and TBA is not considered genotoxic. No classification is required.

The carcinogenic potential of TBA has been investigated in rat and mouse. Although there were tumours observed in both species, the renal tumours in male rats were thought to occur via a male rat-specific mode of action that was not relevant to humans, whereas the benign thyroid tumours in female mice were occurred only at excessively high doses and via a non-genotoxic mode of action are considered of low relevance to humans (see section 7.9.6 for further detail). No classification is required.

No information on fertility or developmental toxicity is available in humans. No adverse effects on fertility were observed in an extended rat reproductive screening study conducted up to doses of 1000 mg/kg bw/day. With regards developmental effects, information from the one good quality study did not show any developmental toxicity. No classification required.

7.10. Assessment of endocrine disrupting (ED) properties

Not assessed.

7.11. PBT and VPVB assessment

Persistence assessment
In an inherent biodegradation study using adapted inoculum, TBA achieved 66% degradation by day 56 and is considered inherently degradable not fulfilling criteria. A half-life in water is not available. Based on available data, TBA is not considered persistent (P) or very persistent (vP).

Bioaccumulation assessment
TBA has a low measured logKow of 0.317. No experimental bioaccumulation data are available. Based on available data, TBA is not considered to screen as bioaccumulative (B) or very bioaccumulative (vB)

Toxicity assessment
There are no acute L(E)C_{50} values <0.1 mg/l and no chronic ecotoxicity NOEC or EC_{10} values ≤0.01 mg/l. TBA is not considered to meet the classification criteria for CMR or STOT 1 or 2. Based on available data, TBA is not considered to meet toxicity (T) criteria.

Summary and overall conclusions on PBT and vPvB Properties
Based on available data, TBA is not considered PBT or vPvB.

7.12. Exposure assessment

TBA is manufactured in the EU. The majority of the tonnage supplied to the EU is used as an intermediate under strictly controlled conditions (SCC). A small percentage is used as a solvent in a range of products used at industrial sites and by professionals. TBA may also be present in certain consumer products, but TBA as such is not supplied to consumers. By January 2019, 7 companies had submitted registrations for TBA covering in total 1 – 10 million tpa. This includes tonnage that is used as an intermediate under strictly controlled conditions (SCC).

7.12.1. Human Health

Worker

The initial evaluation performed in 2013 identified several areas of the exposure assessment that required further work. Although information was provided during the initial evaluation and the decision making process, this did not resolve all of the concerns identified by the eMSCA and therefore requests for information were made in the decision issued in May 2015.

Despite these requests, at the time that this report was drafted (August 2018), there remained a mismatch between the PROC codes listed on ECHA's dissemination site and the claims that TBA is manufactured under SCC. Insufficient contextual information was available to demonstrate that the use situation for the analogous substance is representative for the TBA exposure scenario to which it is being applied. Also, there remains uncertainty about whether or not some registrants are continuing to support scenarios the lead registrant claims are obsolete.

Given these uncertainties, the eMSCA carried out its own exposure assessments where it disagreed with the approach taken by the registrants.

Note to registrants:

To ensure accurate information is available to authorities in relation to the uses and the conditions of use that are supported, all registrants should ensure that they update their CSRs promptly when they receive new information. The comments provided by the eMSCA in this report about the use and exposure information presented in registrations constitutes new information. The eMSCA expects that all registrants, including those whose registrations were not included in this evaluation, will ensure that they clearly identify which of the uses they are supporting in their registrations are covered by the joint submission and which they are covering separately. All registrants should ensure that an exposure scenario is available for each of the uses that they cover in their registration and should take account of the findings from this substance evaluation in their own chemical safety assessments.

This assessment is based on the information available in registrations in August 2018. Updates submitted after this date have not been taken into account.

Manufacture and use as an intermediate

TBA is manufactured in the EU and is used as an intermediate in the manufacture of other substances. The eMSCA had been informed that these processes take place under SCC. However, this is inconsistent with the PROC codes listed on ECHA's dissemination site (PROCs 1, 2, 3, 4, 8a (to cover equipment maintenance), 8b and 15 are listed). Since the disseminated PROC codes are taken from registrations, it is important that each registrant checks that they have listed the correct PROC codes for manufacture and use as an intermediate (where relevant). If stages of these processes are not performed under SCC, exposure scenarios should be provided in an update of the CSR. If all stages

of the process are carried out under SCC, any registrant that has not provided a description of the SCC that are in place at their manufacturing sites within the EU and evidence that confirmation has been obtained from relevant downstream users that they handle TBA under SCC should update their registration with this information without delay.

Given the expectation that these processes are performed under SCC, the eMSCA has not performed a quantitative exposure assessment for these scenarios.

Formulation

TBA is used as a solvent in cleaning agents and coatings. Formulation is described by PROCs 1, 2, 3, 4, 5, 8a (to cover equipment maintenance), 8b, 9, 14 and 15. These processes are stated to take place in predominantly closed systems which are only breached for material additions and sampling. However, based on the selected PROC codes, certain process stages may be performed under more open conditions.

The registrants have used the ECETOC TRA tool versions 2 or 3 for their exposure assessments. For several activities, to achieve an RCR < 1 limitations have been placed on the length of time that an activity should be performed unless respiratory protective equipment (RPE) is used. When time reductions are applied, the exposure estimate generated by the modelling tool assumes there is no further exposure to the substance during the shift. Worker exposure could therfore be underestimated if several short duration tasks are performed in a shift.

Note to registrants:

To ensure that companies receiving exposure scenarios including tasks assessed on a reduced duration basis implement sufficient measures to protect their workers, clarification should be provided with the scenario that the risk management measures (RMMs) identified apply where the worker does not have further exposure to TBA during the day. Where RPE is identified as a necessary RMM, REACH requires information to be provided on suitable types of protective equipment including information on the proper air purifying element to be used. All registrants should ensure that this information is provided for any expsorue scenario that identifies a need for RPE. Ideally this information should be provided in the IUCLID dossier and the CSR.

Dermal exposure estimates have not taken into account use of gloves. A qualitative assessment indicates that gloves should be worn to manage the risks from skin defatting where there is a potential for dermal exposure. The dermal exposure assessment may therefore overestimate the potential for skin contact under normal operating conditions.

The eMSCA has been able to replicate the exposure values calculated by the registrants and will use the registrants' values for the quantitative risk characterisation.

