Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

Tetrafluoroethylene

EC Number: 204-126-9
CAS Number: 116-14-3

CLH-O-0000006727-64-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

5 December 2019

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

International Chemical Identification:

Tetrafluoroethylene

EC Number: **204-126-9**

CAS Number: **116-14-3**

Index Number: **-**

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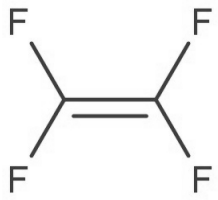
Version number: 2.0 **Date: November 2018**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Tetrafluoroethene.
Other names (usual name, trade name, abbreviation)	Tetrafluoroethylene, TFE.
ISO common name (if available and appropriate)	Not applicable.
EC number (if available and appropriate)	204-126-9
EC name (if available and appropriate)	Tetrafluoroethylene.
CAS number (if available)	116-14-3
Other identity code (if available)	Not applicable.
Molecular formula	C2F4
Structural formula	
SMILES notation (if available)	FC(=C(F)F)F
Molecular weight or molecular weight range	100.02.
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable.
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable.
Degree of purity (%) (if relevant for the entry in Annex VI)	Not applicable.

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Tetrafluoroethylene	Mono-constituent substance	None	Flam. Gas 1; H220 Press. Gas (Comp.); H280 Carc. 1B; H350 STOT SE 2; H371

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling

No impurities relevant for classification.

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling

No additives relevant for classification.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	Tetrafluoroethylene	204-126-9	116-14-3	Carc. 1B	H350	GHS08 Dgr	H350	-	-	-
Resulting Annex VI entry if agreed by RAC and COM	TBD	Tetrafluoroethylene	204-126-9	116-14-3	Carc. 1B	H350	GHS08 Dgr	H350	-	-	-

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier	No
Oxidising gases	Hazard class not assessed in this dossier	No
Gases under pressure	Hazard class not assessed in this dossier	No
Flammable liquids	Hazard class not assessed in this dossier	No
Flammable solids	Hazard class not assessed in this dossier	No
Self-reactive substances	Hazard class not assessed in this dossier	No
Pyrophoric liquids	Hazard class not assessed in this dossier	No
Pyrophoric solids	Hazard class not assessed in this dossier	No
Self-heating substances	Hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	No
Oxidising liquids	Hazard class not assessed in this dossier	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not assessed in this dossier	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity via oral route	Hazard class not assessed in this dossier	No
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	Hazard class not assessed in this dossier	No
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Hazard class not assessed in this dossier	No
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Hazard class not assessed in this dossier	No
Germ cell mutagenicity	Hazard class not assessed in this dossier	No
Carcinogenicity	Harmonised classification proposed	Yes
Reproductive toxicity	Hazard class not assessed in this dossier	No
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	Hazard class not assessed in this dossier	No
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Hazard class not assessed in this dossier	No
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

There is no harmonised classification and labelling for tetrafluoroethylene and it was not previously discussed by the Technical Committee for Classification and Labelling under Directive 67/548/EEC.

Tetrafluoroethylene has been assessed by several expert committees including the National Toxicology Programme (NTP) at the U.S. Department of Health and Human Services and the International Agency for Research on Cancer (IARC). The NTP concluded that under the conditions of the 2 year carcinogenicity studies there is "*clear evidence of carcinogenic activity"* of tetrafluoroethylene in F344/N rats and B6C3F1 mice but that there is inadequate evidence available in humans (NTP, 1997). IARC concluded in their monograph that there is sufficient evidence in experimental animals for the carcinogenicity of tetrafluoroethylene, concluding it is "*probably carcinogenic to humans" (Group 2A)* (IARC, 2016).

RAC general comment

There is no harmonised classification and labelling for tetrafluoroethylene (TFE) and it was not previously discussed by the Technical Committee for Classification and Labelling under Directive 67/548/EEC. TFE has been classified by several expert committees including the National Toxicology Programme (NTP, 1997) and the International Agency for Research on Cancer (IARC, 2016). However, RAC notes that more than two thirds of notifiers to the classification and labelling inventory do not self-classify TFE for carcinogenicity.

TFE is a halogenated olefin that occurs as a colourless, odourless gas at room temperature. It is practically insoluble in water. TFE is used primarily as a monomer in the industrial production of polymers. TFE is very flammable and at high pressures it may polymerize easily without a stabiliser, especially if heated or in the presence of oxygen (IARC, 1979; NTP, 1997). Because of its instability, *d*-limonene is added as a stabiliser and it requires tight control when handling. Registrants under the REACH Regulation report that it is transferred to on-site polymerisation units by direct pipeline at EU manufacturing sites.

RAC notes that impurities are not addressed in the CLH report. According to IARC (2016), industrial-grade TFE generally has a purity of > 99.7 % and TFE for making fluoropolymers usually contains only 1 to 10 ppm (w/w) as impurities (ECETOC, 2003). However, NTP (1997) reported that during 2-year studies, gas chromatography indicated peaks for perfluorocyclobutane (the most abundant dimer produced during TFE decomposition) and *d*-limonene with areas less than or equal to 1.21 % and 0.56 % (respectively) relative to the major peak (TFE). In addition, trifluoroethylene, methylene fluoride, vinyl fluoride, and vinylidene fluoride were present at ≤ 1.7 ppm. None of these chemicals has a harmonised classification but RAC notes that some are self-classified as carcinogens or considered as such by IARC and/or NTP. However, considering their low concentrations in TFE and that *d*-limonene and perfluorocyclobutane are less volatile than TFE, minimising these chemicals in the exposure chambers, RAC does not consider impurities relevant for classification.

The DS included repeated dose toxicity, toxicokinetics and mutagenicity data as supporting information for the assessment of carcinogenicity but the scope of the

proposal was limited to the carcinogenicity endpoint in accordance with Article 36(1) of CLP. RAC also considers the above available information useful for classifying substances for carcinogenicity.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level.

In accordance with article 36(1) of CLP, justification for action is not required for carcinogenic, mutagenic and reproductive toxicity (CMR) substances.

5 IDENTIFIED USES

Tetrafluoroethylene is used primarily in the manufacture of polymers.

6 DATA SOURCES

Data for tetrafluoroethylene are taken from:

- NTP Technical report on the toxicology and carcinogenesis studies of tetrafluoroethylene in F344/N rats and B6C3F1 mice (NTP TR 450 (NIH Publication No. 97-3366), April 1997).
- Publically disseminated REACH registration dossier (ECHA, 2018).
- Publically available literature and unpublished study reports as cited in the reference list (see section 14).

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Colourless, odourless gas.	ECHA, 2018.	-
Melting/freezing point	- 131.15 °C.	ECHA, 2018.	-
Boiling point	- 75.95 °C.	ECHA, 2018.	Measured at 1013 hPa.
Relative density	4.16 kg/m^3.	ECHA, 2018.	Estimated from molecular weight and ideal gas laws at 20 °C.
Vapour pressure	32395 hPa.	ECHA, 2018.	Measured at 24.3 ^{0}C.
Surface tension	No data.		
Water solubility	110 mg/L.	ECHA, 2018.	Measured at 28 °C and pH 7.
Partition coefficient n-octanol/water	Log Kow (Pow) 1.21.	ECHA, 2018.	QSAR estimate at 20 °C and pH 7.
Flash point	No data.		
Flammability	Extremely flammable gas. Limits of flammability in air: 13 % – 43.4 % v/v.	ECHA, 2018.	Measured at 1 atm pressure in air at 20 °C.

Property	Value	Reference	Comment (e.g. measured or estimated)
Explosive properties	Minimum Ignition Temperature of explosive decomposition: 205 °C at 30 bara for stabilised TFE, 230 °C at 20 bara for non-stabilised TFE, and 220 °C at 20 bara for air contaminated TFE.	ECHA, 2018.	Measured.
Self-ignition temperature	240 ^{0}C.	ECHA, 2018.	Measured.
Oxidising properties	Not applicable.		
Granulometry	Not applicable.		
Stability in organic solvents and identity of relevant degradation products	No data.		
Dissociation constant	Not applicable.		
Viscosity	Not applicable.		

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
235 workers employed in a polytetrafluoroethylene (PTFE) production facility were split into three groups: 129 “exposed”, 32 “removed from exposure” (described as exposed to organic fluorides for > year but then separated from exposure for > year) and 74 “control”. Urinary inorganic fluoride levels were measured. Blood samples were taken to determine cholinesterase activity.	Significantly elevated levels of urinary inorganic fluoride (1.35 ± 0.66 mg/L) were detected in the urine of workers exposed to organic fluorides during PTFE production compared to controls (1.05 ± 0.5 mg/L). Cholinesterase activity in blood was increased in workers with current and previous exposure to PTFEs.	Workers were exposed to several organic fluorides, including TFE, chlorodifluoromethane, PTFE and its thermal decomposition products. No air monitoring was undertaken.	Xu *et al.*, 1992.
Liver supernatant (pre incubated with and without carbon monoxide), microsomal and cytosolic fractions from Wistar rats were incubated with TFE and	Incubation of liver fractions with TFE resulted in depletion of glutathione and release of fluoride ions. Reaction rates for glutathione depletion in microsomes and cytosol were 3 nmol/mg protein and 0.7 nmol/mg protein, respectively. Pre-incubation with carbon monoxide to	The paper notes that due to the inherent instability of TFE it could not be radiolabelled, which limited some of the quantitative aspects of the study.	Odum and Green, 1984.

Method	Results	Remarks	Reference
samples taken at intervals up to 60 minutes. Glutathione and fluoride ion levels were determined. The resulting glutathione conjugate was identified by thin layer chromatography.	inactivate cytochrome P450 did not affect levels of glutathione depletion or fluoride ion release. The glutathione conjugate was identified as tetrafluoroethylglutathione (TFE-GSH).		
The cysteine conjugate of TFE, S-(1,1,2,2-tetrafluororethyl)-L-cysteine (TFE-Cys) was incubated with Wistar rat renal cortex slides or a β-lyase solution. Pyruvate and ammonia levels were determined.	TFE-Cys was metabolised by renal cortex slices to pyruvate and ammonia. Incubation of TFE-Cys with β-lyase released pyruvate and ammonia.		
[^{35}S]cysteine was administered to 4 male Wistar rats to label the glutathione pool in the liver prior to exposure to 6000 ppm TFE via inhalation for 6 hours. Bile was collected for 24 hours and analysed for metabolites.	Tetrafluroethylcysteinylglycine was identified in bile. No TFE-GSH was found.		
Groups of 4 male Wistar rats were exposed to 0, 1000, 2000, 3000, 4000 or 6000 ppm TFE via inhalation for 6 hours and 6 male Wistar rats were administered TFE-Cys orally at 100 mg/kg bw. Urine was collected for 24 hours and analysed. Kidneys from TFE exposed animals were subject to histopathological examination.	Marked renal tubular necrosis in the pars recta of the proximal tubule was observed in rats treated with 6000 ppm TFE. Analysis of urine from rats treated with 6000 ppm TFE or TFE-Cys found an increase in renal urea and urine volume, glucose, protein, alkaline phosphatase, N-acetyl-β-D-glucosaminidase, γ-glutamyltranspeptidase and alanine aminopeptidase. Plasma glucose levels were unchanged and no effect on liver function enzymes, plasma alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase, was observed.		
Groups of 4 male Wistar rats were administered 0, 25, 50 and 75 µmol/kg of the mercapturic acid of TFE, N-acetyl-S-(1,1,2,2-tetrafluoroethyl)-l-cysteine (TFE-NAc) via i.p. Urine was collected for 48 hours after treatment and analysed for the presence of protein, glucose and halogenated acetic acids. At 48 hours post	At ≥ 50 µmol/kg TFE-NAc a statistically significant increase in plasma urea, urinary protein and glucose and relative kidney weights was observed. Necrosis of the inner cortex of the kidney was noted. Difluoroacetic acid (DFAA) was detected in the urine at all doses, with the majority of DFAA excreted in first 24 hours. At 48 hours, 4.8 ± 1.0 %, 10 ± 1.2 % and 17.6 ± 2.8 % of the administered dose was excreted as DFAA at 25, 50 and 75 75 µmol/kg, respectively.	Study investigated the toxicity of three halogenated alkenes including TFE. Only results for TFE reported here.	Commandeur *et al.*, 1988.

