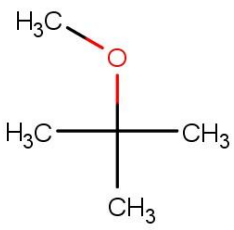
Draft Regulatory Management Option Analysis (RMOA)

Authority: FR- MSCA

Date: April 2022

Substance name: MTBE

General structure:

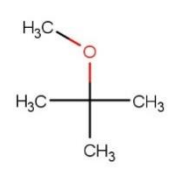


Revision history

Version	*Date*	*Description*
1	May 2022	Initial Version

DRAFT

Substance under consideration:

EC/List number	CAS number	Substance name [and Substance name acronyms (*)]	Chemical structures	Registration type (full, OSII or TII, NONS), highest tonnage band among all the registrations (t/y)
216-653-1	1634-04-4	MTBE		full, 1 000 000-10 000 000 ton/y

DRAFT

Contents

Glossary 7

1 Overview of the substance 8

EC name (public): 8

IUPAC name (public): 8

Index number in Annex VI of the CLP Regulation: 8

Molecular formula: 8

Molecular weight or molecular weight range: 8

2 Overview of regulatory processes 9

2.1 REACH Regulation 9

2.2 Other processes/EU legislation 10

3 Information on uses 11

3.1 Overview of registration dossiers 11

3.2 Overview of uses 11

3.3 Summary 14

4 Hazard Information (including classification) 14

4.1 Classification 14

4.2 Assessment of PMT and vPvM properties 15

4.2.1 Persistence 15

4.2.2 Mobility 17

4.2.3 Toxicity criteria according to Annex XIII of REACH 25

4.2.4 Concern for contamination of water resources related to vPvM properties 25

4.2.5 Summary 29

4.3 Assessment of ED properties relevant for human health 30

4.3.1 Absorption, distribution, metabolism and elimination 30

4.3.2 Carcinogenicity studies 31

4.3.3 Reproductive toxicity studies 34

4.3.4 Endocrine disrupting properties 35

4.3.5 Summary- Analysis of MTBE mode of action in view of ED characterisation 45

5 Environmental exposure & risk assessment 48

5.1 Environmental exposure assessment 50

5.1.1 Tonnage 50

5.1.2 Hazard information 50

5.1.3 Physicochemical properties 50

5.1.4 Degradation in Waste Water Treatment Plants (WWTPs) 51

5.1.5 Emission scenario parameters: description 52

5.2 Environmental risk assessment 56

5.2.1 Local assessment 56

5.2.2 Regional assessment 60

DRAFT

5.2.3 Conclusion ... 60
5.3 Survey of MTBE: monitoring data vs calculated data from risk assessment ... 60
5.4 Conclusion ... 62
6 Justification for the need for regulatory risk management action at EU level ... 64
6.1 Identification as SVHC/Candidate Listing without Inclusion in Annex XIV ... 64
6.2 Restriction ... 66
6.3 Other risk management measures: Drinking water directive (EU 2020/2184) and Industrial Emissions Directive (2010/75/EU) ... 66
6.4 Conclusion ... 67
7 Conclusions and actions ... 68
Annex 1: Harmonised classifications and self-classifications reported by registrants ... 69
Annex 2: Overview of uses based on information available in registration dossiers ... 70
Annex 3: Overview of completed or ongoing regulatory risk management activities ... 71
Annex 4: Human health data : description of sub acute, subchronic and chronic studies (not for publication) ... 72
8 Sub-acute, subchronic and chronic studies including examination of endocrine organs ... 72
8.1 Summary of effects as reported in EU-RAR ... 72
8.1.1 General effects as reported in EU-RAR ... 72
8.1.2 ED effects from the studies reported in EU-RAR ... 73
8.2 Presentation of the studies performed after EU-RAR ... 75
8.2.1 New repeated-toxicity studies (or analysis) after RAR and major RAR studies described ... 75
8.2.2 Reproductive toxicity studies (fertility) ... 88
8.2.3 Reproductive toxicity studies (development) ... 92
9 Endocrine disrupting properties ... 93
9.1 Literature search ... 93
9.2 In silico studies (level 1) and in vitro guideline or non guideline studies (level 2) ... 93
9.2.1 (Q)SAR data, ToxCast and EDSP 21 data ... 93
9.2.2 Other in vitro data ... 93
9.3 In vivo mechanistic data (OECD level 3/4) ... 95
9.3.1 Steroidogenesis ... 95
9.3.2 Effects on oestrogens ... 98
10 OECD level 1 and 2 summary tables ... 100
QSARs ... 100
Models ... 100
Battery ... 100

DRAFT

Case Ultra 100
Leadscope 100
SciQSAR 100
Danish QSAR (Battery, Case Ultra, Leadscope, SciQSAR) 100
Annex 5: References 104

DRAFT

DISCLAIMER

The author does not accept any liability with regard to the use that may be made of the information contained in this document. Usage of the information remains under the sole responsibility of the user. Statements made or information contained in the document are without prejudice to any further regulatory work that ECHA, the Member States or other regulatory agencies may initiate at a later stage. Assessment of regulatory needs and their conclusions are compiled on the basis of available information and may change in light of newly available information or further assessment.

DRAFT

Glossary

CLH	Harmonised classification and labelling
ED	Endocrine disruptor
OEL	Occupational exposure limit
PBT/vPvB	Persistent, bioaccumulative and toxic/very persistent and very bioaccumulative
PMT	Persistent, mobile in water and toxic
RMOA	Regulatory management options analysis
RRM	Regulatory risk management
SEv	Substance evaluation
SVHC	Substance of very high concern

DRAFT

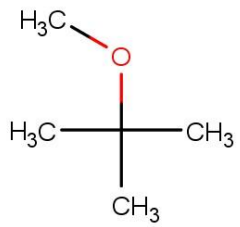
1 Overview of the substance

Table 1: Other substance identifiers

EC name (public):	216-653-1
IUPAC name (public):	tert-butyl methyl ether
Index number in Annex VI of the CLP Regulation:	603-181-00-X
Molecular formula:	$C_5H_{12}O$
Molecular weight or molecular weight range:	88.15
Synonyms:	2-methoxy-2-methylpropane 2-Methyl-2-methoxypropane Ether, tert-butyl methyl Methyl 1,1-dimethylethyl ether Methyl t-butyl ether METHYL tert-BUTYL ETHER Methyl tertiary-butyl ether MTBE Propane, 2-methoxy-2-methyl- tert-butyl methyl ether

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

Structural formula:



In March 2022, the conclusion document related to the evaluation of the MTBE was published on the ECHA website[1] . MTBE was originally selected for substance evaluation in order to clarify concerns about:

- Human health/Potential endocrine disruptor;
- Exposure/Wide dispersive use,
- High (aggregated) tonnage.

During the evaluation, other concerns were identified which were:

- Mutagenicity
- Biodegradability and persistency in the environment,
- Risk for the environment.

[1] https://echa.europa.eu/documents/10162/dbafd544-5671-8b03-980c-37a2bc9291b0

In the following table are gathered the outcome/conclusions of the evaluation:

Table 2 : Evaluated endpoints in the MTBE evaluation

Endpoint evaluated	Outcome/conclusion
Potential endocrine disruptor	For the Environment: potential estrogenic effect but without apical effect. Based on evaluated data, the current endocrine disruptor definition is not fulfilled for environment. Human health: ED related effects seen at high doses. Not sufficient to request additional study. New data may require further consideration in an upcoming RMOA
Mutagenicity	Based on the available database and weight-of-evidence, no classification is proposed for MTBE.
Biodegradability and persistency in the environment	MTBE is not inherently biodegradable and not readily biodegradable. Therefore, MTBE is considered as potentially persistent.
Exposure/Wide dispersive use and aggregated tonnage	High production volume, with a possibility to decrease in future due to an increased use of ETBE.
Risk for the environment	Considering uncertainties about the degradation of MTBE in industrial and municipal wastewater treatment plants, no degradation in STP is taking into account by the eMSCA when refining exposure scenarios, leading to unacceptable risks for the environment (see confidential annex). An RMOA is needed to clarify parameters to be used for risk characterisation and to discuss the need for risk management measures.

France, through ANSES, decided to pursue its investigation on MTBE according to the outcome/conclusions of the evaluation. To do so, and as recommended in the SEv conclusion document, France decided to build a RMOA on particular endpoints: endocrine disruption relevant for human health, PMT/vPvM properties and the risks for the environment. These particular endpoints are detailed in the document and potential need for further regulatory risk management actions is further discussed. For the other endpoints, please refer to the SEv conclusion document.

2 Overview of regulatory processes

2.1 REACH Regulation

Table 3 : Completed or ongoing processes (date of checking: 03 January 2022)

EC/List number	Other REACH related work	RMOA	Evaluation			Authorisation		Restriction	CLH	Actions not under REACH/ CLP(*)
			CCH	TPE	SEV	Candidate List	Annex XIV	Annex XVII	Annex VI (CLP)	
216-653-1	OEL, ED, PBT	x	X[2]		X					

2 https://echa.europa.eu/fr/information-on-chemicals/dossier-evaluation-status/-/dislist/substance/100.015.140

2.2 Other processes/EU legislation

In the EU, the following directive applies to MTBE:

Directive 2009/30/EC amending Directive 98/70/EC as regards the specification of petrol, diesel and gas-oil and introducing a mechanism to monitor and reduce greenhouse gas emissions and amending Council Directive 1999/32/EC as regards the specification of fuel used by inland waterway vessels and repealing Directive 93/12/EEC (**maximum concentration of MTBE for market fuels to be used for vehicles equipped with positive-ignition engines: 22%)**

MTBE may also be affected by other regulations due to its harmonised classification under the CLP regulation:

- Regulation 305/2011/EC on Construction products,
- EU Ecolabel / Restrictions for Hazardous Substances/Mixtures,
- Directive 75/324/EEC on aerosols, flammable contents,
- Directive 2000/53/EC on end-of-life vehicles,
- The general product safety directive 2001/95/EC,
- The inland transport of dangerous goods directive,
- The marine environmental policy framework directive,
- The pressure equipment directive 2014/68/EU,
- The protection of young people directive 94/33/EC,
- The safety and health of workers at work directive,
- The waste framework directive.

Occupational Exposure Limits:

This list contains the indicative occupational exposure limit values (IOELVs) from Commission Directive 2009/161/EU. Member States are required to bring into force the laws, regulations and administrative provisions necessary to comply with this Directive, which establishes values for reference period of eight-hour time weighted average and for a short-term period of 15 minutes.

Long-term Exposure (LTEL)Values		Short-term Exposure Limit (STEL) Values	
mg/m^3	ppm	mg/m^3	ppm
183.5	50	367	100

In the Anses opinion published in 2021 about the biological values for monitoring occupational exposures (Anses, 2021), the working group concluded that " *the available data did not allow to derive a BLV[3] for MTBE based on a dose-effect relationship between the biological indicators concentrations and the health effects.*

The alternative of deriving a BLV corresponding to exposure to the OEL-8h of MTBE does not appear relevant. Indeed, the current OEL-8h is based on irritant effects and subjective signs of subacute toxicity (headaches, fatigue, etc.) and does not protect against all the systemic effects of chronic exposure.
Furthermore, the search for available data concerning impregnation in a general population of adults did not identify any biological exposure indicators making it possible to propose a biological reference value (RBV) for the biological monitoring of occupational exposure to MTBE that would be relevant for the current French

[3] Biological limit value

population (ie from populations whose exposure to MTBE is close to that of the current French population).
The "Biological indicator of exposure" working group concludes that it is not feasible in the current state of knowledge to produce a BLV and/or to identify a RBV for MTBE. The possible continuation of the work is subject to the prior revision of the current OEL-8h.
[Anses] recommends revising the OEL-8h and conducting studies to assess the impregnation of the general population residing in France.

3 Information on uses

3.1 Overview of registration dossiers

Table 4 : Overview of registrations (intermediate registrations versus article 10 full registration)

EC /List number	CAS Number	Substance name	REACH Annex	Article 10 Registrations (active)	Intermediate Registrations (active)	Not-updated NONS
216-653-1	1634-04-4	MTBE	X	1 000 000-10 000 000 ton/y	-	-

3.2 Overview of uses

In the following table are gathered all the uses of MTBE that have been retrieved from registration dossiers based on information available on ECHA website. They are classified according to the technical function and the product or article type.

Table 5 : Overview of uses

Main types of applications structured by product or article types	MTBE	Technical function
PROC 1: Chemical production or refinery in closed process without likelihood of exposure or processes with equivalent containment conditions	A,P,I,F,M	Fuel use (P, I,F,M), Use in coatings (P,I),use in cleaning agents (P,I), Process solvent, extraction agent, intermediate, industrial use (I,M), Distribution of substance (I,M), Rubber production(I), Manufacture of other substance(I,M), Formulation & (re)packing(F)
PROC 2: Chemical production or refinery in closed continuous process with occasional controlled exposure or processes with equivalent containment conditions	P,I,F,M	Fuel use (P,I,F,M), Use in coatings (P,I),use in cleaning agents (P,I), Process solvent, extraction agent, intermediate, industrial use (I,M), Distribution of substance (I), Rubber production(I), Manufacture of other substance(I,M), Formulation & (re)packing(F), Transport (M), Distribution (M)
PROC 3: Manufacture or formulation in the chemical industry in closed batch processes with occasional controlled exposure or processes with equivalent containment conditions	P,I,F,M	Fuel use (P,I,F,M), Use in coatings (P,I),use in cleaning agents (P,I), Process solvent, extraction agent, intermediate, industrial use (I,M), Distribution of substance (I,M), Rubber production(I), Manufacture of other substance(I), Formulation & (re)packing(F), Blending (F), Transport (M), Distribution (M)
PROC 4: Chemical production where opportunity for exposure arises	P,I,F,M	Use in coatings (P,I),use in cleaning agents (P,I), Process solvent, extraction agent, intermediate, industrial use (I,M), Distribution of substance (I,M), Rubber production(I), Fuel(F,M), Formulation & (re)packing(F), Blending (F), Manufacture (M), Transport (M)
PROC 5: Mixing or blending in batch processes	P,I	Use in coatings (P,I), Rubber production(I), Formulation & (re)packing(F)
PROC 6: Calendering operations	I	Rubber production(I)
PROC 7: Industrial spraying	I	Use in coatings (I), Rubber production(I), use of cleaning agents (I)
PROC 8a: Transfer of substance or mixture	P,I,F,M	Fuel use (P,I,F,M), Use in coatings (P,I), Use in cleaning

DRAFT

Main types of applications structured by product or article types	MTBE	Technical function
(charging and discharging) at non-dedicated facilities		agents (P,I), transfer (I,M), Process solvent, extraction agent, intermediate, industrial use (I,M), Distribution of substance (I,M), Rubber production(I), Manufacture of other substance(I,M), Formulation & (re)packing(F),
PROC 8b: Transfer of substance or mixture (charging and discharging) at dedicated facilities	A, P,I,F,M	Fuel use (P,I,F,M), Use in coatings (P,I) ,Use in cleaning agents (P,I),transfer (I,M), Process solvent, extraction agent, intermediate, industrial use (I,M), Distribution of substance (I,M), Rubber production(I), Manufacture of other substance(I,M), Formulation & (re)packing(F),
PROC 9: Transfer of substance or mixture into small containers (dedicated filling line, including weighing)	P,I,F,M	Fuel use (P,M,F), Process solvent, Extraction agent, intermediate, industrial use (I), Distribution of substance (I,M), Rubber production(I), Formulation & (re)packing(F), Transport (M)
PROC 10: Roller application or brushing	P,I	use in coatings (P,I) ,Use in cleaning agents (P,I)
PROC 11: Non industrial spraying	P	use in coatings (P) ,Use in cleaning agents (P)
PROC 13: Treatment of articles by dipping and pouring	P,I	use in coatings (P,I) ,Use in cleaning agents (P,I), Rubber production(I)
PROC 14: Tabletting, compression, extrusion, pelletisation, granulation	I,F	Rubber production(I), Formulation & (re)packing(F)
PROC 15: Use as laboratory reagent	P,I,F,M	Professional use, Use in coatings (P, I,M), Process solvent, extraction agent, intermediate, industrial use (I), Distribution of substance (I), Rubber production (I), sampling (I,M), Manufacture of other substance(I,M), Quality control (I),Fuel(F,M), Formulation & (re)packing(F), Sampling & laboratory activities(F)
PROC 16 : Use of fuels	P,I	Fuel use (P,I), Process solvent, Extraction agent, intermediate, Industrial use (I)
PROC 19: Hand-mixing with intimate contact and only PPE available.	P	Use in coatings (P)
PROC21 : Laboratory chemicals	I	Process solvent, extraction agent, intermediate, industrial use (I),Rubber production(I)
PROC28: Manual maintenance (cleaning and repair) of machinery	I	Fuel(I)
ERC1: Manufacture of the substance	I,F,M	Process solvent, extraction agent, intermediate, industrial use (I,M), Manufacture of other substance(I,M), Fuel(F,M), Sampling & laboratory activities(F,M), Transport (M), Distribution (M),Transfer(M)
ERC2 : Formulation into mixture	P, F,M	Blending (P,F), Fuel (F,M), Formulation & (re)packing(F), Sampling & laboratory activities(F,M), Transport (M), Distribution (M)
ERC4: Use of non-reactive processing aid at industrial site (no inclusion into or onto article)	I,M	Transfer (I) Process solvent, Extraction agent, Intermediate, Industrial use (I,M), Distribution of substance (I), Use in coatings (I), Rubber production(I), Quality control (I), Use of cleaning agents (I)
ERC5: Use at industrial site leading to inclusion into/onto article	I	Distribution of substance (I)
ERC6a: Use of intermediate	I,M	Transfer (I) Process solvent, Extraction agent, intermediate, industrial use (I,M), Distribution of substance (I), laboratories activities, sampling (I)
ERC6b: Use of reactive processing aid at industrial site (no inclusion into or onto article)	I	Fuel (I), Process solvent, Extraction agent, Intermediate, Industrial use (I), Distribution of substance (I)
ERC6c: Use of monomer in polymerisation processes at industrial site (inclusion or not into/onto article)	I	Distribution of substance (I)
ERC6d: Use of reactive process regulators in polymerisation processes at industrial site (inclusion or not into/onto article)	I	Distribution of substance (I), Rubber production(I)
ERC7: Use of functional fluid at industrial site	I	Fuel (I), Process solvent, Extraction agent, intermediate, industrial use (I), Distribution of substance (I)
ERC 10a : Widespread use of articles with low release (outdoor)	A	
ERC8a: Widespread use of non-reactive processing aid (no inclusion into or onto article, indoor)	C,P	Use in cleaning agent (C,P) , Professional use (P), use in coatings (P)
ERC8d: Widespread use of non-reactive processing aid (no inclusion into or onto article, outdoor)	C,P,I	Use in cleaning agent(C,P), Use in coatings (P), Fuel (I)
ERC8e: Widespread use of reactive	C,P	Fuel use (C,P) , Use of engine, including refuelling of

DRAFT

Main types of applications structured by product or article types	MTBE	Technical function
processing aid (no inclusion into or onto article, outdoor)		vehicles by consumers (C)
ERC8b: Widespread use of reactive processing aid (no inclusion into or onto article, indoor)	C,P	Fuel use (C,P), professional use (P)
ERC9a: Widespread use of functional fluid (indoor)	C,P	Fuel use (C,P)
ERC9b: Widespread use of functional fluid (outdoor)	C,P	Fuel use (C,P)
PC 13: Fuels	C,F,P	Fuel use (C,F,P), Blending (F)
PC 3: Air care products	C	Use in cleaning agent
PC 4: Anti-freeze and de-icing products	C	Use in cleaning agent
PC 9a: Coatings and paints, thinners, paint removes	C	Use in cleaning agent
PC 21 : Laboratory chemicals	P	Professional use
PC 24: Lubricants, greases, release products	C	Use in cleaning agent
PC 35: Washing and cleaning products	C	Use in cleaning agent
PC 38: Welding and soldering products, flux products	C	Use in cleaning agent

F: formulation, I: industrial use, P: professional use, C: consumer use, A: article service life

An ANSES opinion published in 2021 on biological values for monitoring occupational exposures also provides valuable information on uses (ANSES, 2021):

In France, in the oil industry until 2005, MTBE was mainly used as an additive to rise the octane number in order to replace lead. Even if MTBE has not been forbidden, it has been replaced by ETBE in the oil industry. Indeed, following the adoption of Directive 2003/30/EC encouraging the use of biofuel through tax reduction, French factories adapted to synthesize ETBE.
Between 2000-2009, the market of the additive to rise octane number was dominated by ETBE in France. The BRGM indicated that France was the first country to introduce ETBE in commercialized fuels.
MTBE can also be used as an extraction solvent and a copolymerization reagent. High purity MTBE can be used in pharmaceutical industry, in chromatography and as a therapeutic agent for dissolving cholesterol deposits (gallstone removal) in humans.
In 2014, Anses, (ANSES, 2014) in its sector study reported that France did not produce anymore MTBE, the last plant ceased the production of MTBE in 2005 and switched to ETBE. In this sector study, distributors or importers have declared to import/distribute few litres of MTBE per year. Nevertheless, nowadays, it seems that plants re-produce MTBE, one of them declaring more than 600,000t/y.
At EU level, the proportions of MTBE, ETBE, ethanol in fuels vary according to the countries (Coftier et al. 2013, see Fig. 1 below).

DRAFT

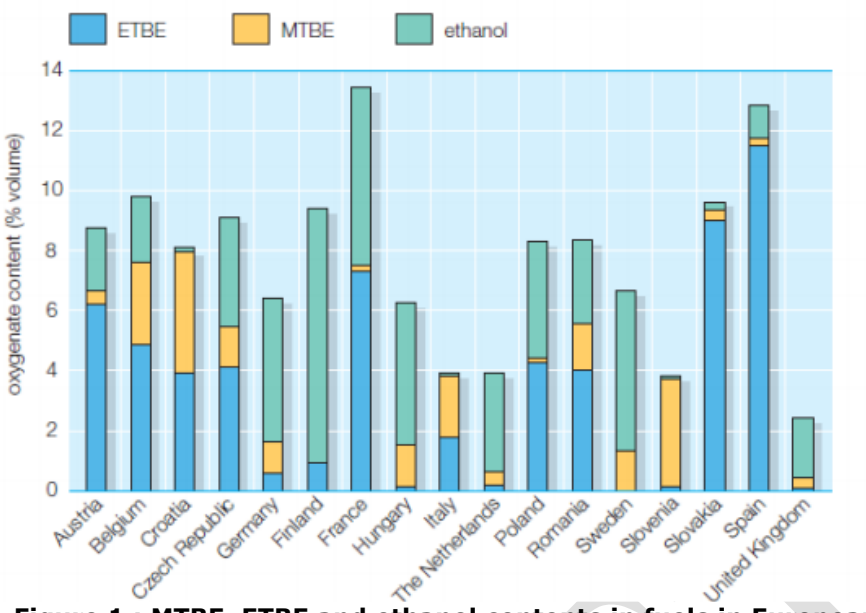


Figure 1 : MTBE, ETBE and ethanol contents in fuels in European countries (Cofier et al. 2013)

Finally, according to ANSES (ANSES, 2021), the EU is the MTBE main production area in the world.

3.3 Summary

As a summary, **MTBE is mostly used in fuels** as an additive to rise the octane number especially in unlead petrol. Fuel is indicated to be used either for consumer or professional or industrial use. It can also be used as a cleaning agent, a solvent in various industries or a laboratory reagent (in health services and scientific research and development). MTBE is listed to be used in coatings, in formulation or, more rarely, in manufacture of chemicals.

This substance is also used in the following activities or processes at workplace: transfer of chemicals, closed batch processing in synthesis or formulation, closed processes with no likelihood of exposure, closed, continuous processes with occasional controlled exposure, in materials as fuel sources.

4 Hazard Information (including classification)

4.1 Classification

Table 6 : Harmonised classification and reported self-classification

EC/ List No	CAS number	Substance name	Harmonised classification(*)	Classification in registrations	Classification in C&L notifications
216-653-1	1634-04-4	tert-butyl methyl ether MTBE 2-methoxy-2-methylpropane	Flam. Liq. 2 H225 Skin Irrit. 2 H315	Flam. Liq. 2 H225 Skin Irrit. 2 H315	2092 notifiers

DRAFT

4.2 Assessment of PMT and vPvM properties

During SEv, MTBE was concluded to be potentially persistent but of low bioaccumulation potential. In this RMOA, the analysis focuses on the assessment of the ability of MTBE to contaminate water resources (PMT and/or vPvM potential) as a follow-up to the SEv Conclusion document (ANSES, 2022).

4.2.1 Persistence

MTBE is stable to hydrolysis at the environmentally significant pH range as concluded in the SEv conclusion document (ANSES, 2022). Concerning the biodegradation in water, three reliable studies testing the ready biodegradability of MTBE according to OECD 301D were available. Based on the conclusion of the Conclusion document, MTBE is not ready biodegradable supporting that MTBE is potentially P/vP. An inherent biodegradation study (unpublished study report, 2005) was also available with an inoculum considered adapted to the MTBE. It was concluded that MTBE is not inherently biodegradable. Additional studies were provided and disregarded because of the use of pure culture inoculum or the use of selected microorganisms originated from contaminated sites.

Simulation tests for the water/sediment compartment were also available. These studies (Bradley *et al.* (1999); Sulfita and Mornile (1993) ; Mornile and Sulfita (1994)) indicated no degradation of MTBE under anaerobic conditions. These studies also show that biodegradation occurs but only in aerobic conditions with adapted microorganisms. In a weight of evidence approach, no degradation of MTBE in water/sediment system is considered for the assessment of the P criterion.

Concerning the biodegradation in soil, MTBE showed a biphasic degradation with a graphically DT_{50} of 112 days at 12°C for the fast phase then there was no degradation (DT_{50} > 180 days at 12°C) in Borden *et al*. (1997), however there is no information concerning the concentration of the microorganisms or the mass balance. Yuan (2006), showed that MTBE is degraded in the soil with a graphically estimated DT_{50} of 212 days and 127 days at 12°C with respectively 100% organic planting soil and 60% organic planting soil. However, it has to be noticed that the concentration of microorganisms in the soil before and after the test and the mass balance were missing. Yeh and Novak (1995) showed that MTBE is not degraded in soil over 250 days in anaerobic conditions. High DT_{50} can also be observed in contaminated soil, with assumed adapted microorganisms (Allard *et al*. (1996)). Although they are not carried out according to OECD guideline, both studies of Borden (1997) and Yuan (2006) support that MTBE can be concluded as vP based on the DT_{50} (DT_{50} > 180 days at 12°C).

As MTBE is not considered bioaccumulative (B) because of the low Kow and supported by the study of Fujiwara *et al.* (1984) (whole-body bioconcentration factors of 1.4 and 1.5 for *Cyprinus caprio)*, the P criterion was initially not further investigated. Therefore, MTBE was considered as potentially P/vP based on the available data.

However, other concerns are emerging, such as the concern related to PMT (persistency, mobility, toxicity) or vPvM properties and the capacity of the substance to contaminate water resources. Such properties could be considered as equivalent to PBT even if currently there are no validated threshold.

As MTBE was considered P/vP based on the soil studies which are not carried out according to OECD guideline, additional literature review on the persistence has been carried out in 2021 to better investigate the P criterion and to confirm that

DRAFT

the substance is vP. Studies with not adapted microorganisms or adapted microorganisms but with still high DT_{50} were focused.

The selected publications from the literature review (with an * in the table 7 below) were in the groundwater compartment except one publication for the sediment compartment with DT_{50} between approximatively 70 to 200 days at 12°C which support that MTBE is very persistent. However, from a regulatory point of view, degradation data in groundwater are not sufficient to conclude on the P criterion, but can be used in a weight of evidence. Scientifically, there is less microorganisms in the groundwater compartment compared to surface water, therefore if MTBE is degraded very slowly in the groundwater, it is not excluded that it can be faster degraded in surface water. It has been investigated if DT_{50} of other contaminants from the selected publications for groundwater compartment are available. They could have been compared to their DT_{50} in water compartment in order to determine in which extent data from the groundwater can be used for the P assessment.

Unfortunately, data on other contaminants were reported only in one study. In the publication of Martienssen *et al*. (2006), the DT_{50} of tert-butyl alcohol (TBA which is a biodegradation product of MTBE) has been graphically determined to be 2 days with 30 $mg.L^{-1}$ of oxygen and 17 days with 160 $mg.L^{-1}$ of H_2O_2. According to the registration dossier, several studies on ready biodegradability of TBA have been conducted with results varying from little or no degradation to 99% degradation. A subsequent inherent biodegradability study using adapted inoculum proved that TBA is inherently biodegradable. However, no simulation study was available, therefore it is difficult to compare and conclude on the similarity or not between the groundwater and surface water compartment. In another study, degradation of MTBE has been compared to benzene; the half-life of MTBE in groundwater is on the order of 2 to 3 years while it is 2 to 3 months for benzene (Wilson, 2003)

Table 7 : Persistency data from the registration dossier and from additional literature search

Authors	Date	Compartment	Adapted ?	Aerobic or Anaerobic	DT50
Bradley *et al.*	1999	Water / sediment	Adapted	Aerobic and anaerobic	73% of deg. after 105 d At room temperature 0% of deg.
Borden *et al.*	1997	Soil	?	?	≈ 112 d at 12°C for the fast phase, **then no degradation**
Yuan	2006	Soil	Not adapted	Aerobic	≈ 212 d at 12°C (100% organic) ≈ 127 d at 12°C (60% organic)
Bradley *et al*. *	2001	Sediment	Not adapted	Aerobic	> 50 d (temperature unknown)
Fischer *et al*. *	2005	Groundwater	Adapted	Aerobic and anaerobic	> 35 d at room temperature (aerobic)
Schirmer *et al.* *	1999	Groundwater	Adapted	Aerobic	> 60 and > 100 days (temperature unknown)
Shah *et al*. *	1999	Groundwater	Not adapted	Aerobic	> 125 days at 10°C

DRAFT

Martienssen *et al.* *	2006	Groundwater	Not clear	O2 / H2O2 addition	> 53 days at 12°C (aerobic)

The concentration of MTBE in publications were compared to the OECD guidelines 308 and 309. In all the publications, concentrations were greater than the recommended concentration in the guideline (> 100 µg.L^{-1}), however theses concentrations are still relevant as they are below the EC10 of microorganisms (710 mg.L^{-1}) and similar to concentrations found in Europe. The conditions in these studies are realistic and similar to the environment, there is no toxicity (EC_{10} for microorganisms = 710 mg.L^{-1}) but it has to be noticed that there are less microorganisms in the groundwater compartment compared to the surface water compartment. These results seems sufficient to conclude that the MTBE is very persistent (vP).

The available data in the registration dossier and the literature review, support that MTBE can be considered as very persistent (vP).

4.2.2 Mobility

4.2.2.1 Log Koc

The organic carbon-water partitioning coefficient (Koc) calculated from the octanol-water partition coefficient (log Kow = 1.06) using the equation from the TGD (Technical Guidance Document on Risk assessment part III - 2003 – Table 4) is 9 L.kg^{-1} (log value = 0.93).

MTBE has therefore a low tendency to adsorb to organic matter and is expected to remain in the water phase, once the substance is released to the environment.

The MTBE can be considered as very mobile (vM) and it is assumed that the substance will contaminate the aqueous environment. Consequently, an analysis of the monitoring data in the aquatic compartment is performed below to determine the occurrence of the substance in this media.

4.2.2.2 Monitoring data

4.2.2.2.1 Surface water and wastewater

The data reported below come from the Norman network. The database contains 39,910 samples registered for MTBE across five countries (Belgium, Denmark, France, Germany, Netherlands, Norway) between 2000 and 2017.

The sample matrix mainly includes data for surface water (river: n= 38 387; 96% of data).

The majority of the data comes from France (41%), Germany (34.5%) and the Netherlands (23.5%). Finally, 79.33% of data are below the LOD or LOQ. The range of the limit of detection values is 0.01 µg/L to 1 µg.L^{-1}. The range of the limit of the quantification is 0 to 3 µg.L^{-1}.

The distribution of data above the LOD/LOQ is shown is the table below.

DRAFT

Table 8 : Distribution of individual value above the LOD/LOQ.

Range of values [µg.L^{-1}]	n
[0.01;0.1[	1,710
[0.1;1[	5,224
[1;10[	956
> 10	44
TOTAL	**7,934**

Analysis of the data indicates that very few values exceed 10 µg.L^{-1} (n=44). Two values are above the hazard threshold value (PNEC value of 304 µg.L^{-1}) for aquatic organisms. Unfortunately it is not possible to explain these high MTBE values.

The Norman network database provide several data on the concentrations on MTBE in European surface water, some examples are provided in the figures here under.