Industrial and professional use in cleaning agents

TBA may be a component in cleaning agents supplied for a wide range of different cleaning tasks. Examples include products supplied for automated cleaning in place (CIP) of industrial process equipment, industrial and professional degreasing, high pressure industrial cleaning, floor maintenance products for use in semi-automatic application devices, trigger sprays, hand wiping of surfaces and sterilization reagents. In order to understand the concentration of TBA that is typically present in cleaning products, the registrants performed a survey of safety data sheets which suggested that the maximum concentration of TBA likely to be found in any cleaning product is 5%. Exposure calculations have been adjusted using the relevant default concentration modifier in the ECETOC TRA tool.

Industrial

Industrial use is described by PROCs 2, 3, 4, 7, 8a, 8b, 10 and 13. It is assumed that LEV is in use for activities covered by PROCs 2, 3, 4, 8a, 8b and 13 or mechanically assisted dilution ventilation for PROCs 7 and 10. In the case of PROCs 7, 10 and 13 (cleaning with high or low pressure washers, manual surface cleaning and degreasing small objects at a cleaning station), use of RPE (Assigned Protection Factor (APF) 10 or 20) is also recommended.

For the dermal route, the use of gloves (APF 5) has only been taken into account for activities covered by PROCs 7, 8a and 10.

Note to registrants:

The IR and CSA Guidance Chapter R14, section R.14.5.3 states that "It is an absolute requirement that the barrier properties of the glove material are known to be adequate to ensure the substance does not migrate through the material of the glove during the proposed use. It is important that gloves are sufficiently described in the IUCLID dossier and the CSR so that there is assurance that the suppliers of substance and formulations can effectively communicate (in section 8 of the Safety Data Sheet) the correct information to downstream users. Important information on gloves relates to those materials that are effective and over what duration they are effective. It is also useful to provide information on common glove materials that are known not to be effective as a barrier". In accordance with the IR and CSA guidance, registrants should ensure that any personal protective equipment (PPE) that is required is sufficiently described in their registrations.

The eMSCA has been able to replicate the registrants' exposure values and will use these for the quantitative risk characterisation for industrial use in cleaning agents.

Professional

Professional use is described by PROCs 1, 2, 3, 4, 8a, 8b, 10, 11 and 13. Use in degreasing formulations is identified as the main professional cleaning use. Exposure scenarios indicate LEV and or mechanically assisted dilution ventilation is required which may need to be supplemented with RPE. Gloves (APF 5 or 10) are also recommended for PROCs describing open handling activities.

The ECETOC TRA tool has been used to estimate exposure with the exception of two scenarios for which analogous measured data has been used. The eMSCA is satisfied that the analogous substance is sufficiently similar to TBA. Insufficient contextual information has been presented in the CSR to demonstrate that the activities covered by the analogous data are representative for the activities the data are being applied to. Further information is needed to confirm that the analogous measurements have been obtained using personal sampling. It is necessary to have descriptions of the controls that were in use at the time the samples were collected. Information is also needed to characterise emissions during the analogous tasks e.g. the duration of the tasks covered by the analogous measured data, the sampling time and the quantities of products that were used. In the absence of this information, the eMSCA cannot verify that the analogous data is representative for solvent emissions during low pressure spraying of TBA and has generated its own exposure estimates for these contributing scenarios (see table 21). For the remaining contributing scenarios, the eMSCA will rely on the exposure estimates derived by the registrants.

Note to registrants:

Currently insufficient contextual information has been presented in the CSR to confirm that the analogous measured data meets the requirements for the use of such data outlined in Chapter R14, section R.14.6.3 of the Information Requirements and

Table 21: Exposure estimates obtained by the eMSCA using the ECETOC TRA tool version 3 for contributing scenarios covered by PROC 11.

Contributing scenario	Assessment parameters	Inhalation value (mg/m³)		Dermal value (mg/kg/day)
		Short term (15 minute TWA)	Full shift (8-hr TWA)	
PROC 11* Professional spraying of concentrated and dilute TBA-containing cleaning products using hand-held trigger sprays.	Activity performed for up to 1 hour with no further exposure to TBA during the shift, maximum concentration in a mixture 5%, work performed indoors, LEV and good general ventilation are in operation, glove efficiency is 80% and the potential for of LEV to reduce dermal exposure is taken into account in the assessment for dermal exposure.	173	8.65	0.17

*This estimate assumes that the vapour phase dominates exposure. The TRA tool does not take into account the aerosol fraction.

The calculations in table 21 do not take account of the effect that other components in a liquid mixture may have on the volatility of TBA. Since the eMSCA does not have information on the composition of liquid mixtures containing TBA it is not possible to refine these calculations further.

Industrial and professional use in coatings

TBA may be used as a solvent in a wide range of coating products. This includes products intended for manual or automated application using hand spreaders, brushes, rollers, spray equipment and dip tanks. Products where TBA has been found include stain protectors, concrete sealants, fire-fighting foam coatings, epoxys, primers, penetrants and paints. Based on a survey of safety data sheets performed by registrants, the maximum concentration likely to be present in any product is 20% with the highest concentrations found in fire-suppressant coatings. Exposure calculations have been adjusted using the appropriate default concentration modifier in the ECETOC TRA tool.

Industrial

Industrial use is described by PROCs 1, 2, 3, 4, 5, 7 (automated and manual spraying), 8a, 8b, 9, 10 and 13. LEV and/or mechanically assisted dilution ventilation may be required supplemented with RPE (APF 10 or 20) for spraying and other open handling situations.

For the dermal route, the use of gloves (APF 5 or 20) has been taken into account for activities covered by PROCs 7, 9, 10 and 13.

The eMSCA has been able to replicate the registrants' exposure values and will use these for the quantitative risk characterisation for industrial use in coatings.

Professional

Professional use is described by PROCs 1, 2, 3, 4, 5, 8a, 8b, 10, 11, 13 and 19. Activities may be performed indoors or outdoors. Gloves (APF 5 or 10) are alrecommended for PROCs describing open handling activities. It is assumed that LEV is in use for indoor activities covered by PROCs 2, 3 and 4. For other indoor activities mechanically assisted dilution ventilation may be in use. RPE (APF 10 or 20) is also required for all activities (both indoor and outdoor) with the exception of PROC 1. The eMSCA has discussed this extensive reliance on RPE with the registrants but it has not been possible to refine the exposure assessments based on the information currently available. Several activities have been assessed on the basis that the activity is performed for part of the shift only (e.g. less than 1 hour) and there is no further exposure during the shift. As indicated previously, downstream users need to be made aware of this limitation in the exposure scenario and prompted to take this into account in their site specific risk assessments.