Method	Results	Remarks	Reference
treatment, blood was collected for analysis of plasma urea and alanine transaminase (ALT). Kidneys were weighed and subject to histologic examination. Renal cytosolic fractions prepared from male Wistar rats were incubated with 4 mM TFE-Cys or TFE-Cys with aminooxyacetic acid (to inhibit β-lyase) for 15 minutes. The presence of halogenated acetic acids was determined.	Incubation with TFE-Cys resulted in the formation of pyruvate, hydrogen sulphide, thiosulphate and DFAA. Addition of aminooxyacetic acid inhibited the formation of pyruvate.		
Renal and hepatic sub-cellular fractions prepared from male Wistar rats were incubated with 4 mM TFE-Cys (with or without aminooxyacetic acid) or TFE-NAc (with and without acetyl-CoA). Reactive intermediates formed from TFE-Cys were trapped with addition of different nucleophilic amines. Reaction products were determined. Male Wistar rats were administered 75µmol/kg bw TFE-NAc via i.p. Urine and faeces was collected and analysed.	Incubation with TFE-Cys resulted in the formation of reactive intermediates DFAA and difluorothio(no)acetic acid (DFTA) which bound to the free amino group of TFE-Cys to form N-difluorothionoacetyl-S-(1,1,2,2-diflurorrgtl)-l-cysteine (TFE-PMS) and N-difluoroacetyl-S-(1,1,2,2-difluorothyl)-L-cysteine (TFE-PMO). Higher amounts of TFE-PMS than TFE-PMO were measured. Addition of aminooxyacetic acid inhibited the metabolism of TFE-Cys. Incubation with TFE-NAc resulted in a higher rate of N-deacetylation in renal than in hepatic supernatant, which corresponded to the formation of TFE-Cys in the same incubations. TFE-PMS was identified in urine at 0.2 % of the administered dose. TFE-PMO was not detected. Unchanged TFE-NAc was excreted at 3-5 % of the administered dose.		Commandeur *et al.*, 1989.
Isolated human proximal tubule cells were incubated with 25-500 µM TFE-Cys in the presence and absence of aminooxyacetic acid for 24 hours. Extracellular lactate dehydrogenase (LDH) activity was measured to determine cell death.	A statistically significant increase in LDH release was observed in TFE-Cys treated cultures when compared with controls. Cytotoxicity was blocked in the presence of aminooxyacetic acid.	Study investigated the toxicity of a number of glutathione and cysteine conjugates of halogenated alkenes including TFE. Only results for TFE reported here.	Chen *et al.*, 1990.
3 male Wistar rats/group were administered 50 µmol/kg of deuterium-labelled TFE-NAc (TFE-NAc-d_3) or TFE-Cys via i.p. Urine was collected	> 90 % of the administered dose of TFE-NAc-d_3 was excreted within 8 hours of treatment. Up to 24 hours after treatment labelled (TFE-NAc-d_3) and unlabelled (TFE-NAc) were found in the urine at 0.4 ± 0.2 % and 1.6 ± 0.5 % of the administered dose,	Study investigated the metabolism of cysteine conjugates and mercapturic acids of four halogenated alkenes including TFE. Only	Commandeur *et. al.*, 1991.

Method	Results	Remarks	Reference
for 48 hours after treatment and analysed for the presence of mercapturic acids. Renal and hepatic cytosolic fractions prepared from male Wistar rats were incubated with 4 mM TFE-Cys or TFE- NAc for 10 minutes. The activities of cysteine S-conjugate β-lyase and N-deacetylase were determined.	respectively. Following treatment with TFE-Cys, the corresponding mercapturic acid was present at 3 ± 1 % of the administered dose. The specific activity of β-lyase in renal cytosol was higher than in hepatic cytosol (24 ± 3 nmol/min/mg versus 5.7 ± 0.8 nmol/min/mg, respectively). The specific activity of N-deacetylase in renal cytosol was higher than in hepatic cytosol (116 ± 20 nmol/min/mg and 15 ± 4 nmol/min/mg, respectively).	results for TFE reported here.	
15 mM TFE-Cys was incubated in a β-lyase mimetic model system with and without a trapping agent, o-phenylenediamine (OPD), for 16 hours and then analysed by ^{19}F-NMR and GC-MS. 4 mM TFE-Cys was incubated with rat renal cytosol for 30 minutes and then analysed by GC-MS.	TFE-Cys was not detected. Several fluorine containing products were detected: fluoride ions, DFAA, DFTA and TFE-PMS. In the presence of OPD, unchanged TFE-Cys was detected in addition to 2-(difluoromethyl)benzimadazole. Fluoride ions, DFAA, DFTA, TFE-PMS and dimethylated S-(1,1,2,2-tetrafluoroethyl)-3-mercapto-2-oxopropionic acid (TFE-MOP) were detected.	Study investigated a number of cysteine conjugates halogenated alkenes including TFE. Only results for TFE reported here.	Commandeur *et. al.*, 1996.
Blood samples from Sprague Dawley and Fischer 344 rats and a human male volunteer were exposed to TFE at 5000 ppm for 3 hours. Blood: air partition coefficients were calculated. PB-PK modelling was used to determine the uptake of 0 - 10000 ppm TFE passing from the alveolar airspace into blood for a 6 hour exposure.	The blood:air partition coefficients for TFE were 0.83 and 0.85 for rats and humans, respectively. It was estimated that < 1.2 % of TFE in the airways is absorbed into systemic circulation regardless of dose.	Limited detail in the report including no information on the number of animals or subjects used in the determination of the blood: air partition coefficient and no table of outputs from modelling.	Central Toxicology Laboratory, 1998.
Liver and kidney microsomal and cytosolic fractions prepared from female F344 rats and B3C3F1 mice were incubated with TFE or TFE-Cys. Samples were taken at intervals up to 60 minutes for analysis of glutathione and fluoride ion levels (TFE) and fluoride ions and pyruvate levels (TFE-Cys). At the end of the incubation	Incubation of liver microsomal fractions with TFE resulted in a time dependent depletion of glutathione with reaction rates of 1 to 1.3 nmol/mg protein. No increase in fluoride ions was observed. F-19 NMR identified signals consistent with the formation of TFE-GSH. Incubation of cytosolic fractions with TFE-Cys resulted in the formation of fluoride ions and pyruvate. In liver fractions, the rate of fluoride and pyruvate production was higher in mouse liver (108 nmol/min/mg protein and 40 nmol/min/mg protein, respectively) than rat liver (5.6	Numbers of animals used for each experiment was not clearly reported. The authors note that due to the inherent instability of TFE it could not be radiolabelled and for this reason F-19 NMR and other physical methods were used to detect and quantify the metabolites	Central Toxicology Laboratory, 2000.

Method	Results	Remarks	Reference
period, the fractions were analysed for metabolites of TFE or TFE-Cys using F-19 NMR.	nmol/min/mg protein and 5.9 nmol/min/mg protein, respectively). In kidney fractions, the rate of fluoride and pyruvate production was higher in rat kidney (38.8 nmol/min/mg protein and 21.9 nmol/min/mg protein, respectively) than mouse kidney (5.9 nmol/min/mg protein and 4.0 nmol/min/mg protein, respectively). Analysis of incubation mixtures identified fluoride ions, DFAA, N-acetyl TFE-Cys and difluororacylated cysteine conjugates.	and intermediates	
Rat and mouse liver S9 fractions were incubated with TFE-Cys with and without aminooxyacetic acid for 60 minutes and analysed for fluoride ions.	Following incubation with aminooxyacetic acid, fluoride ion production was decreased in liver S9 fractions from rats and mice.		
Blood samples from rats and 1 human volunteer were exposed to TFE at 5000 ppm for 4 hours. Blood:air partition coefficients were calculated using PB-PK modelling.	The blood:air partition coefficients for TFE were calculated as 0.83 in rats and 0.85 in humans.		
Groups of 2 or 4 rats and 5 mice were administered 2000 or 6000 ppm TFE via inhalation for 6 hours or a single oral dose of 100 mg/kg TFE-Cys. Urine and faeces were collected and analysed for metabolites and biochemical markers of kidney damage.	An increase in urine volume, glucose, protein, γ-glutamyltranspeptidase, N-acetyl glucosamindase and alkaline phosphatase was observed in the urine of TFE and TFE-Cys treated rats and mice, with the increase more significant in rats than mice. Urinary excretion of fluoride was two times higher in the rat compared to the mouse following TFE or TFE-Cys exposure. Both species excreted difluoroaceylated cysteine conjugates, DFAA and fluoride, and excretion was complete within 48 hours post dosing.		
Liver and kidney cytosolic and microsomal fractions prepared from female F344 rats, female B6C3F1 mice and 3 human tissue samples were used for the following investigations:		No information on numbers of animals used to prepare kidney and liver fractions.	Central Toxicology Laboratory, 2001.
Hepatic glutathione conjugation of TFE was measured in microsomal fractions.	Glutathione conjugation of TFE was comparable across species (94, 79 and 66-100 nmol/min/mg for rat, mouse and humans, respectively).		
Renal β-lyase metabolism of TFE-Cys was measured kidney cytosolic fractions and in a human liver	The rate metabolism in kidney fractions was 21.9, 4.0 and 3.4 nmol/min/mg for rat, mouse and humans, respectively. The rate in human liver was 1.7 nmol/min/mg.		

Method	Results	Remarks	Reference
cytosolic fraction. Renal N-acetylase transferase metabolism of TFE-Cys to TFE-NAc was measured in microsomal kidney and liver fractions. Renal acylase metabolism of TFE-NAc to TFE-Cys was measured in cytosolic fractions.	The rate of metabolism in kidney fractions was comparable across species (91, 48 and 56 nmol/min/mg for rat, mouse and humans, respectively). In the liver, the rate in mouse was significantly higher than in rat or human tissues (3.9, 69 and 3.5 nmol/min/mg for rat, mouse and humans, respectively). The rate of metabolism in liver fractions was comparable across species (37, 18 and 23 nmol/min/mg for rat, mouse and humans, respectively). The rates in kidney were significantly higher than those for liver (216, 248 and 91 nmol/min/mg for rat, mouse and humans, respectively).		
40 female B6C3F1 mice were exposed to 0 or 5000 ppm TFE for 6 hours/day for 10 days. Liver, spleen and kidney damage was analysed by staining for cell differentiation markers and oxidative damage in all organs, and cell proliferation and apoptosis in liver. Blood was collected for analysis and mRNA was extracted from snap frozen liver samples for mouse gene array analysis. Groups of 5 female F344 rats and B6C3F1 mice were administered TFE-GSH via i.p: at 0 or 50 mg/kg bw/day for 5, 10 or 15 days; at 10 or 50 mg/kg bw/day for 5 or 10 days ; or as a single dose at 50 mg/kg bw/day. Liver sections were analysed for apoptosis and endothelial cell markers. The number of BrdU positive nuclei was assessed in liver and kidney. Snap frozen liver samples were subject to genomic analysis Groups of 5 female F344 rats and B6C3F1 mice were administered a single dose of 30 mg/kg bw ^{35}S-TFE-Cys. Groups of 5 female B6C3F1 mice were also administered a single	Minimal reduction in hepatocyte glycogen, without an associated histopathological change, was observed in livers of rats and mice treated with TFE. A decrease in the number of apoptotic hepatocytes in rats and mice was noted. Slight tubular basophila with scattered single apoptotic epithelial cells was observed in the proximal tubule of kidneys of treated mice. An increase in BrdU labelling of cells of proximal tubule of kidney in mice was also observed. No significant changes in cell differentiation markers or in the genomic analysis were noted. Tubular dilation, basophilia and degeneration were observed in kidneys of rats and mice treated with 50 mg/kg. In mouse liver, the frequency of apoptosis was increased. No significant changes in cell differentiation markers were observed in the livers of treated mice or rats. In rats treated with 10 mg/kg TFE-GSH and mice treated with 50 mg/kg TFE-GSH the number of BrdU labelled cells was slightly (not statistically significant) increased in hepatocytes, decreased in sinusoidal cells of the liver and increased in the medulla of the kidney. No changes were reported in genomic analysis. Radiolabelling was similar in livers of rats and mice and there was no evidence of accumulation in endothelial cells. In kidneys, the highest concentration of radiolabelling was in the outer stripe of the outer zone of the medulla and tubular regions of the cortex, with higher intensity	GLP compliant. A number of *in vitro* and *in vivo* experiments were conducted to investigate the possible modes of action and biological responses of TFE and its metabolites in kidney and liver.	Central Toxicology Laboratory, 2003.