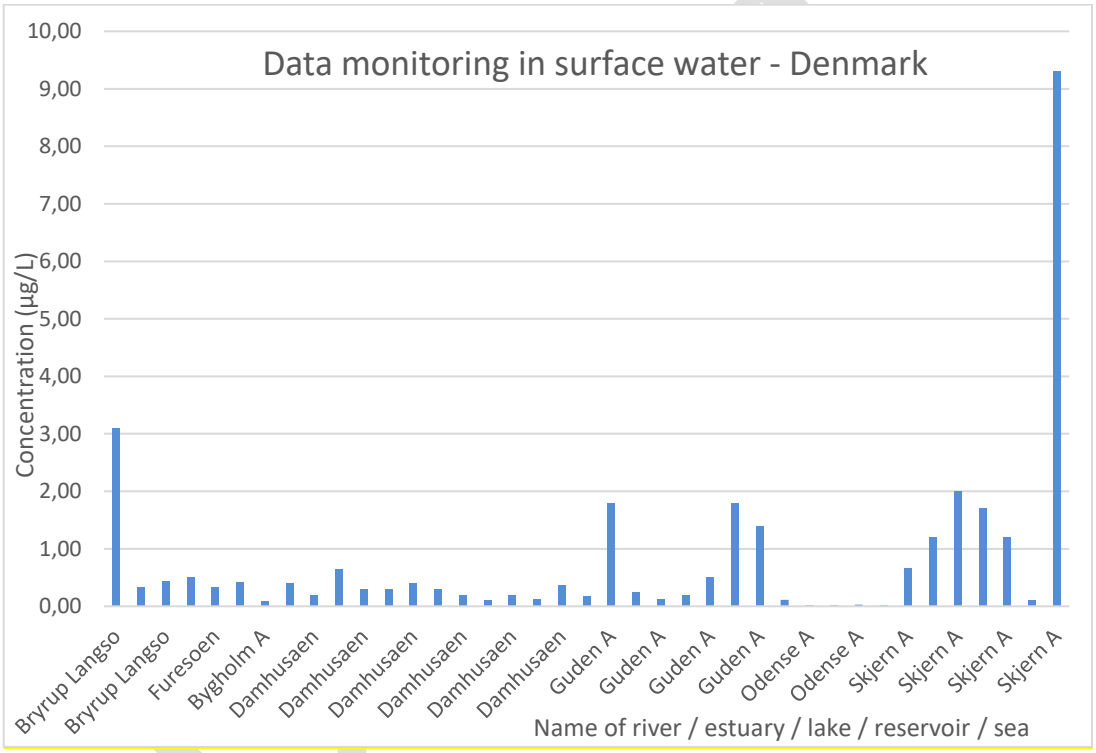


Figure 2 : Data monitoring in surface water in Denmark between 2000 and 2013

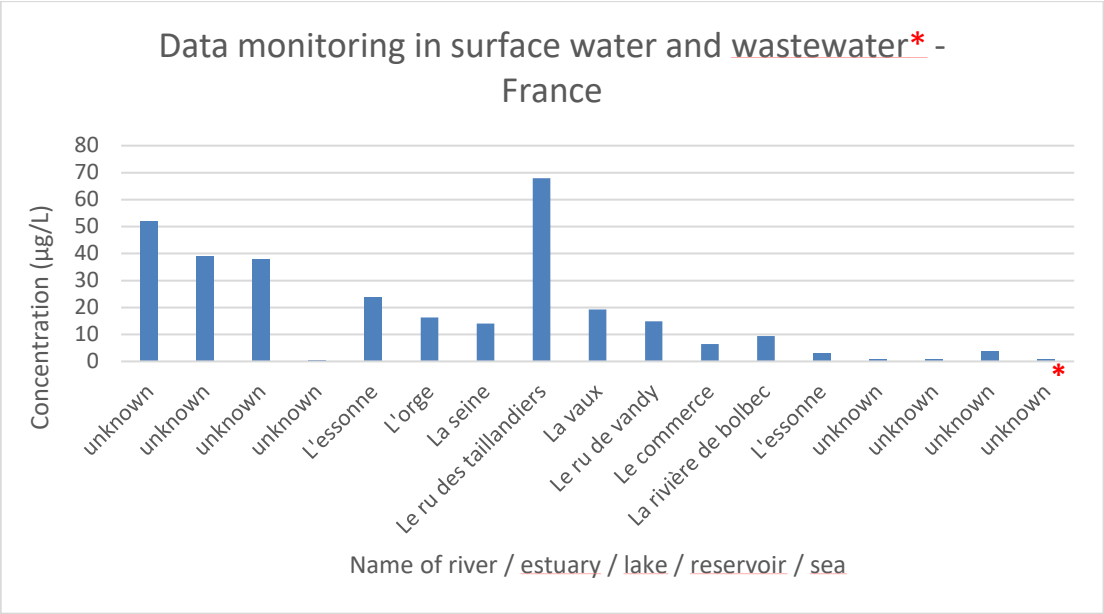


Figure 3 : Data monitoring in surface water and wastewater* in France between 2008 and 2014

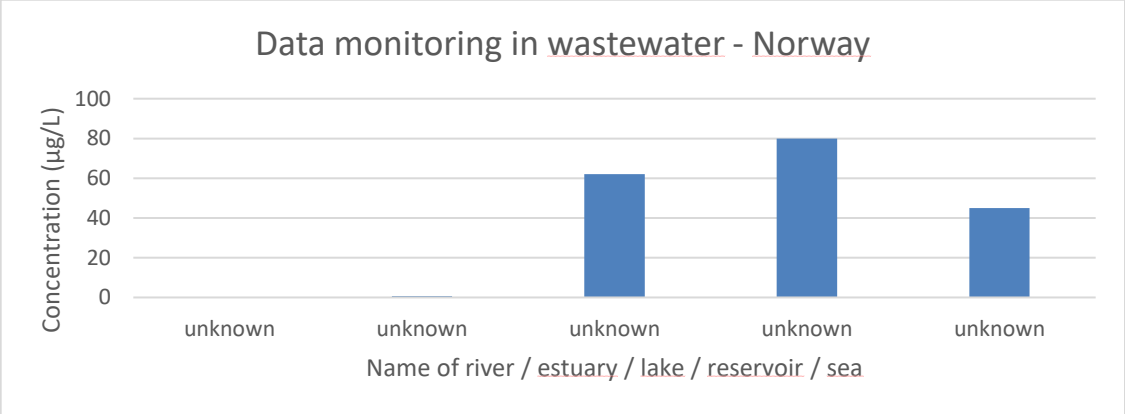


Figure 4 : Data monitoring in municipal waste effluent in Norway – No information concerning the year of sample

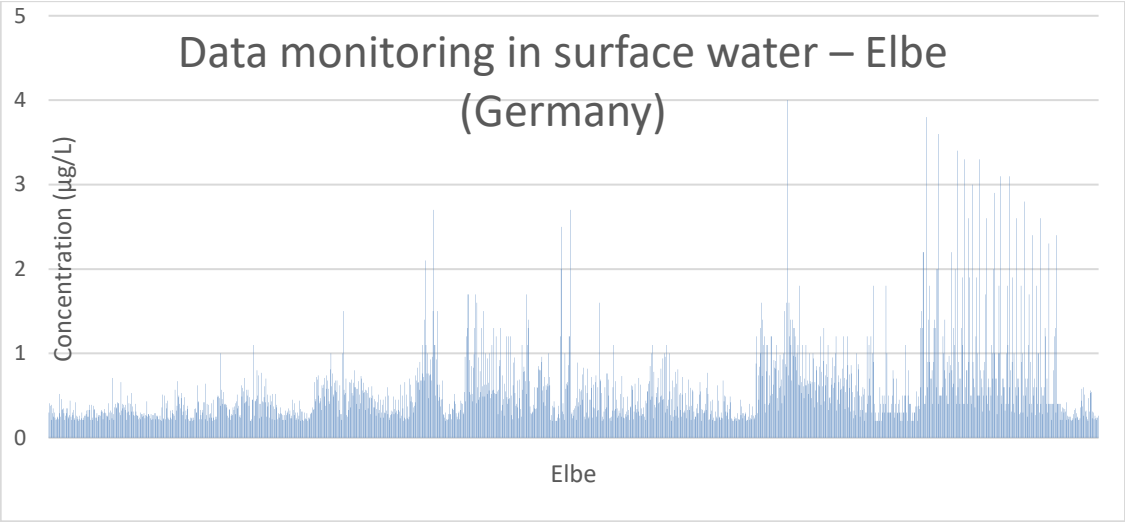


Figure 5 : Data monitoring in Elbe River in Germany between 2002 and 2012

For Germany, only the Elbe River is presented because of the huge amount of data available. In other rivers in Germany, the concentration of MTBE range between 0.2 to 620 µg.L^{-1} with a mean value of 29 µg.L^{-1} when excluding the value below the LOQ and LOD. Moreover, it has to be noticed that there is no information regarding a potential contamination source near the sampling point. The other selected data show that high concentrations are often detected in the European surface water (until 80 µg.L^{-1}). However, it is not reported if the sampling were carried near or far from a potential source of emission of MTBE.

Concerning the STP effluent less data are available. Among 243 data, there are 6 data where the concentration exceed LOQ. For France, only one data is above the LOQ with a concentration of 0.9 µg.L^{-1}. For Norway, the concentration of MTBE can reach 80 µg.L^{-1} in municipal wastewater effluent.

4.2.2.2.2 **Groundwater**

The monitoring data on groundwater were already assessed in the EU-RAR (European Commission, 2002) of the MTBE. According to the EU-RAR, the concentration of MTBE in groundwater display a wide range from high levels (of up to 500,000 µg.L^{-1} near the source to background levels farther down the aquifer) in Europe. In the USA, MTBE has been monitored extensively in groundwater. Median value (of detected concentration only) in the almost 3,000 wells in untreated groundwater sampled by USGS in 1985 – 1995 was 600 ng.L^{-1} in urban areas and 500 ng.L^{-1} in rural areas, the maxima being above 20,000 µg.L^{-1} and 150 µg.L^{-1} respectively.

The table below from the EU-RAR showed the concentration of MTBE in µg.L^{-1} in groundwater.

Please refer to the EU-RAR for the complete assessment.

DRAFT

Table 9 : Summary of measurements (µg.L^{-1}) of MTBE in groundwater from the EU-RAR (European Commission, 2002)

Country	Type of groundwater and loading	Med	Mean	Max	Information source
A	101 groundwater aquifers	0.01-0.1		>20	BMLF (2000)
D	3 groundwater aquifers at petrol stations, leaking tanks		270 (one aquifer)	185-2,000	UBA (1999)
DK	Shallow groundwater aquifers at service stations, leaks			ND-30,000	Miljostyrelsen (1998)
FI	Urban aquifers, Helsinki	<DL		0.72	Municipalities (unpubl. Reports)
FI	Urban aquifers, Tampere	1.9		3.7	
FI	Shallow aquifers/potable water wells near service stations, leaks			16-330,000 [1)]	Regional authorities, firms (unpubl.)
NL	Groundwater at 4 petrol station sites			120	TNO-report (Langenhoff, 2000)
S	A groundwater aquifer, petrol station leak			>>20	KEMI (2000)
UK	Groundwater at 59 petrol station sites			832,500	UK Environment Agency (Dottridge et al., 2000)
UK	251 public water supply wells		1.1	12.7	UK Environment Agency (Dottridge et al., 2000)
UK	Extractable aquifers, mixed loading	55-480	1,100 (one aquifer)	530-2,900	Wrc (unpubl.), various surveys
USA	Urban, mixed loading	0.6		20,000	USGS surveys,
USA	Rural, mixed loading	0.5		150	Squillace et al. (1999)

[1)] range of maxima in the various local contamination cases

As indicated in the EU-RAR (European Commission, 2002) of MTBE, it has been monitored extensively in groundwater in the USA. Detection frequencies were 16.9% of the samples in urban and 3.4% in rural aquifers, making MTBE the second most often detected volatile organic contaminant in groundwater in the USA.

In a more recent report by Concawe (2012), a European survey on available information on the occurrence of MTBE in groundwater has been reported in different literature sources and authority information. A summary of these information has been reported in the table below.

DRAFT

Table 10 : European survey on the occurrence of MTBE in groundwater

	Observation period	No. of sampling locations	No. of Samples in total	Locations > limit of detection (%)	Detection limit ($\mu g.L^{-1}$)	Max. value ($\mu g.L^{-1}$)	Median value of positive samples $\mu g.L^{-1}$	Comments	Source
Austria	2002		95			10.6	0.036	Additional data: 6 samples of the contaminated sites with concentration ranged from 0.129 up to 1 594 $\mu g.L^{-1}$, with a median value of 369 $\mu g.L^{-1}$.	Federal Environmental Agency of Austria
Austria (karst/fissure aquifers)	2004	217	565	14.7%	0.01	1.2			Federal Environmental Agency of Austria
Austria (intergranular porosity aquifers)	2004	1673	5000	56.2%	0.01	31			Federal Environmental Agency of Austria
Swiss	2002-2006	1958		15.5%	0.015-1[1]	6.4	0.52	Additional data: Laboratorio cantonale (2008) reported contamination of groundwater in Morbio Inferiore. The MTBE concentration in 5 groundwater wells at one site was 83.9 to 273 µg/L.	Kilchmann et al, 2009/ NAQUA, BUWAL (2004)
Swiss (Basel)	2002-2009	129	703	4.65%	0.04-1	0.2	0.0775		Agency for Environment and Energy of the city of Basel
Swiss (St Gallen)	2001-2009	65	472	10.80%	Not provided	2.12	0.1		Not reported
German (Bavaria)	2001-2010	626	1408	11.98%	0.004-4	1600	0.1		Environmental Agency of Bavaria
German (Saxony)	2002-2008	135	374	3.70%	0.1-0.2	5	0.93		Environmental Agency of Saxony

DRAFT

German (Thuringia)	2003-2009	30	37	56.70%	0.2-0.1	31	1.3	observation in Thuringia mostly on sites with known / assumed incidents	Not reported
Netherlands			89	60%	0.01	0.3-0.41	0.06-0.1	groundwater contamination	Morgenstein et al (2003)
UK (Scotland)	2005-2009	23	52	0%	1	0			Energy Institute
UK (Northern Ireland)	2000-2006	106	513	0.97%	0.2-10	10			Energy Institute
UK (England and Wales)	1990-2006	2566[1] / 771[2]	6885[1] / 2415[2]	3.2%[1] / 15%[2]	0.1 – 100 (majority 0.5 since 2003)	3900[1] / 103000[2]	0.52[1] / 5.3[2]		Energy Institute

[1] ….. for routine monitoring of groundwater resources
[2] ….. for targeted investigations at potentially contaminated sites

DRAFT

Results show the presence of MTBE in groundwater at the monitored locations. The median MTBE concentration was around 0.1 µg.L^{-1} to 1 µg.L^{-1} except in contaminated sites where concentration are higher. Several reports indicate that higher concentrations were more often found in areas with a higher grade of urbanisation. Moreover, more significant contamination of industrial sites and former storage facilities were reported in German reports (up to 2,000 µg.L^{-1}).

As indicated in the EU-RAR, a typical concentration cannot be meaningfully defined.

4.2.2.3 Conclusion on monitoring data

The Norman Database shows 38,387 data for the surface water in Europe between 2000 and 2017. Finally, 79.33% of data are below the LOD or LOQ (LOD: between 0.01 and 1 µg/L – LOQ: between 0 and 3 µg.L^{-1}). Therefore, 7934 (20.7%) data are above the LOQ/LOD. Among this value, 6180 (16.1%) monitoring data are between 0.1 and 10 µg/L.
For comparison purpose, these values are above the quality standards set for the phytosanitary and biocidal substances (0.1 µg.L^{-1} threshold for water intended for human consumption (Article 4.1.b Directive (EU) 2020/2184 of the European parliament and of the council on the quality of water intended for human consumption)). However, such thresholds are not set for every chemical substance under REACH (some are set in Appendix I of the above mentioned Directive, to values between 0,01 and 100 µg/L depending on the substance). Such concentration could occur in drinking water and some concern are arising for contamination of water resources (cf 4.2.2)

Lower values are available in groundwater according to the Concawe survey (2012); the median value ranges from 0.036 to 0.93 µg.L^{-1} in groundwater in Europe. Nevertheless, the median value can reach 5.3 µg.L^{-1} in groundwater in a potentially contaminated site.

Higher values are however reported in groundwater according to the EU-RAR (European Commission, 2002), the median value ranges from 0.01 to 1.9 µg.L^{-1} and the maximum value ranges from 0.72 to 2900 µg.L^{-1} in groundwater for uncontaminated sites in Europe . The maximum value ranges from 20 to 330,000 µg/L in groundwater for contaminated sites in Europe. However, it is not clearly indicated if this last high value corresponds to point / accidental contamination or a diffuse contamination. The second highest concentration in this report is 30,000 µg.L^{-1}. Higher concentrations can be found under petrol station sites (until 832,500 µg.L^{-1})

Wide range of MTBE concentration in groundwater were also reported for the USA in the EU-RAR, the median value ranges from 0.5 to 0.6 µg.L^{-1} and the maximum value ranges from 150 to 20,000 µg.L^{-1} (limited information regarding the potential contamination).

It should be reminded that the occurrence of mobile substance in the (ground) water results of both its intrinsic property and from emission to the environment. The reported concentration of MTBE in (ground) water supports that the mobility of the substance allows to contaminate aqueous compartment.

DRAFT

4.2.3 Toxicity criteria according to Annex XIII of REACH

4.2.3.1 Human Health

MTBE does not meet the T criteria of annex XIII according to its current harmonized classification.

4.2.3.2 Ecotoxicity

According to the SEv report, MTBE does not fulfill the T criteria for aquatic toxicity, therefore **MTBE is not considered as toxic (T).** Indeed, the (lowest) NOEC for freshwater is 3.04 $mg.L^{-1}$.

4.2.4 Concern for contamination of water resources related to vPvM properties

According to the report "Late lessons from early warnings: the precautionary principle 1896 – 2000" from the European Environment Agency in 2001, the use of MTBE poses an ongoing, everlasting risk of having irreversible adverse effects because of its persistency in groundwater. These adverse effects could come in the form of known effects such as taste and odor in contaminated drinking water, as well as in the form of presently unknown adverse effects.

The monitored concentrations (see 4.2.1.2.2) can be compared to the organoleptic detection limits. The organoleptic detection limits are in the range of 15 to 180 $\mu g.L^{-1}$ for MTBE (Young et al. 1996, Prah et al. 1994). Besides, according to the report of the US EPA "Drinking water Advisory: Consumer Acceptability advice and health effects analysis on methyl tertiary-butyl ether (MTBE), (1997)", the recommended concentration of MTBE in drinking water should be in the range of 20 to 40 $\mu g.L^{-1}$ or below to protect consumer acceptance of the water (with a large margin of exposure (safety) from toxic effects). This threshold value is also based on the estimation linked to the organoleptic effects. The concentration of MTBE monitored in surface water and groundwater are in the same range and sometimes higher than these organoleptic threshold values, supporting a concern for the drinking water quality.

Besides, although not meeting T criteria of annex XIII, the following elements need further consideration in the assessment of the overall concern:

- MTBE is considered by ANSES as a suspected ED based on current state of analysis (see section 4.2.2).
  Additional data have been published after the finalisation of the analysis (Bus et al., 2022; Zhu et al., 2022). They have not been analysed in detail and may provide a different perspective to the existing data. The conclusion may need reconsideration in the future.

- In addition, MTBE showed some carcinogenic effects in several species namely rat and mice and in different strains of rat which leads to consider MTBE as a borderline case between non classification and Carc. Cat. 2. MTBE has no harmonised classification for carcinogenic properties. This conclusion may need further consideration based on a group approach and could provide supportive evidence in a SVHC identification under article 57(f) (equivalent level of concern).

Finally, it appears that PMT substances are difficult to remediate because of their physico-chemical properties, therefore a literature review on the existing methods

DRAFT

of depollution in water has been performed. These methods are described in the table below.

Table 11 : Exhaustive literature review of remediation methods

References	Technical used	Efficacy?
Removal of BTEX, MTBE and TAME from aqueous solutions by adsorption onto raw and thermally treated lignite Aivalioti et al. (2012)	Raw and thermally treated lignite at 250, 550 and 750°C	Lignite samples were quite effective in removing BTEX, MTBE and TAME from aqueous solutions at 750°C
Evaluation of granular activated carbon technology for the removal of MTBE from drinking water Shih *et al.* (2003)	Granular activated carbons (GACs) using rapid small-scale column tests (RSSCTs) on MTBE with or without the presence of BTX	-
Laboratory column study for remediation of MTBE-contaminated groundwater using a biological two-layer permeable barrier Liu *et al.* (2006)	In situ biological two-layer permeable reactive barrier system consisting of an oxygen-releasing material layer followed by a biodegradation layer	MTBE could be removed, and its metabolic intermediate, TBA, could also be further degraded in this passive system
Remediation of groundwater contaminated with MTBE and benzene: the potential of vertical-flow soil filter systems Van Afferden *et al.* (2011)	Two pilot-scale vertical-flow soil filter eco-technologies	The results demonstrate the feasibility of vertical-flow soil filter systems for treating groundwater contaminated with MTBE and benzene
Adsorption of MTBE from contaminated water by carbonaceous resins and mordenite zeolite Hung *et al*. (2006)	Adsorption of MTBE onto two carbonaceous resins and one zeolite	-
Removal of MTBE with Nafion Lien *et al*. (2007)	Solid organic polymer, Nafion, is tested for the removal of MTBE in water	As Nafion is insoluble in water, chemically stable, and regenerable its use in packed-bed reactors for MTBE removal looks promising
Adsorption of MTBE from aqueous solution by porous polymeric adsorbents Ji *et al.* (2009)	Postcrosslinked polymeric adsorbent and a nonpolar porous polymer	Adsorption of MTBE from aqueous solution is dependent on both pore structure and surface chemistry of polymeric adsorbents
Solvent impregnated resins for MTBE removal from aqueous environments Burghoff *et al.* (2010)	The alternative technology of solvent impregnated resins is investigated. From solids screening and impregnation experiments macroporous polupropylene (MPP) particles appear as a suitable solid support	The MTBE capacity of impregnated MPP is lower than that of a carbonaceous resin, but the selectivity of the SIR between MTBE and humic acid is significantly higher
Removal of MTBE from water by polymer-zeolite composites Zadaka-Amir (2012)	The removal of MTBE from water by high-silica zeolite, high-silica zeolite composites, and by granular activated carbon (GAC) was investigated in suspension and by filtration	The removal of MTBE from water was more efficient by composites of polyacrylamide (PAM)-zeolite than by untreated zeolite or GAC
MTBE in drinking water production – occurrence and efficiency of treatment	Aeration, GAC, adsorption, ozonation and advance	The removal of MTBE by conventional technologies is not easily achieved. MTBE is only removed by aeration at

DRAFT

References	Technical used	Efficacy?
technologies Baus *et al.* (2005)	oxidation processes (AOP)	high expense. Ozonation at neutral pH value did not prove to be effective in eliminating MTBE at all. The use of ozone/H2O2 (AOP) may lead to a partly elimination of MTBE. However, the ozone/H2O2 concentrations required for a complete removal of MTBE from natural water is much higher than the ozone levels applied nowadays in waterworks. MTBE is only poorly adsorbed on activated carbon, thus GAC filtration is not efficient in eliminating MTBE. A comparison with real-life data from German waterworks reveals that if MTBE is detected in the raw water it is most often found in the corresponding drinking water as well due to the poor removal efficiency of conventional treatment steps.
Hydrophobic Fe-zeolites for removal of MTBE from water by combination of adsorption and oxidation Gonzalez-Olmos *et al.* (2013)	Fe-zeolites	ZSM5 zeolites with SiO2/Al2O3 ration > 200 were found to provide the best sorption properties for MTBE
Removal of MTBE and other organic contaminants from water by sorption to high silica zeolites Anderson (2000)	Zeolites with high SiO2/Al2O3 ratios	High Si large-port mordenite and ZSM-5 (silicalite) were found to have sorption properties for MTBE and TCE superior to activated carbon
Treating MTBE-contaminated water using sewage sludge-derived activated carbon Liadi *et al.* (2018)	Sewage sludge-derived activated carbon (SDAC) was synthetized and tested for its potential as an absorbent of MTBE	The MTBE removal efficiency of 70% was achieved after 60 min with 2 g/L of SDAC at pH 6, and initial MTBE concentration of 1 ppm. There is a beneficial use of a bio-waste material (sewage sludge) in water treatment technologies
Treatment of MTBE by air stripping, carbon adsorption, and advanced oxidation: technical and economic comparison for five groundwater Sutherland *et al.* (2004)	Air stripping, granular activated carbon (GAC) adsorption and the O3/H2O2 and UV/H2O2 advanced oxidation processes	Air stripping was shown to have the lower unit treatment costs for higher flowrates, although relatively tall towers were required for greater treatment requirements. At low flowrates, advanced oxidation provided the lowest treatment costs for four to five waters Both the O3/H2O2 and UV/H2O2 processes were more efficient at pH 7 versus 9 due in part to increased scavenging at higher pH. GAC was effective at most conditions, although it was also the most costly alternative.
O_3/H_2O_2 treatment of methyl-tert—butyl ether (MTBE) in contaminated waters Safarzadeh-Amari. (2001)	Oxidation employing O_3/H_2O_2	Remediation by O3/H2O2 process is more efficient and less costly than by the UV/H_2O_2 process

DRAFT

References	Technical used	Efficacy?
In situ chemical oxidation of BTEX and MTBE by ferrate: pH dependence and stability Minetti *et al.* (2017)	In situ chemical oxidation (ISCO) potassium ferrate at different pH	High degree of degradation at pH 7 for Benzene and Toluene, and at pH 9 for Ethyl benzene and Xylenes, while MTBE proved recalcitrant to degradation by ferrate
Photocatalytic degradation of MTBE in contaminated water by ZnO nanoparticles Eslami *et al.* (2008)	UV-visible/ZnO/H2O2 photocatalytic process	UV-visible/ZnO/O2 as an advanced oxidation process provides an efficient treatment alternative for the remediation of MTBE-contaminated waters
Laser-based photo-oxidative degradation of MTBE using zinc oxide (ZnO) catalyst Siddiqui *et al.* (2011)	Laser-induced photo-catalytic process	At pH value of 8, 300 mg of ZnO catalyst, 60 min laser irradiation time and 200 mJ of laser energy, delivered best results with maximum degradation of MTBE in water
Ex situ treatment of MTBE-containing groundwater by a UV peroxide system Jamal *et al.* (2006)	Ex situ ultraviolet/hydrogen peroxide (UVP) system	The UVP modification was designed to reduce the operation and maintenance costs of an existing groundwater pump-and-treat treatment system that relied on air stripping and carbon adsorption. The UVP system is relatively inexpensive and can easily be scaled to cope with different groundwater extraction rates up to 80 gpm by adding UV lamps in series or in parallel at the higher groundwater extraction rates. Incorporation of this UVP modification as a second-stage treatment to the groundwater pump-and-treat/soil vapor extraction system, after the air stripper and prior to the carbon vessels, significantly increased the usable life of the carbon (from two months previously to about two years after installation) and completely resolved the issue of frequent MTBE breakthroughs of the carbon that had plagued the remediation system since its inception.
Degradation of MTBE in dilute aqueous solution by gamma radiolysis Hsieh *et al.* (2004)	Radiolytic degradation in air-equilibrated dilute solution	The removal of MTBE can be significantly decreased with increasing concentration of benzene. Gamma radiolysis can be a potentially effective treatment
Electrochemical removal of MTBE from water using the iridium dioxide coated electrode Wu (2011)	An iridium dioxide (IrO2) coated electrode was utilized to perform electrochemical removal of MTBE in a lab-scale bath electrolyzer	The matric effect of iron enhances electrochemical oxidation of MTBE to provide about 2 times improvement on MTBE removal
Toxicity assessment of electrochemical advanced oxidation process-treated groundwater from a gas station with petrochemical contamination Chao *et al.* (2020)	Electrochemical advanced oxidation process (EAOP)	High degradation efficiencies were observed for MTBE (100%)

DRAFT

References	Technical used	Efficacy?
Effect of residual chlorine on the analysis of geosmin, 2-MIB and MTBE in drinking water using the SPME technique Lin *et al*. (2003)	Fibers for solid-phase microextraction (SPME) were employed for the extraction with and without free residual chlorine present	Sodium thiosulfate should be used to chlorinate the water to increase the efficiency
Overview of technologies for removal of MTBE from water Levchuk *et al.* (2014)	GAC - Zeolites - Ion exchanges resins - photocatalysis – Ozone/H2O2 – Ozone/UV – Fenton process – High energy electron beam irradiation – Caviation – Hybrid AOPs	-
Application of persulfate-releasing barrier to remediate MTBE and benzene contaminated groundwater Liang *et al.* (2011)	In situ oxidation barrier system to remediate gasoline-contaminated groundwater	Significant amounts of MTBE and benzene were removed through the oxidation process due to the release of persulfate, and the produced TBF and TBA, by-products of MTBE, were further oxidized in the system. Results suggest that the oxidation rate would be affected by the oxidant reduction potential and concentrations of ferrous iron and persulfate

This exhaustive literature review showed that there are several publications concerning the remediation of MTBE in groundwater supporting the fact that there is a concern about the water contamination of MTBE. This supports that the methods to remediate contaminated ground water are complexes, most of the time not applicable on site but only feasible in laboratory and probably expensive.

4.2.5 Summary

MTBE is considered to be potentially P/vP based on screening studies. As MTBE has been concluded to be not B, no simulation studies have been required at the beginning of the evaluation process. Moreover, MTBE does not fulfil the T criterion based on ecotoxicity data.
Despite the studies are not following OECD guideline, **the soil degradation tests support that MTBE should be considered as P/vP**. Additionally, several degradation tests in groundwater show a very low degradation rate in this compartment. However, it could not be determined if these low degradation rates result from a poor degradability of MTBE of a low microbial activity in groundwater.

Log Koc and monitoring data indicate that MTBE can be considered as very mobile (vM). The monitoring data show that several European surface and ground water are contaminated by MTBE at concentrations which could lead to concern for drinking water quality (from 0.1 to several tens of hundreds of µg/L) and which support that MTBE is able to disseminate into aquatic compartment due to its mobility. Besides, literature data report that remediation of water could be complex, not applicable directly on site and probably expensive.

Therefore, the high persistency and the high mobility of **MTBE could lead to a long term and not remediable contamination of drinkable water, leading to concern which could be considered as equivalent to the PBT/ vPvB substances.**

DRAFT

4.3 Assessment of ED properties relevant for human health

The analysis below focuses on the assessment of the endocrine disruptive properties of MTBE for human health as follow-up to the SEv Conclusion document (ANSES, 2022). Indeed, it was concluded in the SEv Conclusion document published in March 2022, that though vitellogenin concentration was significantly increased in male fish at 2, 4 and 11 $mg.L^{-1}$, "*endocrine disruption cannot be demonstrated for fish*" (ANSES, 2022) as no effects were observed for the Hatching success, larval survival, sex ratio and growth. However, the assessment of endocrine disruption for human health had not been performed during SEv and is therefore performed in this document. In the Annex 4, a detailed review of few key points on human health together with detailed ED assessment is available.

New studies published after the release of the EU-RAR, 2002 (European Commission, 2002) are included in this analysis. Additionally, in order to get a much comprehensive picture of MTBE potential endocrine activity, some previous analysed studies such as Day et al., Moser et al.; Billitti et al. and Williams et al. were included in this report (Billitti et al., 1999; Day et al., 1998; Moser et al., 1998; Williams et al., 2000).

4.3.1 Absorption, distribution, metabolism and elimination

According to ECETOC, 2003:
"MTBE is absorbed by all routes of exposure, though with quantitative differences. Absorbed material is distributed uniformly in all tissues, according to the relevant tissue/blood partition coefficient, which for most tissues is around one. The fat/blood partition coefficient is in the region of 10 and consequently the fat concentrations are about 10-fold higher than blood concentrations at steady state. Overall, due to rapid removal via exhalation and metabolism, there is no tendency for MTBE to accumulate. Metabolism proceeds via two principal metabolites, i.e. TBA and formaldehyde, both of which are further transformed and also show no tendency to accumulate. The products resulting from TBA metabolism are mainly eliminated via urine, whereas formaldehyde and its breakdown products enter the normal physiological pathways. This general description of the MTBE toxicokinetics appears to be valid for all investigated species, including man.

MTBE is rapidly absorbed from the gastro-intestinal and respiratory tract.
(ECETOC, 2003).

MTBE enters the body most notably through oral and inhalation and is metabolized to tert-butyl alcohol (TBA), 2-methyl-1,2-propanediol (MPD or 2M2P), and 2-hydroxyisobutyrate as MTBE metabolites by cytochromes P450 2E1 (CYP2E1) and P450 2B1 (Brady et al., 1990). These metabolites are excreted in urine in rats (Bernauer et al., 1998; Miller et al., 1997). TBA and MPD, the major metabolites of MTBE and ethyl tert-butyl ether (ETBE), were also recovered in the brain, liver, fat and testis of mice exposed to ETBE *via* inhalation (Lin et al., 2020).

DRAFT

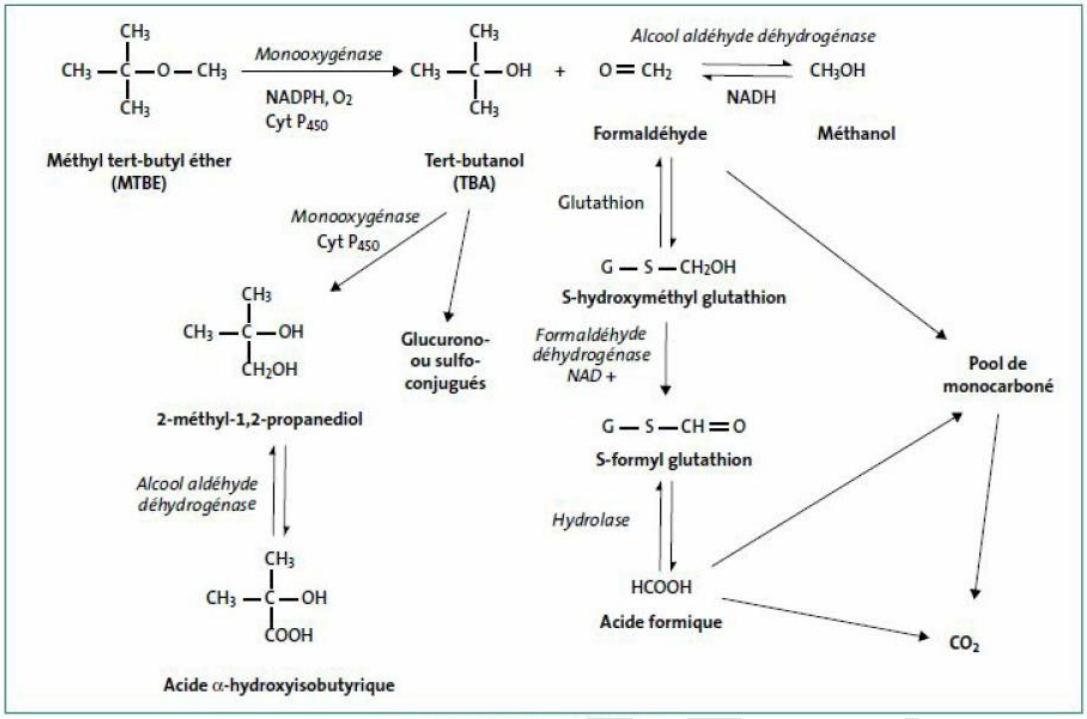


Figure 6: Metabolism of MTBE quoted from (INRS, 2002)

4.3.2 Carcinogenicity studies

Conclusions on carcinogenicity from the EURAR, 2002 are reported below as well as a summary table of tumours observed in rodents exposed to MTBE (see table below).

"MTBE produces tumours in mice and rats at doses ≥ 3,000 ppm after inhalation exposure. Tumours have been reported in rats at oral doses ≥ 250 mg/kg. There is no evidence of a direct genotoxic mode of action. Therefore, respiratory NOAEC of 400 ppm and oral LOAEL of 250 mg/kg are derived. There are indications of carcinogenicity in two species. However the treatment relation of the occurred tumours is equivocal in some studies (mouse adenoma) and the relevance of the mode of action is questionable in others (Leydig cell). Moreover, the tumours appear mostly at very high and systemically toxic doses, and MTBE is not genotoxic *in vitro* or *in vivo*. On the other hand, the human relevance of the testicular interstitial adenomas observed in rats on two separate rat strains cannot be neglected. In addition, certain uncertainty remains as to the significance of the lymphatic tumours found, in the light of the limitations of the study and inadequate reporting. The rapporteur considers MTBE as a borderline case between non classification and Carc. Cat. 3."*

*Carc. Cat. 3 mentioned here under the previous Directive 67/547/EEC is equivalent to Carc 2 under the CLP regulation.

Table 12 : Summary of tumours in rodents exposed to MTBE (quoted from EU RAR, 2002)

Animal/Sex	Dose	Tissue	Tumour	Reference
Fisher-344 rat/Male	3,000 ppm (11,000 mg/m³)	Kidney	Renal tubular adenoma and carcinoma	Bird et al. (1997)
Fisher-344 rat/Male	3,000 ppm	Testes	Interstitial cell adenoma*	Bird et al. (1997)
Sprague-Dawley/Male	1,000 mg/kg	Testes	Interstitial cell adenoma*	Belpoggi et al. (1995)
Sprague-Dawley/ Female	250 mg/kg	Haemo-lymphoreticular	Lymphoblastic lymphoma and Lymphoblastic leukaemia, lymphoimmunoblastic lymphoma	Belpoggi et al. (1995)
CD-1 Mouse/Male and Female	8,000 ppm	Liver	Hepatocellular adenoma* and hepatocellular carcinoma	Bird et al. (1997)

* = Statistically significant

Since EU-RAR, 2002, four additional studies (Bermudez *et al*., 2012; de Peyster et al., 2003; Dodd *et al.*, 2013; Goodman, 2008; Kissling, 2008) were published. An overview of these additional studies is given in Table 13.

Kissling et al. and Goodman et al. concern the statistical analysis of the experimental carcinogenicity study from Belpoggi *et al*, 1995; 1997; 1998. Goodman et al, 2008 showed no significant association between the administration of MTBE and the appearance of Leydig cell tumours (LCTs). In the contrary, the reanalysis of Kissling *et al.,* 2008 confirmed the significant incidence of LCTs in the animals treated with the highest dose of MTBE. Although the relevance of LCTs in humans has been questioned and considered rare, recent data showed that they are the most common stromal tumour of the testis accounting for 3-22% of incidentally found small testicular tumours. In humans, LCTs are strongly associated with male infertility, cryptorchidism, and gynecomastia, supporting the hypothesis that testicular dysgenesis syndrome (TDS) plays a role in the development of these tumours (Tarsitano *et al*., 2018).