The registrants have used the ECETOC TRA tool to estimate exposure with the exception of the contributing scenarios identified in table 22. These contributing scenarios have been assessed using analogous measured data. The eMSCA is satisfied that the analogous substance is sufficiently similar to TBA. As before, insufficient contextual information has been provided to demonstrate that the activities covered by the analogous data are representative for the activities that the data are being applied to and the eMSCA has generated its own exposure predictions using the ECETOC TRA tool version 3. For the remaining scenarios, the eMSCA will rely on the exposure estimates derived by the registrants.

Table 22: Exposure estimates obtained by the eMSCA using the ECETOC TRA tool version 3 for specific contributing scenarios.

Contributing scenario	**Assessment parameters**	**Inhalation value (mg/m³)**		**Dermal value (mg/kg/day)**
		Short term (15 minute TWA)	Full shift (8-hr TWA)	
Industrial use				
PROC 7* Industrial manual spraying of coatings (indoors)	Activity performed for up to 8 hours, maximum concentration in a mixture 25%, LEV and enhanced mechanically assisted ventilation is in use, RPE (APF 10) is used, glove efficiency is 80% and the use of LEV is taken into account in the assessment for dermal	2.78	0.70	0.26

	exposure.			
Professional use				
PROC 10* Professional application of coatings using brushes, rollers or spreaders (indoors)	Activity performed for up to 8 hours, maximum concentration in a mixture 25%, LEV and good general ventilation is in use, RPE (APF 20) is used, glove efficiency is 80% and the use of LEV is taken into account in the assessment for dermal exposure.	5.19	1.3	3.29
PROC 10* Professional application of coatings using brushes, rollers or spreaders (outdoors)	Activity performed for up to 8 hours, maximum concentration in a mixture 25%, work performed outdoors, RPE (APF 20) is used, glove efficiency is 80%.	25.9	6.49	3.29
PROC 11* Professional manual spraying of coatings (indoors)	Activity performed for up to 8 hours, maximum concentration in a mixture 5%, LEV and good general ventilation is in use, RPE (APF 20) is used, glove efficiency is 90% and the use of LEV is taken into account in the assessment for dermal exposure.	8.65	2.16	0.43
PROC 11* Professional manual spraying of coatings (outdoors)	Activity performed for up to 8 hours, maximum concentration in a mixture 5%, work performed outdoors, RPE (APF 20) is used, glove efficiency is 90%.	43.2	10.8	2.14
PROC 13 Professional application of coatings by dipping, immersion and pouring (indoors)	Activity performed for up to 8 hours, maximum concentration in a mixture 25%, LEV and good general ventilation is in use, RPE (APF 10) is used, glove efficiency is 80% and the use of LEV is	10.4	2.59	3.29

	taken into account in the assessment for dermal exposure.			
PROC 13 Professional application of coatings by dipping, immersion and pouring (outdoors)	Activity performed for up to 8 hours, maximum concentration in a mixture 25%, work performed outdoors, RPE (APF 20) is used, glove efficiency is 80%.	25.9	6.49	1.65

*This estimate assumes that the vapour phase dominates exposure. The TRA tool does not take into account the aerosol fraction.

The parameters assumed for modelling have been selected to generate a risk characterisation ratio (RCR) value below 1, or the lowest value if an RCR below 1 cannot be achieved using the options available in the TRA tool for products containing the levels of TBA suggested in registrations. These exposure estimates may not therefore reflect the operating conditions and risk management measures that are typically applied for these activities. More detailed information about the TBA content of products used for these activities and about the work activities covered in each contributing scenario would enable a more refined assessment to be performed.

Use as a laboratory reagent

TBA may be used as a laboratory reagent and also as a general purpose solvent in a laboratory (this scenario also covers quality control analyses performed during product formulation). Laboratory use is described using PROCs 9, 13 and 15. Inhalation exposure has been assessed using the Advanced Reach Tool (ART). The dermal exposure assessment has been performed using the ECETOC TRA tool and it is assumed that gloves will be used. The eMSCA is satisfied that the conditions of use described for laboratory handling of a substance are appropriate and has been able to replicate the registrants exposure estimates. The eMSCA will use the registrant's exposure estimates.

Exposure assessments for the potentially obsolete scenarios reported on ECHA's dissemination site

Two additional uses are listed on ECHA's dissemination site. Information provided by the lead registrant suggests these uses may be obsolete. However, these uses still appear on ECHA's dissemination site and the eMSCA therefore assumes some members in the consortium continue to support these uses.

Use in waste water treatment

It is assumed that this scenario covers the potential for exposure to TBA where it is present in products that are supplied for waste water treatment in industrial settings or by professionals. However, limited information is available about this use. The scenario describes a situation where products appear to be added to enclosed vessels using dedicated transfer lines. However, there may be situations where more open handling methods are adoped e.g. during sampling activities. PROC 13 has also been identified as relevant for both industrial settings and professionals (further characterised as pouring from small containers). For several activities, it appears to have been necessary in exposure modelling calculations to limit the duration of the activity to periods of less than 1 hour. This raises the possibility that workers may be exposed to greater amounts than have been estimated if they perform several tasks involving exposure to TBA containing products during the day. This may be of concern because the RCRs that are reported are in many cases are close to 1. Since it is not certain that this is a relevant exposure scenario for TBA, the eMSCA has not attempted to carry out its own exposure assessment based on the limited information available. However, it expects that if any

registrant does intend to support this use in the future, they will provide more information about the way exposure arises during the activities covered in the scenario.

Use in fuels

The scenario covering use in fuels appears to describe the situation where TBA is present as an impurity in the fuel mixture. Exposure arises from incidental contact with the fuel during refuelling activities and maintenance activities on fuel equipment. As such, the eMSCA expects that level of TBA that an individual is expected to be exposed to will be minimal and does not consider that it is necessary to attempt to quantify this source of exposure.