Method	Results	Remarks	Reference
dose of 10mg/kg bw ^{35}S-TFE-GSH. Livers and kidneys were removed and subject to microautoradiography.	in rats than mice.		
Blood samples from female F344 rats and B6C3F1 mice were incubated with TFE. Fluoride ion and glutathione levels were measured over 60 minutes.	No increase in fluoride or GSH was observed.		
Hepatic microsomes from control female F344 rats and B6C3F1 mice or female rats and mice pre-treated with naphthoflavone, phenobarbitone or ethanol were incubated with TFE. Fluoride levels measured over 3 hours.	An increased rate of fluoride release was observed. The rate was decreased in the absence of NADPH. The rate was further increased when microsomes were pre-treated with phenobarbitone.		
Hepatocytes prepared from female B6C3F1 mice were incubated with 50,000 or 400 μM TFE-GSH, TFE-NAc or TFE-Cys. In some assays, aminooxyacetic acid was added. LDH levels were measured over 4 hours.	An increase in LDH release was only observed in rat and mouse hepatocytes treated with TFE-Cys, which was reduced in the presence of aminooxyacetic acid.		
Microsomes prepared from female F344 rats and B6C3F1 mice were incubated with TFE-Cys for up to 3 hours and analysed for the presence of sulphoxides.	No measurable formation of sulphoxides of TFE-Cys was observed.		

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Systemic toxicity observed in the available repeated dose toxicity studies indicate that tetrafluoroethylene is absorbed following inhalation exposure (see section 11 for summaries of the available data). Blood:air partition coefficients for tetrafluoroethylene were calculated as 0.83 and 0.85 for rats and humans, respectively. PB-PK modelling estimated that <1.2% of tetrafluoroethylene in the airways is absorbed into systemic circulation regardless of the dose administered.

The available inhalation repeated dose toxicity and carcinogenicity studies with tetrafluoroethylene identified the kidney, liver and haematopoietic system as target organs, confirming the distribution of tetrafluoroethylene or its metabolites to these organs.

In vitro and *in vivo* studies indicate that tetrafluoroethylene is metabolised by glutathione-S-transferases to S-(1,1,2,2-tetrafluoroethyl)glutathione (TFE-GSH) in the liver, which is released into the bile or recirculated to the kidneys where it is further metabolised to S-(1,1,2,2-tetrafluoroethyl)-L-cysteine (TFE-Cys). TFE-Cys is either activated by β-lyases to toxic species including difluoroacetic acid and difluorothio(no)acetic acid which form covalent adducts with renal cellular proteins leading to nephrotoxicity. It may also be deactivated by N-acetyltransferases to form N-acetyl-S-(1,1,2,2-tetrafluoroethyl)-L-cysteine (TFE-NAc). TFE-NAc may be eliminated in the urine or undergo N-deacetylation, possibly reforming TFE-Cys which can subsequently be activated via β–lyases. *In vitro* and *in vivo* studies with TFE-Cys demonstrated similar nephrotoxicity to that observed with tetrafluoroethylene and therefore it is postulated that this metabolic pathway is relevant for the renal toxicity observed in rodents. Isolated human proximal tubule cells were shown to be sensitive to TFE-Cys toxicity and therefore it cannot be excluded that this pathway is relevant for humans.

In vitro, glutathione conjugation of tetrafluoroethylene was comparable between rats, mice and human hepatic fractions. Renal β-lyase activities were shown to be higher in rat than mouse or human kidney fractions whereas hepatic β-lyase activities were higher in mouse than rat or human liver fractions, which correlates with the target organs in rat and mouse studies. N-acetylase transferase activity was comparable in rat, mouse and human kidney fractions.

The available data did not identify a role for cytochrome P450 or other pathways in the metabolism of tetrafluoroethylene.

Workers at a PTFE production site which handled a number of organic fluorides including tetrafluoroethylene had increased urinary levels of inorganic fluoride. Analysis of urine of rats and mice exposed to 6000 ppm tetrafluoroethylene for 6 hours found an increase in fluoride, cysteine conjugates (either TFE-Cys or TFE-NAc) and difluoroacetic acid. In both species excretion was complete within 48 hours. Similar urinary metabolites were observed when rats or mice were administered TFE-Cys.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Not evaluated as part of this dossier.

10.2 Acute toxicity - dermal route

Not evaluated as part of this dossier.

10.3 Acute toxicity - inhalation route

Not evaluated as part of this dossier.

10.4 Skin corrosion/irritation

Not evaluated as part of this dossier.

10.5 Serious eye damage/eye irritation

Not evaluated as part of this dossier.

10.6 Respiratory sensitisation

Not evaluated as part of this dossier.

10.7 Skin sensitisation

Not evaluated as part of this dossier.

10.8 Germ cell mutagenicity

No classification is proposed. The studies on genotoxicity are reported for the purpose of background information only. The findings are of relevance to identify whether a genotoxic mechanism of action is relevant for carcinogenicity.

Table 9: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Similar to OECD 471: Bacterial reverse mutation assay. Duplicate plates. GLP compliant. Study did not meet current guideline requirements to include a 5th strain (*S. Typhimurium* TA102 or E.coli WP2 uvrA or WP2 uvrA (pKM101).	TFE, (purity > 99 %).	*S. typhimurium* strains TA 97, TA 98, TA 100, TA 1535 and TA 1537. 0, 0.5, 3, 4 or 5 % TFE. With and without metabolic activation with rat liver S9 (Aroclor induced). Vehicle: air. Positive controls: 9-aminoacridine; N-methyl-N'-nitrosoguanidine; and 2-nitrofluorene (without S9); 2-aminoanthracene and vinyl chloride (with S9).	Result: negative with and without metabolic activation. No information on cytotoxicity.	ECHA, 2018.
Non-guideline: Bacterial reverse mutagenicity assay. Not conducted according to GLP. Limited reporting in the registration dossier.	TFE (purity not stated).	No information reported on the bacterial strains tested, the use of metabolic activation, doses tested or duration.	Result: negative. No information on cytotoxicity.	ECHA, 2018.
Non-guideline: Bacterial reverse mutagenicity assay. Duplicate plates, only two strains tested. GLP compliant.	TFE (purity 99.9 %).	*S. typhimurium* strains TA 1535 and TA 100. 0, 5000, 10000, 20000, 30000 or 40000 ppm TFE over 3 days in the presence of metabolic activation (a microsome mix prepared from phenobarbitone-induced female B6C3F1 mice). Positive controls: 2-aminoanthracene, cyclophosphamide.	Result: negative. No information on the cytotoxicity.	Central Toxicology Laboratory, 2004.
Non-guideline: Bacterial reverse mutagenicity assay. Not conducted to GLP. Limited reporting on method	S-(1,1,2,2-tetrafluoroethyl)-L-cysteine (TFE-Cys) (purity not stated).	*S. typhimurium* strain TA 1537, TA 97, TA 98, TA 1538, TA 1535 and TA 100. 0, 20, 50, 100, 200 or 500 µg/plate TFE-Cys. With and without metabolic activation with rat kidney S9 (Aroclor 1254	Result: negative. No information on cytotoxicity.	Green and Odum, 1985.

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
and results. No positive controls.		induced).		
Non-guideline: Mammalian cell mutagenicity assay. GLP compliant. No information on selection time or number of cells evaluated.	TFE (purity > 99 %).	Chinese hamster ovary cells (HPRT locus). 0, 20, 40, 60, 80 or 100 % TFE for 5 hours in the presence and for 18 – 19 hours in the absence of metabolic activation with rat liver S9 (Aroclor induced). Negative control: 100% nitrogen. Positive control: Ethylmethanesulphonate.	Result: negative with and without metabolic activation. No cytotoxicity observed.	ECHA, 2018.
Non-guideline: Mammalian cell mutagenicity assay. No information is provided on GLP compliance. Limited reporting in the registration dossier.	TFE (purity not stated).	Chinese hamster ovary cells. 0, 20, 40, 60, 80 or 100 % TFE for 5 hours in the presence of metabolic activation (activation system not specified). No information is provided on positive or negative controls.	Result: negative. No information on cytotoxicity.	ECHA, 2018.
OECD 473: Mammalian chromosome aberration assay. GLP compliant. Limited reporting in the registration dossier.	TFE (purity 99 %).	Chinese hamster ovary cells. 0, 25, 50, 75 or 100 % TFE for 2 hours in the presence and for 5 hours in the absence of metabolic activation with rat liver S9 (induction substance not specified). Negative control: 100 % nitrogen. Positive control: Ethylmethanesulphonate.	Result: negative with and without metabolic activation. No cytotoxicity with metabolic activation, cytotoxicity results reported as inconclusive without metabolic activation.	ECHA, 2018.

Table 10: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
OECD 474: Mammalian erythrocyte micronucleus test. GLP compliant. Study did not meet current guideline requirements requiring cell counts of at least 4000 erythrocytes per animal.	TFE (purity 99 %).	15/sex/dose C57BL/6jfC-1/Alpk mice. 0, 5000, 12000 or 19000 ppm (males) and 0, 7000, 17000, 28000 ppm (females) TFE for 6 hour single inhalation exposure. Bone marrow samples taken at 24, 48 and 72 hours post exposure. Positive control: vinyl chloride. 1000 polychromatic erythrocytes (PE) per slide were evaluated for the presence of micronuclei (MN), and 1000 PE were counted to determine PE % of total mature erythrocyte population.	Result: negative. Statistically significant decrease in PE in both sexes, indicating exposure to the bone marrow. A statistically significant increase in MN in males at 5 000 and 12 000 ppm only at 72 hours. A count of a further 3000 PEs for 0, 5000 and 12000 ppm males indicated an increase in MN which was not statistically significant.	ECHA, 2018.
Similar to OECD 486: Unscheduled DNA synthesis assay. GLP compliant. No information on number of cells scored per animal.	TFE (purity 99.9 %).	5/dose male CD-1 mice. 0, 20000 or 40000 ppm TFE for a 6 hour single inhalation exposure. Positive control: N-nitrosodimethylamine.	Result: negative. No significant increase in the mean net nuclear grain count or the percentage of cells in repair.	ECHA, 2018. .
Non-guideline: Mammalian erythrocyte micronucleus test. No information on GLP compliance. Study did not meet current guideline requirements requiring cell counts of at least 4000 erythrocytes per animal. Limited reporting in the registration dossier and NTP report.	TFE (purity > 99 %).	10/sex/group B6C3F1 mice. 0, 312, 625, 1250, 2500 or 5000 ppm TFE for 6 hours inhalation exposure per day, 5 days/week for 13 weeks. 1000 PE were scored and 10,000 normochromatic erythrocytes (NCE) were counted to determine the ratio of PE to NCE. No information on positive control.	Result: negative.	NTP, 1997.
Non-guideline study:	TFE (purity	Male & female B6C3F1 mice.	No increase in	NTP, 1997.

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Expression of H-ras codon 61 *in vivo*. No information on GLP compliance.	> 99 %).	0, 312, 625 or 1250 ppm TFE for 6 hours inhalation exposure per day, 5 days per week over 95-96 weeks (see carcinogenicity study reported in Table 13 below for further details). DNA was isolated from sample groups of hepatocellular adenomas and carcinomas (17, 4, 29 and 29 neoplasms from 0, 312, 625 and 1250 ppm, respectively) and the frequency of H-*ras* mutations at codon 61 was evaluated. No positive control. No information on number of animals sampled.	activated H-*ras* frequency in TFE treated groups (total across groups 15%) when compared with concurrent (59%) or historical controls (56%). K-ras mutations were not detected. The results indicate TFE may induce liver tumours by a ras-independent pathway.	