In the meantime, another carcinogenicity study (Dodd *et al.*, 2013) was published where increased trend of astrocytomas was described with an incidence within historical control ranges for Wistar rats , and the brain has not been identified as a target organ following chronic administration of MTBE, ethyl tert-butyl ether, or tertiary butyl alcohol (in drinking water) to mice and rats. Thus, Dodd et al. 2013 conclude that the astrocytomas observed in this study are not associated with exposure to MTBE. Thyroid dysplasia was observed in the 13-week study from Bermudez et al. but no increase of thyroid adenoma or carcinoma was observed in the one-year study (Dodd *et al*., 2013). Lastly, Dodd *et al*., 2013 did not report any testicular tumor in the tested strain of rat namely Wistar rat.

Finally, an additional study was published investigating mechanisms of MTBE-induced Leydig cell cancer in subchronic studies (de Peyster, 2003) but as the study is more about the mode of action of these leydig cell tumours, it is reported in the endocrine disrupting paragraph.

DRAFT

Table 13: Summary of tumours in rodents exposed to MTBE (study published after the EU-RAR, 2002)

Duration / route	Animal	Dose	Tissue	Tumour	Reference
One-year oral (drinking water)	Male Wistar rat	29 and 166 mg/kg bw/day (0.5 and 3 mg.mL^{-1})	Thyroid	Astrocytoma* Thyroid dysplasia**	(Bermudez *et al.*, 2012)
	Female Wistar rat	54 and 258 mg/kg bw/day (0.5 and 3 mg.mL^{-1})	Thyroid	Thyroid dysplasia**	
	Male Wistar rat	300 mg/kg bw/day (7.5 mg.mL^{-1})			
	Female Wistar rat	900 mg/kg bw/day (15 mg.mL^{-1})		Thyroid dysplasia**	
743 days (drinking water)	Male Wistar rat	25 and 140 mg/kg bw/day corresponding to 0.5 and 3 mg.mL^{-1}	Brain	Astrocytoma*	(Dodd *et al.*, 2013)
	Female Wistar rat	49 and 232 mg/kg bw/day corresponding to 0.5 and 3 mg.mL^{-1}			
	Male Wistar rat	330 mg/kg bw/day (7.5 mg.mL^{-1})	Brain	Astrocytoma* Systemic hemangiosarcoma, Lymph node hemangiosarcoma	
	Female Wistar rat	1042 mg/kg bw/day (14.96 mg.mL^{-1})	Brain	Astrocytoma	

* Statistically significant trend.
**Observed in the 13 week study

Only two experimental studies on the potential carcinogenic effect of MTBE completes the data reported in EURAR, 2002 in which male and female Wistar rats have been exposed with MTBE in drinking water for 13 weeks and one year (Bermudez *et al.*, 2012) and for two years (Dodd *et al.*, 2013). It should be noted that these studies did not report testicular tumours. No histopathologic data were shown in these two studies coming from the same research laboratory.

Conclusion:
These new studies have shown that MTBE increases the incidence of brain (i.e. astrocytoma) and thyroid tumours (i.e. thyroid dysplasia) with no clues about its mode of action. Tumours were observed from oral dose ≥ 25mg/kg bw/day. Male Wistar rats are more likely to develop tumours at low doses than female rats. These new data confirmed the potential carcinogenicity of MTBE at low dose but bring no new element on its mode of action. Leydig cell adenoma observed in rats together with decreased testosterone levels in sub-chronic studies may be relevant to human, LCT represents an increasing part of small testicular tumours. In humans, increasing Leydig cell dysfunction has been shown to be associated with decreased levels of total testosterone and decreased total testosterone/LH ratio (Tarsitano et al., 2018). Although Dodd et al. did not observe testicular tumours, this new study raised additional concerns in particular on brain. But these effects were quite limited in term of incidence. Overall, MTBE showed some carcinogenic effects in several species namely rat and mice and in different strains of rat which leads to consider

DRAFT

MTBE as a borderline case between non classification and Carc. Cat. 2. MTBE has no harmonised classification for carcinogenic properties. This conclusion may need further consideration based on a group approach.

4.3.3 Reproductive toxicity studies

4.3.3.1 One and two-generation studies

No new studies were done since 2002 when the EU-RAR (European Commission, 2002) concluded that there are general toxicity signs at 3,000 and 8,000 ppm (corresponding to 10,800 and 28,880 mg.m^{-3} respectively) in both generations of parental animals (Bevan, 1997b; Biles, 1987).

Table 14 : Summary of the effects of MTBE on fertility (quoted from EU-RAR, 2002)

Study definition	Dosing	Effects in P-animals	Effects in F1-animals	Effects in F2-animals
1-gen. reproduction in Sprague-Dawley rat (Biles et al., 1987)	Inhalation 250, 1,000, 2,500 ppm (900 –9,000 mg/m^3)	Renal dilated pelvis at 250 & 2,500 ppm (non-significant) + slightly lower pregnancy rate at 1,000 ppm, (non-significant)	Lowered Pup viability index at 1,000 and 2,500 ppm*, lowered survival at two lowest doses but not at high dose *	-
2-gen. reproduction in Sprague-Dawley rat (Bevan et al., 1997)	Inhalation 400, 3,000, 8,000 ppm (1,500-29,000 mg/m^3)	8,000 ppm: body weights and gain lower in males at PMP* In females body wt gain increased in PND 21-28* 3,000 ppm: CNS depression, increased relative liver weight*.	8,000 ppm: increase of dead pups on PND4*, male and female body weight reduced at PND 14-28 and from week 0 to end of PMP*, abs. liver wt increased* in both sexes, relative in males*. No histological change in any tissue. 3,000 ppm: Reduced female body weight on PND 14 & PMP wks 0-4*, males PMP wks 0-3*, rel. Liver wt incr. in males*.	8,000 ppm: increase of dead pups on PND4*, reduced female body weight on PNDs 7-28*. 3,000 ppm: reduced male body weight on PNDs 14-28*.

PMP= Pre-mating period
PND = Post natal day
* = statistically significant
+ = toxicological significance unclear

Table 15: Summary table of the reproductive toxicity studies on MTBE published after the EU-RAR, 2002

Duration / route / type of study	Animal	Doses	Effects	Reference
Untreated females mated with males preliminary treated during 30 day paternal Oral (gavage)	Adult male Sprague-Dawley rats (weighing 223 ± 20 g.)	0, 400, 800 and 1600 mg/kg bw/day	- No significant effect on offspring sex ratio. - ↘ male fertility at 1600 mg/kg bw/day (not stat. signif. merely due to the limited sample size, n=5).	(Khalili *et al.*, 2015)

4.3.3.2 Developmental toxicity

The EU-RAR (European Commission, 2002) concluded that: "Although malformations are seen at 8,000 ppm in CD-1 mice, they are considered to occur at a dose level of marked maternal toxicity. When there is significant maternal toxicity, the probability of the occurrence of non-specific developmental effects in the offspring increases. The sternebrae malformations seen in CD-1 mice at 250-2,500 ppm are not considered treatment related. Therefore, based on the available data, MTBE is not considered toxic to fetal development."

Table 16: Summary of effects on reproductive toxicity (development) of MTBE (quoted from EU-RAR, 2002)

Study definition	Dosing	Maternal Effects	Embryo/foetal effects	Reference
Sprague-Dawley rat	Inhalation 250, 1,000, 2,500 ppm	Reduction in food consumption in all treatment groups during the treatment interval during days 9-12*.	A preponderance of male pups over females at 1,000 ppm*	Conaway et al. (1985)
CD-1 Mice	Inhalation 250, 1,000, 2,500 ppm	A slight, non-significant dose-related decrease in food consumption on days 12-15 in the treated groups and in water consumption during days 9-12 in the treated groups (no change in body wt)	A slight increase of sternebrae malformations (4th & 5th fused) in all treated groups, 0.6 (low), 1.2 (mid) and 2.1% (high). (Investigators stated that historically seen with low incidence in control animals with 0.16% incidence. They concluded this not treatment related since there where no increase of vertebral or rib effects usually associated with this malformation).	Conaway et al. (1985)
CD-1 Mice	Inhalation 0, 1,000, 4,000, 8,000 ppm	8000 ppm: hypoactivity, ataxia, prostration, laboured respiration, reduced body wt on GDs 12, 15, 18 and wt gain during GDs 6-15 (treatment), 15-18 (gestational), 0-18 (gestational corrected for uterine)* incr. liver wt, reduced uterine wt*, colour changes in the lungs. 4000 ppm: Ataxia, hypoactivity, reduced food cons. during GD6-10, colour changes in the lungs	8,000 ppm: incr. post impl. loss due to late resorptions and dead foetuses*, lower pct. of live and male foetuses/litter*, lower foetal body wt/litter*, Malformations: cleft palate*, Variations: reduced ossification* 4,000 ppm Reduced foetal body wt/litter* Variations: skeletal (reduced ossification in various sites*, Sternebrae no. 5&6 split)	Bevan et al. (1997)
NZW rabbit	Inhalation 1,000, 4,000, 8,000 ppm	4,000 ppm: >70% reduction in food consumption during GDs 6-10	-	Bevan et al. (1997)

GD = gestation day
* statistically significant

Since EU-RAR, 2002, one new study available displayed malformations after inhalation of complex mixtures containing MTBE (Roberts et al., 2014). Another study dedicated to the evaluation of the anti-angiogenic properties of MTBE in Fisher 344 rats did not show vascular disrupting effects (Kozlosky et al., 2013).

4.3.4 Endocrine disrupting properties

4.3.4.1 Information sources and strategy for endocrine disruptor identification

Although MTBE is an industrial chemical, assessed under the Regulation (EC) No. 1907/2006 concerning the registration, evaluation, authorisation and restriction of

chemicals (REACH), the method used for identification of its ED properties was adapted from the EFSA/ECHA guideline document developed under the pesticide and biocide regulations. To evaluate the potential concern for endocrine disrupting effects induced by MTBE all available data were assessed throughout all levels (1-5) of the OECD conceptual framework for Endocrine Disrupters of the Guidance.

4.3.4.2 In silico and in vitro studies (level 1 & 2)

Based on the in silico (level1) and *in vitro* data (level 2) available (see Annex 4 for details), MTBE and/or its metabolite TBA were considered as non-active on the EATS modalities: According to the Danish (Q)SAR database on endocrine disruption, all models are negative. **VEGA[4] did not indicate any endocrine activity of MTBE**. According to the production of Endocrine disruptome, MTBE displays low to low-medium probability of binding any receptor. In vitro, MTBE is non-binder to AR and ER, non-active on ER, AR, TR, TSHR, negative in the steroidogenesis assay, and non-inhibitor of aromatase activity. However it should be highlighted that the *in vitro* data set on MTBE is rather limited.

4.3.4.3 In vivo mechanistic data (OECD level 3/4)

Hershberger and uterotrophic like-studies have been conducted since the EU-RAR ((Okahara, 1999) [Unpublished data, reported in Supp. Data from (de Peyster, 2014) and (de Peyster, 2003)]. Hormones were measured in intact and orchiectomized rats, with testosterone implants in some castrated rats. Okahara study raises some alert on steroidogenic potential of MTBE (see Annex 4 for more detailed analysis).

4.3.4.4 Summary on the data regarding the Mode of action

An overview of *in vitro* and *in vivo* results extracted from literature search according to EATS and non-EATS criteria is provided in the table below as defined in the Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009.

Table 17 : Overview of endpoints and studies investigating MTBE endocrine activity and effects. Results are ordered by endocrine pathway and OECD levels. The number represents the available results for a given endpoint. Adapted from Brown et al. (2017).

	In vitro Level 2									*In vivo* Level 3		*In vivo Level 3/4*	
	Binding	ER Transactivation assay	AR transactivation assay	Cell proliferation	Steroidogenesis	ER gene expression	AR gene expression	TR transactivation assay	TSHR transactivation assay	Uterotrophic assay	Hershberger assay	Fish sexual development tet	*In vivo* short term and chronic toxicity assay on testicular fonctions

[4] Virtual models for property Evaluation of chemicals within a Global Architecture (VEGA).

ER agonism		0/3										0/1	
ER antagonism		0/3										0/1	
AR agonism			0/2									0/1	
AR antagonism			0/3									0/1	
T agonism								0/1	0/1				
T antagonism								0/1	0/1				
S					0/3					1/1	0/1	1/1	

Based on the *in vitro* data available, MTBE and/or its metabolite TBA were considered as non-active on the EATS modalities: non-binder to AR and ER, non-active on ER, AR, TR, TSHR, negative in the steroidogenesis assay, and non-inhibitor of aromatase activity. .

Based on the *in vivo* data, MTBE is considered to have an effect on the steroidogenesis pathway. Detailed analysis can be found in annex 4.

4.3.4.5 In vivo adverse effect data (OECD level 3/5) and/or mechanistic data

As a summary of the endocrine effects, the EU-RAR stated that: *"At high dose, MTBE produces a number of effects on endocrine system in female mice. Although MTBE causes an increased metabolism of oestrogen in mouse liver, this does not affect the level of free hormone. Thus, MTBE seems to have a slight antioestrogen-like activity at very high doses. Consequently, a weight loss and morphologic changes are seen in uterine. Oestrous cycle length and stages were also altered. Increased interstitial testosterone level was found in rats after a 28-day exposure. (Williams et al., 2000a) reported decreased serum testosterone and LH but the results of this study did not indicate the hypothetical mode of action. Corticosterone and aldosterone levels are elevated after continued exposure to high doses of MTBE. A clear decrease in serum corticosterone level is seen in the later phase of the chronic studies. This is an expected finding, as the microscopy shows a disruption of zona reticularis in the same time. The significance of the altered morphology of adrenal gland and adenohypophysis in the estrogenic effects is not clear. Because the data are not considered sufficient, no NOAEL is assigned."*

Since new studies were published after the release of the EU-RAR report, 2002 (European Commission, 2002), they were evaluated and included in this analysis. Additionally, in order to get a much comprehensive picture of MTBE potential endocrine activity, some previous analyzed studies in the EU-RAR such as Day *et al*., Moser *et al.;* Billitti *et al.* and Williams *et al.* were included in this report (Billitti *et al.*, 1999; Day *et al.*, 1998; Moser *et al.*, 1998; Williams *et al.*, 2000). These studies covered short periods of exposure (eg. 3 days of treatment) up to chronic exposure (eg. 2 months) *via* the oral (eg. gavage or drinking water) or the inhalation route as well as reproductive toxicity studies (see Annex 4 for a full overview).

4.3.4.5.1 Male reproductive system

Adult exposure

All studies reported that one-week exposure to 400 – 1,500 mg/kg bw/day did not change testis weight, testicular histology, serum testosterone and LH levels (Allgaier and de Peyster, 1999; Billitti *et al.*, 1999; de Peyster *et al.*, 2008) It is not clear whether 2,000 mg/kg bw/day alter (Billitti *et al.*, 2005) or not (de Peyster *et al., 2008)* testicular histology.

When exposure lasted around one month, no significant changes in male reproductive organs and hormones were reported with doses equal to or lower than 800-1000 mg/kg bw/day in de Peyster et al. (2008) at the exception of the study from Day *et al* (1998) where a decrease of plasma testosterone was observed at 800 mg/kg bw/day. With 1500-1600 mg/kg bw/day, several studies (Gholami *et al., 2015; Khalili et al., 2015; Williams et al., 2000)* reported decrease of serum testosterone level and dose-dependent decrease of LH and FSH (Khalili *et al.,* 2015), decrease of LH only (Williams *et al.*, 2000) and alteration of the seminiferous epithelium (Gholami *et al.*, 2015). One paper reported a decrease in male fertility but it can be fortuitous as there were only 5 animals per groups (Khalili *et al.*, 2015).

When exposure lasted around 3 or 12 months (Bermudez *et al*., 2012), no clear-cut changes in male reproductive organs weight and in testicular histology were observed with 0.5 to 15 $mg.mL^{-1}$ MTBE added in the drinking water (i.e around 50 to 1,500 mg/kg bw/day).

Thus, on the basis of these observations which are lacking sensitivity (no sperm analysis), MTBE male reprotoxicity in adult remains doubtful. If it exists, it appears at very high doses (1,500 mg/kg bw/day).

Juvenile exposure

Low doses (0.0004 to 0.04 mg/kg bw/day) for 51 days had no effect on the reproductive organ weights and serum testosterone and estradiol levels (de Peyster *et al.*, 2008).

After 4-weeks exposure with doses from 400 to 1,600 mg/kg bw/day, testis and epididymis weights and sperm number in epididymis were unchanged (Li *et al*., 2008). Episodical variations in serum testosterone level were observed, whereas LH and FSH concentrations were unchanged. Importantly, augmentation of the percentage of abnormal sperm and dose-dependent alterations in testicular histology were observed from 400 and 800 mg/kg bw/day respectively. This effect was observed without significant systemic toxicity (Li *et al.,* 2008). A decrease of the relative testes organ weight was also observed in Dong-Mei et al. (2009) from 400 to 1,600 mg/kg bw/day. But this effect although statistically significant from the lowest tested dose level did not show any dose-response relationship and was limited to the two week treatment group.

Thus, exposure of juvenile rats to 400 mg/kg bw/day and higher doses of MTBE altered male reproduction function whereas 0.04 mg/kg bw/day had no detectable effects. No clear relationship with hormonal changes has been evidenced. Data on the reprotoxicity of doses ranged between 0.04 and 400 mg/kg bw/day are lacking.

Two generations studies

No new studies were done since 2002 when the EURAR (Risk assessment report, Finland, 2002) concluded that there are general toxicity signs at 3,000 and 8,000 ppm (corresponding to 10,800 and 28,880 $mg.m^{-3}$ respectively) in both generations of parental animals (Bevan, 1997b; Biles, 1987).

4.3.4.5.2 Female reproductive system

No effect on the ovaries and uterus weights were reported in previously assessed rat MTBE subacute and subchronic studies (see Table 33). In a more recent study conducted by Bermudez *et al*., 2012, adult male and female Wistar rats were exposed to MTBE *via* drinking water for 13 weeks and one-year study. In this non-

guideline study, MTBE exposure did not lead to effect on the wet weight of pituitary gland, ovaries and uterus (data not shown at one-year). On the other hand, (Moser *et al.*, 1998) conducted a previous study on B6C3F1 mice exposed to MTBE by inhalation and they concluded that MTBE-induced endocrine alterations in female mice through a mechanism not mediated by ER (increase estrous cycle length, decrease in the number of uterine glands, alteration of uterine, cervix and vaginal structure while ovaries are not affected. Note that the concentrations of MTBE used in this study are very high and that the relevance for human health is unknown. One and two-generation studies on Sprague-Dawley rats, previously assessed in the EU-RAR, 2002, did not reveal histopathological effects in the reproductive organs nor significant difference in fertility (Bevan, 1997a; Biles, 1987). It should be noticed that estrous cyclicity was not evaluated in these two latter studies. In a non-guideline study on juvenile Sprague-Dawley rats exposed to MTBE and to 2M2P (a metabolite) via the oral route, the authors conclude to an absence of effect on oocyte quality due to MTBE (Berger and Horner, 2003). However, these results must be taken with caution because the dose of MTBE exposure cannot be calculated and the study was carried out on a very small number of animals. Lastly, the carcinogenicity study from Belpoggi et al., did not observe MTBE treatment-related non-oncological pathological changes by gross inspection and histological examination of the reproductive organs (Belpoggi, 1997). A decrease of the mean ovary weight was observed in the Dodd et al. carcinogenicity study but this effect did not follow a dose-response relationship and was not statistically significant. Although the number of animals per group was high (n=32-42), a high variation in each group did not allow to conclude on the ovary weight significance (Dodd *et al.*, 2013). **Overall, since the previous assessment carried on in the EU-RAR, 2002 some of the above mentioned effects have been identified estrogen-mediated (see EFSA/ECHA guidance document). However the evidence of an adverse effect of MTBE on the female reproductive system was judged not robust enough at that time and the additional evidence was not clear enough to modify the conclusion after a first look.**

4.3.4.5.3 Cholesterol, metabolism and obesity

A summary of the 6 references reporting analysis of cholesterol levels is given in the tables below while the studies from Tang, Ren *et al.* 2019 and Ren, Xie *et al.* 2021 are fully described in Annex 4.

Table 18: MTBE sub-acute and subchronic studies with adulthood exposure analyzed in the RAR report and information related to cholesterol

Duration / route	Animal	Doses	Reference	Additional information related to endocrine endpoints	Original data available
14 days / oral	Sprague-Dawley Rat	357-1,428 mg/kg*	(Robinson M. *et al.*, 1990)	No effect on adrenal weights (absolute and relative). Statistically significant increase of relative testes weight at 714 and 1071 mg/kg bw/day. Statistically significant increase of cholesterol levels in most females from 714 mg/kg* and in male rats at 1428 mg/kg. No examination of the thyroid gland.	Yes
28 days oral	Sprague-Dawley Rat (male)	250-1,500 mg/kg	(Williams *et al.*, 2000)	See complementary information in the section 8.1. No histopathological	Yes

				examination of the thyroid gland.	
90 days oral	Sprague-Dawley Rat	100-1,200 mg/kg*	(Robinson M. *et al.*, 1990)	Increased adrenal relative weight in females without a clear pattern. Significant increase of cholesterol levels in most females and in male rats without a clear dose-response relationship. No effect on the (absolute and relative) weights of testes, ovaries and adrenals. No examination of the thyroid gland.	Yes
90 days oral	Sprague-Dawley Rat	200-1,200 mg/kg*	(Zhou and Ye, 1999)	Only males were exposed to MTBE. The following organs were weighed and examined histologically: liver, kidneys, testes, and lungs. No effect on testes weights (absolute and relative) without apparent pathological changes. Cholesterol was not measured.	Yes
13 weeks inhalation	Fisher-344- Rat	800-8,000 ppm	Dodd *et al.* (1989) Lington, Dodd *et al.* 1997	In addition, elevated levels of corticosterone in serum was observed in both sexes from 4000 statistically significant at 8000 ppm MTBE (Lington, Dodd et al. 1997). A higher plasma level of aldosterone was also detected in both sexes. There was also a statistically significant and concentration-related increase in the weight of adrenals but without associated histopathological lesions. Cholesterol level was not assessed. The thyroid gland examination was limited to histological analysis.	Not available for Dodd et al., 1989

* = Gavage administration applied

Table 19: MTBE sub-acute and subchronic studies with juvenile or adulthood exposure published after the EU-RAR, 2002 and including information related to cholesterol

Duration / route	Animal	Doses	Effects	Reference
15 or 28 days Oral (gavage)	Male Sprague-Dawley rats (28–30 day old)	0, 400, 800 and 1600 mg/kg bw/day	After 2 weeks of treatment: -↗ Cholesterol at 1600 mg/kg bw/day. After 4 weeks of treatment: -↗ Cholesterol at the lowest tested dose level (400 mg/kg bw/day).	(Dong-mei *et al.*, 2009)
3 months /drinking water	Male Sprague-Dawley rats	0; 40 , 200 or 1000 µg/L (MTBE) corresponding approximately to 5, 25 and 125 µg/kg bw/day); + 1 group with N, N, N', N'-Tetrakis (2-pyridylmethyl) ethylene diamine (TPEN) only	MTBE enhanced the Cu^{2+}/Zn^{2+} plasmatic ratio, C-reactive protein dose-dependently and fasting glycaemia, induces dyslipidemia with enhanced TG, cholesterol and LDL plasma levels. These effects were counteracted by Zn supplementation. Other effects such as the increased calcium levels observed at the MTBE highest tested dose and the decreased HDL were	(Saeedi *et al.*, 2017a)

		+ 1 group receiving 1000 µg/L (MTBE) + TPEN + 1 group receiving 1000 µg/L (MTBE) + 7.5 mg/L zinc acetate;	not counteracted by Zn supplementation. Lastly, ins1 and ins2 (coding for Insulin) gene expression were decreased with MTBE groups (except at the lowest dose). To be noticed that some of the parameters affected by MTBE were not modified with TPEN (eg. dyslipidemia and C-reactive protein).	

When cholesterol levels were evaluated in juvenile or adult rat in subacute or sub-chronic studies (Dong-mei *et al.*, 2009; Robinson M. *et al.*, 1990; Saeedi *et al.*, 2017a), a rather consistent increase of cholesterol levels were observed. This increase was mainly observed at high dose levels (Dong-mei *et al.*, 2009; Robinson M. *et al*., 1990). Interestingly, a more recent study from Saeedi *et al.* (2017a) examined the effects of MTBE on glucose homeostasis and dyslipidemia at rather low dose levels (5, 25 and 125 µg/kg bw/day). They show a dose-related increase of cholesterol levels from 25 µg/kg bw/day in treated male Sprague Dawley. These results should be put in perspectives with the work from Ren, Xie *et al.* (2021) which showed that MTBE promoted atherosclerosis in the male APoE - / - mouse model by reducing the efflux of cholesterol and promoting the formation of foam cells. However, this latter study did not show a consistent hyperlipidemia in wild-sinsulin sensitivity in males fed a high-fat diet.

4.3.4.5.4 Thyroid

Among the 7 studies analysed, only 6 studies will be considered (Bermudez *et al.*, 2012; Chun *et al.*, 1992; Dodd *et al.*, 2013; Dodd and Kintigh, 1989; Sarhan *et al*., 2019; Williams *et al.*, 2000). Indeed, the data summarized in the abstract presented at SOT 1998 (Williams *et al*., 1999) have not been published. Therefore, they could not be taken into consideration.

The effects on thyroid hormones were only measured in two studies (Chun and Kintigh, 1993; Williams *et al.*, 2000) and were significantly altered at very high dose in Williams et al. (2000) with a drop in T3 without T4 or TSH changes at 1,500 mg/kg bw/day (via gavage) and in Chun and Kintigh (1993) with a drop in T4 at 400 or 8000 ppm (corresponding to 1,440 and 28,880 mg/m^3) via inhalation. Only scarce effect on thyroid gland weight were seen but a recent study show that a chronic respiratory exposure of 60 µl MTBE/day (corresponding 245-296 µg/kg bw) during 3 minutes per day for 12 months induced thyreocytes degeneration (Sarhan *et al.,* 2019). Bermudez *et al.* (2012) observed thyroid changes, namely thyroid dysplasia, after 13-week oral exposure (via drinking water) from the lowest tested 54 mg/kg bw/day in females and at the lowest or medium dose level (29 and 166 mg/kg bw/day) in males. No significant histopathological effects were reported after one-year exposure period (Bermudez et al. (2012)) nor two-year exposure period (Dodd *et al.*, 2013). **Overall, merely due to incomplete testing analysis and lack of sensitive endpoints, actual data are not sufficient to conclude that MTBE has an effect on thyroid but we cannot conclude either that MTBE has no effect on thyroid gland.**

Table 20: MTBE sub-acute, subchronic and chronic studies with adulthood exposure including the examination of the thyroid gland and/or thyroid hormone levels.

Duration / route	Animal	Doses	Effects	Reference
28 days inhalation	CD-1 Mouse	400-8,000 ppm corresponding to 1,440 and	The only change in clinical chemistry attributable to MTBE was an increase in total	(Chun and Kintigh, 1993)

		28,880 mg/m^3 respectively.	T4 and TSH in high dose males. The biological significance of this alteration is, however, unknown, because no parameter in clinical pathology indicated an effect. The female high-dose mice had a dose-related decrease in their total T4 at day 5, which was also seen in the mid-dose group at day 31. However, this effect was only detected in the proliferation groups (5 mice/group) and not in the main groups (10 mice/group).	
13 weeks inhalation	Fisher-344- Rat	800-8,000 ppm	Elevated levels of corticosterone in serum was observed in both sexes from 4000 statistically significant at 8000 ppm MTBE (Lington, Dodd et al. 1997). A higher plasma level of aldosterone was also detected in both sexes. There was also a statistically significant and concentration-related increase in the weight of adrenals but without associated histopathological lesions. Cholesterol level was not assessed. No significant histopathological effects on the thyroid gland.	Dodd *et al.* (1989) Lington, Dodd *et al.* 1997
3, 6 and 12 months Inhalation (vapor)	Adult male Wistar albino rats	60µl/day (corresponding to 245-296 µg/kg bw) during 3 minutes per day	-Increased tracheal and lung histopathological alterations mostly after 6 months of exposure. -Increased degenerated thyroid follicles at 12 months. -Carbonic anhydrase 1, carbonic anhydrase 2; and peroxiredoxin 2 detected in blood sera by mass spectrometry	(Sarhan *et al.*, 2019)
28 days oral	Sprague-Dawley Rat (male)	250-1,500 mg/kg bw/day	-↘serum T3 at 1000 and 1500 mg MTBE/kg bw/day. - No histopathological examination of the thyroid gland.	(Williams *et al.*, 2000)
One-year oral (drinking water)	Male Wistar rat	29 and 166 mg/kg bw/day (0.5 and 3 mg/ml)	Thyroid dysplasia**	(Bermudez *et al.*, 2012)
	Female Wistar rat	54 and 258 mg/kg bw/day (0.5 and 3 mg/ml)	Thyroid dysplasia**	
	Male Wistar rat	300 mg/kg bw/day (7.5 mg/ml)		
	Female Wistar rat	900 mg/kg bw/day (15 mg/ml)	Thyroid dysplasia**	
743 days (drinking water)	Male Wistar rat	25, 140 and 330 mg/kg bw/day corresponding to 0.5,3 and 7.5 mg/l	No thyroid adenoma	(Dodd *et al.,* 2013)
	Female Wistar rat	49, 232 and 1042 mg/kg bw/day corresponding to 0.5,3 and 14.96 mg/lmg/l		

**Observed in the 13 week study

DRAFT

4.3.4.5.5 Adrenals

To our knowledge, no recent scientific data has been published since the EU-RAR, 2002. Among the 6 studies analysed in the EU-RAR, (Bird *et al.*, 1997; Day *et al*., 1998; Lington *et al.*, 1997; Moser *et al.,* 1998; Robinson M. *et al.*, 1990; Williams et al., 2000) only 5 studies will be considered. Indeed, the data summarized in the abstract presented at SOT 1998 (Day et al. 1998) have not been published. Therefore, they could not be taken into consideration. For a summary of the experimental data available on the adrenal gland and mineralo or glucocorticoid, please refer to the following table (Table 21).

Table 21: MTBE sub-acute and subchronic studies including the examination of the adrenal gland and/or mineralo or glucocorticoid hormone levels

Duration / route	Animal	Doses	Reference	Additional information related to endocrine endpoints	Original data available
14 days / oral gavage	Sprague-Dawley Rat	357-1,428 mg/kg*	(Robinson M. *et al.*, 1990)	No effect on adrenal weights (absolute and relative). Statistically significant increase of relative testes weight at 714 and 1071 mg/kg bw/day. Statistically significant increase of cholesterol levels in most females from 714 mg/kg* and in male rats at 1428 mg/kg. No examination of the thyroid gland.	Yes
14-28 days oral gavage	Sprague-Dawley Rat	40-800 mg/kg	(Day *et al.* 1998)	The highest dose at day 28 produced a significantly reduced plasma testosterone level and an increased level of corticosterone while corticosterone level was increased in all MTBE levels at 14 days of treatment.	Abstract
90 days oral gavage	Sprague-Dawley Rat	100-1,200 mg/kg*	(Robinson M. *et al*., 1990)	Increased adrenal relative weight in females without a clear pattern. Significant increase of cholesterol levels in most females and in male rats without a clear dose-response relationship. No effect on the (absolute and relative) weights of testes, ovaries and adrenals. No examination of the thyroid gland.	Yes
28 days oral gavage	Sprague-Dawley Rat (male)	250-1,500 mg/kg	(Williams *et al.,* 2000)	Increased absolute and relative adrenal weight at 1,500 mg/kg bw/day at 15 days only. An increase of the relative pituitary weight was also observed. Decreased serum prolactin levels at 1,500 mg MTBE/kg/day for 15 days.	Yes
13 weeks Inhalation Vapor	Fisher-344- Rat	800-8,000 ppm	Dodd *et al.* (1989) Lington, Dodd *et al.* 1997	In addition, elevated levels of corticosterone in serum was observed in both sexes from 4000 ppm statistically significant at 8,000 ppm MTBE (Lington, Dodd et al. 1997). A higher plasma level of aldosterone was also detected in both sexes. There was also a statistically significant and concentration-related increase in the weight of adrenals but without associated histopathological lesions. Cholesterol level was not assessed. No significant	Not available for Dodd *et al*., 1989

DRAFT

				histopathological effects on the thyroid gland.	
18 months Inhalation Vapor	CD-1 Mice	400, 3000, 8,000 ppm 6 h a day, 5 days per week	(Bird e*t al.,* 1997).	Increased absolute and relative adrenal weights at 8,000 ppm (only in males) Increased corticosterone values observed at the 8000 ppm at 79 weeks for males (212 ± 160 vs 69 ± 56 ng/ml, respectively).	Yes
24 months Inhalation Vapor	F-344 Rats	400, 3000, 8,000 ppm 6 h a day, 5 days per week	(Bird *et al.,* 1997).	An increase in serum corticosterone levels was seen in male rats from the 3000 ppm at week 97 (352 ± 167 vs 176 ± 96 ng/ml, respectively), but a decrease was seen in the 8,000 ppm male rats at week 81 (69 ± 69 vs 176 ± 96 ng/ml, respectively). No changes seen in females.	Yes
4 and 8 months Inhalation Vapor	B6C3F1 mice (female)	8000 ppm, 6 h a day, 5 days per week	Moser *et al.*, 1998	MTBE-exposed mice had a decrease in the zona reticularis of the cortex of the adrenal glands and an increased number of hyaline droplets in the pars intermedia of the pituitary as compared to air-exposed control mice.	Yes

* = Gavage administration applied

Increased adrenal relative weight is observed after a 90 day oral exposure of female Sprague Dawley rat (not in males) to MTBE at high dose levels without a clear pattern (Robinson M. *et al*., 1990). The study of Lington *et al.* (1997) shows that sub-chronic inhalation exposure of Fisher-344 rats to MTBE resulted in an elevated levels of corticosterone, the major glucocorticoid in rodents, in serum from 4,000 ppm (corresponding to 14,400 mg/m^3), with a statistically significance at 8,000 ppm MTBE (corresponding to 28,880 mg/m^3) in both sexes (Lington, Dodd *et al.* 1997). A higher plasma level of aldosterone, the mineralocorticoid derivative of corticosterone, was also observed in both sexes. However this increase was not statistically significant nor considered by the authors as biologically significant. A statistically significant and concentration-related increase in the weight of adrenals was observed from 4000 ppm but without any associated histopathological lesions. A similar trend of increasing serum corticosterone can be seen in the male CD-1 mice (not female) at 8000 ppm in the 18-month study by Bird *et al* (1997). The same study also investigated Fisher-344 rats in a two-year carcinogenicity study but corticosterone plasma levels were either found to increase at 3000 ppm (corresponding to 10,800 mg/m^3) and decrease at 8,000 ppm compared to the respective controls (Bird *et al.,* 1997). Lastly Moser *et al.* (1998) observed an altered morphology of the adrenals in female B6C3F1 mice exposed to 8,000 ppm (corresponding to 28,880 mg/m^3) MTBE for 8 months, but it is rather unclear which part of the adrenal was altered (see also section 8.1). **In conclusion, when the adrenal weight or corticosterol/ aldosterone levels were evaluated in subacute, sub-chronic or chronic studies in rat or mice, in the different studies (see Table 24), data were not consistent and only observed at high dose levels which causes systemic toxicity.**

4.3.4.5.6 Effects on prolactin

There is no new study on prolactin since the EU-RAR, 2002 publication. However some studies displayed somme effects on prolactin that are reported below.