Conclusions about worker exposure

The worker exposure assessment is mainly based on modelled data and the eMSCA has been able to reproduce the registrant's modelled estimates. Where registrants have used analogous measured data, the eMSCA is concerned that insufficient information has been provided to demonstrate that the analogous use situation is representative for the use situation to which it is being applied. The eMSCA has therefore chosen to use modelled data rather than rely on uncertain analogous measured data, but recognises several sources of uncertainty that are associated with its own modelled estimates. During the course of the evaluation, the eMSCA identified areas of the registrants' exposure assessments that need further work. All registrants should:

- check which of the uses that they currently identify in their CSR are still relevant and that an exposure scenario is available for each identified use;
- for any use that takes place under SCC at registrants' own sites ensure that the SCC are described in the registration;
- for any use that takes place under SCC at downstream user sites, ensure that there is evidence that confirmation has been received from the downstream user that SCC are implemented at their site;
- ensure that sufficient information is provided on any PPE that is required; and,
- ensure that wherever analogous measured data is used in the exposure assessment, sufficient contextual data is available to allow the suitability of the data to be examined.

Table 23 summarises the exposure information that the eMSCA will use as the basis for its quantitative risk characterisation.

Table 23: Summary of the exposure estimates that the eMSCA is using for its own risk characterisation

Scenario		Inhalation
Manufacture and use as an intermediate		A quantitative exposure assessment has not been performed on the basis of information indicating these uses take place under SCC
Formulation		eMSCA relying on modelled data from registrations
Use in cleaning agents	Industrial	eMSCA relying on modelled data from registrations
	Professional	eMSCA relying on modelled data from registrations for all contributing scenarios

		except professional use of concentrated and dilute TBA-containing cleaning products in low pressure sprays (see table 21).
Use in coatings	Industrial	eMSCA relying on modelled data from registrations for all contributing scenarios except industrial manual spraying of coatings (see table 22).
	Professional	eMSCA relying on modelled data from registrations for all contributing scenarios except the professional use scenarios in table 22.
Use as a laboratory reagent	Professional	eMSCA relying on modelled data from registrations
Use in waste water treatment	Industrial	eMSCA has not carried out a quantitiative exposure assessment for this scenario because it is not clear if this use is applicable for TBA
	Professional	
Use in fuel	Industrial	eMSCA has not carried out a quantitiative exposure assessment for this scenario because it is not clear if this use is applicable for TBA
	Professional	

Consumer

The eMSCA has examined the exposure assessments provided in registrations. For several scenarios, the eMSCA could not replicate the reigstrants calculations. Where the eMSCA has been able to replicate the registrant's assessments, it does not always agree with the modelling approach that has been chosen. Rather than rely on the registrants' consumer exposure assessments, the eMSCA has chosen to perform its own assessments using ConsExpo web v 1.0.5. The eMSCA relied on the RIVM fact sheet defaults for the various product types along with the registrants' stated weight fraction of the substance in each product type as the basis for its calculations.

Consumer use of washing and cleaning products

TBA may be present in washing and cleaning products supplied for consumer use but it is not clear how frequently TBA occurs in such products. The following assessment covers product types that the registrants have identified as potentially containing TBA. Since the eMSCA does not have information that will allow it to independently verify the range of product types or levels of TBA that may be present in these products, it is not certain that the estimated exposures will arise in practice.

Table 24: Exposure estimates calculated by the eMSCA for consumer cleaning products where TBA may be found.

Scenario	Contributing scenario (where applicable)	Inhalation (mg/m³) (mean concentration on day of exposure)		Dermal (mg/kg) (external dose on day of exposure)	
		Contributing scenario	Total	Contributing scenario	Total
Laundry detergent[13]	Pouring with caps	1.5 E-3	3.5 E-3	7.6 E-1	0.93

max. 10% TBA	Hand washing	2 E-3		1.7 E-1	
Hand dishwash liquid[13] max.10% TBA			6.7 E-2		5.3 E-2
All purpose cleaners max.6% TBA in undiluted product	Mixing and loading	2.3 E-4	1.7 E-2	8.7 E-3	1.3 E-2
	Cleaning using diluted product.	1.7 E-2		4.2 E-3	
Sanitary cleaner (liquid) max. 5% TBA	Mixing and loading	3.1 E-4	3.2 E-3	7.3 E-3	1.2 E-2
	Cleaning using diluted product	2.9 E-3		5.8 E-3	
Floor cleaner (liquid) max. 0.75% TBA	Mixing and loading	4.6 E-5	7.7 E-3	1.1 E-3	3.7 E-3
	Cleaning using diluted product	7.7 E-3		2.6 E-3	
Carpet cleaner (liquid) max. 5% TBA	Mixing and loading	2.6 E-4	6.1 E-1	7.3 E-3	6.2 E-2
	Cleaning using diluted product	6.1 E-1		5.5 E-2	
Metal cleaner (liquid)* max. 10% TBA	Cleaning		3.5		1.6
All purpose cleaner (spray) max. 2% TBA	Spraying (volatile substances)	0.45	0.45	6.2 E-3	9.6 E-2
	Rinsing	N/A		9.0 E-2	
Sanitary cleaner (spray) max. 1% TBA	Spraying (volatile substances)	1.1	1.1	1.8 E-2	0.1
	Rinsing	N/A		9.0 E-2	
Glass cleaner (spray)	Spraying (volatile substances)	3.6	3.6	4.0 E-2	1.1
	Cleaning	N/A		1.1	

[13] Vapour pressure of TBA at elevated temperature calculated using the Clausius-Clapeyron equation and assuming the enthalpy of vapourisation for TBA is 46 kJ/mol (data obtained from: https://webbook.nist.gov/cgi/cbook.cgi?ID=C75650&Mask=4. Site accessed Dec 2018). The following vapour pressure values have been used:

- For the scenario laundry detergent, the application temperature is assumed to be 40°C during hand washing. The vapour pressure of TBA has been calculated to be 13.178 KPa.
- For the scenario hand dishwash liquid, the application temperature is assumed to be 45°C during washing. The vapour pressure of TBA has been calculated to be 17.401 KPa.

max. 10% TBA					

* Infrequent use scenario to be assessed against the consumer infrequent use DNEL.
N/A: RIVM cleaning products fact sheet does not identify inhalation exposure as relevant for the rinsing/cleaning phase following spraying of these products on the basis that this is already covered by inhalation estimates for spraying[14].