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

The genotoxicity of tetrafluoroethylene has been investigated *in vitro* and *in vivo.*

In a GLP compliant bacterial reverse mutation assay conducted using a method similar to OECD 471, five strains of *S. typhimurium* (TA 97, TA 98, TA 100, TA 1535 and TA 1537) were exposed to tetrafluoroethylene at concentrations up to 5% (50,000 ppm). No increase in mutation frequency was observed with or without microsomal metabolic activation. In a non-guideline bacterial reverse mutation assay conducted in accordance with GLP, *S. typhimurium* strains TA 1535 and TA 100 were exposed to concentrations of tetrafluoroethylene of up of 4% (40,000 ppm). No increase in mutation frequency was observed.

In a non guideline bacterial reverse mutation assay, the cysteine conjugate of tetrafluoroethylene, S-(1,1,2,2-tetraflurorethyl)-L-cysteine, was not mutagenic in bacterial strains TA 1535, TA 1537, TA 100, TA 98 or TA 97 with or without metabolic activation.

In a GLP compliant non-guideline *in vitro* mammalian mutagenicity assay, Chinese hamster ovary cells (HPRT locus) were exposed to up to 100 % tetrafluoroethylene for 5 hours, in the presence of metabolic activation, and 18 to 19 hours in the absence of metabolic action. No information is reported on the expression time, cell number or cell viability. No increase in gene mutation frequency was observed. In a second non-guideline *in vitro* mammalian mutagenicity assay, Chinese hamster ovary cells were exposed to concentrations of tetrafluoroethylene up to 100 % in the presence of metabolic activation for 5 hours. No information is provided on the expression time, cell numbers, cell viability or positive and negative controls. The study is reported to be negative.

In a GLP compliant *in vitro* chromosome aberration assay conducted in accordance with OECD 473, tetrafluoroethylene did not increase the frequency of chromosomal aberrations in Chinese hamster ovary cells at nominal atmospheric concentrations of between 25 % and 100 % in the presence or absence of metabolic activation.

In an *in vivo* mammalian erythrocyte micronucleus assay, conducted in accordance with OECD 474 and GLP, tetrafluoroethylene was administered via inhalation at 0, 5000, 12000 and 19000 ppm (males) and 0, 7000, 17000 and 28000 ppm (females) to C57BL/6jfC-1/Alpk mice for a single 6 hour exposure. Bone marrow was sampled at 24, 48 and 72 hours post dosing. 1000 polychromatic erythrocytes (PCEs) per slide were evaluated for the presence of micronuclei. No information on clinical signs is reported. A dose dependent statistically significant decrease in PCEs was observed indicating exposure of the bone marrow. A

statistically significant increase in micronuclei was observed in males in the low and mid dose groups at 72 hours only. In a confirmatory count of a further 3000 PCEs, no statistically significant increase was observed and thus the study is considered to be negative. In a second *in vivo* erythrocyte micronucleus assay, peripheral blood samples were taken from male and female B6C3F1 mice at the end of a 13-week repeated dose toxicity study. No increase in the frequency of micronucleated erythrocytes was observed. It is noted that the two available *in vivo* micronucleus assays were conducted prior to the update of OECD 474 (2016), requiring that at least 4000 immature erythrocytes per animal are scored for micronucleation.

In an unscheduled DNA synthesis (UDS) assay, male CD-1 mice were exposed to tetrafluoroethylene for 6 hours by inhalation at concentrations of up to approximately 160 g/m^3. No increase in UDS induction was observed.

The involvement of the H- and K-*ras* oncogenes in the development of hepatic tumours in mice following inhalation exposure to tetrafluoroethylene was investigated as part of a 2-year carcinogenicity study. No difference in the mutation frequency of the H- and K-*ras* oncogenes in the tetrafluoroethylene exposed groups was observed when compared to controls.

There are some limitations to the data presented in Tables 9 and 10 including limited reporting, the use of older testing guidelines and the use of non-standard tests. Nevertheless, the results of the available data demonstrate that tetrafluoroethylene is not mutagenic in *in vitro* bacterial and mammalian cell assays and is not genotoxic *in vivo* in the liver of mice, a known target tissue for tetrafluoroethylene-mediated toxicity in repeated dose and carcinogenicity studies. The available information also indicates that tetrafluoroethylene does not induce chromosomal damage *in vitro* or in bone marrow or peripheral erythrocytes *in vivo*. A direct assessment of the involvement of *ras* oncogenes in the formation of hepatic tumours further suggests induction of liver tumours in mice may occur via a *ras* independent pathway.

A weight of evidence approach indicates that the available data support the conclusion that tetrafluoroethylene is not genotoxic.

10.8.2 Comparison with the CLP criteria

Not evaluated as part of this dossier.

The information is provided as supportive information for the carcinogenicity assessment (see section 10.9).

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Not evaluated as part of this dossier.

The information is provided as supportive information for the carcinogenicity assessment (see section 10.9).

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Overall information

The genotoxic data are used for information only whether a genotoxic mechanism of action is to be assumed for the induction of carcinogenic effects.

In vitro tests

TFE induces neither gene mutations in bacterial cells nor in a mammalian cell culture (CHO cells) with or without S9-mix. In a chromosomal aberration test no clastogenic

effects are induced with or without S9-mix (CHO cells).

In vivo tests

Micronucleus tests (mice; inhalation) are negative in bone marrow cells as well as in peripheral blood samples.
The available UDS test (indicator test; mice; inhalation) shows no increase in unscheduled DNA synthesis.
Additional information is provided by a 2-year carcinogenicity study (mice; inhalation) regarding the involvement of H- and K-ras oncogenes in development of hepatic tumours. The mutation frequency of H- and K-ras oncogenes is not different to those of controls. Therefore liver tumours in mice may occur via a ras-independent pathway.

Summary

The Dossier Submitter (DS) concluded that tetrafluoroethylene is not genotoxic despite some limitations to the quality of the available data (none of the tests has been carried out according to the current OECD test guideline; the reproducibility of the test data is sometimes inadequate or incomplete in the publications.)
This conclusion is provided as supportive information for the carcinogenicity assessment only.

Comments received during public consultation

No comments were submitted during the public consultation.

Assessment and comparison with the classification criteria

Following tests for genotoxicity with TFE are available:

In vitro tests

- Bacterial gene mutation test (four tests): negative
- Mammalian cell gene mutation test (two tests; HPRT; CHO cells): negative
- Mammalian chromosome aberration test (CHO cells): negative

In vivo tests

- Mammalian erythrocyte micronucleus test (bone marrow cells): negative
- Mammalian erythrocyte micronucleus test (peripheral blood): negative
- Unscheduled DNA synthesis (DNA): negative

Additional information

- Involvement of the H- and K-ras oncogens in the development of hepatic tumours in mice as a part of a 2-year carcinogenicity study: negative

RAC follows the view of the dossier submitter that despite deficiencies regarding the data presentation and test quality the genotoxic data of TFE can be assessed.

A comparison of the genotoxic data with the classification criteria is not necessary because the DS has informed that the evaluation of these data is used for information

only whether a genotoxic mechanism of action is to be assumed for the induction of carcinogenic effects.

Conclusion

Based on the available data RAC supports the conclusion of the DS that TFE does not induce genotoxic effects either *in vitro* or *in vivo* and therefore **does not warrant classification for germ cell mutagenicity**.

10.9 Carcinogenicity

Table 11: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Similar to OECD 451: Carcinogenicity study. Fischer 344/N rats. 60 rats/sex/dose. GLP compliant. Extended evaluation of the kidney: 6-10 additional sections were taken from each kidney by step sectioning at 1mm intervals.	TFE (purity > 98 %). Whole body inhalation. 0, 156, 312 or 625 ppm (males) and 0, 312, 625 or 1250 ppm (females) 6 hours/day, 5 days/week for 104 weeks. Interim assessment at 15 months: 10 rats/sex/dose group. Post-exposure period of 11 days prior to necropsy.	<u>15 month interim assessment:</u> ↑ absolute kidney weight in males at 625 ppm and females at ≥ 625 ppm; ↑ relative kidney weight in males at ≥ 312 ppm and females at 1250 ppm. ↑ incidence of renal tubule degeneration in males at ≥156 ppm & females at ≥625 ppm. ↑ absolute liver weight in females at ≥ 625 ppm; ↑ relative liver weight in females at 1250 ppm; ↑ incidence of clear cell foci in males at ≥ 312 ppm and mixed cell foci in females at ≥ 625 ppm. <u>Study termination:</u> Terminal survival rates of the control, low, mid and high dose groups were 17/50, 12/50, 17/50 & 1/50 for males and 28/50, 16/50, 15/50 & 18/50 for females. Terminal body weights were decreased males at 625 ppm and females at 1250 ppm. *Non-Neoplastic Lesions:* ↑ incidence of renal tubule degeneration in males at ≥ 156 ppm & females at ≥ 625 ppm. ↑ incidence of hepatic cystic degeneration in males at ≥ 156 ppm and hepatic angiectasis in females at ≥ 312 ppm. ↑ incidence of cataracts in females at 1250 ppm. Kidney: ↑ incidence renal tubule hyperplasia in males at 625 ppm and females at 1250 ppm. Liver: ↑ incidence of eosinophilic foci in males at ≥ 156 ppm, basophilic foci in males at ≥ 312 ppm and of mixed cell foci in males at ≥ 312 ppm and females at 1250 ppm. *Neoplastic Lesions:* Kidney: ↑ incidence of renal tubule adenoma in males at 312 ppm and of combined renal tubule adenoma and carcinoma in females at 1250 ppm. Following the evaluation of additional step sections of the kidney, ↑ incidence of renal tubule adenoma and of combined renal tubule adenoma and carcinoma was noted in males at 625 ppm and females at 1250 ppm. Liver: ↑ incidence of hepatocellular adenomas in females at ≥ 312 ppm; in hepatocellular carcinomas in males at 312 ppm and females at 312 ppm and 625 ppm, and in combined hepatocellular adenoma and carcinoma in males and females at ≥ 312 ppm. ↑ incidence of haemangiosarcoma in females at 625ppm. Leukaemia: ↑ incidence of mononuclear cell leukaemia in males at 156 ppm and females at ≥ 312 ppm. Testes: ↑ incidence of interstitial cell adenoma in males at 312 ppm and 625 ppm.	NTP, 1997.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		Mammary gland: ↓ incidence of fibroadenoma in females at ≥ 312 ppm.	
Similar to OECD 451: Carcinogenicity study. B6C3F1 mice. 58 mice/sex/dose. GLP compliant.	TFE (purity > 98 %). Whole body inhalation. 0, 312, 625 or 1250 ppm. 6 hours/day, 5 days/ week for 95 - 96 weeks. Interim assessment at 15 months: 10 mice/sex/dose group. Post-exposure period of 11 days prior to necropsy.	<u>15 month interim assessment</u>: ↑ incidence of renal tubule dilation in males at ≥ 625 ppm and renal tubule karyomegaly in males and females at ≥ 625 ppm. In the liver, ↑ incidences of haemangiosarcoma in males at 1250 ppm, angiectasis in all treated males and females and ↑ eosinophilic foci in females at ≥ 625 ppm. <u>Study termination:</u> Terminal survival rates of the control, low, mid and high dose groups were 38/48, 11/48, 2/48 and 1/48 for males and 36/48, 4/48, 6/48 & 4/48 for females. Study was terminated during week 96 due to reduced survival. *Non-Neoplastic Lesions:* ↑ incidence of renal tubule dilation in males at ≥ 312 ppm and renal tubule karyomegaly in males at ≥ 625 ppm and females at 1250 ppm. In the liver, ↑ angiectasis in males and females at ≥ 312 ppm and coagulative multifocal necrosis in males at ≥ 625 ppm. ↑ haematopoietic cell proliferation in liver in females at ≥ 312 ppm and in spleen in males and females at ≥ 312 ppm. Liver: ↑ incidence of eosinophilic foci in males at ≥ 625 ppm and females at 312 ppm and 625 ppm. *Neoplastic Lesions:* Liver: ↑ incidences of haemangioma in males at 312 & 625 ppm and in females at 312 ppm; haemangiosarcoma in males & females at ≥ 312 ppm; hepatocellular adenomas in females at 625 ppm; and hepatocellular carcinoma in males & females at ≥ 312 ppm. Haematopoietic system: ↑ incidence of histiocytic sarcoma in males and females at ≥ 312 ppm.	NTP, 1997.