In the study conducted by Williams et al. (Williams *et al.*, 1999; Williams *et al.*, 2000) male Sprague-Dawley rats exposed to 250, 500, 1000 or 1500 mg MTBE/kg bw/day via gavage for 15 or 28 consecutive days exhibited a decrease of the prolactin levels at 15 days but not at 28 days. Even though the role of prolactin in the maintenance of Leydig cell function is still debated, investigators accept that prolactin exerts a stimulatory effect on testicular LH, human chorionic gonadotropin, and prolactin receptors in many species (Saez, 1994). Because the negative feedback control of prolactin secretion is governed by the interaction of prolactin with the prolactin receptor, the observed decrease in prolactin in this study would likely lessen the response of circulating LH on the testis. Typically, alterations in prolactin levels are associated with several factors, including exercise, stress, and circadian rhythms, and result in the increased release of prolactin from the anterior pituitary (Reichlin, 1998). It is unclear from these studies if and how the decrease in prolactin levels following MTBE administration contributes to the disruption of the HPT axis regulation.

4.3.5 Summary- Analysis of MTBE mode of action in view of ED characterisation

Most of the past studies analysed in the EU-RAR have used high doses of MTBE which triggered cell toxicity, thereby not allowing firm conclusions on potential EATS endocrine effects thought EATS-mediated effects have been described. Decreased testicular weights have been reported, but not consistently. Decreased testosterone levels have also been reported, but it is not clear whether this was related to improved metabolism and clearance of testosterone. Recent data from a fertility study (Khalili *et al.,* 2015) confirm the doubt regarding potential effects on male fertility, but no clear conclusion can be drawn. The hypothesis that MTBE might exert anti-estrogenic effects in female mice causing liver tumors has been suggested by Bird *et al.* (1997), but no other estrogen-related effects were described in any of the articles reviewed. Further studies would be necessary to determine if MTBE at non-toxic doses can target endocrine organs such as the adrenals, and in this case identify the underlying mechanisms of action. However, in line with the conclusion previously reached under SEv (ANSES, 2022), it has not been considered possible to request additional data. Specially, about adrenals, it should be recalled that mice do not have a "zona reticularis" as described in humans (Dumontet and Martinez, 2021) and thus it is not clear which part of the adrenal showed the altered morphology described by Moser *et al.* (1998). It should also be recalled that high doses of MTBE will probably generate a stress with consequences on the plasma levels of corticosterone and possibly cross-interactions with other endocrine system(s) including that involving estrogens.

Regarding the metabolic properties of MTBE, Valipour *et al.* (2015) showed a modification of the tertiary structure and enhanced aggregation of insulin by MTBE. However, Valipour *et al*. (2015) did not investigate the functionality of such modified insulin and it is rather unlikely that this effect might be sex dependent as shown by Tang *et al.* (2019). Using environmental concentrations and both *in vitro* and *in vivo* approaches, Tang *et al.* (2019) concluded that MTBE exposure could favor adipogenesis especially if male mice were fed a high-fat diet, causing increased visceral fat mass and decreasing insulin sensitivity (Tang *et al.*, 2019). Regarding the adverse effect on artherosclerosis, another recent study from the same team (Ren *et al.*, 2021) showed that MTBE promoted atherosclerosis in the male APoE $^{-/-}$ mouse model by reducing the efflux of cholesterol and promoting the formation of foam cells. However, this latter study did not show a consistent hyperlipidemia in wild-type or ApoE$^{-/-}$ male mice. In this field of research, the team of Du Y. *et al.* (Wen *et al.*, 2019; Xie *et al.*, 2020) conducted other previous experiments on metabolic properties of hexabromocyclododecane and polybrominated diphenyl ether 99 which increases the confidence on these results.

Diabetes and atherosclerosis are both of interest in relation to human relevance and adversity while adipogenesis itself can be seen as a physiological mechanism. Their underlying MoA can be considered separately. Whether the animals in the Tang et al. study fed a high energy diet is an intact model can be questioned as well as the use of the APoE - / - mouse model in Ren *et al.* (2021). However the high energy diet and the use of the APoE - / - mouse model can be considered relevant as Western human population is known to feed with high energy diet and ApoE4 mutations is common in Western population. The apparent no significant weight gain of the animals in the obesity model can be questioned as well but it may also be due to a possible re-distribution of fat to other tissues. The fact that only the mid-range dose was presented for certain measurements in the Tang et al. study could also be pointed out as well as the unclear dose-response relationship and lack of positive control. However, the absence of a positive control is not critical in a study with a positive outcome (i.e. test substance related effects are seen). Lastly, it could be pointed out that there is no agreed set of adverse parameters relating to decreased insulin sensitivity (see also discussion below).

Based on the human relevance of these two effects, the available data on MTBE were presented and discussed during ED-EG 21 see official minutes (ECHA, 2021). Following the ED-EG consultation, an expert elicitation procedure was conducted with ANSES experts from the Endocrine Disruptor (ED) expert Working Group according to the procedure developed in the Anses report (ANSES, 2021). Based on the available studies and the effects described from low doses onward, three endpoints have been assessed: atherosclerosis, lipogenesis and increased resistance to insulin. The one linked to atherosclerosis, although a known human relevant adverse effect could not be categorized due to the absence of data on the targeted nuclear receptors and specific mechanistic parameters in relation to endocrine disruption. Regarding the effects on lipogenesis and increased resistance to insulin, the ANSES ED expert Working Group considered the latter endpoint as being an adverse effect with much relevance compared to an effect as adipogenesis which may be reversible. It should be noticed nevertheless, that adipocyte dysfunction may have also consequences to sensitivity to insulin. Thus, the following questions were submitted to expert elicitation :

Q1 : What is the plausibility that MTBE has the potential to induce resistance to insulin? *Please consider the models used for identifying these effects and the entire database available.*

Q2: What is the plausibility that MTBE acts through adipocyte dysfunction via FABP4 and PPAR gamma pathways?

Q3: What is the biological plausibility that the identified MOA namely via FABP4 and PPAR gamma in adipocytes induces a resistance to insulin?

Q4: Knowing the plausibility of QUESTION 1, QUESTION 2 and QUESTION 3, what is the plausibility that the studied substance has the potential to cause the adverse effect through the identified endocrine MOA (i.e. be considered as an endocrine disruptor for human health (HH))?

During the elicitation process, additional literature was provided with Gayoso-Diz et al. (2013) (Gayoso-Diz et al., 2013), Julien B. et al. (2019)(Julien et al., 2019), and Naville D. et al. (2019)(Naville et al., 2019) for question 1 and Ahmadian et al. (2013)(Ahmadian et al., 2013) for question 3.

The available data were analysed to evaluate the plausibility that MTBE has the potential to induce insulin resistance? The data were also used to decide if MTBE

DRAFT

acts through adipocyte dysfunction via the FABP4 (previously called aP2) and PPAR gamma pathways? The biological plausibility that the proposed mode of action, namely activation by MTBE of FABP4 and PPAR gamma in adipocytes, induces insulin resistance was questionned? Finally, the plausibility that MTBE has the potential to induce insulin resistance via the endocrine mode of action (*via* FABP4 and PPAR gamma in adipocytes) for human health was assessed.

For question 1, the following points were thoroughly discussed: the role of the development of hepatic steatosis in relation to insulin resistance and consequently the lack of liver histopathological analysis to objectify (or not) an hepatic steatosis in Tang *et al.* (2019), the adequacy of the statistical analysis conducted in Tang *et al.* (2019), the apparent inconsistency of HOMA-IR (females versus males), the difference in sensitivity between humans and rodents and the translation of HOMA-IR results from rodents to humans. Overall, the experts ranked plausibility as suspected for question 1.

For question 2, although in vitro experimental data were considered robust and well documented, some uncertainties were expressed regarding the limited number of genes analyzed, namely PPARG and FABP4 with mRNA analysis. Additional analyses including protein expression measurements, or gene expression using microarray assays or SiRNA would have been beneficial. Therefore, the plausibility that MTBE acts through adipocyte dysfunction via FABP4 and PPARγ pathways was considered suspected.

For question 3, the literature available on FABP and in particular FABP4, its negative feedback loop exerted to control PPARγ receptor signaling may well explain the loss of insulin sensitivity, as PPARγ induces insulin sensitivity (Ahmadian et al., 2013; Hotamisligil and Bernlohr, 2015). Furthermore $Fabp4^{-/-}$ mice are protected from obesity-induced insulin resistance, whereas exogenous FABP4 administration impairs insulin sensitivity. Although this article is quite strong, an additional article such as Ahmadian *et al*. (2013) was considered useful as supplemental material. Thus the biological plausibility that the identified mode of action (via FABP4 and PPARγ in adipocytes) induces insulin resistance is considered presumed.

Finally, the plausibility that MTBE has the potential to induce insulin resistance via the endocrine mode of action (via FABP4 and PPARG in adipocytes) for human health led to a suspected category (median at 0.6). Uncertainty was mainly related to the availability of a single study even if this study was considered of good quality and documenting the adverse effect with vivo, vitro and mechanistic data. Finally, to strengthen the case, an additional in vivo study with the Knock-Out model would have been appreciated which would allow a revision of this proposed categorization.

Overall, all data are in favor of a clear effect between Fabp4, PPARγ and insulin resistance. However, due to the limited number of experimental studies available (only one study although robust and well-conducted), the plausibility that MTBE has the potential to induce insulin resistance via the endocrine mode of action (FABP4 and PPARγ in adipocytes) for human health leads to a suspected category. As a conclusion, MTBE is considered by ANSES as a suspected Endocrine disruptor for human health.

However, additional data have been published after the finalisation of the analysis (Bus et al., 2022; Zhu et al., 2022). They have not been analysed in detail and may provide a different perspective to the existing data. The conclusion may need reconsideration in the future.

DRAFT

5 Environmental exposure & risk assessment

The SEv Conclusion document (ANSES, 2022) identified environmental risks for several emission scenarios. Therefore, a better consideration of the behaviour of the substance in wastewater treatment plants(WWTPs) has been carried out. Subsequently, calculated local concentration in the different environmental compartments have been compared to existing environmental monitoring data in Europe.

MTBE has a high production volume (total tonnage band: 1 000 000 - 10 000 000 tonnes per annum) and is mainly used in fuel production to enhance the octane index (fuel oxygenate). MTBE is also used as an intermediate (to produce isobutylene) and as a process solvent or extraction agent (for laboratory chemical analyses, research and development purposes…).

An environmental exposure assessment is carried out by following the life cycle of the substance.

Table 22 - Life-Cycle Emission scenario

Life Cycle Stage (LCS) M: Industrial manufacture of MTBE	
ES 1	Manufacture of MTBE
MTBE is manufactured for use as a fuel additive in closed, continuous, processes. MTBE is typically manufactured in petroleum refineries but production can also occur in facilities that specialize in the large-scale synthesis of organic chemicals. MTBE is prepared principally by reacting isobutene with methanol over an acidic ion-exchange resin catalyst at 38-93 °C and 100-200 psi. It can also be prepared from methanol, tert-butyl alcohol (TBA), and diazomethane. The manufacture of MTBE occurs outdoors in an automated and principally closed wet or dry process with a connection to central waste gas system to minimise release to environment.	
Life Cycle Stage (LCS) F: Formulation or re-packing	
ES 2	Formulation and re-packaging of MTBE
Formulation covers the blending of petrol with MTBE both on site and off site. The formulation of MTBE into petrol is an automated that occurs principally in closed outdoor systems with a connection to central waste gas system. There are two formulation techniques for blending petrol with MTBE, in-line blending and batch blending. With in-line blending, petrol components such as MTBE are pumped from their storage tanks to a common line which leads to a product storage tank. The components are blended both during the pumping through the common line and in the product tank. In batch blending the petrol components are pumped through separate lines to the storage tank. The blending of the components hence takes place only in the product tank.	
ES 3	Transport of MTBE-containing fuels - Tank storage of MTBE-containing fuels
Following blending and transfer to the appropriate vehicle, the MTBE-containing fuel is transported and distributed for use. The blended petrol products are transported from the refinery to depot-terminals then distributed from the bulk storage areas to service stations. The fuel products can be transported by air, rail car, truck and/or ship. Emissions during transport and distribution are mainly atmospheric, with emissions to all environmental compartments a possibility during storage, loading/reloading, transportation and delivery of petrol at service stations. Release to the aquatic environment may occur during transportation of petrol via the waterways and during the refueling of watercraft. Environmental exposures resulting from storage in floating roof tanks is also considered.	
Life Cycle Stage (LCS) IS: Use at industrial sites	

DRAFT

ES 4	Industrial use of MTBE-containing fuels
MTBE-containing fuels are used in industrial fuel applications. Industrial uses cover emissions from the use of petrol in spark ignition engines. In addition, this scenario includes activities associated with fuel transfer and use, equipment maintenance, and the handling of waste. Emissions to all environmental compartments are possible, although emissions to the environment are mainly atmospheric.	
ES 4	Accidental release of an MTBE-containing fuel to groundwater from an underground storage tank
The primary use of MTBE in the European Union is as a fuel additive that can be present in gasoline at levels up to 15% (v/v). The formulated gasoline finds wide dispersive use in industrial, professional and consumer use settings. The MTBE-formulated gasoline is often stored onsite in underground storage tanks (USTs) whose construction and design are strictly controlled by the individual Member States (European commission, 2001). In general, the UST systems currently in use within many Member States have corrosion, spill, and overfill protection that provide safeguards against the accidental release or leakage of fuel to soil or groundwater. The corrosion protection includes the use of a double-walled tank design or the use of a containment system. In addition, corrosion resistant coatings or the connection to a sacrificial anode have been legislated in some Member States. Leak detection systems are often employed that alert operators to the release of fuel from the primary tank. Accidental overfill of the tanks is prevented with a device that shuts off the flow of fuel once the tank has filled to its maximum capacity. Finally, regular inspection of the tanks is often required as an added risk management measure that protects against the accidental release of fuel. A release scenario therefore focuses on the indirect exposure of humans via drinking water. The leakage occurs directly into an underground aquifer containing groundwater that will subsequently be used to generate drinking water. The scenario assumes a moderately-sized industrial refuelling site that dispenses quantity in tons/day of an MTBE-containing fuel.	
ES 7	Use of MTBE as an intermediate
A small percentage of MTBE is used as an intermediate for the production of high purity isobutylene. The process uses a proprietary catalyst to decompose MTBE in a reactor that is heated with steam. The process yields isobutylene and methanol, which is extracted via a water wash. Air and water releases are possible with this use.	
ES 8	Use of MTBE as a process solvent or extraction agent
Although MTBE is used almost exclusively as a fuel additive, limited amounts of very pure (97.5%) material are produced for use as a solvent. MTBE finds use as a solvent under a variety of conditions (solvent for laboratory chemical analyses, or for research and development purposes and for the dissolution of gallstones in patients and laboratory).	
Life Cycle Stage (LCS) PW: Widespread use by professional workers	
ES 5	Professional use of MTBE-containing fuels
MTBE-containing fuels also find use in professional applications. Emissions to all environmental compartments are possible, although emissions into environment are mainly atmospheric. Emissions to air from the use of petrol are the main source of MTBE released to the environment. This emission source contributes a majority of the total emitted mass volume. These emissions are divided into two main categories: evaporative emissions and exhaust emissions.	
Life Cycle Stage (LCS) C: Consumer use	
ES 6	Consumer use of MTBE-containing fuels
MTBE-containing fuels are available for use by consumers. Emissions to all environmental compartments are possible although emissions into environment are mainly atmospheric. Emissions to air from the use of petrol to fuel automobiles, garden equipment, motorcycles, recreational vehicles, and watercraft are all considered. In each of these cases, the primary interest is the air	

DRAFT

emissions, which is the main source of the MTBE released to the environment since it covers the majority of the total emitted mass volume. Emissions sources can be divided into two categories: evaporative and exhaust-related.

5.1 Environmental exposure assessment

5.1.1 Tonnage

The evaluation is based on a local tonnage used in the assessment for each environmental contributing activity, corresponding to a tonnage at the site for uses taking place at industrial sites and to a tonnage assumed for a town of 10,000 inhabitants for widespread uses.

5.1.2 Hazard information

Hazard information required in the risk characterisation are described in the following table and are based on the SEv Conclusion document (ANSES, 2022).

Table 23 - PNEC derivation

Compartment	Hazard conclusion	Justification
Freshwater	$PNEC_{freshwater}$ = 0.304 $mg.L^{-1}$	NOEC zebrafish: 3.04 $mg.L^{-1}$ (reproduction endpoint) Assessment factor: 10 Unpublished study report,2012
Sewage treatment plant	$PNEC_{stp}$ = 710 $mg.L^{-1}$	Growth inhibition test with *Pseudomonas putida* EC_{10} = 710 $mg.L^{-1}$ [nominal] Assessment factor: 1 Unpublished study report,1991d
Soil	$PNEC_{soil}$ = 142.8 $\mu g/kg_{dw}$	Extrapolation method: EPM derivation

5.1.3 Physicochemical properties

Table 24 - Physicochemical properties used for exposure estimation

Input	Value	Unit
Molecular weight	88.15	$g.mol^{-1}$ [25°C]
Vapour pressure	33000	Pa [25°C]
Henry's law constant	64.9	$Pa.m^3.mol^{-1}$ [25°C]
Water solubility	41850	$mg.L^{-1}$ [20°C]
Organic carbon/water partition coefficient (Koc)	9.12	$L.kg^{-1}$
Octanol-water partition coefficient	1.06	[log10]
Biodegradability	Not readily biodegradable	[-]

DRAFT

Soil-water partition coefficient	0.476	[$m^3.m^{-3}$]
Total rate constant for removal from agricultural top soil	1.10E-02	d^{-1} [12°C]
Total rate constant for removal from grassland top soil	2.02E-02	d^{-1} [12°C]

5.1.4 Degradation in Waste Water Treatment Plants (WWTPs)

Abiotic decomposition can be considered as a non-significant degradation route for MTBE.

Three reliable studies testing the ready biodegradability of the MTBE according to the standard guideline OECD 301D are available (table 9).

In an unpublished report (1991a), a ready biodegradability test was performed on MTBE. The test substance (2 $mg.L^{-1}$) was incubated during 28 days with a **non-adapted inoculum** sampled from a municipal treatment plant. Test substance degradation was estimated based on O_2 consumption. **After 28 days of incubation, 0% of the test substance was mineralised**, which indicated that MTBE should be considered as not readily biodegradable, as the percentage of degradation after 28 days is below the regulatory threshold (i.e. 60% of degradation based on O2 consumption).

In an unpublished report (1996) a ready biodegradability test on MTBE was also performed. The test substance (2 $mg.L^{-1}$) was incubated during 28 days with a **non-adapted inoculum**. Test substance degradation were estimated based on O_2 consumption. **After 28 days of incubation, 1.8% of the test substance was mineralised**, which indicated that MTBE should be considered as not readily biodegradable, but not have inhibitory effects on the bacteria inoculum at the concentration tested (2 $mg.L^{-1}$).

In addition, a biodegradability test on MTBE was performed according to the standard guideline OECD 301D (non GLP-compliant) (unpublished study report 2005, RI = 2). The test substance (2.5 $mg.L^{-1}$) were incubated during 28 days with an inoculum (5 $mL.L^{-1}$) originated from an industrial wastewater treatment plant that received effluent from manufacture of MTBE. No data is available about adaptation of the inoculum to the MTBE. However, the **inoculum** should be considered as **adapted** to the MTBE. Test substance degradation was estimated based on O_2 consumption. After 7 days of incubation, 9.24% of the test substance was mineralised. The biodegradation result achieved after day 7 was not increased in the following days of the test (i.e. 28 days). As a consequence MTBE should be considered as **not inherently biodegradable** by adapted inoculum originated from industrial wastewater treatment plant that received effluent from manufacture of MTBE.

A study shows that degradation of MTBE (wastewater removal efficiency of 97%) could occur in wastewater treatment plants with adapted inoculum; using the non-linear Monod kinetics rather than linear first order kinetics. This result has been considered as relevant for all industrial use scenarios in registration dossiers. However, such result should be considered with caution for a regulatory purpose as the MTBE is considered as not inherently biodegradable. The previous study (according to the OECD 301D guideline) using inoculum originated from an industrial wastewater treatment plant shows a degradation below 10% after 28 days. In addition, effluent measurements have been carried out from 13

DRAFT

productions and formulations facilities with removal efficiencies ranged from 35.6% to 99.9%.

Consequently and considering uncertainties about the degradation of MTBE in industrial and municipal wastewater treatment plant, no degradation in STP is taking into account.

Below the output model of EUSES for the MTBE used for municipal waste water treatment in the exposure assessment.

Table 25: Calculated fate and distribution in STP -EUSES

Compartment	Percentage [%]
Air	34.8
Water	65.1
Sludge	0.104
Degraded in STP	0

5.1.5 Emission scenario parameters: description

The concentrations of substances released from a single point source (industrial site or standard municipal biological STP) are to be assessed after release to the environment.

Table 26 - Life-Cycle Emission scenario – description of key parameters

Life Cycle Stage (LCS) M: Industrial manufacture of MTBE	
ES 1	Manufacture of MTBE
Percentage of EU tonnage used at regional scale: 25 % **Water Release factor**: 0.03% The default worst-case release factors resulting from the conditions of use described in the ERC 1 is 0.06. In the RAR EU, a value of 0.0003 has been proposed taking into account site-specific information (production/formulation) from 28 sites (highest emission factor). The registrants apply a degradation of 97% on the ERC value, considering a site specific biological STP. The refinement proposed by registrants is 0.0018. Nevertheless, this value of 97% is not sufficiently argued. Consequently, results from the RAR EU value (f=0.003) are kept. **Discharge rate of STP**: >= 2E3 $m^3.day^{-1}$ (site specific biological STP) **Receiving surface water flow rate:** >= 1.8E4 $m^3.day^{-1}$ **Dilution factor to freshwater:** >= 10 **Application of the STP sludge on agricultural soil**: No	
Life Cycle Stage (LCS) F: Formulation or re-packing	
ES 2	Formulation and re-packaging of MTBE
Number of days emission per year: 300 $days.year^{-1}$ **Percentage of EU tonnage used at regional scale**: 25 % **Water Release factor**: 0.03% The SpERC value taken from ESVOC 2.2.e.v1 (formulation & (re)packing of substances and mixtures (industrial): solvent-borne (vapour pressure > 1000 Pa and water solubility > 1000 mg/L) is 0.5%. In the RAR EU, a value of 0.0003 has been proposed taking into account site-specific information	

DRAFT

(production/formulation) from 28 sites (highest emission factor). The registrants apply a degradation of 97% on the SpERC value, considering a site specific biological STP. The refinement proposed by registrants is 0.00015. Nevertheless, this value of 97% is not sufficiently argued. Consequently, results from the RAR EU value (f=0.003) are kept.

Discharge rate of STP: >= 2E3 $m^3.day^{-1}$ (site specific biological STP)
Receiving surface water flow rate: >= 1.8E4 $m^3.day^{-1}$
Dilution factor to freshwater: >= 10

Application of the STP sludge on agricultural soil: No

ES 3	Transport of MTBE-containing fuels

Number of days emission per year: 300 $days.year^{-1}$

Percentage of EU tonnage used at regional scale: 25 %

Water Release factor: 1E-03%
The SpERC value taken from ESVOC 1.1b.c.v1 (Distribution of substance - Industrial (solvent-borne) (vapour pressure > 100 Pa and water solubility > 10 mg/L)) is 1E-03%.
The registrants apply a degradation of 97% on the ERC value, considering a site specific biological STP. The refinement proposed by registrants is 3E-05%. Nevertheless, this value of 97% is not sufficiently argued. Consequently, results from the SpERC value (f=1E-03) are kept.

Discharge rate of STP: >= 2E3 $m^3.day^{-1}$ (site specific biological STP)
Receiving surface water flow rate: >= 1.8E4 $m^3.day^{-1}$
Dilution factor to freshwater: >= 10

Application of the STP sludge on agricultural soil: No

ES 3	Tank storage of MTBE-containing fuels

Number of days emission per year: 300 $days.year^{-1}$

Percentage of EU tonnage used at regional scale: 25 %

Water Release factor: 3.85E-04%
The release of MTBE was determined using the following information:

- Consumption of 10 litre of gasoline per inhabitant per day for a city of 100 000 inhabitants, corresponding to 1000 m^3 of gasoline per day.
- This 1000 m^3 of gasoline set free 200 litres of tank bottom water per day or 1400 litres tank bottom water per week;
- According to US Patent (1995) the ratio between MTBE in gasoline and water is 0.039 (v/w);
- Gasoline contains 15% MTBE;

Consequently,

- if the tank bottom water is released every day automatically, an amount of 1.2 kg MTBE is discharged to the sewer, resulting in a concentration of 0.6 $mg.l^{-1}$ in the waste water entering a wastewater treatment plant of 10 000 inhabitant equivalents;
- if the tank bottom water is released on a weekly basis, 8.4 kg MTBE is released in one day to the sewer system, resulting in a concentration of 4.2 $mg.l^{-1}$ in the waste water entering a wastewater treatment plant of 10 000 inhabitant equivalents.

A release of 8.4 kg MTBE in one day to the sewer system corresponds to a water release factor of **3.85E-4%.**
The refinement proposed by registrants of **1.15E-05%** The registrants apply a degradation of 97% on the 3.85E-04% value, considering a site specific biological STP). The refinement proposed by registrant is **1.15E-05%**. Nevertheless, this value of 97% is not sufficiently argued. Consequently, results from the first value (f=3.85E-04%) are kept.

Discharge rate of STP: >= 2E3 $m^3.day^{-1}$ (site specific biological STP)
Receiving surface water flow rate: >= 1.8E4 $m^3.day^{-1}$
Dilution factor to freshwater: >= 10

Application of the STP sludge on agricultural soil: No

Life Cycle Stage (LCS) IS: Use at industrial sites	
ES 4	Industrial use of MTBE-containing fuels

DRAFT

Number of days emission per year: 365 days.year^{-1}

Percentage of EU tonnage used at regional scale: 25 %

Water Release factor: 1E-03%
The SpERC value taken from ESVOC 7.12a.a.v3 (use as a fuel (industrial): solvent-borne) is **1E-03%**. The factor considers the results from a life cycle assessment for heavy fuel use in a power plant. The analysis includes an examination of the release of unspecified hydrocarbons and oils to wastewater.

The registrants apply a degradation of 97% on the SpERC value, considering a site specific biological STP). The refinement proposed by registrants is 3E-05. Nevertheless, this value of 97% is not sufficiently argued. Consequently, results from the SpERC value (f=1E-03%) are kept.

Discharge rate of STP: >= 2E3 m^3.day^{-1} (site specific biological STP)
Receiving surface water flow rate: >= 1.8E4 m^3.day^{-1}
Dilution factor to freshwater: >= 10

Application of the STP sludge on agricultural soil: No

ES 4	Accidental release of an MTBE-containing fuel to groundwater from an underground storage tank

The primary use of MTBE in the European Union is as a fuel additive that can be present in gasoline at levels up to 15% (v/v). The formulated gasoline finds wide dispersive use in industrial, professional and consumer use settings. The MTBE-formulated gasoline is often stored onsite in underground storage tanks (USTs) whose construction and design are strictly controlled by the individual Member States (European Commission, 2001). In general, the UST systems currently in use within many Member States have corrosion, spill, and overfill protection that provide safeguards against the accidental release or leakage of fuel to soil or groundwater. The corrosion protection includes the use of a double-walled tank design or the use of a containment system. In addition, corrosion resistant coatings or the connection to a sacrificial anode have been legislated in some Member States. Leak detection systems are often employed that alert operators to the release of fuel from the primary tank. Accidental overfill of the tanks is prevented with a device that shuts off the flow of fuel once the tank has filled to its maximum capacity. Finally, regular inspection of the tanks is often required as an added risk management measure that protects against the accidental release of fuel.

A release scenario therefore focuses on the indirect exposure of humans via drinking water. The leakage occurs directly into an underground aquifer containing groundwater that will subsequently be used to generate drinking water. The scenario assumes a moderately-sized industrial refueling site that dispenses quantity in tons/day of an MTBE-containing fuel. ERC 7 (Industrial use of substances in closed systems) was judged to be the most appropriate scenario descriptor for use with an estimated emission factor to water of 0.35%.

The scenario assumes no biodegradation of MTBE in the aquifer and a very conservative dilution factor of 10 in the groundwater.

ES 7	Use of MTBE as an intermediate

Number of days emission per year: 300 days.year^{-1}

Percentage of EU tonnage used at regional scale: 25 %

Water Release factor: 1%
The SpERC value taken from ESVOC 6.1a.e.v1 (use as an Intermediate (industrial): solvent-borne) is **1%**.

The registrants apply a degradation of 97% on the SpERC value, considering a site specific biological STP). The refinement proposed by registrants is 0.03. Nevertheless, this value of 97% is not sufficiently argued. Consequently, results from the SpERC value (f=1%) are kept.

Discharge rate of STP: >= 2E3 m^3.day^{-1} (site specific biological STP)
Receiving surface water flow rate: >= 1.8E4 m^3.day^{-1}
Dilution factor to freshwater: >= 10

Application of the STP sludge on agricultural soil: No

ES 8	Use of MTBE as a process solvent or extraction agent

Number of days emission per year: 20 days/yr

Percentage of EU tonnage used at regional scale: 25 %

Water Release factor: 1%

DRAFT

The SpERC value taken from ESVOC 6.1a.e.v1 (use as an Intermediate (industrial): solvent-borne) is **1%**.

The registrants apply a degradation of 97% on the SpERC value, considering a site specific biological STP). The refinement proposed by registrants of 0.03%. Nevertheless, this value of 97% is not sufficiently argued. Consequently, results from the SpERC value (f=1%) are kept.

Discharge rate of STP: >= 2E3 m^3/day (site specific biological STP)
Receiving surface water flow rate: >= 1.8E4 m^3/day
Dilution factor to freshwater: >= 10

Application of the STP sludge on agricultural soil: No

Life Cycle Stage (LCS) PW: Widespread use by professional workers	
ES 5	Professional use of MTBE-containing fuels

Water Release factor: 1E-03%
The SpERC value taken from ESVOC 9.12b-c.a.v1 is **1E-03%** (Use as a Fuel (wide dispersive use): solvent-borne, Vapour pressure > 5000 Pa. Covers the use as a fuel (or fuel additive) and includes activities associated with its transfer, use, equipment maintenance and handling of waste and consumer uses in liquid fuels).

Discharge rate of STP: >= 2E3 m3/day (municipal STP)

Receiving surface water flow rate: >= 1.8E4 m3/day
Dilution factor to freshwater: >= 10

Application of the STP sludge on agricultural soil: Yes

Life Cycle Stage (LCS) C: Consumer use	
ES 6	Consumer use of MTBE-containing fuels

Water Release factor: 1E-03%
The SpERC value taken from ESVOC 9.12b-c.a.v1 is **1E-03%** (Use as a Fuel (wide dispersive use): solvent-borne, Vapour pressure > 5000 Pa. Covers the use as a fuel (or fuel additive) and includes activities associated with its transfer, use, equipment maintenance and handling of waste and consumer uses in liquid fuels).

Discharge rate of STP: >= 2E3 m3/day (municipal STP)
Receiving surface water flow rate: >= 1.8E4 m3/day
Dilution factor to freshwater: >= 10

Application of the STP sludge on agricultural soil: Yes

DRAFT

5.2 Environmental risk assessment

5.2.1 Local assessment

The following table shows results of environmental risk assessment (only for industrial emission scenarios featuring an industrial sewage treatment plant (STP)), considering the refinements proposed by the Registrants for the F_{water} parameter. The Registrants apply a degradation of 97% (site specific biological STP). These results are not the final results used in the environmental risk assessment. They are only shown here to support the fact that risks are also identified according to the registrant's approach. The refinement of 97% cannot be considered as relevant, not being sufficiently argued.

Table 27 : Results of the environmental risk assessment (degradation of 97% in STP as proposed by Registrants)

Exposure Scenario		STP	Aquatic (including sediment for RCR)	Agricultural soil	Groundwater
Life Cycle Stage (LCS) M: Industrial manufacture of MTBE					
ES 1 - Manufacture of MTBE		PEC: 2.27E+03 mg.L^{-1} RCR: **3.19**	PEC: 2.27E+02 mg.L^{-1} RCR: **745**	No application of the STP sludge on agricultural soil (incineration of sludge)*	NR
Life Cycle Stage (LCS) F: Formulation or re-packing					
ES 2 - Formulation and re-packaging of MTBE		PEC: 1.07E+02 mg.L^{-1} RCR: 0.15	PEC: 1.07E+01 mg.L^{-1} RCR: **35.3**	No application of the STP sludge on agricultural soil (incineration of sludge)*	NR
ES 3	Storage and transport of MTBE-containing fuels	PEC: 2.15E-01 mg.L^{-1} RCR: <0.001	PEC: 2.15E-02 mg.L^{-1} RCR: <0.1	No application of the STP sludge on agricultural soil (incineration of sludge)*	NR
	Tank storage of MTBE-containing fuels	PEC: 8.22E-02 mg.L^{-1} RCR: <0.001	PEC: 8.22E-03 mg.L^{-1} RCR: <0.1	No application of the STP sludge on agricultural soil (incineration of sludge)*	NR

DRAFT

Life Cycle Stage (LCS) IS: Use at industrial sites					
ES 4	Industrial use of MTBE-containing fuels	PEC: 1.76E-01 mg.L^{-1} RCR: < 0.001	PEC: 1.76E-02 mg.L^{-1} RCR: < 0.1	No application of the STP sludge on agricultural soil (incineration of sludge)*	NR
	Accidental release of an MTBE-containing fuel to groundwater from an underground storage tank	PEC: Not relevant	PEC: Not relevant	No application of the STP sludge on agricultural soil (incineration of sludge)*	NR
ES 7 - Use of MTBE as an intermediate		PEC: 1.04E+01 mg.L^{-1} RCR: < 0.1	PEC: 1.04E+00 mg.L^{-1} RCR: **3.44**	No application of the STP sludge on agricultural soil (incineration of sludge)*	NR
ES 8 - Use of MTBE as a process solvent or extraction agent		PEC: 3.92E+01 mg.L^{-1} RCR: < 0.1	PEC: 3.92E+00 mg.L^{-1} RCR: **12.90**	No application of the STP sludge on agricultural soil (incineration of sludge)*	NR

* =Implemented in Registration dossiers.
NR: not relevant

The following table shows **final results of environmental risk assessment (all emission scenarios), considering a conservative approach, no degradation in the STP.**

Industrial emission scenarios featuring an industrial STP as well as emission scenarios featuring municipal STP (for professional and consumer uses) are considered.

DRAFT

Table 28 : final results of environmental risk assessment, with no degradation in STP.