Consumer use of a formulated coating product for general repair and maintenance

TBA may be present as a solvent in home maintenance products supplied to consumers for Do-it-Yourself (DIY) activities. This may include paints, aerosol sprays, paint/glue removers, fillers and putty and plasters and floor equalisers. However, it is not known how widely TBA is found in products currently on the market. According to the registrants, TBA is most likely to be present in paint thinners and removers. The other product types have been included in the CSR as a precautionary measure in case downstream formulators have occasionally used this solvent. The following assessment covers product types that the registrants have identified as potentially containing TBA. Since the eMSCA does not have information that will allow it to independently verify the range of product types or levels of TBA that may be present in these products, it is not certain that the estimated exposures will arise in practice.

Table 25: Exposure estimates calculated by the eMSCA for DIY home maintenance products where TBA may be found.

Scenario	Contributing scenario (where applicable)	Inhalation (mg/m³) (mean concentration on day of exposure)		Dermal (mg/kg) (external dose on day of exposure)	
		Contributing scenario	Total	Contributing scenario	Total
Latex wall paint (liquid)* max 0.5% TBA	Application		22		0.26
Solvent rich paint (liquid)* max 1.5% TBA	Application		24		0.78
High solid paint (liquid)* max 1.5% TBA	Application		32		0.78
Waterborne paint (liquid)* max 1.5% TBA	Application		23		0.78

[14] J.A.J. Meesters, M.M. Nijkamp, A.G. Schuur, J.D. te Biesebeek (2018). Cleaning Products Fact Sheet. Default parameters for estimating consumer exposure – Updated version 2018. RIVM Report 2016-0179. Available at: https://www.rivm.nl/publicaties/cleaning-products-fact-sheet-default-parameters-for-estimating-consumer-exposure. Site accessed Dec 2018.

Canned spray paint* max 8% TBA	Application		1.6		1.7
Paint remover (liquid)* max 1.5% TBA	Application		13		0.11
Glue remover (liquid)* max 0.5% TBA	Application		7.7		0.52
Wall paper remover (liquid)* max 0.5% TBA	Mixing and loading		N/A	17.3 E-4	1.4
	Application			1.4	
Sealant remover (liquid)* max 5% TBA	Application		9.9		7.3 E-2
Fillers and putties in tubes (paste)* max 8% TBA	Application		9.9		5.8 E-2
Floor equalisers (paste)* max 5% TBA	Mixing and loading		N/A	3.2 E-4	1.5
	Application			1.5	
Wall plaster (paste)* max 2% TBA	Application		N/A		1.7

* Infrequent use scenario to be assessed against the consumer infrequent use DNEL. N/A: RIVM fact sheet for DIY products does not identify inhalation exposure as relevant for these products in the expectation that they will be supplied in a form that does not create a potential for inhalation exposure . For the purposes of this assessment, the eMSCA will consider the case where a ready to use product may have been formulated using TBA as a component of the liquid matrix in the formulation. The eMSCA will assume that exposures in this situation will be of the same order as those created using paints. A value of 32 mg/m3 will therefore be carried forward for the risk characterisation.

Exposure assessments for the potentially obsolete scenarios reported on ECHA's dissemination site

Two additional uses are listed on ECHA's dissemination site. Information provided by the lead registrant suggests these uses may be obsolete. However, these uses still appear on

ECHA's dissemination site and the eMSCA therefore assumes some members in the consortium continue to support these uses.

Consumer use in adhesives and sealants

This scenario describes hobby and DIY use of glues and use of sealants in which TBA may be present as a component in the glue or sealant. The eMSCA made a comparison between the parameters used by the registrants in their exposure calculations, the parameters recommended by FEICA in the Specific Consumer Exposure Determinants (SCEDs) they have published for use of glues/sealants , and the default parameters used in version 2 of the EGRET tool. The parameters used by the registrants were not always consistent with the parameters that are currently recommended. Since it is not clear if there is any use of TBA in glues and sealants supplied to the consumer market, the eMSCA has chosen not to cover this use in its own exposure assessment. Given that the exposure assessments that the eMSCA has seen for this use do not appear to be suitable, any registrant that wishes to continue to support this use should ensure that they provide clear justifications for any adaptations they make to default parameters in modelling tools and follow the approaches outlined in the latest version of the consumer exposure assessment guidance.

Consumer use in fuels

As for workers, the scenario covering use in fuels appears to describe the situation where TBA is present as an impurity in the fuel mixture. Exposure arises from incidental contact with the fuel during refuelling activities. As such, the eMSCA expects that level of TBA that an individual is expected to be exposed to will be minimal and does not consider that it is necessary to attempt to quantify this source of exposure.

Conclusions about consumer exposure

During the course of the evaluation, the eMSCA identified areas of the registrants exposure assessment that need further work. Several of the eMSCAs concerns have already been resolved. Work still needs to be done by each registrant to:

- check which of the consumer uses currently identified in CSRs are relevant and that an exposure scenario is available for each identified use; and,

- demonstrate that the parameters chosen for modelling and the modelling equations that have been used are appropriate and that a scientific justification is provided for any modifications that are made to default parameters set within the chosen exposure modelling tools.

Given the uncertainties identified by the eMSCA in the consumer exposure assessments provided by registrants, the eMSCA performed its own consumer exposure assessment and will use its own values for the risk characterisation.

Note to registrants:

Based on the information currently available in CSRs, it is not clear how the exposure modelling parameters used for consumers will be converted into operating conditions and risk management measures for downstream communication. For example, where exposure assessments are based on the assumption that products contain a specific weight fraction of TBA, this must be specified as an upper concentration limit in the exposure scenario.

7.12.2 Environment

Environmental exposure assessment

The substance was not nominated as an environmental priority. Due to this, only a brief review of the environmental exposure modelling was conducted. The focus of this was the generic risk management measures and the generic exposure modelling input assumptions.

Aquatic compartment (incl. sediment)

In the 2016 registration update the registrant updated the PNEC, environmental exposure information and RCRs. This included updating the on-site treatment plant effectiveness figure to 98.9%. There is a discussion on the effectiveness of the on-site treatment plant below.

Monitoring data

There was no environmental monitoring data available in the CSR.