Table 12: Summary table of human data on carcinogenicity

Method	Population	Exposure level	Results	Reference
Cohort mortality study (1950-2002) 6 polytetrafluoroethylene production sites in the EU and North America.	5879 male workers categorised into: 4473 'ever' exposed; 1081 'never' exposed; and 25 'unknown' exposure.	Not specified. All plants handled both TFE and ammonium pentadecafluorooctanoate (APFO), and exposure to both was assumed.	↓ mortality rate in TFE exposed workers from all causes (SMR 0.77, C.I. 0.71 - 0.84). ↑ risk of cancer in TFE exposed workers: liver (SMR 1.27, C.I. 0.55 - 2.51), kidney (SMR 1.44. C.I. 0.69-2.65) leukaemia (SMR 1.48, C.I. 0.77 – 2.59). A non-significant upward trend by TFE cumulative exposure was observed for liver cancer, but not for kidney cancer and leukaemia.	Consonni *et. al*., 2013.

SMR : Standardised mortality ratio (SMR). Confidence interval (C.I.) of 95%.

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

Animal studies

Two year carcinogenicity studies are available in which rats and mice were exposed via inhalation to tetrafluoroethylene (NTP, 1997). These studies are considered of a reliable standard and conducted in accordance with GLP. Further details are provided in Annex I to this report.

Fischer 344/N rats were exposed via inhalation to tetrafluoroethylene at 0, 156, 312 and 625 ppm (males) or 0, 312, 625, 1250 ppm (females) for 104 weeks. At study termination, the survival rate was reduced in high dose males and all treated females and there was a decrease in terminal body weight in high dose animals.

There was a statistically significant increase in renal tubule adenomas in males at the mid dose and a non-statistically significant increase in all treated females. In order to further assess the renal lesions, additional step sections of the kidney were prepared and analysed. From this extended evaluation, a statistically significant increase in renal tubule adenomas was observed in high dose males and females. When the standard and extended sections were considered together, there was a statistically significant increase in renal tubule adenomas in mid (9/50, 18 %) and high (13/50, 26 %) dose males and high dose females (8/50, 16%) when compared with the concurrent control (2/50 for male and 0/50 for females). Renal tubule adenomas are considered to be relatively uncommon in rats: the incidence of renal adenomas in the historical control data from NTP 2-year inhalation studies was 0.9 % (6/652) for males and 0.6 % (1/650) for females. Therefore, the dose dependent increase in renal tubule adenomas observed in this study is considered to be treatment related.

An increased incidence of renal tubule hyperplasia was observed in high dose males and females. This was distinguished histopathologically from regenerative epithelial changes often seen with nephropathy and was considered by the study authors to be a pre-neoplastic lesion. The study report does not document the criteria used to distinguish the observed hyperplasia from regenerative epithelial changes but does note that the hyperplasia was focal and consisted of dilated tubules which were lined with increased numbers of epithelial cells which sometimes stained more basophilic than normal cells. The NTPs "Nonneoplastic Lesion Atlas" describes atypical tubule hyperplasia (ATH) as a putative pre-neoplastic lesion, characterised by an increase in the number of epithelial cells within a single tubule, an increase in the proliferative epithelium lining by two or three cell layers and enlarged hyperplastic cells with a slightly basophilic cytoplasmic sheen (NTP, 2018). Although the hyperplasia observed in the current study was not specifically

identified as ATH, it would appear to be consistent with the above definition of ATH and is therefore, considered to be a pre-neoplastic lesion rather than a regenerative change associated with nephropathy.

There was a statistically significant, dose dependent increase in the incidence of hepatocellular adenomas in females in all dose groups which is considered to be treatment related. The reported incidences were 0/50 (0 %), 4/50 (8 %), 5/50 (10 %) and 6/50 (12 %) for the control, low, mid and high dose females, respectively. An increased incidence of hepatocellular adenomas in treated males was also observed although not statistically significant: 3/50 (6 %), 6/50 (12 %), 8/50 (16 %) and 5/50 (10 %) for control, low, mid and high dose groups, respectively. The incidence of hepatocellular adenomas in historical control males in NTP 2-year inhalation studies was 20/653 (3.1%). Therefore, the increase in hepatocellular adenomas in males was considered to be biologically significant.

There was an increase in the incidence of hepatocellular carcinomas in mid dose males (10/50, 20 %) and low (4/50, 8 %) and mid (9/50, 18 %) dose females. The incidence of this neoplasm lacked a positive dose-dependent trend which may have been due to reduced survival in the high dose group. The incidence of hepatocellular carcinomas in the historical control data from NTP 2-year inhalation studies was 1.2% (8/653) for males and 0.2 % (1/650) for females. Therefore, as the incidence of hepatocellular carcinomas exceeded the historical control range it is considered to be treatment related.

There was a statistically significant increase in the incidence of hepatic haemangiosarcomas in mid dose females (5/50, 10 %) when compared with the concurrent control (0/50). The incidence of hepatic haemangiosarcomas observed in this study is above that observed in historical control females (0/650) from NTP 2-year inhalation studies. It is also above the incidence of haemangiosarcomas in all organs (2/653, 0.3 %) in females from NTP 2-year inhalation studies. Therefore, although the increase in hepatic haemangiosarcomas occurred without a dose dependent trend, due to the low background incidence of hepatic haemangiosarcomas in rats, it is considered to treatment related.

In the liver, there was an increased incidence of hepatocellular foci: eosinophilic foci in males and females in the high dose group; basophilic foci in males in the mid and high dose groups; and mixed cell foci in males in the mid dose group and males and females in the high dose group. The presence of hepatocellular foci was considered by the study authors to be a potential pre-neoplastic lesion.

The incidence of hepatic cystic degeneration was statistically significantly increased in males: 17/50 (34 %), 39/50 (78 %), 35/50 (76 %) and 32/50 (64 %) for the control, low, mid and high dose respectively. The study authors report that while hepatic cystic degeneration occurs spontaneously at low incidences in aging rats, it is common following exposure to hepatocarcinogens. In females, the incidence of hepatic angiectasis was statistically significantly increased in all treated groups: 0/50 (0 %), 9/50 (18 %), 9/50 (18 %) and 14/50 (28 %) for the control, low, mid and high dose respectively and the study authors note that these lesions are occasionally associated with hepatocellular neoplasms.

The incidence of mononuclear cell leukaemia was increased in males of the low dose group (43/50, 86 %) and all treated females (31/50 (62 %), 23/50 (46 %) and 36/50 (72 %), for the low, mid and high dose group females, respectively) when compared with the concurrent controls (34/50 (68 %) males and 16/50 (32 %) females). The incidence of this lesion in control males (68 %) exceeded the range observed in male historical control data from 2-year NTP inhalation studies for all types of leukaemia (34 % - 66 and therefore the significance of the increase in low dose males is unclear. However, the incidence in control females was within the range observed in female historical control data from 2-year NTP inhalation studies (30 % - 54 %) and therefore it cannot be excluded that the statistically significant increase in the incidence of this lesion in low and high dose females is related to tetrafluoroethylene exposure.

A significant increase in interstitial cell adenomas in the testes was observed in males of the mid (48/50, 96 %) and high (47/50. 94 %) dose when compared with both the concurrent control (39/50, 78 %) and historical control males in NTP 2-year inhalation studies (450/653, 69 %). It is noted that this type of tumour is common in aging F334/N rats and therefore the biological significance of the increase in exposed males is unclear.

The incidence of fibroadenoma in the mammary gland was decreased in all treated females when compared with the concurrent control: 22/ 50 (44 %), 11/50 (22 %), 9/50 (18 %) and 7/50 (14 %) for the control, low, mid and high dose females, respectively It is noted that fibroadenomas are common benign tumours in

mammary gland of female F344/N rats and therefore the biological significance decreased incidence in this study is unclear.

The tumour incidences from the rat NTP study are summarised in table 13 below. Tumour incidences in historical control rats in 2-year NTP inhalation studies are reported in table 14 below.

Table 13: Incidences of neoplastic lesions in rats following 2-year inhalation exposure to tetrafluoroethylene (NTP, 1997).

	Males				**Females**			
Dose group (ppm)	**0**	**156**	**312**	**625**	**0**	**312**	**625**	**1250**
Number of animals examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Kidney								
Single Sections:								
Renal tubule adenoma	0	0	6*	3	0	3	1	3
Renal tubule carcinoma	1	0	2	0	0	0	0	2
Renal tubule adenoma or carcinoma	1	0	6	3	0	3	1	5**
Step Sections:								
Renal tubule adenoma	2	4	3	11**	0	0	2	5*
Renal tubule carcinoma	0	1	0	0	0	0	0	1
Renal tubule adenoma or carcinoma	2	5	3	11**	0	0	2	5*
Single and Step Sections:								
Renal tubule adenoma	2	4	9*	13**	0	3	3	8**
Renal tubule carcinoma	1	1	2	0	0	0	0	3
Renal tubule adenoma or carcinoma	3	5	9	13**	0	3	3	10*
Liver								
Hepatocellular adenoma	3	6	8	5	0	4*	5**	6**
Hepatocellular carcinoma	1	1	10**	3	0	4*	9**	2
Hepatocellular adenoma or carcinoma	4	7	15**	8	0	7**	12**	8**
Haemangiosarcoma	0	0	0	0	0	0	5*	1
Mononuclear Cell Leukaemia	34	43*	38	31	16	31**	23	36**
Testes: Interstitial cell adenoma	39	40	48**	47*	-	-	-	-
Mammary gland: Fibroadenoma	-	-	-	-	22	11*	9**	7**

** Significantly different from the control group (P≤0.05); ** significantly different from the control group (P≤0.01)*

Table 14 Incidences of neoplastic lesions in historical control rats in 2-year NTP inhalation studies as reported in NTP, 1997.

	Males			Females		
Neoplastic lesion	**Total**	**Mean ± SD**	**Range**	**Total**	**Mean ± SD**	**Range**
Kidney						
Renal tubule adenoma	6/652	0.9% ± 1.3%	0% - 4%	1/650	0.2 ± 0.6 %	0% - 2 %
Renal tubule carcinoma	0/652	-	-	1/650	0.2 ± 0.6 %	0% - 2 %
Renal tubule adenoma or carcinoma	6/652	0.9% ± 1.3%	0% - 4%	2/650	0.3% ± 0.8%	0% - 2%
Liver						
Hepatocellular adenoma	20/653	3.1% ± 2.8%	0% - 8%	9/650	1.4% ± 2.1%	0% - 6%
Hepatocellular carcinoma	8/653	1.2% ± 1.5%	0% - 4%	1/650	0.2% ± 0.6%	0% - 2%
Hepatocellular adenoma or carcinoma	28/653	4.3% ± 2.9%	2% - 9%	10/650	1.5% ± 2.0%	0% - 6%
Mononuclear Cell Leukaemia	356/655	54.4% ± 8.8%	34% - 66%	262/653	40.1% ± 7.2%	30% -54%
Haemangiosarcoma (all organs)	-	-	-	2/653	0.3% ± 0.8%	0% - 2%
Testes: Interstitial cell adenoma	450/655	68.7% ± 8.7%	54% - 83%	-	-	-

B6C3F1 mice were exposed via inhalation to tetrafluoroethylene at 0, 312, 625 or 1250 ppm until week 95-96, when the study was terminated due to reduced survival rates in all exposure groups.

At the 15 month interim assessment, there was a non-statistically significant increase in the incidence in hepatic haemangiosarcomas in males in the high dose group (3/10, 30 %) when compared with the concurrent control (0/10). At study termination, the incidence of hepatic haemangiosarcomas was statistically significantly increased in all treated groups: 0/48 (0 %), 21/48 (44 %), 27/48 (56 %), and 37/48 (77 %) in males and 0/48 (0 %), 27/48 (56 %), 27/47 (57 %) and 34/47 (71 %) in females for the control, low, mid and high dose groups, respectively. The incidence of hepatic haemangiosarcomas observed in this study is also above that observed in historical control males (12/947, 1.3 %) and females (5/937, 0.5 %) from NTP 2-year inhalation studies. Therefore, due to the clear dose response and the low background incidence of hepatic haemangiosarcomas in mice, the increase in this tumour type is considered to be treatment related.