Exposure Scenario		STP	Aquatic (including sediment for RCR)	Agricultural soil	Groundwater
Life Cycle Stage (LCS) M: Industrial manufacture of MTBE					
ES 1 - Manufacture of MTBE		PEC: 3.78E+02 $mg.L^{-1}$ RCR: 0.53	PEC: 3.78E+01 $mg.L^{-1}$ RCR: **124**	PEC: 1.99E+00 $mg.kg_{wwt}^{-1}$ RCR: **18.2** RMM: incineration of sludge*	3.79E+03 $\mu g.L^{-1}$
Life Cycle Stage (LCS) F: Formulation or re-packing					
ES 2 - Formulation and re-packaging of MTBE		PEC: 2.15E+02 $mg.L^{-1}$ RCR: 0.30	PEC: 2.15E+01 $mg.L^{-1}$ RCR: **70.7**	PEC: 1.13E+00 $mg.kg_{wwt}^{-1}$ RCR: **10.4** RMM: incineration of sludge*	2.16E+03 $\mu g.L^{-1}$
ES 3	Storage and transport of MTBE-containing fuels	PEC: 7.16E+00 $mg.L^{-1}$ RCR: <0.1	PEC: 7.16E-01 $mg.L^{-1}$ RCR: **2.36**	PEC: 3.77E-02 $mg.kg_{wwt}^{-1}$ RCR: 0.35	71.9 $\mu g.L^{-1}$
	Tank storage of MTBE-containing fuels	PEC: 2.75E+00 $mg.L^{-1}$ RCR: <0.01	PEC: 2.75E-01 $mg.L^{-1}$ RCR: 0.9	PEC: 1.45E-02 $mg.kg_{wwt}^{-1}$ RCR: 0.13	27.6 $\mu g.L^{-1}$
Life Cycle Stage (LCS) IS: Use at industrial sites					
ES 4	Industrial use of MTBE-containing fuels	PEC: 5.87E+00 $mg.L^{-1}$ RCR: < 0.01	PEC: 5.87E-01 $mg.L^{-1}$ RCR: **1.93**	PEC: 3.58E-02 $mg.kg_{wwt}^{-1}$ RCR: 0.28	58.9 $\mu g.L^{-1}$
	Accidental release of an MTBE-containing fuel to groundwater from an underground storage tank	PEC: Not relevant	PEC: Not relevant	PEC: 5.99E-02 $mg.kg_{wwt}^{-1}$ RCR: < 0.55	114 $\mu g.L^{-1}$
ES 7 - Use of MTBE as an intermediate		PEC: 3.48E+02 $mg.L^{-1}$ RCR: 0.49	PEC: 3.48E+01 $mg.L^{-1}$ RCR: **115**	PEC: 1.83E+00 $mg.kg_{wwt}^{-1}$ RCR: **16.8** RMM: incineration of sludge*	3.49E+03 $\mu g.L^{-1}$

DRAFT

ES 8 - Use of MTBE as a process solvent or extraction agent	PEC: 1.31E+03 mg.L^{-1} RCR: **1.84**	PEC: 1.31E+02 mg.L^{-1} RCR: **429**	PEC: 6.88E+00 mg.kg_{wwt}^{-1} RCR: **63.1** RMM: incineration of sludge*	1.31E+04µg.L^{-1}
Life Cycle Stage (LCS) PW: Widespread use by professional workers				
ES 5- Professional use of MTBE-containing fuels	PEC: 5.86 mg.L^{-1} RCR: < 0.01	PEC: 5.86E-01 mg.L^{-1} RCR: **1.93**	PEC: 3.08E-02 mg.kg_{wwt}^{-1} RCR: < 1	58.8 µg.L^{-1}
Life Cycle Stage (LCS) C: Consumer use				
ES 6- Consumer use of MTBE-containing fuels	PEC: 1.17E-02 mg.L^{-1} RCR: < 0.001	PEC: 1.17E-03 mg.L^{-1} RCR: < 0.01	PEC: 6.17E-05 mg.L^{-1} RCR: < 0.001	0.12 µg.L^{-1}

* Implemented in Registration dossiers. Proposed RMM to control risks

DRAFT

5.2.2 Regional assessment

The total releases to the environment from all the exposure scenarios covered are used to estimate the PECs. This is the sum of the releases to the environments from all exposure scenarios addressed. The regional predicted environmental concentration (PEC regional) and the related risk characterisation ratios are presented in the table below. The exposure estimates have been obtained with EUSES 2.1.2

Table 29 : Predicted regional exposure concentrations (Regional PEC) and risks for the environment

Protection target	Regional PEC	Risk characterisation
Aquatic (surface water)	3.27E-03 $mg.L^{-1}$	0.01
Agricultural soil	2.61E-04 $mg.kg_{wwt}^{-1}$	0.002
Porewater of agricultural soil	0.93 $\mu g.L^{-1}$	Not relevant

5.2.3 Conclusion

Two types of PEC values are derived to be used in further risk characterisation: the regional concentration (PECregional) and the local concentration (PEClocal). These two types of concentrations differ in temporal and spatial scale. The local concentration (PEClocal) close to a point source emission is usually calculated as the sum of the concentration from the point source and the background concentration (the regional concentration (PECregional).

As a conclusion of the calculation performed above, environmental risks are observed for almost all local emission scenarios except for ES3 "Tank storage of MTBE-containing fuels", ES4 "Accidental release of an MTBE-containing fuel to groundwater from an underground storage tank" and ES6 "Consumer use of MTBE-containing fuels". No risks are identified at the regional scale based on calculation.

5.3 Survey of MTBE: monitoring data vs calculated data from risk assessment

Representative monitoring data may be used for the derivation of the regional and/or local concentrations as well. But according to the R.16 guidance and for the purpose of risk assessment, there are distinct aspects to consider: 1) the level of confidence in the results, 2) the representative level of data (local or regional: samples taken at sites directly influenced by the release should be used to describe the local scenario, while samples taken at larger distances may represent the regional concentrations).

Monitoring from industrial wastewater treatment plant

Twenty nine production and/or formulation MTBE sites, known in February 2000 in Europe, are included in the EU-RAR (2002) of the MTBE. Of these sites, 13 production and formulation facilities have actual influent and effluent measurements. The actual removal efficiencies from these sites ranged from 33.3 to 99.9% and averaged 81.4%. In addition, the highest effluent concentration of MTBE was found to be less than 10 $mg.L^{-1}$ (range from 0.00003 $mg.L^{-1}$to 9.98 $mg.L^{-1}$). The mean of measured concentrations in effluent is 0.85 $mg.L^{-1}$.

DRAFT

Wastewater samples (n=82) were collected from two sewage plants (Achten et al. 2002) in Germany in wintertime 2000/2001.

Table 30 : Wastewater treatment plant samples - Germany

	Influents	Effluent	Removal efficiencies
Niederrad sewage plant (Germany)	**Griesheim** 231 000 m^3/d Housing areas Median: 196 $ng.L^{-1}$ (24h) Mean: 214 $ng.L^{-1}$ (24h) **Niederrad** 75 000 m^3/d Primarily industrial Median: 299 $ng.L^{-1}$ (24h) Mean: 384 $ng.L^{-1}$ (24h) Max: 1268 $ng.L^{-1}$ MTBE loading: 10-37 kg/a	Median: 78 $ng.L^{-1}$ (24h) Mean: 265 $ng.L^{-1}$ (24h)	33%
Sindlingen sewage plant (Germany)	**Sindlingen** 154 000 m^3/d Housing areas Median: 96 $ng.L^{-1}$ (24h) Mean: 165 $ng.L^{-1}$ (24h) MTBE loading: 2-5 kg/a	Median: 81 $ng.L^{-1}$ (24h) Mean: 164 $ng.L^{-1}$ (24h)	31%

The reported elimination rate from water of 43.2% in the RAR report is slightly higher than rough estimations of 33% for Niederrad and 31% for Sindlingen.

In the same publication, the results of 19 industrial effluent samples of companies in the Frankfurt/M area showed two exceptional high inputs of 12.4 $\mu g.L^{-1}$and 28.4 $\mu g.L^{-1}$, whereas concentrations in other samples were generally low (median of 45 $ng.L^{-1}$and mean of 82 $ng.L^{-1}$).

Monitoring from Municipal wastewater treatment plant

Monitoring of MTBE in the effluents from municipal wastewater treatment plants performed as part of the national Danish environmental surveillance programme (NOVANA) shows low levels of MTBE, i.e. average values of 0.03-0.04 µ $g.L^{-1}$.

Table 31 : Monitoring data for MTBE outlet from point sources from the National Danish Monitoring and assessment programme

Point source	Number of samples ([1])	Average $\mu g.L^{-1}$	Median $\mu g.L^{-1}$	Year
WWTP (effluent)	40 (6)	0.03	0.00	2011
WWTP (effluent)	26 (8)	0.04	-	2004

[1]: Number of positive samples in brackets

Monitoring in surface Water

Please see Mobility section.

Groundwater

DRAFT

Please see Mobility section.

5.4 Conclusion

Surface water contamination

Many monitoring data have been collected over a large part of European countries (river: n= 38 387). Finally, 79.33% of data are below the LOD or LOQ (LOD range: 0.01 µg.$L^{-1}$to 1 µg.L^{-1}/ LOQ range: 0 to 3 µg.L^{-1}). Analysis of the data indicates that very few values exceed 10 µg.L^{-1} (n=44, 0.11% of data). And only two values are above the hazard threshold value (PNEC value of 304 µg.L^{-1}) for aquatic organisms. Unfortunately it is not possible to explain these high MTBE values.

There is however an uncertainty whether the available monitoring data represent local or regional assessment. Usually, samples taken at sites directly influenced by the release should be used to describe the local scenario, while samples taken at larger distances may represent the regional concentrations. Based on the available information, it is not possible to answer this question.

a) Emission scenarios with Industrial waste water treatment plant - site specific

The range value of removal efficiency rate [30%; 99.9%] found in the monitoring studies indicate that the value of 34.9% used in the environmental risk assessment of MTBE is relevant. Nevertheless, if monitoring data and calculated data are compared for effluent concentration values, it can be concluded that calculated value may be overestimated in some emission scenarios. Two parameters can explain this situation:

1) The daily amount used on a site:
   In the monitoring study included in theEU- RAR (2002), fourteen out of twenty nine production and/or formulation MTBE sites presents a specific value for the daily amount used. The range value is [0.015; 41.1] kg/d, significantly below the value calculated for the first two emission scenarios for the ES1 and for the ES2.

2) The STP effluent discharge rate:
   In the monitoring study included in the EU-RAR (2002), twenty six out of twenty nine MTBE production and/or formulation sites have a specific STP effluent discharge rate. The median value is 9 600 $m^3.d^{-1}$. The default value used in the environmental risk assessment of 2 000 $m^3.d^{-1}$ corresponds to the percentile 10. We can considered that this default value is slightly overestimated for MTBE production and/or formulation sites in Europe.

Monitoring data from industrial waste water treatment plant provides information from several manufacture and formulation sites that indicates that the risks are controlled. Recent data are however not available.

No monitoring data representative for the following industrial uses are available:
- industrial use of MTBE-containing fuels
- use as an intermediate
- use as a process solvent or extraction agent

and risks, as identified for these uses by calculation, cannot be excluded based on the available data.

DRAFT

b) Emission scenarios with municipal waste water treatment plant

The monitoring data collected do not allow to draw a conclusion or to be compared with the calculated values.

Therefore risks related to professional use of MTBE-containing fuels, as identified by calculation, cannot be excluded based on the available data.

Agricultural soil contamination

No monitoring data were collected for the terrestrial compartment. They are lacking or fragmented.

Based on calculated approach, risks occurs for manufacture, formulation and industrial uses. **Therefore, the regulatory action to manage risks for agricultural soil contamination (incineration of sludge) as proposed by registrants is relevant.** For these uses, risks can be controlled by the implementation of systematic incineration of STP sludge as an additional risk management measure.

Groundwater contamination and indirectly drinking water

Please see mobility section above (section 4.2.1).

Final Overview

Risks are identified based on calculated data for aquatic compartment for industrial and professional uses of MTBE. Monitoring data from specific industrial sites show that calculated values may be overestimated in some emission scenarios (manufacturing and formulation) for water concentration values. In addition, surface water monitoring data indicate that PNEC are indeed exceeded in very limited cases (2/38 387). There is however an uncertainty whether the available surface water monitoring data represent local assessment (as closely as possible to the emission source). **Therefore, monitoring data do not allow to exclude the risks identified based on calculation and it is necessary to consider further regulatory action to manage risks for surface water contamination related to the industrial and professional uses of MTBE.**

For agricultural soil, risks can be controlled by the implementation of systematic incineration of STP sludge and should be systematically implemented.

DRAFT

6 Justification for the need for regulatory risk management action at EU level

As shown in table 2, one of the conclusion of the SEv was the need to pursue investigation through a RMOA for ED properties for human health, the PBT/PMT properties and the environmental risks. These investigations lead ANSES to conclude that:

- MTBE is considered as a suspected ED for human health;
- the high persistency and the high mobility of MTBE could lead to a long term contamination of water resources that could be difficult and costly to remediate, leading to concern which could be considered as equivalent to the PBT/ vPvB substances;
- finally, considering calculation and monitoring data, environmental risks cannot be excluded for the aquatic compartment in relation to industrial and professional uses.

Taking into account all the concerns raised above, a need for further regulatory action is identified. Different RMOs have been assessed by ANSES in order to cover all the concerns raised above.

6.1 Identification as SVHC/Candidate Listing without Inclusion in Annex XIV

MTBE is very persistent and very mobile in the environment and could lead to a long term and not remediable contamination of drinking water. Those hazardous properties are considered to trigger an equivalent level of concern (ELoC) according to Art 57 f) of REACH regulation and MTBE is considered as a relevant substance of very high concern for Candidate Listing on this basis.

In addition, MTBE is considered by ANSES as a suspected ED. Although MTBE does not fulfil the SVHC identification criteria *per se* on this basis, it may be further considered in the light on other data as discussed in section 4.2.1.4.

While Candidate Listing is often seen as a first step to Authorisation, it has direct effects even when the substance is not further included in Annex XIV.

Most importantly, Candidate Listing is the only way today to legally acknowledge the vPvM properties of a chemical that has the capacity to reach and contaminate on the long term water resources. The hazard assessment during SVHC identification may therefore support potential subsequent risk management options. It would require the registrants to acknowledge the SVHC status of MTBE and to minimize emissions of this substance to the environment by the help of substance tailored operational conditions and RMMs.

SVHC identification as such is also considered to encourage the substitution of the substance.

DRAFT

Furthermore, Candidate Listing triggers information rights for consumers and the duty to report certain information for industry in relation to the presence of the substance in articles:

- *Requirement to inform customers and consumers under REACH (Art. 33):* Suppliers of articles which contain MTBE in a concentration above 0.1 % (w/w) have to provide sufficient information to allow safe use of the article to their customers within the supply chain. Additionally a consumer can request such information from the supplier as well and it has to be provided within 45 days after the request.
- *Requirement to notify ECHA under REACH (Art. 7(2)):* Producers or importers of articles containing MTBE in a concentration above 0.1 % (w/w) have (under certain conditions) to notify ECHA that their article contains a substance on the Candidate List. This obligation applies if the substance is present in those articles in quantities totalling over one tonne per producer or importer per year.

In addition, this information would be published in the open SCIP database (database for information on Substances of Concern In Articles as such or in complex Products)[5] established under the Waste Framework Directive (2008/98/EC)[6] to make the information available to waste operators and consumers.
It is noticed that most of the information requirements for SVHC substances apply to articles. As shown in Table 5, only a limited number of uses of MTBE covers article service life. Further data may be collected through these obligations.

For suppliers of MTBE as such, as residues in other substances above 0.1 % or in mixtures, the following requirements under REACH will apply also for the PMT properties (in addition to the classification for other hazards), if MTBE is identified as SVHC (Art. 31(1) and (3)):
Suppliers of MTBE as such and in mixtures have to provide their customers with a safety data sheet, which should be updated to reflect the identification of the substance as an SVHC. Suppliers of mixtures containing MTBE with a concentration in the mixture above 0.1 % (w/w) have to provide the recipients, at their request, with a safety data sheet even if the mixture would not need to be classified as hazardous.

Finally, Candidate listing is a further step toward authorisation through inclusion in Annex XIV. The prioritisation for inclusion in Annex XIV from the candidate list is driven by several criteria that are set by Article 58 of REACH and implemented by ECHA following a methodology that has been agreed by the Member States Committee (MSC[7]). The criteria includes considerations related to inherent properties, volume and wide dispersive use.

The authorisation provisions do not apply to uses of a substance as fuel according to Art 56(4) of REACH while fuel is by far the biggest use of MTBE so that priorisation to Annex XIV is unlikely for MTBE and will not be a fully effective measure to address the risks.

[5] Link to SCIP database on ECHA homepage: https://echa.europa.eu/de/scip-database

[6] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32008L0098

[7] https://echa.europa.eu/documents/10162/17232/recom_gen_approach_svhc_prior_2020_en.pdf/fbbd748b-22dc-38c2-9b4c-58c6bc80c930

6.2 Restriction

As described in section 5, the assessment of risks of MTBE for the environment requires to consider further regulatory action. Industrial and professional uses of MTBE that cause an unacceptable risk at the EU level can be restricted and included in Annex XVII of REACH. A restriction may apply to a substance, as such, or to one included in a mixture or an article. The restriction may also apply to substances in imported goods**.** In relation to the uses of MTBE, REACH does not exclude fuels from the scope of restriction.

Restriction under REACH may be designed in different ways in order to reach the highest possible risk reducing effect without having a disproportionate economic impact on the EU market.

Regarding the substance covered by the scope of this RMOA, **the aim of a restriction would be to limit the discharge of MTBE into the environment through its uses for which risks have been identified.** The vPvM properties of MTBE and its impact on drinking water resources, as acknowledged by an SVHC identification would also require consideration in the context of restriction.

An "unacceptable" risk has to be demonstrated. As shown in section 5 of this RMOA, after performing an environmental risks assessment through a modelisation using the lead registrant data, and comparing these results with monitoring data, it can be concluded that risks for the environment could not be excluded.

The scope of the restriction would need to be defined precisely to ensure the effectiveness, the enforceability and the monitorability of the restriction but also its consistency with other existing pieces of legislations which may cover the same or close field. This capacity to target specifically the uses associated with risks highly depends on the quality of the information provided in the registration dossiers and that will be provided in the different phases prior to the restriction submission.

6.3 Other risk management measures: Drinking water directive (EU 2020/2184) and Industrial Emissions Directive (2010/75/EU)

In relation to its capacity to contaminate water resources, MTBE might be addressed within the revised EU Drinking Water Directive (EU 2020/2184)[8]. To be of regulatory relevance, substance specific regulations need to be implemented on a national level in all Member States.
However, implementing substance specific regulatory measures into national level would likely be a time-consuming process. Further, the drinking water legislation would request all drinking water suppliers to control MTBE concentrations at the tap, but not limit the concentration of the substance in the resources of the drinking waters. This would mean both, that the resources are protected inadequately, and that drinking water facilities might have to find solutions of removing MTBE from drinking water without being the source of the MTBE emission. In this case the costs for remediation of MTBE from the raw water are transferred to the society whereas the polluters are not obliged to contribute to the remediation costs.
In addition, this RMM will not be effective to reduce the risks for environment.

[8] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32020L2184

Concerning the risks for environment, releases of MTBE, in particular due to industrial emissions, could be regulated by defining more stringent tolerable release concentrations. By applying such an approach, two important risk management principles would be followed: the upstream limitation of releases, the principle "the polluter pays". Defining stringent release concentrations of MTBE could be of added value to control environmental emission of MTBE and could considered to be a complementary risk management option. However, IED is a directive and implementation may differ across Europe. In conclusion, IED is not considered as the most efficient RMM at a European level.

6.4 Conclusion

The objective of this RMOA is to identify the RMOs with regard to the following conclusions on MTBE:

- the high persistency and the high mobility of MTBE could lead to a long term and costly to remediate contamination of drinkable water resources, leading to concern which could be considered as equivalent to the PBT/ vPvB substances.

- environmental risks cannot be excluded for the aquatic compartment in relation to industrial and professional uses.

- In addition, MTBE is considered as a suspected ED for human health.

Further regulatory risk management actions are therefore needed at the EU level.

As shown in the sections 6.1 and 6.2 , two risk management options were considered as the most appropriate to address the regulatory need concerning MTBE. While considering all the advantages, the drawbacks, the uses, the results of the exposure and risk assessment and the hazards, **ANSES considers that the most appropriate risk management option for MTBE are a combination of a REACH restriction dossier (Article 68.1), in order to cover the environmental risks that can't be ruled out, and an SVHC identification due to the capacity of MTBE to contaminate water resources.**

- An SVHC identification is required to acknowledge the properties of MTBE to contaminate water resources due to its very high persistence and mobility. As the main use of MTBE, i.e. fuel, is not in the scope of authorisation, it is not expected that the SVHC identification will result in the inclusion of MTBE in Annex XIV for authorisation and will not be sufficient to address risk of MTBE for the environment.

- To address risks for the environment, a restriction, according to article 68.1, is required. Its specific scope will need to be further defined.

The SVHC identification prior to restriction will enable to also consider in the restriction risks related to the properties of MTBE to contaminate water resources due to its high persistence and mobility.

DRAFT

7 Conclusions and actions

substance name EC number	Human Health Hazard	Environmental Hazard	Relevant use(s) & exposure potential	Last foreseen action	Action
MTBE (EC : **216-653-1**)	In this RMOA, only Human Health ED properties have been examined : **MTBE is considered by ANSES as a suspected Endocrine disruptor for human health.**	According to the available data and the literature, **MTBE is considered to be vP/vM and equivalent to the PBT/ vPvB** substances.	MTBE is reported to be mainly used in fuel, in coatings, detergents, in manufacture of substances and as a process agent/intermediate/extraction agent	Need for EU RRM Justification: MTBE is considered to fulfil SVHC criteria due to vPvM properties. Moreover, environmental risks cannot be excluded for the aquatic compartment in relation to industrial and professional uses	**First step:** SVHC identification **Next steps:** Restriction

DRAFT

Annex 1: Harmonised classifications and self-classifications reported by registrants

Data consulted on 03 January 2022

EC/ List No	CAS No	Substance name	Harmonised classification	Classification in registrations	Classification in C&L notifications
216-653-1	1634-04-4	MTBE; tert-butyl methyl ether; 2-methoxy-2-methylpropane	Flam Liq. 2 H 225 Skin Irrit. 2 H 315	Flam Liq. 2 H 225 Skin Irrit. 2 H 315	-

DRAFT

Annex 2: Overview of uses based on information available in registration dossiers

Data consulted on January 2022.

Main types of applications structured by product or article types	MTBE
Fuel Use	P,I,F,M,C
Use in coatings	P,I,M,C
Use in cleaning agents	P,I,C
Use as a process agent, extraction agent , intermediate use	I,M
Manufacture of substance	I,M
…Rubber production	I

F: formulation, I: industrial use, P: professional use, C: consumer use, A: article service life

DRAFT

Annex 3: Overview of completed or ongoing regulatory risk management activities

Data consulted on 03 January 2022

EC/List number	RMOA	Authorisation		Restriction	CLH	Other actions
		Candidate list	Annex XIV	Annex XVII	Annex VI (CLP)	
MTBE	On going RMOA	-	-	-	Substance with a harmonised classification & Labelling	- Substance Evaluation : included in the CoRAP (2014 by France)[9]

DRAFT

[9] Conclusion document published on 9 March 2022 https://echa.europa.eu/fr/information-on-chemicals/evaluation/community-rolling-action-plan/corap-table/-/dislist/details/0b0236e18068d70b

Annex 4: Human health data : description of sub acute, subchronic and chronic studies (not for publication)

8 Sub-acute, subchronic and chronic studies including examination of endocrine organs

8.1 Summary of effects as reported in EU-RAR

8.1.1 General effects as reported in EU-RAR

Among the subacute and subchronic studies analyzed in the EURAR European Commission, 2002), no specific effects on the male or the female reproductive organs were observed (see below the EURAR's conclusion on repeated dose toxicity (RDT) as well as Table 32). However, some additional information related to endocrine endpoints can be retrieved from some of the studies previously analyzed in EURAR, 2002 (please refer to Table 33).

"**A NOAEC of 800 ppm for inhalation** exposure is selected, based on the mild liver effects seen in the 13-week rat study by Lington et al. (1997) at 4,000 ppm and in the two-year study at 3,000 ppm (Bird et al., 1997). The absence of these effects at 400 and 500 ppm in the other 13-week (Greenough et al., 1980) and the 28-day study (Chun et al., 1993) on rat supports the selection of this NOAEL value. Moreover, the effects seen at 1,000 ppm are only slight changes in red blood cell parameters and lactate (Greenough et al., 1980). In the previous study, a NOAEL of 500 ppm was chosen due to reduced lung weight seen at 1,000 ppm. However, this finding was not seen except transiently at 14 days in the 90-day oral study. Apart from slightly increased severity and incidence of chronic progressive nephropathy (CPN), there were no significant signs of toxicity in microscopical analysis at 400 ppm in the 2-year carcinogenicity study. CPN is a common pathological event in ageing rats under prolonged exposure to a xenobiotic chemical. The use of the two-year study for other toxicological end-points is somewhat limited since haematological analysis and urinalysis was performed only for the control and high dose animals. In addition, and clinical chemistry was carried out only for corticosterone. In oral exposure, male rats exhibit hyaline droplet formation in the proximal convoluted tubules at 440 mg/kg (IITRI, 1992), an effect that is male-rat specific. In the 90-day Sprague-Dawley study by Robinson (1990), there is an absence of significant findings at 100 mg/kg. At 300 mg/kg only the female rats had a statistically significant increase in kidney weight. However, this was not accompanied by degenerative microscopical findings, which only appeared in the males of the 1,200 mg/kg group. In addition, clinical chemistry of the females showed no adverse signs that would support kidney toxicity at that level (Robinson et al. 1990). Williams al. (2000a) reported seemingly different results. They found increased relative kidney weight and protein droplet nephropathy with significantly increased severity and incidence in the same male rat species (Sprague-Dawley) already at 250 mg/kg. The effects seen in liver, namely weight increase, hypertrophy and slight morphological changes, are mostly seen at doses 500 mg/kg and higher. These effects may be adaptive responses, which is corroborated by the fact that there are few signs of remarkable liver toxicity even in the 2-year carcinogenicity studies (described in detail in the carcinogenicity section). **In any case, less weight is put on the study by Williams et al. (2000a), since it was conducted mainly to observe changes in endocrine homeostasis with limited weight put on statistical analysis of other toxicological end-points.** Based on the findings in the rat liver in the study by Robinson et al. (1990) in the sub-chronic 90-day study, a **NOAEL of 300 mg/kg** is chosen for **oral administration**.

Table 32: MTBE sub-acute and subchronic studies with adulthood exposure (quoted from the EU-RAR, 2002)

Duration / route	Animal	Doses	NOAEL/ LOAEL	Effects at LOAEL	Reference
14 days / oral	Sprague-Dawley Rat	357-1,428 mg/kg*	<357/357mg/kg*	Depressed Lung weight*	(Robinson M. et al., 1990)

28 days oral	Sprague-Dawley Rat	90-1,750 mg/kg*	90/440 mg/kg*	Increased kidney weights, hyaline droplet formation in kidney pct	(IITRI, 1992)
28 days oral	Sprague-Dawley Rat (male)	250-1,500 mg/kg	<250/250 mg/kg*	Kidney protein droplet nephropathy	(Williams et al., 2000)
90 days oral	Sprague-Dawley Rat	100-1,200 mg/kg*	300/900 mg/kg*	Increased liver weight, AST,, increased cholesterol	(Robinson M. et al., 1990)
90 days oral	Sprague-Dawley Rat	200-1,200 mg/kg*	<200/200 mg/kg *	+ Increased Liver weight, Signs of morphological changes to hepatocyte cell structures in electron microscopy	(Zhou and Ye, 1999)
28 days inhalation	Fisher-344 Rat	400-8,000 ppm	400/3,000 ppm	Proliferation of the kidney proximal tubuliepithelial cells	Chun et al. (1993)
28 days inhalation	CD-1 Mouse	400-8,000 ppm	400/3,000 ppm	Liver cell proliferation	Chun et al. (1993)
13 week inhalation	CD-rat	250-1,000 ppm	500/1,000 ppm	Depressed lung weight (females), increased haemoglobin, blood urea nitrogen and ldh (males)	Greenough et al. (1980)
13 weeks inhalation	Fisher-344- Rat	800-8,000 ppm	800/4,000 ppm	Abnormalities in kidney pct morphology, changes in hormone levels, Alterations in red blood cell parameters	(Dodd and Kintigh, 1989) (Lington et al., 1997)

* = Gavage administration applied
+ = LOEL
AST = aspartate amino transferase
LDH = Lactate dehydrogenase
pct = proximal convoluted tubule

8.1.2 ED effects from the studies reported in EU-RAR

Table 33: MTBE sub-acute and subchronic studies with adult exposure analyzed in the EU-RAR report and additional information related to endocrine endpoints

Duration / route	Animal	Doses	Reference	Additional information related to endocrine endpoints	Original data available
14 days / oral	Sprague-Dawley Rat	357-1,428 mg/kg*	(Robinson M. et al., 1990)	No effect on adrenal weights (absolute and relative). Statistically significant increase of relative testes weight at 714 and 1071 mg/kg bw/day. Statistically significant increase of cholesterol levels in most females from 714 mg/kg* and in male rats at 1428 mg/kg. No examination of the thyroid gland.	Yes

DRAFT

28 days oral	Sprague-Dawley Rat	90-1,750 mg/kg*	(IITRI, 1992)		No
28 days oral	Sprague-Dawley Rat (male)	250-1,500 mg/kg	(Williams et al., 2000)	See complementary information in the section below.	Yes
90 days oral	Sprague-Dawley Rat	100-1,200 mg/kg*	(Robinson M. et al., 1990)	Increased adrenal relative weight in females without a clear pattern. Significant increase of cholesterol levels in most females and in male rats without a clear dose-response relationship. No effect on the (absolute and relative) weights of testes, ovaries and adrenals. No examination of the thyroid gland.	Yes
90 days oral	Sprague-Dawley Rat	200-1,200 mg/kg*	(Zhou and Ye, 1999)	Only males were exposed to MTBE. The following organs were weighed and examined histologically : liver, kidneys, testes, and lungs. No effect on testes weights (absolute and relative) without apparent pathological changes. Cholesterol was not measured.	Yes
28 days inhalation	Fisher-344 Rat	400-8,000 ppm	Chun et al. (1993)		No
28 days inhalation	CD-1 Mouse	400-8,000 ppm	Chun et al. (1993)	The only change in clinical chemistry attributable to MTBE was an increase in total T4 and TSH in high dose males. The biological significance of this alteration is, however, unknown, because no parameter in clinical pathology indicated an effect. The female high-dose mice had a dose-related decrease in their total T4 at day 5, which was also seen in the mid-dose group at day 31. However, this effect was only detected in the proliferation groups (5 mice/group) and not in the main groups (10 mice/group).	No
13 week inhalation	CD-rat	250-1,000 ppm	Greenough et al. (1980)		No
13 weeks inhalation	Fisher-344- Rat	800-8,000 ppm	Dodd et al. (1989) Lington, Dodd et al. 1997	Elevated levels of corticosterone in serum was observed in both sexes from 4000 statistically significant at 8000 ppm MTBE (Lington, Dodd et al. 1997). A higher plasma level of aldosterone was also detected in both sexes. There was also a statistically significant and concentration-related increase in the weight of adrenals but without associated histopathological lesions. Cholesterol level was not assessed. The thyroid gland examination was limited to histological analysis.	Not available for Dodd et al., 1989

* = Gavage administration applied

DRAFT

In addition, studies published after the release of the EU-RAR, 2002 were integrated in this review. In order to get a comprehensive picture of all data set, some studies including the assessment of endocrine organs and previously reviewed in the EU-RAR, 2002 were included in the sections below. These studies covered short periods of exposure (eg. 3 days of treatment) up to chronic exposure (eg. 2 months) *via* the oral (eg. gavage or drinking water) or the inhalation route. These studies are described below.

8.2 Presentation of the studies performed after EU-RAR

8.2.1 New repeated-toxicity studies (or analysis) after RAR and major RAR studies described

***In male rodents*:**

- ***By gavage:***

Non-guideline 30 day study on adult male Sprague-Dawley rat, Oral (by gavage):

Adult Sprague-Dawley male rats were treated for 30 days with 400, 800 and 1600 mg/kg bw/day MTBE by gavage (Khalili et al., 2015). At the end of the treatment, serum levels of sex hormones (LH, FSH, testosterone), and of antioxidant enzymes were measured in anesthetized male rats with ether. Then males were mated with unexposed female rats (for results on F1 please also refer to Annex 4). Male fertility index was unchanged in groups 400 and 800 with respectively 5/5 and 4/4 fertile males. Only 3/5 males were fertile in group 1600 ($p = 0.058$). The number of pups per litter and sex ratio were unaffected by treatments. Serum FSH levels were unchanged by the treatments but significant decreasing trends for LH and testosterone were observed in experimental groups. No changes were observed for SOD. Glutathione peroxidase (GPX) was reduced significantly by 4.5 fold in the 400 group. Its reduction in 800 and 1600 groups was not statistically significant although it was equal to 2.5 fold (see table below). This study showed that MTBE exposure could exert dose-dependent changes in serum testosterone and LH in treatment groups. Testosterone decrease with MTBE was statistically significant at 1600 mg/kg bw/day.

Table 34: Comparison of serum luteinizing hormone (LH, mIU/ml), follicle stimulating hormone (FSH, mIU/ml), testosterone (ng/ml), superoxide dismutase (SOD, U/gHb) and glutathione peroxidase (GPX, U/gHb) in MTBE-treated adult Sprague-Dawley quoted from (Khalili et al., 2015)

	MTBE (mg/kg/day)							
	0		400		800		1600	
Parameter	**Mean (SD)a**	**Median**	**Mean (SD)**	**Median**	**Mean (SD)**	**Median**	**Mean (SD)**	**Median**
FSH	4.90 (3.3)	4.10	4.50 (4.4)	2.70	2.98 (3.0)	1.80	2.35 (2.3)	1.40
LH**	3.79 (2.7)	3.50	3.38 (2.9)	2.90	0.91 (0.6)*	0.62	1.36 (1.1)	0.97
Testosterone**	11.66 (4.2)	10.20	9.98 (2.7)	10.50	5.50 (2.9)	4.50	4.98 (3.3)*	4.10
SOD	5.82 (1.0)	5.55	6.19 (1.3)	6.22	5.88 (3.9)	7.21	6.35 (0.9)	6.22
GPX	185 (181.8)	117.77	40.32 (15.0)*	50.40	88.25 (37.3)	84.05	70.60 (32.3)	67.20

[a] Standard deviation

* Significant difference with control group ($P < 0.05$)

** Significant dose-response relationship ($P < 0.05$)

DRAFT

The same authors using the same rats also studied MTBE-induced histologic and histomorphometric testicular changes (Gholami et al., 2015). Absolute and relative testis weights, germinal epithelium height, Sertoli cell numbers were unchanged. Presence of vacuoles in the seminiferous epithelium were observed in all MTBE treatments groups. Statistical trend analysis showed that the seminiferous tubule diameter increased and interstitial cell, spermatogonia, spermatocytes and spermatids numbers decreased. The effect was more prominent in the more mature cell types i.e. spermatocytes and spermatids, and in higher dose groups.

Non-guideline 5 day study (3 days of treatment) on male CD-1 mice, Oral (by gavage)

Four to 6 month-old CD-1 male mice were gavaged on days 1, 3 and 5 with 0, 400, 1000 or 2000 mg/kg of MTBE (n=5/group) in canola oil (Billitti et al., 1999 [abstract only] (Billitti et al., 2005). Additionally, 3 mice were dosed subcutaneously with cadmium chloride (CdCl), a known testicular toxicant. Faecal analysis was used to determine testosterone levels in mice (5/group) before, during and after oral MTBE dose of 400, 1000 and 2000 mg/kg/day for 5 days. A point sample of serum testosterone was taken and testes were investigated using light microscopy. MTBE had little effect on testosterone levels and the other reproductive endpoints used in this study, even at high treatment levels. There was no difference in unstimulated or hCG stimulated fecal testosterone at any MTBE treatment level. Serum testosterone levels did not differ between the untreated control and MTBE dosed animals. Mean body and testes weights also were not different between MTBE dosed and control animals. There were no histological differences between control and 2000 mg/kg MTBE exposed animals in the percent of tubules with seminiferous epithelial vacuolization, marginated chromatin, multinucleated giant cells, and sloughing. A greater number of tubules showed gross disruption in the 2000 mg/kg group (6 ± 2.6%, mean ± SD). No disruption of tubules was observed ($p < 0.05$) in the control group. The authors considered the histopathological damage at the highest dose to be insufficient to warrant examination of the 400 and 1000 mg/kg MTBE groups. In contrast, the CdCl treated positive controls had decreased testis weight, fecal and serum testosterone, and increased histopathological damage compared to the both dosed and control animals ($p < 0.05$).