Modelled data

In the original CSR it was not clear how the registrant had interpreted the biodegradation characteristics of TBA for the environmental exposure modelling. This is important as the choice has a significant impact on the PECs that are derived. The eMSCA requested that the registrant clarified and justified the choice made in the context of the REACH endpoint guidance R.7b (section R7.9.4.1) which states:

> *Inherent biodegradability data may be used for extrapolation to a rate constant in models for estimation of the elimination of chemicals in STP. However, this extrapolation is only allowed, if the pass level of 70% degradation in the Zahn-Wellens/EMPA Test is reached within seven days, including the lag-phase and the log-phase, the log-phase should be no longer than three days, and the percentage removal in the test before biodegradation occurs should be below 15%. The pass level of 70% in the Modified MITI Test (II) must be reached within 14 days, including the lag-phase and the log-phase, and the log-phase should be no longer than three days.*

In subsequent correspondence the registrant confirmed that they consider *inherently degradable not meeting criteria* to be appropriate for the substance and included this in their 2016 CSR update. In the original CSR the specified removal efficiency of the wastewater treatment plant of >80 to >95% was justified by the registrant principally from independent determination of the chemical oxygen demand in effluent streams showing 99% removal of all organics. Specific influent concentrations of TBA have been measured and vary between 10 mg/L and up to 600 mg/L (detection limit = 10 mg/L). There does not appear to be any determination of TBA in the effluent, and so it is unclear where the level of removal of 99% is applicable to TBA. The level of (non-specific) removal indicated by the COD decrease is in contrast to expectations from modelling. For example, based on physico-chemical data and a setting of *inherently degradable not meeting criteria* in EUSES 2.0.3, >99% of TBA would be emitted to water in a standard sewage treatment plant (i.e. there would only be <1% removal).The registrant was asked to justify whether the specified RMM of a removal efficiency of in a wastewater treatment plant can be achieved in practice for TBA, to focus on how the measures described as *activated sludge, anaerobic treatment,* and *dissolved air flotation* would specifically affect TBA.

In 2016 the registrant updated the CSR with influent and effluent measurements of TBA taken during March and April of 2014 at two industrial wastewater treatment plants in France and The Netherlands. A sample size of 206 paired samples were taken and analysed with a limit of quantitation (LOQ) of 10 mg/L with a confidence level of 90%. The samples with TBA levels below the LOQ were halved to obtain a value that could be used in an efficiency calculation. The influent results ranged from <10 to 817 mg/L, the effluent

samples ranged from <10 to 15 mg/L, only three of the effluent samples were above 10 mg/L. The registrant calculated a removal efficiency at the 90th percentile of 98.9%. If the value for the samples below LOQ was 9.9 mg/L the removal efficient at the 90th percentile would be 97.8%. The removal rate of 98.9% is appropriate for industrial waste treatment plants containing adapted microorganisms. The registrant further clarified that the use of TBA in the downstream user sites was on a semi-continuous basis the effluent plants on those sites would also have adapted microorganisms. The eMSCA agrees that, in principle, for the two sites sampled there is good evidence that a significant proportion of TBA is removed in the industrial wastewater treatment plants. The registrant was asked to explain whether TBA is discharged continuously or is periodic and whether this was taken into account in the sampling programme, and to justify the number of sites sampled or the reasons for selecting those sites. This information is needed to show why these data for two sites are representative for all other sites. In particular the registrant needs to justify why an on-site industrial WWPT can be assumed for all formulation and industrial use sites.

The registrant responded in personal communication that the sites chosen for monitoring were two of the three manufacturing plants and that they had higher concentrations of TBA in their effluent streams, as the downstream users would be expected to have lower concentrations in their effluent to registrant confirmed that these two sites were representative of all the users. The discharge is continuous on these manufacturing sites. In the CSR the registrant specified the RMM of on-site treatment of TBA containing effluent prior to discharge to STP for all downstream users.

Terrestrial compartment

Not assessed.

Atmospheric compartment

Not assessed.

7.12.3 Combined exposure assessment

A combined exposure assessment examining exposure where multiple products containing TBA are used in the same day has not been performed.

7.13. Risk characterisation

7.13.1 Human Health

TBA is classified as an eye and respiratory tract irritant. Since it is not possible to identify dose response relationships for either effect, a qualitative risk characterisation has been performed. Appropriate measures are recommended to manage these hazards.

TBA is classified as harmful following single exposure by the inhalation route (Acute tox 4, H332) though the reasons for this classification are not clear from the information provided in registrations. TBA is not classified for effects on repeated exposure. However there is evidence that it may cause adverse effects in the kidneys at high dose levels (above the thresholds that would trigger classification) and this is the effect driving the numerical values for the long term systemic DNELs. There is also evidence that the substance may have a defatting effect in the skin following repeated dermal exposure, For this reason, workers are advised to wear gloves if they are in situations where there is the potential for dermal contact with TBA.

Using information from the registrantions the eMSCA has calculated the following DNELs:

Table 26: DNELs calculated by the eMSCA

	Worker	**Consumer**
DNEL short term local effects	Not quantifiable	Not quantifiable
DNEL short-term inhalation	214 mg/m^3 (15 minute)	159.8 mg/m^3 (15 minute)
DNEL long-term inhalation	2.7 mg/m^3	0.5 mg/m^3
DNEL long-term dermal	5.5 mg/kg bw/day	2.7 mg/kg bw/day
DNEL long-term oral		0.3 mg/kg bw/day

When the exposure estimates calculated by the registrants are compared with these DNELs, all RCRs are below 1.

During its evaluation, the eMSCA identified areas of the registrants exposure assessment that needed further work. In light of the uncertainties that it identified with the registrants' exposure assessment, the eMSCA chose to perform its own exposure assessment for some contributing scenarios for workers and for consumers.

Workers

For all worker scenarios, the RCRs for short-term inhalation are below 1. The eMSCA has obtained RCRs > 1 where it generated its own exposure assessments rather than rely on the analogous measured data provided by the registrants (see table 27).

Table 27: RCRs calculated by the eMSCA for scenarios where the registrants have relied on analogous measured data.