The incidence of haemangiosarcomas in other organs was reported to be low but the study authors note that a few animals with hepatic haemangiosarcomas also displayed haemangiosarcomas in other organs. However, no dose response relationship was observed and it was not clear whether these non-hepatic haemangiosarcomas were metastases or developed concurrently. No haemangiosarcomas in any organ were observed in the control animals. Therefore, the biological significance of the occurrence of haemangiosarcomas in other organs in these animals is unclear.

There was a statistically significant increase in the incidence of hepatic haemangiomas in both sexes in the low dose group and males in the mid dose group: 0/48 (0 %), 10/48 (21 %), 5/48 (10 %) and 2/48 (4 %) in males and 0/48 (0 %), 5/48 (10 %), 2/47 (4 %) and 1/47 (2 %) in females of the control, low, mid and high dose groups, respectively. The incidence of hepatic haemangiomas observed in this study is also above that observed in historical control males (2/947, 0.2 %) and females (1/937, 0.1 %) from NTP 2-year inhalation studies. Although no clear dose response was observed, due to the low background incidence of hepatic haemangiomas in mice, the increase observed in this study is considered to be treatment related. Multiple haemangiomas were also found all treated males and females with the exception of high dose females, however the incidence was only statistically significant in low dose males (7/48, 15 %).

The combined incidence of haemangiomas and haemangiosarcomas was significantly increased in all treated animals. The reported incidences were 0/50, 26/48 (54 %), 30/48 (63 %) and 38/48 (80 %) in males and 0/50,

31/48 (65 %), 28/47 (60 %), 35/47 (74 %) in females of the control, low, mid and high dose groups, respectively.

There was a statistically significant increase in hepatocellular carcinomas in males and females in all dose groups. The reported incidences were 11/48 (23 %), 20/48 (42 %), 33/48 (69 %) and 26/48 (54 %) in males and 4/48 (8 %), 28/48 (58 %), 22/47 (47 %) and 20/47 (43 %) in females of the control, low, mid and high dose groups, respectively. The incidence of this lesion in the treated animals also exceeded the historical control ranges for hepatocellular carcinomas in males (184/947, 19.4 %) and females (103/937, 11 %) in NTP 2-year inhalation studies. The incidence of multiple hepatocellular carcinomas was also statistically significantly increased in all treated animals: 4/48 (8 %), 9/48 (19 %), 9/48 (19 %) and 6/48 (13 %) in males and 0/48 (0 %), 5/48 (10 %), 7/47 (15 %) and 7/47 (15 %) in females of the control, low, mid and high dose groups, respectively.

The incidence of hepatocellular adenomas was significantly increased in mid dose females (20/47, 43 %) when compared with the concurrent control (15/48, 31 %).

The combined incidence of hepatocellular adenomas and carcinomas were also statistically significantly increased in all treated males and females. The reported incidences were 26/50 (52 %), 34/50 (68 %), 39/50 (78 %), 35/50 (70 %) in males and 17/50 (34 %), 33/50 (66 %), 29/50 (58 %) and 28/50 (56 %) in females of the control, low, mid and high dose groups, respectively. The combined incidence of hepatocellular adenomas and carcinomas observed in this study is also above the range observed in historical control male (11 % - 60 %) and female (3% - 54 %) mice from 2-year NTP inhalation studies (As there is a clear dose response in both sexes, it is considered that the increased incidence of these tumours is treatment related.

There was an increase in the incidence of hepatic eosinophilic foci in males in the low dose group and males and females in the mid and high dose groups. The reported incidences were 1/48 (2 %), 6/48 (13 %), 7/48 (15 %) and 7/48 (15 %) in males and 5/48 (10 %), 13/48 (27 %), 12/47 (26 %) and 7/47 (15 %) in females of the control, low, mid and high dose groups respectively. The study authors considered hepatic eosinophilic foci to be a pre-neoplastic lesion.

A statistically significant increase in coagulative multifocal necrosis of the liver was observed in males of the mid (13/48, 27 %) and high (11/48, 23 %) dose groups when compared with the concurrent control (4/48, 8 %). An increase in the incidence of haematopoietic cell proliferation was observed in all treated females, which was statistically significant in the low and high dose groups. The reported incidences were 3/48 (6 %), 19/48 (40 %), 13/47 (28 %) and 15/47 (32 %) for control, low, mid and high dose females, respectively. The study authors note that both lesions are often observed with malignant hepatic neoplasms in mice.

There was a statistically significant increase in histiocytic sarcoma in all organs in all treated males and females, The reported incidences were 0/48 (0 %), 12/48 (25 %), 7/48 (15 %) and 7/48 (15 %) in males and 1/48 (2 %), 21/48 (44 %), 19/47 (40 %) and 18/47 (38 %) in females of the control, low, mid and high dose groups, respectively. The highest incidences were observed in liver and lung, with lower incidence in the spleen, lymph nodes, bone marrow and kidney. The incidence of histiocytic sarcoma in all organs observed in this study is also above that observed in historical control males (6/950, 0.6%) and females (26/941, 2.8%) from NTP 2-year inhalation studies. Therefore, due to the clear dose response and the low background incidence of this tumour in mice, it is considered to treatment related.

The tumour incidences from the mouse NTP study are summarised in table 15 below. Tumour incidences in historical control rats in 2-year NTP inhalation studies are reported in table 16 below.

Table 15: Incidences of neoplastic lesions in mice following 2-year inhalation exposure to tetrafluoroethylene (NTP, 1997).

	Males				Females			
Dose group (ppm)	**0**	**312**	**625**	**1250**	**0**	**312**	**625**	**1250**
Number of animals examined	**48**	**48**	**48**	**48**	**48**	**48**	**47**	**47**
Liver								
Hepatocellular adenoma	17	17	12	20	15	17	20*	15
Hepatocellular carcinoma	11	20**	33**	26**	4	28**	22**	20**
Hepatocellular carcinoma, multiple	4	9**	9**	6*	0	5**	7**	7**
Combined hepatocellular adenoma or carcinoma	26	34**	39**	35**	17	33**	29**	28**
Haemangioma	0	10**	5*	2	0	5*	2	1
Haemangioma, multiple	0	7**	2	1	0	1	1	0
Haemangiosarcoma	0	21**	27**	37**	0	27**	27**	34**
Haemangiosarcoma, multiple	0	16**	17**	18**	0	8**	12**	15**
Combined haemangioma or haemangiosarcoma	0	26**	30**	38**	0	31**	28**	35**
Haematopoietic system								
Histiocytic sarcoma (all organs)	0	12**	7**	7**	1	21**	19**	18**

** Significantly different from the control group (P≤0.05); ** significantly different from the control group (P≤0.01)*

Table 16: Incidences of neoplastic lesions in historical control mice in 2-year NTP inhalation studies as reported in NTP, 1997.

	Males			Females		
Neoplastic lesion	**Total**	**Mean ± SD**	**Range**	**Total**	**Mean ± SD**	**Range**
Liver						
Hepatocellular adenoma	200/947	21.1% ± 11.6%	4% - 46%	114/937	12.2% ± 9.7%	0% - 40%
Hepatocellular carcinoma	184/947	19.4% ± 5.8%	9% - 29%	103/937	11% ± 6.7%	0% - 30%
Hepatocellular adenoma or carcinoma	358/947	37.8% ± 12.5%	11% - 60%	200/937	21.3% ± 11.9%	3% - 54%
Haemangioma	2/947	0.2% ± 0.7%	0% - 2%	1/937	0.1% ± 0.5%	0% - 2%
Haemangiosarcoma	12/947	1.3% ± 1.7%	0% - 6%	5/937	0.5% ± 1.0%	0% - 3%
Haematopoietic system						
Histiocytic sarcoma	6/950	0.6% ± 1.2%	0% - 4%	26/941	2.8% ± 3.1%	0% - 10%

Human data

A cohort mortality study examined the cancer risk in workers exposed to tetrafluoroethylene at six polytetrafluoroethylene production sites across Europe and the USA from 1950 to 2002 (Consonni *et. al*., 2013). All sites handled tetrafluoroethylene and ammonium pentadecafluorooctanoate (APFO). No exposure monitoring data were available. Instead, the exposure assessment was undertaken using a job-exposure matrix based on yearly semi quantitative estimates of tetrafluoroethylene exposure. The number of workers who were "ever exposed" to tetrafluoroethylene was 4,773 and the number "never" exposed amounted was 1,081. Standardised mortality ratios (SMR) were calculated for selected causes of death, including all causes and a number of cancers.

In comparison with national rates, the mortality rate from all causes and all cancers, were lower than expected in the tetrafluoroethylene exposed workers. SMRs were increased for liver, oesophageal, pancreatic and kidney cancers and for leukaemia in the tetrafluoroethylene exposed workers. A non-significant upward trend by cumulative tetrafluoroethylene exposure was observed for liver cancer, but not for kidney cancer and leukaemia.

Table 14: Standardised Mortality Ratios for selected causes of death in workers exposed to TFE (Consonni et. al., 2013)

Cause of death	Observed deaths	Expected deaths	SMR	95% C.I.
All causes	632	821.7	0.77	0.71 - 0.84
All cancers	187	241.6	0.77	0.67 – 0.89
Liver	8	6.3	1.27	0.55 – 2.51
Kidney	10	6.9	1.44	0.69 – 2.65
Leukaemia	12	8.1	1.48	0.77 – 2.59
Pancreas	13	11.3	1.15	0.61 – 1.97
Oesophagus	11	8.9	1.23	0.62 – 2.21

SMR: Standardised mortality ratio; C.I: Confidence interval

It is noted that the calculated SMR estimates in this study had large confidence intervals and therefore there is some uncertainty regarding their reliability. In addition, all production sites also handled APFO and therefore it is not possible to exclude APFO as a cofounding factor. APFO has a harmonised classification as a category 2 carcinogen and a category 1B reproductive toxicant.

The study authors also noted a number of other limitations in the study including the low statistical power to detect an increase in mortality rate for rare cancers, the possible misclassification of tetrafluoroethylene exposure and the semi-quantitative exposure assessment employed in the study. However, the study authors note that none of the potential confounders for liver or renal cancers such as alcohol consumption, hepatitis or tobacco smoking were considered as influencing the observed increases in the occurrence of these cancers in this study.

The increases in SMRs identified in this study related to cancers of the same organs as those observed in the NTP studies in rats and mice (leukaemia and cancers of liver and kidney). However, due to the limitations discussed above a direct correlation between worker exposure to tetrafluoroethylene and the development of cancer cannot be made.

Relevance of the information for human carcinogenicity

Based on the available information, tetrafluoroethylene is not considered to be genotoxic (see section 10.8).

A carcinogenic mode of action has not been definitively identified for the neoplasms identified in the available experimental studies.

The available toxicokinetic data indicates that tetrafluoroethylene is metabolised by glutathione conjugation in the liver and then further metabolised to S-(1,1,2,2-tetrafluoroethyl)-L-cysteine (TFE-Cys). TFE-Cys is activated by β-lyases to toxic species including difluoroacetic acid and difluorothio(no)acetic acid which form covalent adducts with renal cellular proteins leading to nephrotoxicity. It is postulated that this pathway may be responsible for kidney tumours observed in the rodent studies. Glutathione conjugation and subsequent β-lyase activation of tetrafluoroethylene was demonstrated in human liver and kidney fractions *in vitro* and, therefore, it cannot be excluded that this pathway, and possible mechanism of kidney tumour formation, is relevant for humans.

No mechanism of tumour formation has been identified for the other tumour types identified in the experimental studies. Therefore, it cannot be excluded that the increase in the incidence of mononuclear cell leukaemia and in neoplasms of the liver and haematopoietic system observed in rats and mice are relevant for humans.

The available human data, while limited, demonstrated an increase in SMR for cancers of the same organs observed in the animal studies and thus can be used as supporting evidence.