Non-guideline 7 day study (3 days of treatment) on adult male CD-1 mice, Oral (by gavage)

De Peyster et al. (2008) applied a protocol similar to Billiti et al. (1999). Male CD-1 mice (96 day old) were gavaged with 400–2000 mg/kg MTBE on days 1, 3, and 5, injected i.p. with hCG (2.5 IU/g) on day 6, and necropsied on day 7. No changes were observed in testis, epididymide, absolute and relative weights. No effect was seen in testis histology while Billiti et al. (1999) reported disruption of seminiferous tubules in MTBE-gavaged mice. No effect of MTBE-treatment was observed on hCG-stimulated serum testosterone levels but values showed a puzzling considerable variability within groups. More convincingly, the weight of seminal vesicles, an androgen-sensitive organ, was not affected by the treatments.

One month and 14 weeks study using ApoE-/- male mice and C57bl/6J male and female mice respectively, Oral (via gavage):

Tang et al. (2019) investigated the effect of MTBE on obesity and obesity related metabolic disorders by using both *in vitro* and *in vivo* studies. *In vitro* studies using the 3T3-L1 cell model showed that MTBE did not affect hyperplasia but adipogenesis at concentrations of 0.1 and 1 mM with an increase in the red O oil staining, increased FABP4 and PPARG mRNA levels as well as reduced glucose uptake

indicating decreased insulin sensitivity (decreased index of insulin sensitivity). *In vivo* studies using 9 week-old C57bl/6J male and female mice exposed for 14 weeks to MTBE at environmentally relevant concentrations in the range of 1 mmol/L (approximately 0.9 mg/kg bw/day) increased visceral white adipose tissue (WAT) weight resulting from adipocyte hypertrophy with induced adipocyte differentiation, reducing insulin sensitivity in males when fed a high-fat diet. These outcomes were not modified in males fed a standard diet suggesting that MTBE could aggravate the deleterious metabolic effects of a high-fat diet. In females, the situation is less clear. On the one hand, plasma TG and insulin levels are lower in females fed a standard diet, but the glucose tolerance and insulin sensitivity metabolic tests gave identical results between mice exposed to MTBE and unexposed mice, which suggests that MTBE is not deleterious to the metabolic profile. On the other hand, a significant hypertrophy of the visceral adipocytes is described in females fed a high-fat diet in response to MTBE exposure at doses of 0.1 and 100 mg/kg bw/day together with increased sensitivity to insulin. How rodent HOMA-IR data are transposable to humans may be discussed as well as the absence of liver pathological examination.The authors have also examined gut microbiota because obesity is accompanied by gut dysbiosis characterized by reduced diversity and changes in the composition of the gut microbiota. They observed changes, but the data cannot be taken into account to confirm the effects described on glucose and lipid metabolism. In conclusion, MTBE exposure affects adipogenesis and triggered reduced insulin sensitivity in males fed a high-fat diet. The plausibility that MTBE has the potential to induce insulin resistance is considered suspected. Further studies will be required to determine the specificities of the effects in females (Tang et al., 2019).

The effect of MTBE in the initiation and progression of atherosclerosis was studied by using both *in vitro* and *in vivo* studies (Ren et al., 2021). Indeed, atherosclerosis is considered as the major pathophysiological basis for many cardiovascular diseases. Apolipoprotein E is a multifunctional protein that is synthesized by the liver and several peripheral tissues and cell types, including macrophages. The protein is involved in the efficient hepatic uptake of lipoprotein particles, stimulation of cholesterol efflux from macrophage foam cells in the atherosclerotic lesion, and the regulation of immune and inflammatory responses. Apolipoprotein E deficiency in mice leads to the development of atherosclerosis, which does not spontaneously develop in mice. Macrophage-derived foam cell formation within the vascular wall is the initiating event of atherosclerosis and it is favored by disturbances between cholesterol uptake and efflux in macrophages. This outcome was regarded *in vitro* using THP-1 cells at doses not toxic for cells (the 100 nM dose was toxic). Ren et al. (2021) demonstrated that 10 nM MTBE decreased the expression of cholesterol efflux transporters ATP binding cassette transporter A1 (ABCA1) and G1 (ABCG1) in THP-1 macrophage. In addition, the same dose of 10 nM MTBE inhibited cholesterol efflux from THP-1 macrophage to HDL. A daily gavage of MTBE 0.1 or 1 mg/kg bw/day for a month using ApoE-/- male mice fed a high fat diet significantly increased oil red O staining and the lesion area; and promoted foam cell formation in the atherosclerotic plaques as assessed through quantification of Mac-2 cells in lesions by immunohistochemistry without affecting lipidemia or inflammation. The authors also suggested based on their former study on the dysregulation of gut microbiota in MTBE-treated mice (Tang et al., 2019) that the effects of MTBE could be driven by reduction of Akkermansia in particular (Ren et al., 2021).

Non-guideline 5, 14 or 28 day studies on adult male Sprague Dawley rats (no indication given about the age of the animals), Oral (by gavage) or by inhalation

Day et al. (1998) dosed Sprague-Dawley rats (no indication given about the age of the animals) *via* gavage at doses ranging from 40-800 mg/kg for 28 days (Day et al. 1998). The highest dose at day 28 produced a significantly reduced plasma testosterone level without change in plasma LH. Corticosterone level was increased in all MTBE levels at 14 days of treatment. Mean testicular mitochondrial and microsomal total P450 content and liver microsomal P450 were similar in all groups at 28 days. This study is reported as abstract.

Allgaier and de Peyster (1999) studied the impact of high MTBE gavage doses (800 mg/kg) or inhalation (8000 ppm corresponding to 28,880 mg/m^3) on circulating LH level in gonadectomized Sprague-Dawley rats with or without testosterone levels maintained by silastic implants containing testosterone propionate. No consistent or statistically significant differences in the circulating LH level was observed after 2-5 h or again 5 days after daily MTBE treatment. This study is reported as abstract (Allgaier and de Peyster, 1999)

Non-guideline 15 or 28 day study on adult male Sprague-Dawley rat, Oral (by gavage):

Williams et al. (1999 and 2000) conducted a study with male Sprague-Dawley rats (231–383 g) that was divided in three parts. In one part (A), rats were treated with 0, 250, 500, 1000 or 1500 mg MTBE/kg bw/day for 28 consecutive days. To compare hormonal changes at day 15 and 28, another experiment (B) was initiated on the same day. Rats were given 1500 mg MTBE/kg bw/day or vehicle for 15 days. Based on the findings of these two studies, an additional dose-response study (C) was conducted in which the rats (258-324 g) received 0, 250, 500 or 1000 mg MTBE/kg bw/day for 15 consecutive days. The previously used 1500 mg dose was not used because of adverse clinical effects observed in the two other experiments. Body weights were recorded every 3-4 days throughout the study. For a positive control, 10 male rats were injected with either vehicle (1,2-propane) or 10 mg/0.25 ml flutamide, which increases testosterone and LH secretion. After decapitation, serum was separated from the trunk blood and testicular interstitial fluid (TIF) was collected. Radioimmunoassay kits were used to determine 17β-estradiol, testosterone, dihydrotestosterone (DHT), triiodothyronine (T3) and thyroxine (T4) from serum. Testosterone was measured also from TIF. In experiment C, serum testosterone, LH, prolactin and TIF testosterone were measured. Liver, epididymis, testes, kidneys, dorsal lateral and ventral prostate, pituitary gland adrenal glands and seminal vesicles were weighed. Right testis and kidney, adrenal glands and liver were stained with H&E and lesions seen under microscopy were graded.

During the last two weeks, the highest dose group had a statistically significantly lower (7-12%) body weight gain compared to controls. After 15 days of dosing, relative kidney, adrenal, pituitary weight was increased in the highest dose group. Rats dosed for 28 days with 1000 and 1500 mg/kg showed statistically significant dose-related increase in relative liver weight. Similar increase with dose was seen in relative kidney weight at all doses. Relative testis but not absolute weight was increased only at the highest dose level. Absolute and relative weights of all other male reproductive organs were unchanged for the doses. No testicular lesions were observed at any dose level. After 15 days of treatment, the high dose rats had a lower serum and intratesticular testosterone levels (statistically significant) without LH changes. Rats of the same dose group treated for 28 days had a significant serum LH decrease whereas testosterone level in the serum and in the testicular interstitial fluid showed no change. The rats in the 1500 mg/kg dose group had a decreased DHT after 28 days. No changes in LH and testosterone levels were observed in all other groups. Serum FSH concentration was unchanged in any

DRAFT

groups. Serum prolactin level was decreased only in animals treated with 1500 mg MTBE/kg/day for 15 days. In addition, no change was observed in serum TSH or T4 but T3 was significantly decreased (19% of control) in rats dosed with 1000 and 1500 mg/kg bw/day for 28 days. Estradiol level was below the limit of detection in control and MTBE-treated rats. Centrilobular hypertrophy of the liver was characterized by a dose related severity, which varied from minimal to moderate. Kidneys lesions included enlarged eosinophilic bodies within epithelial cytoplasm, and individual cell necrosis, characteristic to protein droplet nephropathy.

Overall, mild changes were observed in male reproductive function only at high doses accompanied by some endocrine changes. No clear scheme on the mode of action of MTBE on the reproductive system can been drawn. However, since Leydig cells tumor is often attributed to an increase in LH levels which is never observed here, the present data, suggest that the effect of MTBE on Leydig cells tumor does not result from an action on the hypothalamus-pituitary axis. Adrenal gland, liver, and kidney weights were increased. Histologic changes included protein droplet nephropathy of the kidney and centrilobular hypertrophy of the liver. At 28 days, serum **T3** was significantly decreased at 1000 and 1500 mg MTBE/kg bw/day compared to control animals, These results indicate that MTBE causes mild perturbations in **T3 levels** (Williams et al., 1999; Williams et al., 2000)**.**

- ***Orally, through drinking water:***

Non-guideline 28 days study on adult male Balb/c mice, Oral (in drinking water) [abstract only]

Almeida et al. (2004) exposed male adult Balb/c mice to 80, 800 or 8000 ppb (= µg/L) MTBE in drinking water for 28 days. The authors reported in an abstract a significant dose dependent increase in mean combined testis weight, mean seminal vesicle weight, mean seminiferous tubule diameter, and incidence of abnormal seminiferous tubules with all the doses from 80 ppb (µg/L). Serum testosterone was substantially decreased by exposure to 800 and 8000 ppb in male Balb/c mice [(Almeida L et al., 2004); Abstract not available]. According to a personal communication between E. Hall and de Peyster et al. the age of the animals at the start of Almeida et al. study was 52 days meaning that these mice could still have been just reaching sexual maturity during treatment].

Non-guideline 28 days study on adult male Balb/c mice, Oral (in drinking water)

With the same exposure protocol as Almeida et al. (2004), de Peyster et al. (2008) exposed male adult *Balb/*c mice (127 day old) to 80, 800 or 8000 ppb (= µg/L) MTBE in drinking water for 28 days (de Peyster et al., 2008). Drinking water consumption was similar in all groups for this experiment and the mean MTBE consumed calculated were 0.011, 0.111 and 1,178 mg/kg bw/d for each group. No significant treatment-related differences were seen in group mean body weight or organ weights (testes, epididymides, seminal vesicles, liver, brain). No clear MTBE dose-related effect was seen in group mean serum testosterone, sperm per mg cauda, or testis histology. Testicular histology was similar in control and treated groups.

DRAFT

Non-guideline 13 week study on adult male Sprague-Dawley rat, Oral (via drinking water):

In the study by Saeedi et al. (2017a), male Sprague-Dawley rats (6/group) received MTBE (40, 200 or 1000 µg/L) in drinking water for 3 months (i.e., around 5, 25 and 125 µg/kg bw/day). This study interrogated if MTBE had any pancreatic toxicity effects impacting glucose homeostasis. Motivations were based on the effects of MTBE exposure on zinc deficiency, on the role of zinc in pancreas along with the findings of lower zinc plasma levels in type 2 diabetes (TD2) *versus* not TD2 individuals reported in literature (Jansen et al., 2012).Within this study, pancreas was collected for biochemical analyses (fasting glycaemia and measurement of the plasma levels of triglycerides (TG), cholesterol, HDL and LDL) and molecular examinations (RT-qPCR of genes that encode major proteins and enzymes related to zinc and glucose homeostasis) at the end of the 3-month treatment. The authors have also used TPEN as a zinc chelator and zinc supplementation for restoring the MTBE-induced effects. Consistently, the ratio of C_u^{2+} on Z_n^{2+} plasma levels significantly enhanced with the highest dose of MTBE and TPEN, an effect that was counteracted with zinc supplementation. The authors also reported that the highest dose of MTBE and TPEN enhanced fasting glycaemia, an effect not observed in the group having received zinc supplementation indicating that zinc deficiency could cause hyperglycaemia. They have demonstrated dyslipidemia with enhanced TG, cholesterol and LDL plasma levels in response to MTBE and that the effect was counteracted with zinc supplementation. However, the effects were not dose-dependent and treatment with TPEN had no effect on these outcomes. Besides, HDL plasma levels while reduced with MTBE exposure and TPEN were not restored following zinc supplementation. The marker of inflammation C-reactive protein enhanced dose-dependently with MTBE exposure, an effect counteracted by zinc supplementation but TPEN had no effect. Ca levels were also measured and were significantly elevated in the groups exposed to TPEN and the highest dose of MTBE but zinc supplementation did not restore Ca levels. Lastly, the authors analyzed gene expression in pancreatic tissues. RT-qPCR analysis of ins1 and ins2 genes (coding for Insulin) show decreased expression levels in TPEN and MTBE groups (not the lowest dose). Not intuitively, the expression was highly increased in the TPEN- and MTBE- exposed group for Ins1. In addition, Ins1 and Ins2 mRNA levels were further reduced in the MTBE and zinc-supplemented group. MT1A and Slc30a8 mRNA levels had a similar profile of expression among groups as the one described for Ins genes. These inconsistencies have not yet received any explanation by authors. The 2 last groups may have possibly been inverted. In conclusion, this paper is less conclusive than Saeedi et al. (2017b). The positive control TPEN is not really a positive control according to the parameters looked at and the addition of zinc does not systematically restore the parameters impacted by MTBE; suggesting that not all effects of MTBE are the consequence of zinc depletion but the point is not discussed further by the authors. The reviewer also has a problem with the way the authors have calculated the doses. Indeed, they indicate "MTBE concentrations were 20–40 µM, which is 1–25 times greater than the EPA's suggested consumer acceptability level". Already 20 to 40 does not make 1 to 25 times. In addition, based on a body weight of 400 gr at the end of the experimentation and a consumption of 50 ml of tap water, the levels of exposure are of 5, 25 and 125 µg/kg bw/day. These values are even lower than the one used in Tang et al. These inconsistencies have not yet received any explanation by authors. In the meantime, they are several reports that zinc deficiency is associated with a higher risk of non-alcoholic fatty liver disease (NAFLD) and an impaired lipid profile through mechanisms linked to oxidative stress and reduced lipid oxidation (Jansen et al., 2009). Interestingly, Yang et al. (2016) investigated the potential hepatic toxicity of MTBE in humans, and did not find significant evidence on an association between MTBE exposure and the prevalence of NAFLD in humans (Yang et al., 2016).

DRAFT

Non-guideline 13 weeks and one-year studies on adult male and female Wistar rat, Oral (via drinking water):

In Bermudez et al. (2012), male and female Wistar rats (6-8 week old; n= 10/15) were exposed *via* drinking water for 13 weeks to 0.5, 3, 7.5 and 15 mg/ml (corresponding to 37, 209, 514, and 972 mg/kg bw/day in males and to 50, 272, 650 and 1153 mg/kg bw/day in females) and to 0.5, 3 and 7.5 mg/ml (corresponding to 24, 130 and 300 mg/kg bw/day) in males or to 0.5, 3 and 15 mg/ml (corresponding to 44, 200, 900 mg/kg bw/day) in females for one year. Body weights of male rats, but not female rats, were significantly depressed approximately 10% at the end of the 13-week study in the 7.5 and 15 mg/ml MTBE exposure groups. Male terminal body weight was also reduced by around 10% in 0.5, 3 and 7.5 groups in the one-year study. A dose dependent decrease in water consumption was observed in both sexes for the two duration of exposure. At the lowest dose level, the animals reduced their intake of water by approximately 20% up to a reduction of 48% in the high dose females. Concomitantly, a decrease of the urine volume and an increase of specific gravity, osmolality and creatinine levels were observed. Finally, as the daily water consumption decreased as a function of the age in both sexes when it is refered to body weight, the daily absorption refered to body weight decreased from around 90 to 28, 350 to 200, 1250 to 370 and 2500 to 750 mg/kg bw/day in males throughout the first 13 weeks of exposure to 0.5, 3, 7.5 and 15 mg/ml in the drinking water respectively. It decreased also in female but values were around 25 % higher than in males. Mean values during this period were 37, 209, 514 and 972 mg/kg bw/day in males and 50, 272, 650, 1153 mg/kg bw/day in females for the 0.5, 3, 7.5 and 15 mg/ml groups respectively. Thereafter, it continued to decrease to reach around 24, 130 and 300 mg/kg bw/day in 0.5, 3 and 7.5 mg /ml male groups respectively and 44, 200, 900 mg/kg bw/day in 0.5, 3 and 15 mg/ml female groups respectively after one year treatment.

After 13 weeks and one year exposure, multiple organs (adrenal glands, brain, heart, liver, lungs, pituitary gland, spleen, thymus, thyroid or uterus) were collected and the wet weight was determined.

Following 13 weeks of exposure, relative ovary weights was significantly decreased at 0.5 mg/ml, but the authors considered that this had no biological significance. Thyroid dysplasia was observed in both male (a single animal in each of the groups receiving 0.5 and 3mg/ml) and female (a single animal in each of the control, 0.5, and 3 mg/ml dose groups and two animals in the 15mg/ml dose group) rats. According to (Shimoi et al., 2001), this thyroid dysplasia is sporadically observed in Wistar Hannover rats. Additionally, retinal atrophy with focal lesion with loss of some of the retinal layers to a very extensive lesion with total loss of retinal structures was observed in more female rats (16%) than males (4%). Since it was distributed among exposure groups, this effect was considered by the authors to be unrelated to treatment. No differences in absolute or relative organ wet weights (adrenal glands, brain, epididymis, heart, liver, lungs, pituitary gland, prostate gland, seminal vesicles, spleen, testes, thymus, thyroid or uterus) were observed between control rats and treated rats (data not shown). No histopathologic findings was observed in testis of any dose group (data not shown). Kidney wet weights were increased in males from 7.5 mg/ml and in females at 15 mg/ml. Kidney cell replication and α2u-globulin levels in males were increased at 1 and 4 weeks of MTBE exposure and tubular cell regeneration was increased in male kidneys exposed to MTBE concentrations from 7.5 mg/ml for 13 weeks. No hyaline droplets in the kidney tubular epithelium were evident in female rats.

DRAFT

Following one year of exposure to MTBE, relative but not absolute weight of the left testis (and not the right one) was increased in the males at 3 and 7.5 mg/ml without any histopathologic findings (data not shown). Although testis cell replication was analyzed at 1, 4 or 13 weeks, no information is reported by the authors. In addition, cardiovascular, respiratory, digestive, glandular, nervous, urogenital and skeletomuscular tissues were collected, weighed and fixed in a manner identical to that used for the core subgroup of the 13 week study. However, these data were not shown nor discussed by the authors. Kidney cell replication and α2u-globulin levels in males were increased at 1 and 4 weeks. Wet weights of male kidneys were increased following 6 months and one year of exposure to MTBE concentrations of 7.5 mg/ml or greater. CPN, of minimal to mild severity, increased in males, but not females, with one year of MTBE exposure. Lastly, tertiary-butyl alcohol blood levels, a metabolite of MTBE, was increased linearly with dose in males and females following one year of exposure. In summary, exposure of Wistar rats to MTBE resulted in minimal exposure-related effects including limited renal changes in male rats suggestive of α2u globulin nephropathy following 13 weeks of exposure and an exacerbation of CPN in males at the end of one year of exposure (Bermudez et al., 2012).

- ***By inhalation:***

Non-guideline one-year study on adult male Wistar rat, Inhalation (vapor):

In Sarhan et al. (2019) adult male Wistar rats (15/group) exposed to vapor of MTBE (60µl/L corresponding 245-296 µg/kg bw) [10], 3mn per day during 3, 6 or 12 months exhibited tracheal and lung histological anomalies of trachea and lung mostly after 6 months of exposure. Degenerated thyroid follicles were observed. No statistical analysis is shown for histopathology and no further indication is given about the examination of other organs such as the reproductive organs. Lastly the authors analyzed the blood sera at the end of treatment for the presence of carbonic anhydrase and peroxiredoxin. The blood serum samples were analyzed by SDS-Page and visualized by Coomassie blue staining. The composition of protein bands only present in treated samples compared to control was analyzed by mass spectrometry. Carbonic anhydrase I and II (*CA1* and *CA2*) were recovered among the most abundant proteins in MTBE-treated samples leading the authors to suggest that these enzymes may be linked to cancer potency in rat exposed to MTBE. This study does not provide any evidence concerning the expression of the carbonic anhydrase under MTBE exposure, as it lacks validation of the results obtained by LC-MS/MS analysis (Sarhan et al., 2019).

Non-guideline chronic studies on adult Fischer-344 rats and CD-1 mice, inhalation (vapor):

The studies of Bird et al. show that chronic inhalation exposure of mice to MTBE at doses of 8000 ppm (corresponding to 28,880 mg/m^3) for 18 months resulted in a decrease of both absolute body weight and body weight gain in both sexes with 49 and 33% mortality in males and females, respectively (caused probably by obstructive uropathy). In males exposed to the highest dose (not in females), absolute and relative adrenal weights were significantly increased and there was an increase in corticosterone values at 79 weeks compared to controls (212 ±160 vs 69 ±56 ng/ml, respectively). At the highest dose (8000 ppm), hepatocellular

[10] If we consider the density of MTBE to be 0.7405 (https://www.osha.gov/chemicaldata/291) and an exposure to MTBE in its pure state, the concentration in µg/L corresponds to 44.43 µg/day. The rats weighing 150 à 180 gr, this leads to 245 -296 µg/kg per exposure.

hypertrophy was increased in both sexes. Female mice showed this hepatocellular hypertrophy from the mid dose (3000 ppm corresponding 10,800 mg/m^3) as well as an increased incidence of hepatocellular adenomas accompanied by hepatocellular hyperplasia at 8000 ppm. As for mice, rats exposed for two years to MTBE 8000 ppm showed a decrease of both absolute body weight and body weight gain in both sexes) and decreased mean survival rate mainly due to the chronic progressive nephropathy (confirmed by histology). The authors also described that females at 400 ppm (lowest dose tested corresponding to 1,440 mg/m^3) and 3000 ppm group showed a decrease in the weight of adrenals but corticosterone values did not consistently change with MTBE exposure (increase in males from the 3000 ppm group (week 97) and decrease in the 8000 ppm male group (week 81); no change in females). The authors also noted an increase of absolute and relative liver and kidney weight from 3000 ppm (in females). Finally, male rats displayed renal tubular cell tumors and increased incidence and size of interstitial cell adenomas of the testes from 3000 ppm. The authors concluded that the NOEL for chronic toxicity is 400 ppm, and the NOEL for carcinogenic effects is 3000 ppm in mice (liver tumors in female) and 400 ppm in rats (kidney tumors in male). Regarding the values of the weight of adrenal glands and plasma corticosterone levels, no conclusion can be drawn about a possible endocrine effect of MTBE.

Non-guideline sub-chronic/chronic studies on adult female B6C3F1 mice, inhalation (vapor):

Moser al., 1998 investigated the endocrine effects of MTBE in female B6C3F1mice. Similar test conditions to the mouse carcinogenicity study by Bird et al. (1997) were used. Twelve female B6C3F1-mice were exposed to air or to a target concentration of 8,000 ppm (corresponding to 28,880 mg/m^3) MTBE for 4 or 8 months, 6 hours per day, five days a week. MTBE exposure significantly decreased body weight gain and ovary and pituitary weight at 4 and 8 months and uterine weight at all time points. After 8 months of exposure, MTBE significantly increased the length of the estrous cycle by increasing the mean number of days in both the estrus and the nonestrus stages. Histological evaluation of H&E-stained tissues after 4 or 8 months MTBE exposure showed a decrease in the number of uterine glands, a decreased number of convolutions and epithelial layers and less branched tubular glands in the cervix and vagina while ovaries are not affected. MTBE-exposed mice had an increased number of hyaline droplets in the pars intermedia of the pituitary. The authors also reported a decrease in the zona reticularis of the cortex of the adrenal glands. Such area described in humans produces precursor androgens including dehydroepiandrosterone (DHEA) and androstenedione from cholesterol. However, the existence of such a zone is controversial in mice (Dumontet and Martinez, 2021). In addition, histological differences between mice exposed to air only, or to MTBE are not obvious. The authors also claim that ovariectomized animals did not show alterations but no data are provided. An increased number of hyaline droplets in the pars intermedia of the pituitary was observed in MTBE exposed mice compared to air-exposed control mice (Moser et al., 1998). No significant differences in circulating estrogen levels were found after MTBE exposure (data not shown). Estrogen receptor (ER) immunoreactivity of MTBE-exposed uteri, cervices, and vaginas did not reveal difference in location or intensity compared to control (Moser et al., 1998). In addition, MTBE was shown to not activate human ER transiently transfected in HepG2 cells (luciferase assay), nor antagonize a maximally inducing dose of estradiol. Lastly, MTBE, TBA and formaldehyde did not inhibit the specific binding of estradiol to human ER. (Moser et al., 1998). The authors concluded that MTBE-induced endocrine alterations in female mice which did not occur through a mechanism mediated by ER.

Table 35: MTBE sub-acute, subchronic and chronic studies with adulthood exposure including the examination of endocrine organs (studies published after EU-RAR, 2002)

Duration / route	Animal	Doses	Effects	Reference
30 days Oral (gavage)	Adult male Sprague-Dawley rats (weighing 223 ± 20 g.)	0, 400, 800 and 1600 mg/kg bw/day	Dose-dependent ↘ of serum testosterone level stat. signif. at 1600 mg/kg bw/day Dose-dependent ↘ of LH , FSH No changes were observed for SOD . ↘ glutathione peroxidase (GPX) in all treated groups significant at 400 mg/kg/day (P = 0.016) and near to significant at 1600 mg/kg bw/day.	(Khalili et al., 2015).
			Presence of vacuoles in the seminiferous epithelium in all MTBE treatments groups Trend analysis showed: ↑ seminiferous tubule diameter; ↘ interstitial cell, spermatocyte and spermatid cell numbers in MTBE treated groups (P<0.05).	(Gholami et al., 2015)
3 months / drinking water	Male Sprague-Dawley rats	0; 40 , 200 or 1000 µg/L (MTBE) corresponding approximately to 5, 25 and 125 µg/kg bw/day); + 1 group with N, N, N', N'-Tetrakis (2-pyridylmethyl) ethylene diamine (TPEN) only + 1 group receiving 1000 µg/L (MTBE) + TPEN + 1 group receiving 1000 µg/L (MTBE) + 7.5 mg/L zinc acetate;	MTBE enhanced the Cu^{2+}/Zn^{2+} plasmatic ratio, the marker of inflammation C-reactive protein dose-dependently and fasting glycaemia, induces dyslipidemia with enhanced TG, cholesterol and LDL plasma levels. These effects were counteracted by Zn supplementation. Other effects such as the increased calcium levels observed at the MTBE highest tested dose and the decreased HDL were not counteracted by Zn supplementation. Lastly, ins1 and ins2 (coding for Insulin) gene expression were decreased with MTBE groups (except at the lowest dose). To be noticed that some of the parameters affected by MTBE were not modified with TPEN (eg. dyslipidemia and C-reactive protein).	(Saeedi et al., 2017)
13 week and one year oral (drinking	Wistar Rat (6-8 week old)	13-week study: 0.5, 3, 7.5 and 15mg/ml Average daily MTBE doses in	Increased relative weight of the left testis but not the right one from 3 mg/ml without	(Bermudez et al., 2012)

DRAFT

water)		treated: -males: 37 ± 10, 209 ± 61, 514 ± 142, and 972 ± 288 mg/kg bw/day. - females: 50 ± 12, 272 ± 51, 650 ± 142 and 1153 ± 191 mg/kg bw/day.	histopathologic findings (data not shown). Increased wet kidney weights in males from 7.5mg/ml and at 15mg/ml in females. In males, increased kidney cell replication and α2u-globulin levels at 1 and 4 weeks following MTBE exposure and tubular cell regeneration at 7.5mg/ml or greater for 13 weeks.	
		<u>One-year study</u>: -0.5, 3 and 7.5 (males) or 0.5, 3 and 15 mg/ml (females). Average daily MTBE doses in treated: -males 24, 130 and 300 mg/kg bw/day - females: 44, 200, 900 mg/kg bw/day.	In male rats, absolute weights of heart, liver and thyroid/ parathyroid were significantly less than control, but normalization to body weight indicated that this was due to a body weight effect. Increased relative weight of the left testis but not the right one from 3 mg/ml without histopathologic findings (data not shown). Increased wet weights of male kidneys at 6 months and one year of exposure to MTBE concentrations of 7.5 mg/ml or greater. No data shown on female reproductive organs, thyroid or adrenal gland). TBA blood levels were found to be linear with MTBE exposure concentration in both sexes.	(Bermudez et al., 2012)
3, 6 and 12 months Inhalation (vapor)	Adult male Wistar albino rats	60µl/day (corresponding to 245-296 µg/kg bw) during 3 minutes per day	-Increased tracheal and lung histopathological alterations mostly after 6 months of exposure. -Increased degenerated thyroid follicles at 12 months. -Carbonic anhydrase 1, carbonic anhydrase 2; and peroxiredoxin 2 detected in blood sera by mass spectrometry	(Sarhan et al., 2019)
14 weeks (*via* gavage)	C57bl/6J male and female mice (8 week-old)	0 (PBS*), 100 or 1000 µg/kg bw/day or 100 mg/kg bw/day (corresponding to 114 mmol/L).	**In males fed a high-fat diet :** - ↗ visceral WAT weight resulting from adipocyte hypertrophy with induced adipocyte differentiation and reduced insulin sensitivity. **In males fed a standard diet** : -no metabolic effects.	(Tang et al., 2019)

DRAFT

			In females fed a standard diet: - ↘ plasma TG and insulin levels without positive results on the glucose tolerance and insulin metabolic tests. **In females fed a high-fat diet:** -signif. hypertrophy of the visceral adipocytes at 0.1 and 100 mg/kg bw/day together with increased sensitivity to insulin (metabolic test). -Changes on the gut microbiota but the data cannot be taken into account to confirm the effects described on glucose and lipid metabolism.	
One month Oral (gavage)	ApoE-/- male mice	0.1 or 1 mg/kg bw/day	In males fed a high fat diet - signif. ↗ oil red O staining, - promoted foam cell formation in the atherosclerotic plaques, - without affecting lipidemia or inflammation. - the effects could be driven by reduction of Akkermansia in particular.	(Ren et al., 2021)
743 days Oral (drinking water)	Male and female Wistar rat (6-8 week old)	Males: 0.5 (0.04), 3 (0.29), 7.44 (0.45) mg/l in drinking water (SD in brackets). Females: 0.5 (0.04), 3 (0.29), 14.96 (0.79) mg/l in drinking water (SD in brackets) (analytical conc.) Males: 25 (11), 140 (63), 330 (139) mg/kg/day in drinking water (SD in brackets). Females: 49 (14), 232 (66), 1042(280) mg/kg/day in drinking water (SD in brackets). (actual ingested) Equivalent or similar to OECD Guideline 451 (Carcinogenicity Studies)	NOAEL (carcinogenicity): 330 mg/kg bw/day (actual dose received) (male) based on findings deemed significant. No biologically significant findings seen at maximum tested dose. NOAEL (carcinogenicity): 1042 mg/kg bw/day (actual dose received) (female) (No significant findings seen at maximum tested dose.) Neoplastic effects: yes (Only statistically significant finding in brain).	(Dodd et al., 2013)

*PBS = Phosphate buffered saline.

Other studies conducted with tert-butyl alcohol (TBA):

Lin et al. (2020) studied the toxicity of the tert-butyl alcohol (TBA) in wild-type (WT) and *Aldh2* knocked-out (KO) C56BL/6 male mice (**8 week old**). In this study, WT and KO mice (6/group) directly received TBA (5 and 20 mg/ml) in drinking water for **6 weeks**. At the end of treatment, animals were killed, several organs

(liver, kidney, testis, epididymis, spermaduct) were collected and weighted and submitted to histopathological examination (liver and testis). Blood and urine samples were collected for hepatic biochemical, and DNA damage and oxidative stress biomarkers. Direct treatment with 20 mg/ml TBA caused a decrease of the relative weight of epididymis in both WT and KO mice, whereas it causes an increase in liver weight only in KO mice. Exposure to 20 mg/ml TBA also resulted in histopathologic defects in liver and increased levels of genetic damages in WT mice. These defects were seen in both WT and KO mice but are enhanced in KO mice even when exposed to a lower TBA concentration. In the testis, significant degeneration in the spermatogenic process was only seen in KO mice exposed with 20 mg/ml TBA. This study showed the TBA-induced toxicity in the high-dose group related to oxidative stress and suggest that inactive *Aldh2* could be a risk factor for this TBA-induced toxicity (Lin et al., 2020).

New Carcinogenicity studies (or re-analysis) after RAR

Kissling et al. and Goodman et al**.** concern the statistical analysis of the experimental carcinogenicity study from Belpoggi et al, 1995; 1997; 1998. The experimental carcinogenicity study published by Belpoggi et al., consisted in administering MTBE by gavage at doses of 250 and 1000 mg/kg bw/day to females and males Sprague-Dawley rats (60/group). After daily administration (4 days/week) for two years (104 weeks), the animals were kept until spontaneous death and histopathological examination was realized on all organs and tissues (including thyroid and brain). The original study of Belpoggi et al. reported a significant increase in Leydig cell tumors (LCT) for the highest dose of MTBE and a dose-related increase in lymphomas and leukemias in females and concluded that MTBE must be considered carcinogenic. The statistical analysis of this study was refuted by Goodman et al, 2008 who proposed a statistical method including a poly-3 survival adjustment and not showed any significant association between the administration of MTBE and the appearance of LCTs. Based on the definitive data of the Belpoggi's study and arguing that the Poly-3 adjustment has not been validated for two-year studies, the reanalysis of Kissling et al, 2008 confirmed the significant incidence of LCTs in the animals treated with the highest dose of MTBE.

Another carcinogenicity study (Dodd et al., 2013) was published where MTBE was administered in drinking water at doses of 25, 140, 330 mg/kg bw/day in male and at doses of 49, 232, 1042 mg/kg bw/day in female Wistar rats (see also Table 35). After daily administration for two years (106 weeks) (Bermudez et al., 2012), the animals were euthanized and a complete necropsy was performed. At the end of two years of exposure, cardiovascular, respiratory, digestive, glandular, nervous, urogenital and skeletomuscular were analyzed. Brain was the only tissue with a statistically significant finding of neoplasms. One astrocytoma (1/50) was found in a female rat (1042 mg/kg bw/day). The incidence of brain astrocytomas in male rats was 1/50, 1/50, 1/50 and 4/50 for the 25, 140, 330 mg/kg bw/day exposure groups, respectively. This was a marginally significant statistical trend, but not statistically significant when pairwise comparisons were made or when multiple comparisons were taken into account. A significant increase in Chronic progressive nephropathy (CPN) was reported, observed in both male and female rats and with more severity in males, but was not considered relevant to human tumors. Beside CPN, thyroid dysplasia was observed in male and female rats in the 13 week-study in all groups of exposure together with a statistically significant reduction of the absolute thyroid weight at one year from the exposure with 0.5 mg/ml MTBE (corresponding to 29 and 54 mg/kg bw/day in male and female respectively).. Astrocytomas were observed after one-year exposure from 0.5 mg/ml MTBE (Dodd et al., 2013) together with increased relative brain weight (Bermudez, 2011) in

male rats and after two year of 15 mg/ml MTBE (corresponding to 1042 mg/kg bw/day) exposure in female rats (Dodd et al., 2013). Increased tumor incidence of other organs was reported in male rats exposed to 7.5 mg/ml MTBE (phaechromocytoma, hemangiosarcoma, pancreas adenoma, pituitary adenoma, hemangiosarcoma) and in female rats exposed to 15mg/ml MTBE (mammary gland adenocarcinoma and fibroadenoma, ovary stromal tumor, thymoma, uterus polyp) (Dodd et al., 2013) but statistical comparisons with the control group showed no significant differences.