Scenario	**Parameters used to estimate exposure**	**Inhalation RCR**	**Dermal RCR**	**Combined RCR**
Professional cleaning and degreasing				
PROC 11 Professional	Activity performed for up to 1 hour with no further exposure to	**3.2**	0.03	**3.2**

spraying of concentrated and dilute TBA-containing cleaning products using hand-held trigger sprays.	TBA during the shift, maximum concentration in a mixture 5%, work performed indoors, LEV and good general ventilation are in operation, glove efficiency is 80% and the potential for of LEV to reduce dermal exposure is taken into account in the assessment for dermal exposure.			
Industrial use in coatings				
PROC 7 Industrial manual spraying of coatings (indoors)	Activity performed for up to 8 hours, maximum concentration in a mixture 25%, LEV and enhanced mechanically assisted ventilation is in use, RPE (APF 10) is used, glove efficiency is 80% and the use of LEV is taken into account in the assessment for dermal exposure.	0.26	0.047	0.3
Professional use in coatings				
PROC 10 Professional application of coatings using brushes, rollers or spreaders (indoors)	Activity performed for up to 8 hours, maximum concentration in a mixture 25%, LEV and good general ventilation is in use, RPE (APF 20) is used, glove efficiency is 80% and the use of LEV is taken into account in the assessment for dermal exposure.	0.48	0.6	**1.08**
PROC 10 Professional application of coatings using brushes, rollers or spreaders (outdoors)	Activity performed for up to 8 hours, maximum concentration in a mixture 25%, work performed outdoors, RPE (APF 20) is used, glove efficiency is 80%.	**2.4**	0.6	**3**
PROC 11 Professional manual spraying of coatings (indoors)	Activity performed for up to 8 hours, maximum concentration in a mixture 5%, LEV and good general ventilation is in use, RPE (APF 20) is used, glove efficiency is 90% and the use of LEV is taken into account in the assessment for dermal exposure.	0.8	0.08	0.88
PROC 11 Professional manual spraying of coatings (outdoors)	Activity performed for up to 8 hours, maximum concentration in a mixture 5%, work performed outdoors, RPE (APF 20) is used, glove efficiency is 90%.	**4**	0.39	**4.39**

PROC 13 Professional application of coatings by dipping, immersion and pouring (indoors)	Activity performed for up to 8 hours, maximum concentration in a mixture 25%, LEV and good general ventilation is in use, RPE (APF 10) is used, glove efficiency is 80% and the use of LEV is taken into account in the assessment for dermal exposure.	0.96	0.06	**1.02**
PROC 13 Professional application of coatings by dipping, immersion and pouring (outdoors)	Activity performed for up to 8 hours, maximum concentration in a mixture 25%, work performed outdoors, RPE (APF 20) is used, glove efficiency is 80%.	**2.4**	0.3	**2.7**

Although several of the RCRs for these scenarios are above 1, most are only marginally above 1. Since these RCRs are based on worst case assumptions about the percentage of TBA in formulations and about the length of time a worker will spend using these formulations, it is likely that any risks in practice will be lower than is suggested by these RCRs. In reaching this conclusion, the eMSCA also reflects that RCRs based on the analogous measured data proposed by the registrants for these scenarios are all less than 1. The eMSCA therefore does not consider that the RCRs it has calculated provide evidence for an unacceptable risk and concludes that no further regulatory action is necessary. However, there may be actions that can be taken in the supply chain to provide further evidence that will help the identification of safe use conditions for TBA.

Looking at these RCRs it can be seen that the inhalation route is the dominant route of exposure. Currently the registrants are making extensive use of RPE to limit inhalation exposure. The eMSCA considers that alternative control solutions should also be explored. For example, it may be possible to refine the exposure assessments with better information about the concentration of TBA in products which are used for the activities covered by these PROC codes. This information could be provided to registrants by downstream formulators, or formulators may choose to carry out product specific assessments to clarify if RPE is a necessary RMM for that product. It may also be possible for the registrants to set limits on the concentration of TBA in products that are intended for use in situations where it is not feasible to apply containment or other engineering controls.

Consumers

There are two distinct use patterns for consumer products which may contain TBA. Products supplied for washing and cleaning activities, with the possible exception of specialized cleaning products e.g. metal cleaners, are likely to be used on a daily or weekly basis. The exposures that may arise from the use of these washing and cleaning products can be assessed using the previously calculated systemic short- and long-term DNELs (see table 26). TBA may also be present in paints and coatings supplied for DIY household maintenance. These products are likely to be used only occasionally during the year and it is important to understand the risks that such uses pose during each use event. Version 3.0 of Chapter 15 of the IR & CSR guidance describes methods that can be used to calculate an infrequent event DNEL to be used where it is necessary to assess risks from infrequent events and the eMSCA has followed this guidance to calculate a systemic, inhalation, infrequent use DNEL for its own risk characterisation.

Infrequent use DNEL

Use NOAEC of 1385 mg/m^3 from 18-day inhalation study in rats (exposure for 6h/day) as a starting point (Table R.15-1 of ECHA's guidance R.15).

Corrected NOAEC adjusted for duration of exposure (6h to 24 h for consumers) = 1385*(6/24) = 346.25 mg/m^3

Apply assessment factors = 346.25 / 25 = 13.85 mg/m^3

DNEL consumer infrequent exposure = 13.9 mg/m^3 (24 hours).

Inhalation exposures for infrequent events will be compared with this DNEL.

Tables 28 and 29 present RCRs calculated by the eMSCA using its own exposure estimates and its own DNELs. With the exception of the cleaning products listed in table 28, all RCRs for washing and cleaning products are below 1 (metal cleaner was assessed against the infrequent use DNEL).

Table 28: RCRs for consumer washing and cleaning products based on the eMSCA's exposure calculations and the long-term systemic inhalation and dermal DNELs.

Scenario	Inhalation RCR	Dermal RCR	Combined RCR
Carpet cleaner (max. 5% TBA)	**1.2**	0.02	**1.22**
Sanitary cleaning spray (max. 1% TBA)	**2.2**	0.04	**2.24**
Glass cleaning spray (max.10% TBA)	**7.2**	0.41	**7.61**

Table 29: RCRs for consumer paints and coatings based on exposure values on the day of exposure calculated by the eMSCA. Inhalation exposure has been assessed against the infrequent use DNEL calculated by the eMSCA. Dermal exposure has been assessed against the long-term systemic DNEL.