Table 18: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Response in single or both sexes	Confounding effect by excessive toxicity?	MoA and relevance to humans
Rat (Fischer F344/N).	↑ Renal tubule adenoma and carcinoma.	Yes Tumours observed in kidney, liver and blood.	Yes. ↑ Incidence of renal tubule hyperplasia (pre-neoplastic lesion). Renal tubule hyperplasia, adenoma carcinoma are a morphologic continuum.	-	Both sexes.	No. The occurrence of regenerative epithelial changes associated with degenerative nephropathy were distinguished from hyperplasia.	Non-genotoxic MoA assumed. TFE is metabolised in rat by glutathiones and β-lyases to nephrotoxic thiols. While the relevance of this pathway for human toxicity has not been fully investigated, its relevance for humans cannot be excluded. For the remainder of the tumour types, the MoA has not been elucidated and therefore are assumed to be relevant for humans.
	↑ Mononuclear cell leukaemia.		Yes. Mononuclear cell leukaemia (malignant).				
	↑ Hepatocellular adenoma and carcinoma.		Yes. ↑ Incidence of hepatocellular foci (pre-neoplastic lesion). Both adenoma (benign) and carcinoma (malignant) hepatic tumours observed.				
	↑ Hepatic haemangiosarcoma (mid dose females only). Not observed in NTP historical control females.		Yes. Hepatic haemangiosarcomas (malignant).		Female.		
	↑ Interstitial cell adenoma (mid and high dose males).		No.		Male.		
B6C3F1 mice	↑ Hepatocellular adenoma and Carcinoma. ↑ Hepatic haemangiomas. ↑ Hepatic haemangiosarcomas.	Yes. Tumours observed in liver and haematop-oietic system	Yes. ↑ Incidence of adenoma and haemangiomas (benign) and carcinoma and haemangiosarcoma (malignant) hepatic tumours observed.	Yes ↑ Hepatic haemangi-osarcomas in high dose males at 15 months	Both sexes.	No	MoA has not been elucidated and therefore is assumed to be relevant for humans.
	↑ Histiocytic sarcoma (all organs).		Yes. Histiocytic sarcoma (malignant).	-			

In conclusion, the available data in rats and mice demonstrate a statistically and biologically significant increase in the incidence of benign and malignant tumours in multiple organs in both sexes. A mechanism of tumour formation in kidney has been postulated and this mechanism is considered relevant for humans.

The mechanism of tumour formation for the remaining tumour types observed in rats and mice has not been elucidated. However, based on the available data, the relevance for humans cannot be excluded.

10.9.2 Comparison with the CLP criteria

According to Annex I to the CLP Regulation, substances may be classified as category 1A carcinogens *"if they are known to have carcinogenic potential for humans, classification is largely based on human evidence"*. The available cohort mortality study by Consonni *et. al.* (2013) has a number of limitations which do not allow the demonstration of a causal relationship between tetrafluoroethylene exposure and the development of cancer. Therefore, classification in category 1A is not warranted.

Classification as a category 1B carcinogen is generally based on evidence in experimental animals demonstrating "*a causal relationship between the agent and an increased incidence of malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study…or a single study in one species might provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites"* (CLP Annex I, Section 3.6.2.2.3 (b)).

The available experimental carcinogenicity data demonstrate a causal relationship between tetrafluoroethylene exposure in rats and mice and increased incidence of neoplasms. In rats, a statistically and biologically significant increase in the incidence of multiple tumour types in the kidney and liver of both sexes was observed. In addition, an increase in mononuclear cell leukaemia was observed in female rats. In mice, a statistically and biologically significant increase in the incidence of histiocytic sarcoma and in the incidence of multiple tumour types in the liver was observed in both sexes. Both benign (e.g. renal tubule and hepatocellular adenomas and hepatic haemangiomas) and malignant (e.g. renal and hepatocellular carcinomas, hepatic haemangiosarcoma and histiocytic sarcoma) neoplasms were observed in both species and in both sexes. The tumour types observed were considered relevant for humans. Therefore, classification in category 1B is warranted.

It is noted that tetrafluoroethylene is a gas and the available animal carcinogenicity studies were conducted via whole body inhalation. However, based on the available data it is not possible to conclusively prove that cancer is caused only by the inhalation route of exposure. For this reason, the hazard statement H350: May cause cancer, without specifying the route of exposure, is warranted.

According to the CLP Regulation substances may be classified as category 2 carcinogens based on *limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies*. The available experimental information provides evidence of carcinogenicity in multiple organs of both sexes in two species. Therefore, classification in category 2 is not warranted.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Based on the available data, classification of tetrafluoroethylene as carcinogen category 1B is warranted.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Human data

A cohort mortality study examined the cancer risk in workers exposed to TFE at six polytetrafluoroethylene production sites across Europe and the USA from 1950 to 2002 (Consonni et. al., 2013). All sites handled TFE and ammonium pentadecafluorooctanoate (APFO). No exposure monitoring data were available. Instead, the exposure assessment was undertaken using a job-exposure matrix based on yearly semi quantitative estimates of TFE exposure. The number of workers who were "ever exposed" to TFE was 4 773 and the number "never" exposed amounted was 1 081. Standardised mortality ratios (SMR) were calculated for selected causes of death, including all causes and a number of cancers.

The calculated SMR estimates in this study had large confidence intervals and therefore there is some uncertainty regarding their reliability. In addition, all production sites also handled APFO and therefore it is not possible to exclude APFO as a cofounding factor. APFO has a harmonised classification as a category 2 carcinogen and a category 1B reproductive toxicant.

In comparison with national rates, the mortality rate from all causes (combined) and all cancers (combined) were lower than expected in the TFE exposed workers. SMRs were increased for liver (SMR = 1.27; 95 % CI 0.55-2.51), oesophageal (SMR = 1.23; 95 % CI 0.62-2.21), pancreatic (SMR = 1.15; 95 % CI 0.61-1.97) and kidney cancers (SMR = 1.44; 95 % CI 0.69-2.65), and for leukaemia (SMR = 1.48; 95 % CI 0.77-2.59) in the TFE exposed workers. A non-significant upward trend by cumulative TFE exposure was observed for liver cancer, but not for kidney cancer and leukaemia.

Animal studies

Two year carcinogenicity studies (similar to OECD TG 451) are available in which rats and mice were exposed via inhalation to TFE (NTP, 1997).

60 Fischer 344/N rats were exposed via whole-body inhalation to TFE (purity > 98 %) for 6 hours per day, 5 days per week for 104 weeks. 10 males and 10 females were assigned to the 15 month interim evaluation. The target chamber concentrations were at 0, 156, 312 and 625 ppm for males and 0, 312, 625, 1250 ppm for females. The measured chamber concentrations were found to be within 10 % of the range of the nominal concentration. For the kidney, a single section of each kidney was initially prepared for each animal. However, a further six to ten sections at 1 mm intervals were then prepared and assessed for each animal.

At the 15-month interim assessment, no effects on haematological, clinical chemistry or urinalysis parameters was observed. There was an increase in kidney weight in high dose animals. A statistically significant increase in incidences and severity of renal tubule degeneration was observed in males in all dose groups and in females in the mid and high dose groups. Renal tubule hyperplasia was also observed in 1/10 males in the mid and high dose groups and in 1/10 females in the mid dose group. An increase in liver weight was observed in females of the mid and high dose group. In the liver of all dose groups of males, there was also an increased incidence of clear cell foci and in the mid and high dose groups of females an increase of mixed cell foci was observed.

At study termination, the survival rates of the control, low, mid and high dose groups were 17/50, 12/50, 17/50 & 1/50 in males and 28/50, 16/50, 15/50 & 18/50 in females, respectively. There was a decrease in terminal body weight in high dose males and females (slight effect here). The terminal body weight as a percentage of the controls was 99 %, 99 % and 79 % for males and 97 %, 102 % and 91 % for females of the low, mid and high dose groups, respectively.

The only exposure related clinical finding was opacity of the eyes in females of the high dose group observed in 45/50 females (compared with 15/50 in the concurrent control), which was identified microscopically as cataracts.

In rats, a statistically and biologically significant increase in the incidence of multiple tumour types in the kidney and liver of both sexes was observed. In addition, an increase in mononuclear cell leukaemia was observed in female rats.

Detailed information on the incidences of neoplastic and non-neoplastic lesions seen in the kidney, liver and blood is given in Annex I of the CLH report.

Tumour incidences from the rat NTP study are summarised in Table 13 of the CLH report. Tumour incidences in historical control rats in 2-year NTP inhalation studies are reported in Table 14 of the CLH report.

Table 15 of the CLH report*: Incidences of neoplastic lesions in rats following 2-year inhalation exposure to TFE (NTP, 1997).*

	Males				**Females**			
Dose group (ppm)	0	156	312	625	0	312	625	1 250
Number of animals examined	50	50	50	50	50	50	50	50
Kidney								
Single Sections:								
Renal tubule adenoma	0	0	6*	3	0	3	1	3
Renal tubule carcinoma	1	0	2	0	0	0	0	2
Renal tubule adenoma or carcinoma	1	0	6	3	0	3	1	5**
Step Sections:								
Renal tubule adenoma	2	4	3	11**	0	0	2	5*
Renal tubule carcinoma	0	1	0	0	0	0	0	1
Renal tubule adenoma or carcinoma	2	5	3	11**	0	0	2	5*
Single and Step Sections:								
Renal tubule adenoma	2	4	9*	13**	0	3	3	8**
Renal tubule carcinoma	1	1	2	0	0	0	0	3
Renal tubule adenoma or carcinoma	3	5	9	13**	0	3	3	10*
Liver								
Hepatocellular adenoma	3	6	8	5	0	4*	5**	6**
Hepatocellular carcinoma	1	1	10**	3	0	4*	9**	2
Hepatocellular adenoma or carcinoma	4	7	15**	8	0	7**	12**	8**
Haemangiosarcoma	0	0	0	0	0	0	5*	1
Mononuclear Cell Leukaemia	34	43*	38	31	16	31**	23	36**

Testes: Interstitial cell adenoma	39	40	48**	47*	-	-	-	-
Mammary gland: Fibroadenoma	-	-	-	-	22	11*	9**	7**

* Significantly different from the control group (P ≤ 0.05); ** significantly different from the control group (P ≤ 0.01)

***Table 14 of the CLH report**: Incidences of neoplastic lesions in historical control rats in 2-year NTP inhalation studies as reported in NTP, 1997.*

	Males			**Females**		
Neoplastic lesion	Total	Mean ± SD	Range	Total	Mean ± SD	Range
Kidney						
Renal tubule adenoma	6/652	0.9 % ± 1.3 %	0 % - 4 %	1/650	0.2 ± 0.6 %	0 % - 2 %
Renal tubule carcinoma	0/652	-	-	1/650	0.2 ± 0.6 %	0 % - 2 %
Renal tubule adenoma or carcinoma	6/652	0.9 % ± 1.3 %	0 % - 4 %	2/650	0.3 % ± 0.8 %	0 % - 2 %
Liver						
Hepatocellular adenoma	20/653	3.1 % ± 2.8 %	0 % - 8 %	9/650	1.4 % ± 2.1 %	0 % - 6 %
Hepatocellular carcinoma	8/653	1.2 % ± 1.5 %	0 % - 4 %	1/650	0.2 % ± 0.6 %	0 % - 2 %
Hepatocellular adenoma or carcinoma	28/653	4.3 % ± 2.9 %	2 % - 9 %	10/650	1.5 % ± 2.0 %	0 % - 6 %
Mononuclear Cell Leukaemia	356/655	54.4 % ± 8.8 %	34 % - 66 %	262/653	40.1 % ± 7.2 %	30 % -54 %
Haemangiosarcoma (all organs)	-	-	-	2/653	0.3 % ± 0.8 %	0 % - 2 %
Testes: Interstitial cell adenoma	450/655	68.7 % ± 8.7 %	54 % - 83 %	-	-	-

Groups of 58 B6C3F1 mice were exposed via whole body inhalation to TFE (purity > 98 %) for 6 hours per day, 5 days per week for 95-96 weeks. 10 males and 10 females were assigned to the 15 month interim evaluation. The target chamber concentrations were 0, 312, 625, 1 250 ppm (analytical concentrations were within a 10 % range).

At the 15 month interim assessment, no effect on haematological, clinical chemistry or urinalysis parameters was observed. A statistically significant increase in the incidence of renal tubule dilation was observed in males at the mid and high dose and in renal tubule karyomegaly in both sexes in the mid and high dose groups, which occurred in the absence of a change in kidney weight. In the liver, there was an increased incidence of angiectasis in all dosed groups, which was statistically significant in mid-dose males and low-dose females. There was a statistically significant increase in eosinophilic foci in mid and high dose females. There was an increased incidence of hepatic haemangiosarcomas in males in the high dose group (3/10) and in females of the low dose (1/10) when compared with the concurrent controls (0/10). There was also an increased incidence of hepatocellular adenoma and carcinomas in females of all dose groups in comparison to their absence in control females. A single case of histiocytic carcinoma has been observed in one high dose male.