Conclusion:
These new studies have shown that MTBE increases the incidence of brain tumors with no clues about its mode of action. Tumors were observed from oral dose ≥ 25mg/kg bw/day. Male Wistar rats are more likely to develop tumors at low doses than female rats. These new data confirmed the potential carcinogenicity of MTBE at low dose but bring no new element on its mode of action. Leydig cell carcinomas (LCT) observed in rats together with decreased testosterone levels in sub-chronic studies may be relevant to human LCT which represents an increasing part of small testicular tumors. In humans, increasing Leydig cell dysfunction has been shown to be associated with decreased levels of total testosterone and decreased total testosterone/LH ratio (Tarsitano et al., 2018). Overall, many borderline effects are shown which are not sufficient to build a CLP dossier based only on the data on MTBE.

8.2.2 Reproductive toxicity studies (fertility)

8.2.2.1 Parental exposure published after EU-RAR

In addition to the studies from Biles et al. (1987) and Bevan et al.(1997), Khalili, Gholami et al. (2015) studied the impact of a paternal MTBE exposure on the sex-ratio of the offspring born from untreated dams (Khalili et al., 2015). Adult Sprague-Dawley male rats treated for 30 days with 400, 800 and 1600 mg/kg bw/day MTBE *via* gavage were mated with healthy unexposed female rats. At birth, sex-ratio of the offspring was: 0.48, 0.50, 0.43 and 0.50 in 0, 400, 800 and 1600 mg/kg/day MTBE groups respectively (P = 0.91). MTBE exposure did not lead to significant effect on offspring sex ratio but has the potential to alter the fertility in male at 1600 mg/kg bw/day (see results in table below). Although this decrease did not reach statistically significance merely due to the limited sample size, this decrease of fertility is in accordance with the significant decrease in testosterone observed at the same dose level (1600 mg/kg/day MTBE).

Table 36: Reproductive outcomes in MTBE-treated adult Sprague-Dawley male rats (Khalili et al., 2015)

Parameters		MTBE (mg/kg bw/day)		
	0	400	800	1600
Number of males mated	5	5	4	5
Number of fertile males	5	5	4	3
Fertility index, males (%)a	100	100	100	60
Number of females mated	11	11	11	11
Number of pregnant females	9	9	9	6
Fertility index, females (%)b	81.8	81.8	81.8	54.5
Number of alive litters	9	9	9	6
Mean number of pups at birth c (SD)d	10.3 (2.4)	8.9 (2.1)	8.1 (1.5)	10.7 (2.1)
Number of female pups	47	39	42	27

DRAFT

Number of male pups	46	41	31	31
Mean of individual male sex ratio (SD)	0.48 (0.16)	0.50 (0.20)	0.43 (0.24)	0.50 (0.19)

Berger and Horner, 2003 assessed the fertilizability of the oocytes of young female rats (a Sprague-Dawley derived strain, 28–45 days) following exposure to MTBE and to 2M2P which is one metabolite of MTBE. Females were treated with 0.3% of MTBE or 2M2P in drinking water for the 2 weeks preceding oocyte recovery (6 mice/group). The female rats exposed to MTBE had a lower weight gain than the control without statistically significant decrease of the final weight. MTBE and 2M2P appeared to have no effect on the percentage of females ovulating nor on the number of oocytes per ovulating female (number of oocytes recovered per ovulating female (n=6): 30, 28 and 24 for control, MTBE and M2P2 respectively, not significant). There was no effect on oocyte fragility with MTBE although the oocytes from the females treated with 2M2P was more fragile (45% versus 57% remaining after removal of the zona pellucida for oocytes from 2M2P exposed females versus control females, SEM=6; P<0.10). No effect on fertilizability of oocytes was observed with MTBE nor with 2M2P. In this study, oocyte quality following *in vivo* exposure to MTBE is not impaired (Berger and Horner, 2003).

8.2.2.2 Exposure during juvenile period

An overview of the MTBE sub-acute and subchronic studies with juvenile exposure is presented in Table 37.

A non-guideline 51 days study on juvenile male mice, Oral

De Peyster et al. (2008) exposed male juvenile BALB/c mice for 51 days to 80, 800 or 8000 ppb (µg/L) MTBE in drinking water through PND 25-26 until PND 76-77. Daily water consumption was unaffected by the presence of MTBE and daily MTBE intake was 0.38, 3.90 39.17 µg/kg bw/day in 80, 800 and 8000 ppb groups respectively. These doses are very low. Absolute group mean body weights and reproductive organs (testes, epididymides and seminal vesicles) and other organ (brain, liver, kidneys, spleen, heart and lungs) weights were similar across the groups after 51 days of exposure. When expressed as relative organ/body weight ratio, the 80 ppb group relative seminal vesicle mean weight and the 800 ppb group relative lung mean weight were both increased over tap water control weights ($p < 0.05$). Serum testosterone level was unaffected by MTBE but it can be noted that values were more variable than expected. Serum estradiol levels were less variable and did not varied statistically across the groups. Evidence of oxidative stress was examined in liver homogenates from this juvenile study using MDA (malondialdehyde), Trolox Equivalent Antioxidant Capacity (TEAC) and 8-Hydroxy 2-deoxyguanosine 8OH2hG as endpoints. MTBE exposures at the levels examined indicated no significant changes in the male mouse reproductive tract and no signs of hepatic oxidative stress (De Peyster et al., 2008).

Equivalent to OECD TG 407 (repeated dose 28 day- oral toxicity study in rodent), Oral (by gavage)

Li et al. (2008) studied the effects of subacute exposure of MTBE on the reproductive systems of male Sprague-Dawley rats (38–40 day old) by administrating MTBE at dose levels of 0, 400, 800 and 1600 mg/kg bw/day by gavage. After 2 or 4 weeks of treatments, the rats were euthanized, and their serum, epididymis and testes collected. Histopathological examination revealed

DRAFT

alterations in the testes of MTBE treated groups. After 4 weeks of treatment, body weight was unaffected by MTBE treatment. Although liver and kidney were collected and weighed in this study, no specific data are reported. Absolute epididymis weight and sperm number in epididymis were unaffected by MTBE treatments after 4 weeks of treatment. However, significant adverse effects in their reproductive system were observed including: a significant dose-dependent increase in the percentage of abnormal sperm from 400 mg/kg onward, an irregular and disordered arrangement of the seminiferous epithelium. After two weeks of treatment, serum levels of testosterone were decreased in 800 and 1600 groups. Surprisingly, it was increased in 400 group and decreased in 800 groups after 4 weeks of treatment. Luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels were increased from 400 and 800 groups respectively after two weeks of treatment. No any changes in these gonadotropins levels were observed after 4 weeks of treatment. Lastly, decreased levels of mRNA expression of androgen binding protein (ABP) in testes was observed with 1600 mg/kg bw/d after 2 weeks and from 800 mg/kg bw/day after 4-week treatment.

In the oxidative stress study, serum maleic dialdehyde (MDA) content was increased in the 1600 group after 2 weeks of treatment and in the 400 group after 4 weeks of treatment. The total antioxidant ability was significantly higher in the 400, 800 and 1600 groups after 2 weeks of treatment and in the1600 group after 4 weeks of treatment.

ln the testis after 2 weeks of treatment level of mRNA for 8-oxoguanine DNA glycosidase (OGG1) was significantly decreased whereas level of mRNA for extra-cellular form of superoxide dismutase (SOD(EX)) was significantly increased in the 1600 group showing an increase in oxidative stress. After 4 weeks of treatment no changes were observed in these levels (Li et al., 2008).

A non-guideline 15 or 28 day study on male Sprague-dawley rat, Oral (by gavage):

The study of Dong-Mei et al. (2009) investigated the possible subchronic health effects of MTBE exposure (0, 400, 800 and 1600 mg/kg bw/day) by gavage on mortality, body weight, relative organ weight, hematology, and blood biochemistry indicators in male Sprague-Dawley rats. MTBE did not disrupt significantly the growth rate of rats. However, unexpected altered mortality rate (30%) was observed in the 400 group.
After two weeks of treatment, weights of heart and liver were increased in 1600 group and weight of thymus was decreased in 800 and 1600 groups. Testicular weight was decreased in the three treated groups but without any dose-response relationship. Weight of brain, spleen, lung, kidney, epididymis and prostate were unchanged. After four weeks of treatment, all affected organs except thymus and liver recovered a normal weight. The weights of liver and kidneys were increased in the 800 group. Those of thymus and prostate were decreased in the 1600 group. In the 2-week treatment, MTBE exerted toxicity on white blood cell count, including lymphocyte, granulocyte, and eosinophil. This finding was especially strong at 1600 mg/ kg bw/day. An increase of cholesterol was also observed at 1600 mg/kg bw/day. In the 4-week treatment, hemoglobin was significantly increased at high dose The authors concluded that MTBE could impair liver and kidney functions and have adverse effects on lipid metabolism and immune system (Dong-mei et al., 2009).

DRAFT

Table 37: MTBE sub-acute and subchronic studies with juvenile exposure published after the EURAR, 2002.

Duration / route	Animal	Doses	Effects	Reference
51 days through PND 25-26 until PND 76-77 Oral (drinking water)	Male juvenile BALB/c mice (22–23 day old)	80–8000 ppb (µg/L) corresponding to 0.38 to 39.17 µg/kg bw/day	↗ relative seminal vesicle mean weight relative organ/body weight ratio at 80 ppb and lung mean weight at 800 ppb. No statist. changes in testosterone and estradiol serum levels.	De Peyster et al., 2008
2 or 4 weeks Oral (gavage)	Male Sprague-Dawley rats (28–30 day old)	0, 400, 800 and 1600 mg/kg bw/day	No significant body weight changes. Testes histopathological alterations from 800 mg/kg bw/day at 4 weeks and at 1600 mg/kg bw/day after 2 weeks of treatment. Signif.↘ testosterone serum levels ↗ Luteinizing hormone (LH) and ↗ Follicle stimulating hormone (FSH) after 2 weeks oftreatment.	(Li et al., 2008)
15 or 28 days Oral (gavage)	Male Sprague-Dawley rats (28–30 day old)	0, 400, 800 and 1600 mg/kg bw/day	Transient signs of the central nervous system effects, including ataxia, hypoactivity, blepharospasm, and lack of startling reflex were observed during MTBE exposure , especially at 1600 mg/kg/d group. Unexpected altered mortality rate (30 %) observed at 400 mg/kg bw/day at 400 mg/kg bw/day. After 2 weeks of treatment: -↘ Testes and thymus relative organ weights from 400 and 800 mg/kg bw/day respectively (Statist. signif. for testes but without any dose-response relationship). -↗ Liver and heart relative organ weights at 1800 mg/kg bw/day. -↗ Cholesterol at 1600 mg/kg bw/day. <u>After 4 weeks of treatment</u>: -↘ Thymus relative organ weight maintained at 1600 mg/kg bw/day. -↗ Liver and kidney relative organ weights at 800 mg/kg bw/day. -↗ Cholesterol at the lowest tested dose level (400 mg/kg bw/day).	(Dong-mei et al., 2009)

DRAFT

8.2.3 Reproductive toxicity studies (development)

Since EU-RAR, 2002, there are new studies available:

Developmental toxicity study on Sprague Dawley rat, oral (gavage):

In the study by Kozlosky et al. (2013), MTBE in corn oil was given to four groups of five pregnant female rats (Sprague–Dawley), orally by gavage, once daily from day 6 of pregnancy through day 10 of gestation at 500, 1,000, 1,200 or 1,500 mg/kg bw/day. An additional group of five pregnant rats given corn oil alone served as a control. At birth, approximately one-half of the animals of each sex from each litter were sacrificed and necropsied, and any gross observations were recorded. Representative samples of brain, lung, liver, kidney, heart and stomach from these animals were collected and fixed in 10% neutral buffered formalin. Tissues were processed, sectioned, stained with hematoxylin and eosin, and subsequently examined by light microscopy for deviations in vascular development. The remaining animals from each litter were given MTBE in corn oil orally by gavage once daily from birth until post-partum day 10 at the same level as their respective dam. These animals were then sacrificed on post-partum day 11 and examined as described above. Statistical significance from control was determined by Dunnett's test and the results were expressed as means SEM. A value of $P<0.05$ was considered statistically significant (Kozlosky et al., 2013).

Narcotic effects, similar to those observed in the dams, were also observed in the pups receiving 1,200 or 1,500 mg/kg bw/day MTBE. Control pup body weight gain was not significantly different between any of the treatment groups. Control and treated rat pups were necropsied on post-partum day 11 and examined in a similar manner to that described above. No significant changes in organ weights were observed. In addition, no changes in vascular development were observed in any of the rats following either gross or histological examination at the light microscopy level. This new study does therefore not contradict the conclusion of EURAR on development toxicity: *"Although malformations are seen at 8000 ppm in CD-1 mice, they are considered to occur at a dose level of marked maternal toxicity. When there is significant maternal toxicity, the probability of the occurrence of non-specific developmental effects in the offspring increases. The sternebrae malformations seen in CD-1 mice at 250-2500 ppm are not considered treatment related. Therefore, based on the available data, MTBE is not considered to be toxic to foetal development."*

Developmental toxicity study on CD-1 mice, inhalation (vapor)

CD-1 mice were exposed to baseline gasoline vapor condensate (BGVC) alone or to vapors of gasoline blended with methyl tertiary butyl ether (G/MTBE). Inhalation exposures were 6 h/day on GD 5–17 at levels of 0, 2,000, 10,000, and 20,000 mg/m^3. Developmental effects were observed: two uncommon ventral wall closure defects occurred: gastroschisis (1 fetus at 10,000 mg/m^3) and ectopia cordis (1 fetus at 2,000 mg/m^3; 2 fetuses/1 litter at 10,000 mg/m^3). This effect was reproduced in a second study (G/MTBE-2) where instead an increased incidence of cleft palate was observed at 30,000 mg/m^3 G/MTBE. No ectopia cordis occurred in the replicate study, but a single observation of gastroschisis was observed at 30,000 mg/m^3.(Roberts et al., 2014a). Roberts et al. studied also the effects on Sprague-dawley rat of BGVC and condensed vapors from gasoline blended with G/MTBE, ethyl t-butyl ether (G/ETBE), t-amyl methyl ether (G/TAME) diisopropyl ether (G/DIPE), ethanol (G/EtOH), or t-butyl alcohol (G/TBA). However, since this study dealt with a rather complex mixture, those results were not judged

DRAFT

relevant for the evaluation of MTBE and therefore not included in this report (Roberts et al., 2014b).

9 Endocrine disrupting properties

9.1 Literature search

A literature search was conducted up to 23 March, 2021. This search was performed in PubMed without limitations of publication date and in Scopus from 2017-2021. A single concept strategy search was applied to retrieve all relevant information as recommended in the EDC guidance (ECHA & EFSA, 2018). Its scientific chemical or common names (i.e. Methyl tert butyl ether, MTBE, 2-methoxy-2-methylpropane, 2-Methyl-2-methoxypropane, 2-Methyl-2-methoxypropane, Propane, 2-methoxy-2-methyl, Ether, tert-butyl methyl, Methyl 1,1-dimethylethyl ether, Methyl t-butyl ether, Methyl tertiary-butyl ether) was searched for in PubMed and Scopus. Additionally, CASREGNUMBER (1634-04-4) was searched in Scopus. 2987 scientific papers were retrieved and downloaded in Endnotes. Then the following words were searched in:

- the abstract: disrupt, endocrine, kinetic, neurotoxic,
- the title: MTBE, toxicity, endocrine, disrupt, kinetic, toxicokinetic, metabolism, neurotoxic, carcinogenicity;
- the keywords: MTBE or Methyl Tert-Butyl Ether

Only papers linked with human health effects were kept. Supplementary references identified by other sources or related to the interpretation of data were included. A complementary literature search launched the 20th of September, 2021 did not retrieve additional relevant study.

9.2 In silico studies (level 1) and in vitro guideline or non guideline studies (level 2)

9.2.1 (Q)SAR data, ToxCast and EDSP 21 data

According to the Danish (Q)SAR database, VEGA, Endocrine disruptome, ToxCast and EDSP 21 screening results, no particular alert is raised.

9.2.2 Other in vitro data

The data below review the findings for HH and do not take into account the data for ENV for evaluating the ED potential of MTBE.

9.2.2.1 Androgen pathway

Overall, only one study investigated the ability of MTBE and TBA to bind to androgen receptor (AR).
CeeTox, Inc., 2013a investigated the ability of MTBE and its metabolite tert-butyl alcohol (TBA) to interact with AR isolated from rat prostate following U. S. EPA OPPTS 890.1150. Under the conditions of the assay, MTBE and TBA were classified as "non-binders" to AR in all three independent runs at concentrations from 10-10 to 10-3 M, and thus have a final classification of "non-binder" according to the test guideline (see also (de Peyster, 2014b)).

DRAFT

The data available indicate that MTBE and its metabolite TBA do not competitively bind AR in rat prostate homogenate.

9.2.2.2 Estrogenic pathway

A non-guideline study is available that investigated the potential for MTBE to bind with the estrogen receptor in a Yeast Estrogen Screen (YES) assay. Because the YES assay has not been validated at a national or international level, and without full details available on the test system and methods used, there is limited potential utility for the study. S Moser et al., (1998) investigated the activity of MTBE on estrogen receptor (ER) in *vitro*. Specific binding of estradiol to human ER *in vitro* was not inhibited by MTBE or its major metabolites (tertiary butyl alcohol (TBA) and formaldehyde) at concentrations from 10^{-11} to 10^{-4} M. MTBE did not activate human ER transiently transfected in HepG2 cells (luciferase assay), nor antagonize a maximally inducing dose of estradiol. Lastly, MTBE exposure did not alter the location or intensity of ER immunoreactivity in the uterus, cervix or vagina of MTBE-exposed mice (Moser et al., 1998).

9.2.2.3 Steroidogenesis

CeeTox, Inc., 2013b investigated the ability of MTBE and TBA to affect the steroidogenic pathway, specifically by inhibiting catalytic activity of aromatase, the enzyme responsible for the conversion of androgens to estrogens, using a human recombinant test system following U.S. EPA guideline OPPTS 890.1200. Under the conditions of the assay, the test substance and its primary metabolite were classified as non-inhibitors, with mean aromatase activities of 101.6 % (± 1.7 % SD) and 102.3 % (± 1.7 % SD), respectively, at the highest tested concentration at 10^{-3} M.

The ability of the test substance and its main metabolite to affect the steroidogenic pathway, affecting the production of testosterone or estradiol was also investigated in a H295R steroidogenesis assay following U. S. EPA guideline OPPTS 890.1550 (CeeTox, Inc., 2013c). The OECD 456 (H295R Steroidogenesis Assay) guideline was used to provide additional guidance in evaluation of the results. The test substance and its main metabolite at concentrations from 10^{-4} to 100 µM did not cause changes compared to the controls in the production of testosterone or estradiol in accordance with the US EPA guideline at the exception of statistically significant effects observed in only one run of the assay for each test substance which were not reproducible. Based on the OECD guidelines, both substances should be classified as negative for effects on testosterone or estradiol in this H295R steroidogenesis assay.

9.2.2.4 Other mode of actions described with MTBE

MTBE cytotoxicity and oxidative stress were studied in isolated rat spermatogenic and cultured rat Sertoli cells respectively (Li et al., 2009; Li et al., 2007). **MTBE at high doses significantly decreased the cell viability and increased plasma membrane damage and the ratio of necrotic cells at 5 or 50 mM in spermatogenic cells and at 0.5 or 50 mM in Sertoli cells**. The assessment of the MTBE-induced oxidative stress showed increased production of reactive oxygen species (ROS) and enhanced lipid peroxidation in exposed spermatogenic cells which include spermatogonia, spermatocytes and round spermatids without the elongated spermatids and spermatozoa. The enhancement of lipid peroxidation may explain the observed decrease of cytosolic superoxide dismutase (SOD) and the increase of extracellular superoxide dismutase SODEX, at the highest tested

DRAFT

dose (5 mM) of MTBE. Compared with the spermatogenic cells, the Sertoli cells were less susceptible to oxidative damages with only a transient increased ROS activity observed for all doses of MTBE and an increase in lipid peroxidation observed only with the highest dose of MTBE (5mM). **By affecting both spermatogenic cells and Sertoli cells by oxidative stress, MTBE could exert a direct toxic effect in the seminiferous epithelium which may lead to an impaired spermatogenesis**.

Xie et al. investigated MTBE-induced cytotoxicity and protein profile in Chinese hamster ovary cells *in vitro*. In this study, *in vitro* Chinese hamster ovary (CHO) cells were exposed to increased doses of MTBE, ranging from 0.5 to 100 nM. They reported that MTBE significantly decreased the cell viability from 5 nM by inducing oxidative stress in CHO cells (evaluated by lactate dehydrogenase (LDH) release and measurement of lipid peroxidation). The authors performed a proteomic analysis and reported an increase of proteins with catalytic activity when CHO cells were exposed to high doses of MTBE (50nM and over). No adverse effect involving an endocrine pathway was reported by the authors, nor investigated (Xie et al., 2017).
Najdegerami et al. compared the *in vitro* effect of MTBE on human hemoglobin of patients with and without *mellitus diabetes*. The authors reported that high doses of MTBE (100 to 500 µM) were able to increase the heme aggregation rate due to an increase of ROS production. The effects of MTBE were more severe with heme from patients with mellitus diabetes, including heme degradation. The authors explained this special effect by a previous increase of ROS in such patients. No adverse effect involving an endocrine pathway was investigated by the authors (Najdegerami et al., 2017).

Valipour et al. investigated the *in vitro* effects of MTBE on human insulin structure. Human insulin was exposed *in-vitro* to increasing concentrations of MTBE (from 8 to 40 µM). **MTBE was able to modify the tertiary structure and enhance aggregation of insulin. This effect was related to a formation of ROS.** The authors did not investigate the functionality of such modified insulin on its usual targets. It is not possible to deduct an endocrine adverse effect from this study (Valipour et al., 2015).

9.3 In vivo mechanistic data (OECD level 3/4)

9.3.1 Steroidogenesis

9.3.1.1 Testosterone

Several *in vivo* experimental studies showed testosterone levels changes in male rats. However this effect was not consistent across studies. A short summary of the results obtained are reported below.

- *Decreased serum testosterone concentration:*
  - (de Peyster, 2003): strong decrease at 1500 mg/kg bw/day, dose-related, by gavage at day 1 (within 4-5 hour) not retrieved at day14 or 28 (experiment 1 with 14 gavage treatments over 27 days and blood sampling at 16-20 hour after the final treatment). In experiment 2 conducted with daily MTBE treatment with 40, 400 and 800 mg/kg bw/day, no significant testosterone decrease (blood sampling on day 14 occurred before treatment that day and at 16-20 hour after the final treatment) was observed at day 14 or 28 except at 800 mg/kg bw/day where the decrease was statistically significant. In experiment 5, adult male Sprague Dawley

received a daily treatment of MTBE 1200 mg/kg bw/day via gavage for 14 days, a statistically significant decrease of serum testosterone levels was observed. Interestingly, the blood sampling occurred 1 hour after the last treatment.

- (Williams et al., 1999; Williams et al., 2000) : decrease of serum (52% of control value) and interstitial fluid testosterone after 15 days of treatment with MTBE at 1500 mg/kg bw/day in Sprague-Dawley rats. Dihydrotestosterone (DHT) was decreased after 28 days of treatment (Williams 2000).

- (Li et al., 2008) : decrease serum testosterone levels after 2 weeks ($p < 0.01$) of treatment with MTBE at 800 and 1600 mg/kg bw/day by gavage in Sprague-Dawley rat (38–40 day old at the start of treatment).

- (de Peyster, 2014b): decrease of testosterone level (Elisa immunoassay) after 14 days of treatment with MTBE at 800/1000, 1200/1500 mg/kg bw/day by gavage in adult Sprague-Dawley rat.

- (Day et al. 1998) [Abstract only]: decrease of plasma testosterone after 28 days with MTBE at 800 mg/kg by gavage in Sprague-Dawley rats (no indication given about the age of the animals).

- (Almeida L et al., 2004) [Abstract not available]. Serum testosterone was substantially decreased by exposure to 800 and 8000 ppb in drinking water in male Balb/c mice (presumably 52 day old at the start of treatment) after 28 days of treatment.

- (Khalili et al., 2015). Dose-dependent decrease of serum testosterone was observed from 800 mg/kg bw/day and statistically significant at 1600 mg/kg bw/day in adult Sprague-Dawley male rats after 30 days of treatment via gavage.

- *No modification on serum or testicular concentration:*

  - (Billitti et al., 1999 [abstract only] (Billitti et al., 2005) : little effect on serum testosterone concentration ($p<0.05$) at 400, 1000, 2000 mg/kg bw in CD- 1 male mice (4 to 6 month-old) by gavage on days 1, 3 and 5.

  - (de Peyster, 2003): no hormonal modification (eg. serum testosterone and LH) observed in castrated rats 4 hours and 5 day after MTBE gavage administration at 800 mg/kg bw/day in adult Sprague-Dawley rat (experiment 3 from de Peyster et al., 2003).

  - (de Peyster et al., 2008): no changes on serum testosterone level with MTBE at 400, 1000 and 2000 mg/kg bw/day by gavage on days 1, 3, and 5, and on day 6, with hCG (2.5 IU/g) IP on day 6 in adult male CD-1 mice or with 80, 800, 8000 ppb via drinking water in just weaned juvenile BALB/c mice for the 51-days.

  - (de Peyster, 2014b): no statistically change on serum testosterone concentration (with radio immunoassay) nor intra-testicular after 14 days of treatment with MTBE at 600 or 1200 mg/kg bw/day in adult male Sprague-Dawley.

- *Increase:*

DRAFT

- (Li et al., 2008) : Only one publication shows an increase of serum testosterone concentration after 4 weeks ($p < 0.05$) at 400 mg/kg bw/day in Sprague-Dawley (38–40 day old at the beginning of treatment) by gavage.

Where changes in testosterone levels have been seen, these are only in very high exposure levels and may be the result of effects other than P450 activity. The potential relevance of P450 activity to endocrine modulation is considered further in section 4 on specific investigations. Based on the other reproductive toxicity studies available, the observed changes in P450 activity and testosterone do not give rise to observable (adverse) effects.

A specific investigation on steroid hormone levels and measurements of both aromatase activity and aromatase mRNA in liver and testis microsomes was included as part of three 14-day in vivo experiments in male Sprague-Dawley rats with doses of MTBE ranging from 400 to 1500 mg/kg bw/day (de Peyster & Mihaich, 2013). Serum testosterone and estradiol did not dramatically change in the experiments although the general pattern was a decrease in testosterone and either an increase or no change in estradiol.

Across the experiments, there was a lack of definitive and consistent supporting statistically significant findings in steroid hormone measurements and aromatase activity and mRNA measured in liver and testis microsomes (de Peyster et al., 2013). Evidence of other underling systemic effects were also seen, including reduced body weight gain, increased adrenal weights, and elevated corticosterone suggestive of a more general stress response. When considered together with the results of the results of the three guideline in vitro assays, and a general literature review relating to MTBE and potential effects on the steroidogenic pathway, the authors conclude from these studies suggest that MTBE and TBA do not directly impact the steroidogenic pathway (de Peyster et al., 2013).

The investigation of the steroidogenic pathway included examining P450, as the cytochrome P450-dependent mixed function oxidases (CYP) and hydroxysteroid dehydrogenases have a key role in relevant enzymatic conversions. There were no statistically significant changes in either total P450 levels in liver and testis microsomes at two test exposure concentrations of 600 and 1200 mg/kg bw/day (de Peyster & Mihaich, 2013).

These results are consistent with an earlier study in male Sprague-Dawley rats, showing total liver P450 to only be increased at the highest dose group of 1500 mg/kg bw/day over 15 days but not at 250, 500 or 1000 mg/kg bw/day and also not after 28 day exposures (Williams & Burghoff, 2000). When measuring indicators of CYP activity, increases were evident at the higher two exposure concentrations, although statistically differences mostly limited to the highest dose of 1500 mg/kg/bw day (Williams & Burghoff, 2000).

A detailed review of the potential for MTBE to induce its own metabolism through increased P450 activity is available in the ECETOC risk assessment report on MTBE (ECETOC, 2003a). This report concludes that the level of P450 induction following prolonged exposure to MTBE at high test concentration is low and that the status of P450 in animals receiving such repeated high doses of MTBE is unlikely to be predictive of the cytochrome P450 status in animals or humans at lower exposures. Consequently, NOELs relating to total P450 or P450 activity have a limited relevance in human health risk assessment of MTBE. Overall, the reported effects on the endocrine system caused by exposure to MTBE only occur at high doses (typically above limit doses used in guideline studies) and the available evidence indicates that MTBE does not directly interact with the endocrine system, hence MTBE is a low

concern for endocrine effects at occupational and environmental levels. Recent studies have examined potential for effects on the endocrine system resulting from investigations of Leydig cell tumours observed in male rats, which have a very limited or no potential relevance to humans. Moreover, these tumours appear mostly at high systemically toxic concentrations and above metabolic saturation. In turn, high dose levels have been used in the investigative studies in order to elucidate a potential mode of action for previously reported effects.

Direct effect of high dosage MTBE on testosterone has been proposed but that fact that coherent effects on other apolar solvent was not reported.

9.3.1.2 Effects on LH

Contrarily to Williams (Williams et al., 1999; Williams et al., 2000). and Khalili (Khalili et al., 2015), both reporting decrease of LH after MTBE oral exposure, neither Allgaier and de Peyster (Allgaier and de Peyster 1999) [abstract only] nor Day et al., 1998 showed consistent or statistically significant disruption of LH following MTBE exposure.

9.3.2 Effects on oestrogens

The endocrine effects in female CD-1 mice have been investigated by Moser *et al*. (see also section 8 for a comprehensive description). Similar test conditions to the mouse carcinogenicity study by Bird et al. were used (Bird et al. 1997). Twelve female B6C3F1-mice were exposed to air or to a target concentration of 8000 ppm MTBE for 4 or 8 months, 6h/day, five days a week. Results showed several effects consistent with endocrine modulation. The microscopical findings included decreased uterine, ovary and pituitary weight, fewer uterine glands, decreased number of cervical and vaginal epithelial layers. Additionally, alterations in the stages and length of the oestrous cycle and increased ACTH-immunoreactivity of the pituitary and a loss of zona reticularis of the adrenal cortex were seen (Moser et al., 1998) [abstract].

In a previous study, Moser et al have demonstrated that a short term exposure to 1800 mg/kg MTBE by gavage causes a two-fold increase in liver oestrogen metabolism rate in mouse hepatocytes. This was mainly a contribution of increased activity of liver P450 enzymes (Moser et al. 1996). However, no decrease in circulating oestrogen levels was noted. Furthermore, MTBE did not inhibit oestrogen binding to its receptor or alter the receptor immunoreactivity. This evidence together with normal follicular maturation and the presence of oestrous cycle, although longer, suggests that MTBE was not directly toxic to ovary or pituitary in mice (Moser et al. 1998). To study the estrogenic effects, Okahara et al. administrated MTBE to immature CD-1 mice from PND21-25, by gavage at 600 or 1500 mg/kg. Dosing was given with and without a subcutaneous injection of 1 µg estradiol from PND23-25 (n=6-11). A slightly higher uterine/body weight was seen in the mice treated with both substances. Estrogen-treated mice. had an unusual translucent surface. One half of the mice that received 1500 mg/kg MTBE had a delayed vaginal opening on PND 26 (Okahara, 1999) [quoted in (de Peyster, 2014a)]. It should be noted that the sensitivity of the assay could have been higher if blotted weights have also been recorded and not only wet weights. De Peyster and Milhaich concluded on a lack of significant, estrogenic or anti-estrogenic effects in this 5-day mouse immature uterotrophic assay.

Cruzan et al. reviewed the available data on the carcinogenicity of MTBE using the EPA framework (Cruzan, 2007). In this publication, he reviews the changes in estrogen sensitive tissues include: (1) a decrease in the incidence of cystic

DRAFT

endometrial cell hyperplasia in the uterus of mice exposed to 3000 and 8000ppm MTBE for 18 months (Bird et al., 1997)and (2) there is less branching and fewer convolutions and epithelial layers in the tubular glands of the cervix and vagina of female mice exposed to 8000 ppm MTBE for 3 days to 8 months (Moser et al., 1998)
Since MTBE is not considered as direct genotoxicant, Cruzan et al. (2007) propose also different key events for supporting the postulated MoA for MTBE causing liver tumors in female mice is that MTBE: interference of MTBE with the ability of estrogen to suppress liver tumor promotion:
a. Changes in estrogen sensitive tissues include: (1) a decrease in the incidence of cystic endometrial cell hyperplasia in the uterus of mice exposed to 3000 and 8000ppm MTBE for 18 months (Bird et al., 1997); and (2) there is less branching and fewer convolutions and epithelial layers in the tubular glands of the cervix and vagina of female mice exposed to 8000ppm MTBE for 3 days to 8 months (Moser et al., 1998)
b. The conversion of [3H]-17β-estradiol to water soluble metabolites was increased 2.1 fold in hepatocytes from mice treated orally with 1800 mg/kg bw/day MTBE for 3 days, compared to control mice (Moser, 1996). Although MTBE increased the catabolism of estradiol in vitro, no decrease in serum estradiol levels occurred in mice exposed to 8000 ppm MTBE for 3 days to 8 months (Moser et al., 1998).
c. MTBE caused an increase in hepatic cell proliferation in female mice following 3 or 5 days of inhalation exposure to MTBE. However, following 21 or 28 days of inhalation exposure, cell replication rates in the mouse liver were similar to control ((Bird et al., 1997);(Moser, 1996)). At 3 and 21 days, there was no increase in serum aminotransferase activity and no changes in liver histopathology, indicating that MTBE is not hepatotoxic. These data support MTBE acting in the liver as a mitogenic chemical. In mice exposed to 8000 ppm MTBE, the amount of liver CYP450 increased, along with an increase in CYP2B activity (7-pentoxyresorufin-O-dealkylase (PROD)) and CYP 1A activity (7-ethoxyresorufin-O-deethylase (EROD)) (Moser, 1996). The induction of CYP2B has been associated with hepatic tumor promotion (Burke et al., 1985) and EROD is a marker enzyme of CYP 1A that metabolize endogenous estrogens (Aoyama et al., 1990). After 3 or 21 days of exposure to 8000 ppm MTBE for 6h/day, EROD activity was increased 2- to 3-fold and PROD activity 5- or 14-fold, respectively, compared to controls (Moser, 1996).

MTBE did not compete with estradiol for binding to the estrogen receptor, nor did it activate human estrogen receptors transiently transfected in HepG2 cells. Exposure to MTBE also did not alter the location or intensity of the estrogen receptor in the uterus, cervix or vagina of MTBE-exposed mice (Moser et al., 1998).