Scenario	Inhalation RCR	Dermal RCR	Combined RCR
Latex wall paint (max. 0.5% TBA)	**1.58**	0.1	**1.6**
Solvent rich paint (max. 1.5% TBA)	**1.73**	0.29	**2.02**
High solid paint (max. 1.5% TBA)	**2.30**	0.29	**2.59**
Waterborne paint (max. 1.5% TBA)	**1.65**	0.29	**1.94**
Spray paint (max. 8% TBA)	0.12	0.63	0.75
Paint remover (max. 1.5% TBA)	0.91	0.04	0.97

Glue remover (max. 0.5% TBA)	0.55	0.19	0.74
Wall paper remover (max. 0.5% TBA)	N/A **(2.30)**	0.52	0.52 **(2.82)**
Sealant remover (max. 5% TBA)	0.71	0.02	0.73
Fillers and putties in tubes (max. 8% TBA)	0.71	0.02	0.73
Floor equalisers (max. 5% TBA)	N/A **(2.30)**	0.56	0.56 **(2.86)**
Wall plaster (max. 2% TBA)	N/A **(2.30)**	0.63	0.63 **(2.93)**

N/A: RIVM fact sheet for DIY products does not identify inhalation exposure as relevant for these products on the expectation that they will be supplied in a form that does not create a potential for inhalation exposure. For the purposes of this assessment, the eMSCA has considered the case where a ready to use product may have been formulated using TBA as a component of the liquid matrix in the formulation and has assumed that exposures are of the same order as those created from the use of a high solid paint (RCR in brackets).

In considering how to react to RCRs > 1 for consumer products containg TBA, the eMSCA notes that the extent to which TBA may be used in these product types is uncertain as is the concentration in which it may be used. These RCRs probably reflect a worst case situation, but the eMSCA does not have information that will enable it to refine the calculations. For this reason, and taking into account the hazard profile of TBA, the eMSCA does not consider that there is sufficient evidence to conclude that an unacceptable risk exists. However, it is recommended that registrants make further efforts to determine the extent to which TBA may be present in consumer products. Downstream formulators may be willing to assist with this process by providing information on the TBA content of consumer products. If TBA is found to be present in levels that give rise to RCRs > 1, registrants should consider amending the maximum permitted concentrations for these product types.

Conclusions for human health

In summary, the following conclusions have been reached in this evaluation.

TBA is not considered to present an unacceptable risk to workers or consumers from any identified use. However, the eMSCA has identified several areas where registrants can usefully improve the information provided in their registrations to increase the accuracy and transparency of their chemical safety assessments.

To ensure that exposure scenarios and suitable exposure assessments are available for each of the uses identified by registrants, each registrant should:

- check which of the uses that they currently identify in their CSR are still relevant and that an exposure scenario is available for each identified use;
- for any use that takes place under SCC at registrants' own sites ensure that the SCC are described in the registration;
- for any use that takes place under SCC at downstream user sites, ensure that there is evidence that confirmation has been received from the downstream user that SCC are implemented at their site;
- ensure that sufficient information is provided on any PPE that is required;
- ensure that wherever analogous measured data is used in the exposure assessment, sufficient contextual data is available to allow the suitability of the

data to be examined. This has been identified as an area to address in relation to worker exposure assessments, but if in future, analogous data is used for the consumer exposure assessment, it is equally important that supporting contextual data is provided for that analogous data; and,

- provide scientific justifications for the choice of parameters and exposure models used to estimate consumer exposure.

7.13.2 Environment

The substance was not nominated as an environmental priority. Due to this, only a brief review of the environmental risk characterisation values was conducted. The focus of this was any scenarios highlighted from the work in section 7.12.2.

In the 2016 updated CSR the registrant updated the WWTP removal efficiency from 91% to 98.9%. The eMSCA accepts this revised figure.

The aquatic PNEC has also been updated as presented in section 7.8.4. The RCRs for the revised PECs using the updated aquatic PNEC are <1.

The use of the removal rate of 97.8% (as discussed in section 7.12.2) would also result in RCRs <1.

7.14. References

Human Health

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Environment

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7.15. Abbreviations

%	Percentage
B	Bioaccumulative
BCF	Bioconcentration factor
CLP	Classification, labelling and packaging (of substances and mixtures)
cm	Centimetre
CoRAP	Community Rolling Action Plan
CSR	Chemical Safety Report
d	Day
DEA	2,2'-iminodiethanol, CAS No 111-42-2 (EC No 203-868-0)
DMEL	Derived Minimal Effect Level
DNEL	Derived No Effect Level
DSD	Dangerous Substances Directive
ECETOC TRA	European Centre for Ecotoxicology and Toxicology of Chemicals Targeted Risk Assessment
ECHA	European Chemicals Agency
EGRET	European Solvents Industry Group Generic Exposure Scenario Tool
EPA	Environmental Protection Agency
ES	Exposure Scenario
ERC	Environmental release category (ERC)
EU	European Union
g	Gramme
GC	Gas chromatography
GC/MS	Gas chromatography – Flame Ionisation Detection
GC/MS	Gas chromatography – mass spectrometry
GLP	Good laboratory practice
hPa	Hectopascal
IR	Information Requirements
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database

IUPAC	International Union of Pure and Applied Chemistry
kg	Kilogram
kJ	Kilojoule
km	Kilometre
kPa	Kilopascal
Koa	Octanol-air partition coefficient
Koc	Organic carbon-water partition coefficient
Kow	Octanol-water partition coefficient
L	Litre
LEV	Local Exhaust Ventillation
Log	Logarithmic value
LOD	Limit of detection
LOQ	Limit of quantitation
M	Molar
m	Metre(s)
µg	Microgram
mg	Milligram
MTBE	Methyl tertiary butyl ether
min	Minute
mL	Millilitre
mol	Mole
MS	Mass spectrometry
MSCA	Member State Competent Authority
m/z	Mass to charge ratio
nm	Nanometre
NOEC	No-observed effect concentration
OC	Operational condition
OECD	Organisation for Economic Co-operation and Development
p	Statistical probability
P	Persistent

Pa	Pascal
PBT	Persistent, Bioaccumulative and Toxic
PC	Product category
pg	Picogramme
pKa	Acid dissociation constant
PNEC	Predicted no effect concentration
ppb	Parts per billion
PPE	Personal Protective Equipment
ppm	Parts per million
PROC	Process Category
QSAR	Quantitative structure-activity relationship
r2	Correlation coefficient
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals (EU Regulation No. 1907/2006)
RCR	Risk characterisation ratio
RMM	Risk Management Measures
RPE	Respiratory protective equipment
RWC	Reasonable Worst Case
t	Tonne
T	Toxic (hazard classification)
TBA	Tertiary Butyl Alcohol
TG	Test Guideline
UK	United Kingdom
UV	Ultraviolet
vB	Very bioaccumulative
vP	Very persistent
vPvB	Very persistent and very bioaccumulative
wt.	Weight