For consideration of other endocrine-related endpoints examined during repeated-dose exposures, the high exposure inhalation study at 8000 ppm by Moser et al. (1998) in female B6C3F1 mice has shown that MTBE can cause changes in estrogen sensitive tissues without affecting serum estrogen levels: MTBE exposure significantly decreased body weight gain and ovary and pituitary weight at 4 and 8 months and uterine weight at all time points; after 8 months of exposure, there was a significantly increased the length of the estrous cycle by increasing the mean number of days in both the estrus and the nonestrus stages; histological evaluation of H&E stained tissues showed a decrease in the number of uterine glands after subchronic MTBE exposure.

It has been proposed that the female reproductive system is more susceptible to stress than the male system and is particularly sensitive to the stress associated with decreased feed intake and decreased body weight gain, with the most sensitive reproductive parameter being disturbance of the estrous cycle, with stress generally leading to an extended duration of the estrous cycle (Everds et al., 2013). Moser et al. (1998) did not measure feed intake, but noted that decreases in body

weight gain were mild compared to that typically seen in food restriction studies. Moser et al. (1998) did not measure corticosterone levels, although the carcinogenicity study in mice did see increased corticosterone in female mice at 8000 ppm that were not statistically significant (Bird et al., 1997).

Because Moser et al. (1998) did not observe changes in levels of serum estrogen, this indicates that there is not an effect from hepatic catabolism of endogenous estrogen. Moser et al. (1998) suggest that the effects observed may through an anti-estrogenic mode of action. However, a weight of evidence analysis on endocrine interactions presented in section 5.10.3 on specific investigations concludes that there are no clear supportive evidence of MTBE having a direct effect on the estrogen pathway. Therefore the effects observed by Moser et al. (1998) may likely be a result of general or specific toxicity. Although it is not possible to derive a NOAEL from the Moser et al. (1998) because the study exposure was at such a high concentration, the effects on organs weights have also been observed in other studies (pituitary, uterine, ovary), NOAELs from other toxicological studies on MTBE can adequately cover the effects from general or specific toxicity.

In addition, if changes in liver induction of P450 enzymes are resulting in enhanced metabolism of steroid hormones, this type of induction is unlikely to be significant at low doses of MTBE as only minimal levels of enhanced activity are reported at high doses and impact at high doses is unlikely to be reflective of low dose. Furthermore, is this P450 activity is unlikely to impact homeostasis at even high MTBE exposure levels.

10 OECD level 1 and 2 summary tables

Table 38 : Danish QSAR data on MTBE (data extracted in May, 2021)

QSARs	Models	Battery	Case Ultra	Leadscope	SciQSAR
Danish QSAR (Battery, Case Ultra, Leadscope, SciQSAR)	**Estrogen Receptor α Binding, Full training set (Human *in vitro*)**	NEG_IN	NEG_IN	NEG_OUT	NEG_IN
	Estrogen Receptor α Binding, Balanced Training Set (Human *in vitro*)	INC_OUT	NEG_OUT	NEG_OUT	NEG_OUT
	Estrogen Receptor α Activation (Human *in vitro*)	INC_OUT	NEG_OUT	NEG_OUT	NEG_OUT
	Estrogen Receptor Activation, CERAPP data (*in vitro*)	N/A	N/A	NEG_IN	N/A
	Androgen Receptor Inhibition (Human *in vitro*)	NEG_IN	NEG_IN	NEG_IN	NEG_IN
	Androgen Receptor Binding, CoMPARA data (*in vitro*)	N/A	N/A	NEG_IN	N/A
	Androgen Receptor Inhibition, CoMPARA data (*in vitro*)	N/A	N/A	NEG_IN	N/A
	Androgen Receptor Activation, CoMPARA data (*in vitro*)	N/A	N/A	NEG_IN	N/A
	Thyroperoxidase (TPO) inhibition QSAR1 (Rat *in vitro*)	N/A	N/A	NEG_IN	N/A
	Thyroperoxidase (TPO) inhibition QSAR2 (Rat *in vitro*)	N/A	N/A	NEG_IN	N/A

DRAFT

QSARs	Models	Battery	Case Ultra	Leadscope	SciQSAR
	Thyroid Receptor α Binding (Human *in vitro*) - **mg/L** - **µM** - **Positive for IC_{50} ≤ 10 µM** - **Positive for IC_{50} ≤ 100 µM** - **Domain**		 14100.1 159955.8 OUT	 1036.817 11761.96 OUT	 25.88345 293.6296 OUT
	Thyroid Receptor β Binding (Human *in vitro*) - **mg/L** - **µM** - **Positive for IC_{50} ≤ 10 µM** - **Positive for IC_{50} ≤ 100 µM** - **Domain**		 2852.48 32359.38 OUT	 27.58432 312.9248 OUT	 242.7853 2754.229 OUT
	Arylhydrocarbon (AhR) Activation – Rational final model (Human *in vitro*)	N/A	N/A	INC_OUT	N/A
	Arylhydrocarbon (AhR) Activation – Random final model (Human *in vitro*)	N/A	N/A	INC_OUT	N/A
	Pregnane X Receptor (PXR) Binding (Human *in vitro*)	NEG_IN	NEG_IN	NEG_IN	NEG_IN
	Pregnane X Receptor (PXR) Binding (Human *in vitro*) NEW	N/A	N/A	NEG_IN	N/A
	Pregnane X Receptor (PXR) Activation (Human *in vitro*)	N/A	N/A	NEG_IN	N/A
	Pregnane X Receptor (PXR) Activation (Rat *in vitro*)	N/A	N/A	NEG_IN	N/A
	Constitutive Androstane Receptor (CAR) Activation at max. 20 µM (*in vitro*)	N/A	N/A	NEG_IN	N/A
	Constitutive Androstane Receptor (CAR) Activation at max. 50 µM (*in vitro*)	N/A	N/A	NEG_IN	N/A
	Constitutive Androstane Receptor (CAR) Inhibition at max. 20 µM (*in vitro*)	N/A	N/A	NEG_IN	N/A
	Constitutive Androstane Receptor (CAR) Inhibition at max. 50 µM (*in vitro*)	N/A	N/A	INC_OUT	N/A
	CYP3A4 Induction (Human *in vitro*)	N/A	N/A	NEG_IN	N/A

INC: Inconclusive. A definite call within the defined applicability domain could not be made
POS: Positive
NEG: Negative
OUT: Outside the applicability domain
IN: In the applicability domain
NA: Not applicable, because training set data cannot be released for commercial models

DRAFT

Table 39 : OECD QSAR Toolbox data on MTBE (data extracted in May, 2021)

Danish QSAR	**Estrogen Receptor Binding, alerts in:**	**- parent only**	Non binder, non cyclic structure
		- metabolites from *in vivo* Rat metabolism simulator only	Non binder, non cyclic structure
		- metabolites from Rat liver S9 metabolism simulator only	Non binder, non cyclic structure
	rtER Expert System - USEPA, alerts in:	**- parent only**	No alert found
		- metabolites from *in vivo* Rat metabolism simulator only	No alert found
		- metabolites from Rat liver S9 metabolism simulator only	No alert found

OECD QSAR Toolbox v.4.2 profilers

Profiler predictions are supporting information to be used together with the relevant QSAR predictions

Table 40 : Endocrine Disruptome data on MTBE (data extracted in May, 2021)

QSAR	Output	Conclusions
Endocrine Disruptome	AR: -4.2	Low probability
	ER α: -3.3	
	ER α an.: -3.2	
	ER β: -3.5	
	ER β an.: -3.2	
	GR: -3.5	
	GR an.: -3.6	
	LXR α: -3.7	
	LXR β: -3.8	
	MR: -3.9	
	PPAR α: -3.5	
	PPAR β: -3.4	
	PPAR γ: -3.4	
	PR: -1.8	
	RXR α: -3.6	
	TR α: -3.7	
	TR β: -3.7	
	AR an.: -3.8	Low-medium probability
High probability Medium-High Low-medium Low probability		

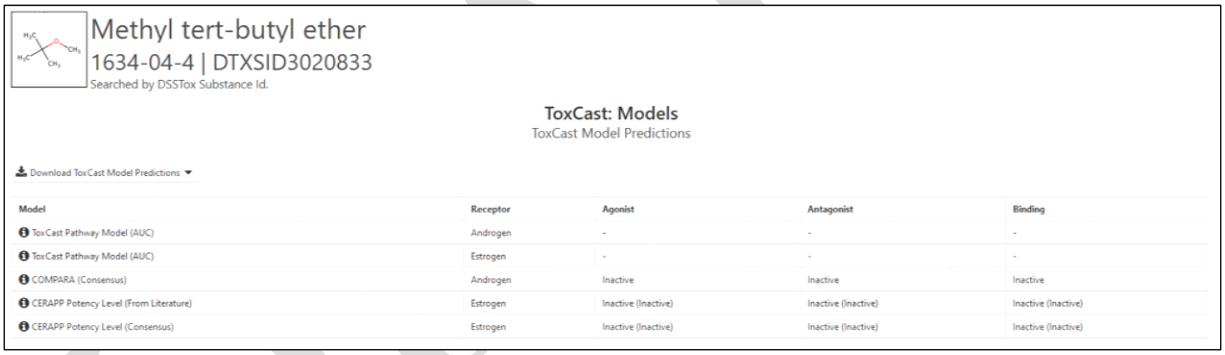
For the threshold calculations sensitivity (SE) was used to obtain corresponding docking scores. Class "red" corresponds to SE < 0.25 and indicates high probability of binding, two intermediate classes "orange" (0.25 < SE < 0.50) and "yellow" (0.50 < SE < 0.75) indicate medium probability of binding, and class "green" (SE > 0.75) corresponds to low probability of binding (Kolšek et al., 2014).

DRAFT

Table 41 : VEGA data on MTBE (data extracted from VEGA QSAR – VEGA HUB in May, 2021

QSAR	Output and conclusions
VEGA v.1.1.4	- Estrogen receptor relative binding affinity model (IRFMN) Prediction is inactive, but the result shows some critical aspects, which require to be checked: ○ Only moderately similar compounds with known experimental value in the training set have been found. - Estrogen receptor-mediated effect (IRFMN/CERAPP) Prediction is not predicted, but the result may be not reliable. A check of the information given in the following section should be done, paying particular attention to the following issues: ○ Accuracy of prediction for similar molecules found in the training set is not optimal ○ Similar molecules found in the training set have experimental values that disagree with the predicted value - Androgen Receptor-mediated effect (IRFMN/COMPARA) Prediction is NON-active. The result appears reliable. - Thyroid Receptor Alpha effect (NRMEA) Experimental value is inactive. Model prediction is inactive (good reliability) - Thyroid Receptor Beta effect (NRMEA) Experimental value is inactive. Model prediction is inactive (good reliability)

Table 42 : ToxCast Predictive Models (data extracted in June, 2021)


Methyl tert-butyl ether
1634-04-4 | DTXSID3020833
Searched by DSSTox Substance Id.

ToxCast: Models
ToxCast Model Predictions

Download ToxCast Model Predictions

Model	Receptor	Agonist	Antagonist	Binding
ToxCast Pathway Model (AUC)	Androgen	-	-	-
ToxCast Pathway Model (AUC)	Estrogen	-	-	-
COMPARA (Consensus)	Androgen	Inactive	Inactive	Inactive
CERAPP Potency Level (From Literature)	Estrogen	Inactive (Inactive)	Inactive (Inactive)	Inactive (Inactive)
CERAPP Potency Level (Consensus)	Estrogen	Inactive (Inactive)	Inactive (Inactive)	Inactive (Inactive)

https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID3020833#bioactivity-toxcast-models

The ToxCast Pathway Models for androgen and estrogen receptors have not been performed. Thus, no results are available.

Table 43 : Summary of EDSP21 assays related to MTBE results on EATS pathway (data extracted in May, 2021)

Intended target			Nb of positive results/nb assays
ER	Agonist activity	TOX21_ERa_BLA_Agonist_ratio TOX21_ERa_LUC_VM7_Agonist)	0/2
	Antagonist activity	TOX21_ERa_BLA_Antagonist_ratio assay TOX21_ERa_LUC_VM7_Antagonist_0.5nM_E2	0/2
	Viability	2 assays	
AR	Agonist activity	TOX21_AR_BLA_Agonist_ratio TOX21_AR_LUC_MDAKB2_Agonist	0/2

DRAFT

	Antagonist activity	TOX21_AR_BLA_Antagonist_ratio, TOX21_AR_LUC_MDAKB2_Antagonist_0.5nM_R1881 TOX21_AR_LUC_MDAKB2_Antagonist_10nM_R1881	0/3
	Viability	3 assays	
Thyroid (ThR, TPO, TR)	TR and TSHR agonist activity	1 TOX21_TR_LUC_GH3_Agonist and 1 TOX21_TSHR_Agonist_ratio	0/2
	TR and TSHR antagonist activity	1 TOX21_TR_LUC_GH3_Antagonist, 1 TOX21_TSHR_Antagonist_ratio	0/2
	Viability	1 assay	0/1
S	Aromatase inhibition	1 TOX21_Aromatase_Inhibition	0/1
	Viability	1 assay	

Annex 5: References

Achten, C. et al. 2002. Methyl tert-Butyl Ether (MTBE) in River and Wastewater in Germany. Environ. Sci. Technol. 2002, 36, 3652-3661

Ahmadian, M., et al., 2013. PPARγ signaling and metabolism: the good, the bad and the future. Nat Med. 19**,** 557-66.

Allard,1-S. et al,1996. The aerobic biodegradation of tert-butyl methyl ether and tert-butanol : an initiator study.Swedish environmental research institute.

Allgaier, B. S., de Peyster, A., Methyl t-Butyl Ether (MTBE) effects on plasma Luteinizing Hormone (LH) in gonadectomized male rats. Society of Toxicology Abstract 1254, 1999.

Almeida L, et al., 2004. The effects of methyl tertiary-butyl ether on mouse testis. . Toxicologist. 78(S-1)**,** 188.

Anderson M.A. 2000. Removal of MTBE and other organic contaminants from water by sorption to high silica zeolites. Environ.Sci. Tech. 34,4,735-727.

ANSES. 2014. Filières, usages et expositions liées à la présence de substances reprotoxiques et/ou perturbatrices endocriniennes dans les produits de consommation : le méthyl tert-butyl éther (MTBE). 50phttps://www.anses.fr/fr/system/files/VSR2019SA0215.pdf

ANSES. 2021. Note d'appui scientifique et technique de l'Agence nationale de sécurité sanitaire de l'alimentaton, de l'environnement et du travail relatif à la recommandation de valeurs biologiques pour la surveillance professionnelle concernant le méthyl-tert-butyl éther. 37p. https://www.anses.fr/fr/system/files/VSR2019SA0215.pdf

ANSES. 2022. Substance evaluation conclusion as required by REACH Article 48 and Evaluation report for tert-butyl methyl ether. 68p.

ANSES. 2021. Elaboration of a method to categorize substances of interest as regards to their potential endocrine disrupting activity: assessment and categorization of prioritized substances.

Aivalioti. M, et al., 2012. Removal of BTEX, MTBE and TAME from aqueous solutions by adsorption onto raw and thermally treated lignite. Journal of hazardous materials 207-208 '2012) 136-146Anderson. M.A. , 2000. Removal of MTBE and other organic contaminants from water by sorption to high silica zeolites. Environ. Sci. technol.2000,34,725-727

Baus, C. et al., 2005. MTBE in drinking water production – occurrence and efficiency of treatment technologies. Acta hydrochim. hydrobiol. 33 (2005) 2, 118-132

DRAFT

Belpoggi, F. S., M.; Filippini, F.; Maltoni, C., 1997. Results of long-term experimental studies on the carcinogenicity of methyl tert-butyl ether. Ann N Y Acad Sci. 837**,** 77-95.

Berger, T., Horner, C. M., 2003. In vivo exposure of female rats to toxicants may affect oocyte quality. Reprod Toxicol. 17**,** 273-81.

Bermudez, E., et al., 2012. Toxicity of methyl tertiary-butyl ether (MTBE) following exposure of Wistar Rats for 13 weeks or one year via drinking water. J Appl Toxicol. 32**,** 687-706.

Bernauer, U., et al., 1998. Biotransformation of 12C- and 2-13C-labeled methyl tert-butyl ether, ethyl tert-butyl ether, and tert-butyl alcohol in rats: identification of metabolites in urine by 13C nuclear magnetic resonance and gas chromatography/mass spectrometry. Chem Res Toxicol. 11**,** 651-8.

Bevan, C. N.-B., T. L.; Tyl, R. W.; Fisher, L. C.; Panson, R. D.; Kneiss, J. J.; Andrews, L. S., 1997a. Two-generation reproductive toxicity study of methyl tertiary-butyl ether (MTBE) in rats. J Appl Toxicol. 17 Suppl 1**,** S13-9.

Bevan, C. T., R. W.; Neeper-Bradley, T. L.; Fisher, L. C.; Panson, R. D.; Douglas, J. F.; Andrews, L. S., 1997b. Developmental toxicity evaluation of methyl tertiary-butyl ether (MTBE) by inhalation in mice and rabbits. J Appl Toxicol. 17 Suppl 1**,** S21-9.

Biles, R. W. S., R.E.; Holdsworth, C.E., 1987. Methyl tertiary butyl ether inhalation in rats: a single generation reproduction study. Toxicol Ind Health. 3**,** 519-534.

Billitti, J. E., et al., 1999. Acute testicular toxicity of MTBE and breakdown products in lab mice. SOT. 266.

Billitti, J. E., et al., 2005. Absence of acute testicular toxicity of methyl-tert butyl ether and breakdown products in mice. Bull Environ Contam Toxicol. 75**,** 228-35.

Bird, M. G., et al., 1997. Oncogenicity studies of inhaled methyl tertiary-butyl ether (MTBE) in CD-1 mice and F-344 rats. J Appl Toxicol. 17 Suppl 1**,** S45-55.

Borden,R.C., et al., 1997. Intrinsic biodegradation of MTBE and BTEX in a gasoline-contaminated aquifer. Water resources research. 33,(. 1105-1115.

Bradley,P.M. et al. 1999. Aerobic mineralization of MTBE and tert-butyl alcohol by steam-bed sediment microorganims. Environ.sci.technol. 1999, 33, 1877-1879.

Bradley,P.M. et al. 2001. Effect of redox conditions on MTBE biodegradation in surface water sediments. Environ. Sci. Technol. 35,4643-4647.

Brady, J. F., et al., 1990. Metabolism of methyl tertiary-butyl ether by rat hepatic microsomes. Arch Toxicol. 64**,** 157-60.

Bus, J. S., et al., 2022. Methyl-tert-butyl ether (MTBE): integration of rat and mouse carcinogenicity data with mode of action and human and rodent bioassay dosimetry and toxicokinetics indicates MTBE is not a plausible human carcinogen. J Toxicol Environ Health B Crit Rev. 1-27.

Burghoff ,B. et al., 2010. Solvent impregnated resins for MTBE removal from aqueous environments. Reactive & functional polymers 70 (2010) 41-47.

BUWAL .2004. NAQUA - Grundwasserqualität in der Schweiz 2002/2003. Bern:
Bundesamt für Umwelt, Wald und Landschaft

Chao, H-R., et al. 2020. Toxicity assessment of electrochemical advanced oxidation process-treated groundwater from a gas station with petrochemical contamination. Environ Monit Assess(2020) 192:473

Chun, J. S., et al., 1992. Methyl Tertiary Butyl Ether: Vapor Inhalation Oncogenicity Study in Fischer 344 Rats. Bushy Run Research Center.

DRAFT

Chun, J. S., Kintigh, W. J. 1993. Methyl Tertiary Butyl Ether: Twenty-Eight Day Vapor Inhalation Study in Rats and Mice. Export, Pennsylvania: Bushy Run Research Center, pp. pp. 387.

Coftier A, Broissard G., Colombano S., avec la collaboration de Caurant A., Amaalric L., et Jarzabek M. 2013. Additifs oxygénés et composés NSO dans les carburants : quelle prise en compte dans la gestion des sites pollués ? – Rapport BRGM/RP -63966-FR, Rapport final, 261p., 44 tab., 12 ann.

Concawe (2012).Gasoline ether oxygenate occurrence in Europe, and a review of their fate and transport characteristics in the environment".

Day, K. J., et al.1998. Methyl t-butyl ether (MTBE) effects on the male rat reproductive endocrine axis. pp. 74.

de Peyster, A., et al., 2003. Subchronic studies in Sprague-Dawley rats to investigate mechanisms of MTBE-induced Leydig cell cancer. Toxicological Sciences. 72**,** 31-42.

de Peyster, A., et al., 2008. Effect of oral methyl-t-butyl ether (MTBE) on the male mouse reproductive tract and oxidative stress in liver. Reprod Toxicol. 26**,** 246-53.

de Peyster, A. M., E., 2014. Hypothesis-driven weight of evidence analysis to determine potential endocrine activity of MTBE. Regul Toxicol Pharmacol. 69**,** 348-70.

Dodd, D., et al., 2013. Two-year drinking water carcinogenicity study of methyl tertiary-butyl ether (MTBE) in Wistar rats. J Appl Toxicol. 33**,** 593-606.

Dodd, D. E., Kintigh, W. J.1989. Methyl Tertiary Butyl Ether (MTBE): Repeated (13-week) Vapor Inhalation Study in Rats with Neurotoxicity Evaluation. Appendix 1A: Methyl Tert Butyl Ether 13-Day Probe Range-Finding in Fischer 344 Rats and CD-1 Mice. Appendix 5: Methyl Tertiary Butyl Ether Repeated Exposure Vapor Inhalation Study in Rats with Neurotoxicity Evaluation. Neurobehavioral Toxicology Report. pp. 165 (Neurobehavioral Toxicology Report). Export, Pennsylvania: Bushy Run Research Center, Union Carbide Corp.

Dong-mei, L., et al., 2009. Effects of subchronic methyl tert-butyl ether ether exposure on male Sprague-Dawley rats. Toxicol Ind Health. 25**,** 15-23.

Dumontet, T., Martinez, A., 2021. Adrenal androgens, adrenarche, and zona reticularis: A human affair? Mol Cell Endocrinol. 528**,** 111239.

ECETOC, 2003. Methyl tert-butyl ether (MTBE). Health risk characterisation. Technical Report 72. . pp. 126.

ECHA, ED-EG 21. 2021. https://echa.europa.eu/documents/10162/1459379/flashreport_edeg-21_en.pdf/e530deb9-5baf-7fd4-dc35-8a4cd7c73f33?t=1639059043393

European Commission. 2002. European Union Risk Assessment Report TERT-BUTYL METHYL ETHER. 1-292.

European Environment Agency. 2001. Late lessons from early warnings: the precautionary principle 1896 – 2000.

Fischer, A., et al., 2005. Biotic and abiotic transformations of methyl tertiary btyl ether (MTBE). Environ Sci & Pollut Res 12 (6) 381 – 386

Gayoso-Diz, P., et al., 2013. Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: effect of gender and age: EPIRCE cross-sectional study. BMC Endocr Disord. 13**,** 47.

Gholami, S., et al., 2015. Histologic and histomorphometric changes of testis following oral exposure to methyl tertiary-butyl ether in adult rat. Iran J Vet Res. 16**,** 288-92.

DRAFT

Gonzalez-Olmos, R., et al., 2013. Hydrophobic Fe-zeolites for removal of MTBE from water by combination of adsorption and oxidation. Environ. Sci. Technol. 2013, 47, 2353-2360

Goodman, J. E. G., D.et al., 2008. Effects of MTBE on the reported incidence of Leydig cell tumors in Sprague-Dawley rats: range of possible Poly-3 results. Regul Toxicol Pharmacol. 50**,** 273-84.

Hotamisligil, G. S., Bernlohr, D. A., 2015. Metabolic functions of FABPs--mechanisms and therapeutic implications. Nat Rev Endocrinol. 11**,** 592-605.

Hung, H-W. et al., 2006. Adsorption of MTBE from contaminated water by carbonaceous resins and mordenite zeolite. Journal of hazardous materials B135 (2006) 210-217.

Hsieh, L-L., et al., 2004. Degradation of MTBE in dilute aqueous solution by gamma radiolysis. Water Research 38(2004)3627-3633

IITRI, 1992. 28 day oral (gavage) toxicity study of methyl tert-butyl ether (MTBE) in rats. . Chicago, Illinois: IIT Research Laboratories.

INRS, 2002. Fiche toxicologique de l'oxyde de tert-butyle et de méthyle (MTBE).

Jamal, I.S. et al., 2006. Ex situ treatment of MTBE-containing groundwater by a UV peroxide system. Wiley Interscience.

Jansen, J., et al., 2009. Zinc and diabetes--clinical links and molecular mechanisms. J Nutr Biochem. 20**,** 399-417.

Ji, B J., et al., 2009. Adsorption of MTBE from aqueous solution by porous polymeric adsorbents. Journal of hazardous material 161.81-87.

Julien, B., et al., 2019. Estrogen withdrawal and replacement differentially target liver and adipose tissues in female mice fed a high-fat high-sucrose diet: impact of a chronic exposure to a low-dose pollutant mixture(☆). J Nutr Biochem. 72**,** 108211.

Khalili, L., et al., 2015. Evaluation of offspring sex ratio, sex hormones and antioxidant enzymes following exposure to methyl tertiary butyl ether in adult male Sprague-Dawley rats. Excli j. 14**,** 75-82.

Kissling, G. E. P., C. J.; Huff, J., 2008. MtBE and cancer in animals: statistical issues with poly-3 survival adjustments for lifetime studies. Regul Toxicol Pharmacol. 50**,** 428-9.

Kolšek, K., et al., 2014. Endocrine Disruptome—An Open Source Prediction Tool for Assessing Endocrine Disruption Potential through Nuclear Receptor Binding. Journal of Chemical Information and Modeling. 54, 1254-1267.

Kozlosky, J., et al., 2013. Methyl tert butyl ether is anti-angiogenic in both in vitro and in vivo mammalian model systems. J Appl Toxicol. 33**,** 820-7.

Laboratorio cantonale. 2008. Rapporto d'esercizio 2008 del Laboratorio cantonale. Bellinzona: Laboratorio cantonale

Levchuk, I. et al.,2014. Overview of technologies for removal of MTBE from water. Science of the Total Environment 476-477 (2014) 415-433

LFU .2003. Grundwasserüberwachungsprogramm. Ergebnisse der Beprobung 2002. Grundwasserschutz 23. Karlsruhe: Landesanstalt für Umweltschutz Baden-Württemberg

Liadi, M.A., et al., 2018. Treating MTBE-contaminated water using sewage sludge-derived activated carbon. Environmental science and pollution research (2018) 25 : 29397-29407.

Li, D., et al., 2009. Cytotoxicity and oxidative stress study in cultured rat Sertoli cells with methyl tert-butyl ether (MTBE) exposure. Reprod Toxicol. 27, 170-6.

DRAFT

Li, D., et al., 2007. Methyl tert-butyl ether (MTBE)-induced cytotoxicity and oxidative stress in isolated rat spermatogenic cells. J Appl Toxicol. 27, 10-7.

Li, D., et al., 2008. The effects of methyl tert-butyl ether (MTBE) on the male rat reproductive system. Food Chem Toxicol. 46**,** 2402-8.

Liang, S.H., et al. 2011. Application of persulfate-releasing barrier to remediate MTBE and benzene contaminated groundwater . Journal of Hazardous Materials 185 1162-1168

Lien, H-L., et al., 2007. Removal of MTBE with Nafion. JOurnal of hazardous materials 144, 194-199

Lin, L., et al., 2020. Aldehyde dehydrogenase 2 deficiency significantly exacerbates tert-butyl alcohol-induced toxicity in mice. Journal of Applied Toxicology. 40**,** 979-990.

Lington, A. W., et al., 1997. Evaluation of 13-week inhalation toxicity study on methyl t-butyl ether (MTBE) in Fischer 344 rats. J Appl Toxicol. 17 Suppl 1**,** S37-44.

Lin, T-F., et al.,2003. Effect of residual chlorine on the analysis of geosmin, 2-MIB and MTBE in drinking water using the SPME technique. Water Research 37 (2003) 21-26.Lington, A. W., et al., 1997. Evaluation of 13-week inhalation toxicity study on methyl t-butyl ether (MTBE) in Fischer 344 rats. J Appl Toxicol. 17 Suppl 1, S37-44.

Liu. S-J., et al., 2006. Laboratory column study for remediation of MTBE-contaminated groundwater using a biological two-layer permeable barrier. Water research 4° 3401-3408.

Martienssen,M., et al., 2006. Determination of naturally occuring MTBE biodegradation by analysing metabolites and biodegradation by-products. Journal of Contaminant Hydrology 87, 37–53.

Miller, M. J., et al., 1997. Pharmacokinetics and disposition of methyl t-butyl ether in Fischer-344 rats. J Appl Toxicol. 17 Suppl 1**,** S3-12.

Minetti, R.C.P., et al., 2017. In situ chemical oxidation of BTEX and MTBE by ferrate: pH dependence and stability. Journal of hazardous materials 324, 448-456.

Mormile.M.R. et al.(1994). anaerobic biodegradation of gasoline oxygenate :Extrapolation of information to multiple sites and redox conditions. Environ.Sci.Technol.28,1727-1732.

Moser, G. J., et al., 1998. Methyl tertiary butyl ether-induced endocrine alterations in mice are not mediated through the estrogen receptor. Toxicol Sci. 41**,** 77-87.

Najdegerami, I. H., et al., 2017. Antichaperone activity and heme degradation effect of methyl tert-butyl ether (MTBE) on normal and diabetic hemoglobins. Journal of Molecular Recognition. 30.

Naville, D., et al., 2019. Chronic exposure to a pollutant mixture at low doses led to tissue-specific metabolic alterations in male mice fed standard and high-fat high-sucrose diet. Chemosphere. 220**,** 1187-1199.

Okahara, N., Investigation of the Antiestrogenic Effects of Methyl Tertiary Butyl Ether Using Immature Female CD-1 Mice., Vol. Masters thesis. San Diego State, 1999.

Reichlin, S., 1998. Neuroendocrinology. In Williams Textbook of Endocrinology

Wilson J et al.Eds.. . W. B. Saunders Co., Philadelphia.

Ren, Q., et al., 2021. Methyl tertiary-butyl ether inhibits THP-1 macrophage cholesterol efflux in vitro and accelerates atherosclerosis in ApoE-deficient mice in vivo. J Environ Sci (China). 101**,** 236-247.

DRAFT

Roberts, L. G., et al., 2014. Health assessment of gasoline and fuel oxygenate vapors: developmental toxicity in mice. Regul Toxicol Pharmacol. 70**,** S58-68.
Roberts, L. G., et al., 2014b. Health assessment of gasoline and fuel oxygenate vapors: developmental toxicity in rats. Regul Toxicol Pharmacol. 70, S69-79.
Robinson M., et al., 1990. Fourteen- and Ninety-Day Oral Toxicity Studies of Methyl Tertiary-Butyl Ether in Sprague-Dawley Rats. 9**,** 525-540.
Saeedi, A., et al., 2017a. Disturbance of zinc and glucose homeostasis by methyl tert-butyl ether (MTBE); evidence for type 2 diabetes. Xenobiotica. 47**,** 547-552.
Saeedi, A., et al., 2017b. Exposure to methyl tert-butyl ether (MTBE) is associated with mitochondrial dysfunction in rat. Xenobiotica. 47**,** 423-430.
Saez, J. M., 1994. Leydig cells: endocrine, paracrine, and autocrine regulation. Endocr Rev. 15**,** 574-626.
Safarzadeh-Amari, A. 2001. O3/H2O2 treatment of methyl-tert—butyl ether (MTBE) in contaminated waters. Wat.Res. Vol.35, No15, pp. 3706-3714.
Sarhan, O. M., et al., 2019. Impact Effect of Methyl Tertiary-Butyl Ether "Twelve Months Vapor Inhalation Study in Rats". Biology (Basel). 9.
Schirmer, M., et al., 1999. Evaluation of biodegradation and dispersion as natural attenuation processes of MTBE and benzene at the borden field site. Phys.Chem.Earth (B), Vol.24,No.6, pp 557-560
Shimoi, A., et al., 2001. Vacuolar Change in the Thyroid Follicular Cells in BrlHan: WIST@Jcl (GALAS) Rats. Journal of Toxicologic Pathology. 14**,** 253-253.
Shih.T-C., et al.,(2003. Evaluation of granular activated carbon technology for the removal of MTBE from drinking water . Water research 37, 375-385
Siddiqui, M. et al. (2011). Laser-based photo-oxidative degradation of MTBE using zinc oxide (ZnO) catalyst. Journal of Environmental Science and Health, PArt A., 46:10, 1154-1159
Sulfita. J., Mormile.M.R. 1993. Anaerobic biodegradation of known and potential gasoline oxygenates in the terrestrial subsurface. Environ. Sci.Technol.27, 976-978
Sutherland,J . et al. 2004. Treatment of MTBE by air stripping, carbon adsorption, and advanced oxidation: technical and economic comparison for five groundwater. Water Research 35, 193-205
Tang, Y., et al., 2019. Effect of methyl tert-butyl ether on adipogenesis and glucose metabolism in vitro and in vivo. Journal of Environmental Sciences (China). 85**,** 208-219.
Tarsitano, M. G., et al., 2018. Quantification of the Leydig cell compartment in testicular biopsies and association with biochemical Leydig cell dysfunction in testicular cancer survivors. Andrology. 6**,** 748-755.
US EPA, CompTox Chemicals Dashboard Vol. 2021. USEPA.
US EPA. 1997. Drinking water Advisory: Consumer Acceptability advice and health effects analysis on methyl tertiary-butyl ether (MTBE).
Valipour, M., et al., 2015. Interaction of insulin with methyl tert-butyl ether promotes molten globule-like state and production of reactive oxygen species. International Journal of Biological Macromolecules. 80, 610-614.
Van Afferden, M. et al., 2011. Remediation of groundwater contaminated with MTBE and benzene: the potential of vertical-flow soil filter systems. Water research 45,5036-5074
Wen, Q., et al., 2019. The brominated flame retardant PBDE 99 promotes adipogenesis via regulating mitotic clonal expansion and PPARγ expression. Sci Total Environ. 670**,** 67-77.

DRAFT

Williams, T. M., et al., 1999. Alterations in endocrine activity in male sprague-dawley rats following oral administration of methyl t-butyl ether. SOT.

Williams, T. M., et al., 2000. Alterations in endocrine responses in male Sprague-Dawley rats following oral administration of methyl tert-butyl ether. Toxicol Sci. 54**,** 168-76.

Wu,T-N. 2011. Electrochemical removal of MTBE from water using the iridium dioxide coated electrode. Separation and Purification TEchnology 79, 216-220

Xie, X., et al., 2020. Exposure to HBCD promotes adipogenesis both in vitro and in vivo by interfering with Wnt6 expression. Sci Total Environ. 705**,** 135917.

Yang, J., et al., 2016. Relationship between Methyl Tertiary Butyl Ether Exposure and Non-Alcoholic Fatty Liver Disease: A Cross-Sectional Study among Petrol Station Attendants in Southern China. Int J Environ Res Public Health. 13.

Yeh,J.T et al.1995. The effect of hydrogen peroxide on the degradation of methyl and ethyl tert-butyl ether in soils.Water environment research. 67,5.828-834.

Yuan, H. 2006. ETBE as an additive in gasoline : advantages and disadvantages. MAster of science thesis, Environmental science programme. 40p.

Zadaka-Amir. D., et al. 2012. Removal of methyl tertiary-ether (MTBE) from water by polymer-zeolite composites. MIcroporous and mesoporous materials 151 (2012) 216-222.

Zhou, W., Ye, S.-H., 1999. Subchronic Oral Methyl Tertiary Butyl Ether (MTBE) Exposure in Male Sprague-Dawley Rats and Effects on Health of MTBE Exposed Workers. J. Occup. Health. 41**,** pp 33-38.

Zhu, Q., et al., 2022. Methyl tert-butyl ether inhibits pubertal development of Leydig cells in male rats by inducing mitophagy and apoptosis. Ecotoxicol Environ Saf. 232**,** 113282.

DRAFT