At study termination, the survival rates of the control, low, mid and high dose groups were 38/48, 11/48, 2/48 and 1/48 for males and 36/48, 4/48, 6/48 and 4/48 for females. Due to the reduced survival the study was terminated during week 96.

A statistically and biologically significant increase in the incidence of histiocytic sarcoma and in the incidence of multiple tumour types in the liver was observed in both sexes. Both benign (e.g. renal tubule and hepatocellular adenomas and hepatic haemangiomas) and malignant (e.g. renal and hepatocellular carcinomas, hepatic haemangiosarcoma and histiocytic sarcoma) neoplasms were observed in both species and in both sexes.

Detailed information on the incidences of neoplastic and non-neoplastic lesions seen in the liver and of histiocytic sarcoma (at several organs) is given in Annex I of the CLH report.

Tumour incidences from the mouse NTP study are summarised in Table 15 of the CLH report. Tumour incidences in historical control mice in 2-year NTP inhalation studies are reported in Table 16 of the CLH report.

***Table 15 of the CLH report**: Incidences of neoplastic lesions in mice following 2-year inhalation exposure to TFE (NTP, 1997).*

	Males				**Females**			
Dose group (ppm)	0	312	625	1 250	0	312	625	1 250
Number of animals examined	48	48	48	48	48	48	47	47
Liver								
Hepatocellular adenoma	17	17	12	20	15	17	20*	15
Hepatocellular carcinoma	11	20**	33**	26**	4	28**	22**	20**
Hepatocellular carcinoma, multiple	4	9**	9**	6*	0	5**	7**	7**
Combined hepatocellular adenoma or carcinoma	26	34**	39**	35**	17	33**	29**	28**
Haemangioma	0	10**	5*	2	0	5*	2	1
Haemangioma, multiple	0	7**	2	1	0	1	1	0
Haemangiosarcoma	0	21**	27**	37**	0	27**	27**	34**
Haemangiosarcoma, multiple	0	16**	17**	18**	0	8**	12**	15**
Combined haemangioma or haemangiosarcoma	0	26**	30**	38**	0	31**	28**	35**
Haematopoietic system								
Histiocytic sarcoma (all organs)	0	12**	7**	7**	1	21**	19**	18**

* Significantly different from the control group (P ≤ 0.05); ** significantly different from the control group (P ≤ 0.01)

***Table 16 of the CLH report**: Incidences of neoplastic lesions in historical control mice in 2-year NTP inhalation studies as reported in NTP, 1997.*

	Males			**Females**		
Neoplastic lesion	Total	Mean ± SD	Range	Total	Mean ± SD	Range
Liver						
Hepatocellular adenoma	200/947	21.1 % ± 11.6 %	4 % - 46 %	114/937	12.2 % ± 9.7 %	0 % - 40 %
Hepatocellular carcinoma	184/947	19.4 % ± 5.8 %	9 % - 29 %	103/937	11 % ± 6.7 %	0 % - 30 %
Hepatocellular adenoma	358/947	37.8 % ± 12.5	11 % -	200/937	21.3 % ±	3 % - 54

or carcinoma		%	60 %		11.9 %	%
Haemangioma	2/947	0.2 % ± 0.7 %	0 % - 2 %	1/937	0.1 % ± 0.5 %	0 % - 2 %
Haemangiosarcoma	12/947	1.3 % ± 1.7 %	0 % - 6 %	5/937	0.5 % ± 1.0 %	0 % - 3 %
Haematopoietic system						
Histiocytic sarcoma	6/950	0.6 % ± 1.2 %	0 % - 4 %	26/941	2.8 % ± 3.1 %	0 % - 10 %

The available inhalation repeated-dose toxicity and carcinogenicity studies with TFE identified the kidney, the liver and the haematopoietic system as target organs, confirming the distribution of TFE or its metabolites to these organs.

The available data in rats and mice demonstrate a statistically and biologically significant increase in the incidence of benign and malignant tumours in multiple organs in both sexes. A mechanism of tumour formation in kidney has been postulated and this mechanism is considered relevant to humans.

In vitro and *in vivo* studies indicate that TFE is metabolised by glutathione-S-transferases to *S*-(1,1,2,2-tetrafluoroethyl)glutathione (TFE-GSH) in the liver, which is released into the bile or recirculated to the kidneys where it is further metabolised to *S*-(1,1,2,2-tetrafluoroethyl)-*L*-cysteine (TFE-Cys). TFE-Cys is either activated by β-lyases to toxic species including difluoroacetic acid and difluorothio(no)acetic acid, which form covalent adducts with renal cellular proteins leading to nephrotoxicity. It may also be deactivated by *N*-acetyltransferases to form *N*-acetyl-*S*-(1,1,2,2-tetrafluoroethyl)-*L*-cysteine (TFE-NAc). TFE-NAc may be eliminated in the urine or undergo N-deacetylation, possibly reforming TFE-Cys which can subsequently be activated via β–lyases. In vitro and in vivo studies with TFE-Cys demonstrated similar nephrotoxicity to that observed with TFE and therefore it is postulated that this metabolic pathway is relevant for the renal toxicity observed in rodents. Isolated human proximal tubule cells were shown to be sensitive to TFE-Cys toxicity and therefore it cannot be excluded that this pathway is relevant for humans.

In vitro, glutathione conjugation of TFE was comparable between rats, mice and human hepatic fractions. Renal β-lyase activities were shown to be higher in rat than mouse or human kidney fractions whereas hepatic β-lyase activities were higher in mouse than rat or human liver fractions, which correlate with the target organs in rat and mouse studies. *N*-acetylase transferase activity was comparable in rat, mouse and human kidney fractions.

Workers at a production site which handled a number of organic fluorides including TFE had increased urinary levels of inorganic fluoride. Analysis of urine of rats and mice exposed to 6 000 ppm TFE for 6 hours found an increase in fluoride, cysteine conjugates (either TFE-Cys or TFE-NAc) and difluoroacetic acid. In both species excretion was complete within 48 hours. Similar urinary metabolites were observed when rats or mice were administered TFE-Cys.

The mechanism of tumour formation for the remaining tumour types observed in rats and mice has not been elucidated. However, based on the available data, the relevance for humans cannot be excluded.

The DS considered the tumour types observed as relevant for humans and the classification in category 1B warranted.

It is noted that TFE is a gas and the available animal carcinogenicity studies were conducted via whole

body inhalation. The DS found that based on the available data it is not possible to conclusively prove that cancer is caused only by the inhalation route of exposure. For this reason, the hazard statement H350: May cause cancer, without specifying the route of exposure, is warranted.

Comments received during public consultation

In their comments Industry REACH Consortium (TFE Subgroup) expressed their agreement with the proposed classification and pointed to the self-classification as Carc. 1B. With regard to the requested harmonised classification proposal including the other hazards of the substance the DS in their response declared to limit the CLH proposal on carcinogenicity only. In their comment the cohort study was not judged as supporting the classification proposal. The consortium disagreed with the DS's proposal not to specify the inhalation route due to the physico-chemical properties of TFE.

One Member State commented on the interpretation of the tumour observed in the rat study which was reflected on by the DS. Another Member States agrees with the proposal on category 1B.

Assessment and comparison with the classification criteria

Comparison with the criteria

RAC agrees with the DS's observation that the available experimental carcinogenicity data demonstrate a causal relationship between TFE exposure in rats and mice and increased incidence of neoplasms. In rats, a statistically and biologically significant increase in the incidence of multiple tumour types in the kidney and liver of both sexes was observed. In addition, an increase in mononuclear cell leukaemia was observed in female rats. In mice, a statistically and biologically significant increase in the incidence of histiocytic sarcoma and in the incidence of multiple tumour types in the liver was observed in both sexes. Both benign (e.g. renal tubule and hepatocellular adenomas and hepatic haemangiomas) and malignant (e.g. renal and hepatocellular carcinomas, hepatic haemangiosarcoma and histiocytic sarcoma) neoplasms were observed in both species and in both sexes. The tumour types observed were considered relevant for humans. Therefore, classification as **carcinogen in category 1B** is warranted.

There is no evidence suggesting a genotoxic mode of carcinogenic action.

A mechanism of tumour formation in kidney has been postulated and this mechanism is considered relevant for humans. No mechanism of tumour formation has been identified for the other tumour types identified in rats and mice.

Therefore, it cannot be excluded that the increase in the incidence of mononuclear cell leukaemia and in neoplasms of the liver and haematopoietic system observed in rats and mice are relevant for humans.

RAC concurs with the factors in Table 18 (below) to be taken into consideration for this hazard assessment.

***Table 18 of the CLH report**: Compilation of factors to be taken into consideration in the hazard assessment*

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Response in single or both sexes	Confounding effect by excessive toxicity?	MoA and relevance to humans
Rat (Fischer F344/N).	↑ Renal tubule adenoma and carcinoma.	Yes Tumours observed in kidney, liver and blood.	Yes. ↑ Incidence of renal tubule hyperplasia (pre-neoplastic lesion). Renal tubule hyperplasia, adenoma carcinoma are a morphologic continuum.	-	Both sexes.	No The occurrence of regenerative epithelial changes associated with degenerative nephropathy were distinguished from hyperplasia.	Non-genotoxic MoA assumed. TFE is metabolised in rat by glutathiones and β-lyases to nephrotoxic thiols. While the relevance of this pathway for human toxicity has not been fully investigated, its relevance for humans cannot be excluded. For the remainder of the tumour types, the MoA has not been elucidated and therefore are assumed to be relevant for humans.
	↑ Mononuclear cell leukaemia.		Yes. Mononuclear cell leukaemia (malignant).				
	↑ Hepatocellular adenoma and carcinoma.		Yes. ↑ Incidence of hepatocellular foci (pre-neoplastic lesion). Both adenoma (benign) and carcinoma (malignant) hepatic tumours observed.				
	↑ Hepatic haemangio sarcoma (mid dose females only). Not observed in NTP historical control females.		Yes. Hepatic haemangiosarcomas (malignant).		Female.		
	↑ Interstitial cell adenoma (mid and high dose males).		No		Male.		
B6C3F1 mice	↑ Hepatocellular adenoma and Carcinoma ↑ Hepatic haemangiomas.	Yes. Tumours observed in liver and haematop-oietic	Yes. ↑ Incidence of adenoma and haemangiomas (benign) and carcinoma and haemangiosarcoma	Yes ↑ Hepatic haemangiosarcomas in high dose males at 15 months	Both sexes.	No	MoA has not been elucidated and therefore is assumed to be relevant

	↑ Hepatic haemangio sarcomas.	system	(malignant) hepatic tumours observed.				for humans.
	↑ Histiocytic sarcoma (all organs).		Yes. Histiocytic sarcoma (malignant).	-			

The available human data, while limited, demonstrated an increase in SMR for cancers of the same organs observed in the animal studies and according to the DS thus can be used as supporting evidence.

RAC agrees with the DS that classification as carcinogen, category 1B is warranted as according to CLP Annex I, Section 3.6.2.2.3., the experimental data on animals are demonstrating "a causal relationship between the agent and an increased incidence of malignant neoplasms in (a) two or more species of animals".

Category 1A classification is not supported as the available cohort study due to its limitations does not allow to concluding on a causal relationship between TFE exposure and the development of tumours.

Category 2 should be considered appropriate if evidence of carcinogenicity in human studies or in animal studies is limited. The available information providing (clear) evidence of carcinogenicity in multiple organs of both sexes in two species does not support category 2.

RAC takes note of the proposal of the DS not to specify the route of exposure. TFE is a gas and the available information is only from whole body inhalation studies on rats and mice (where dermal or oral uptake may have contributed to the systemic availability to an unknown extent). No data on dermal/oral absorption rates are available and exposure via other routes cannot be excluded due to the lack of data.

Consonni *et al*. (2013) stated that the inhalation exposure is the only relevant route at the workplace (without having assessed a possible contribution via dermal exposure). IARC in their monograph (No 110) assessed the exposure of the general population as very low due to its flammability, thus direct dermal or oral exposure to the gaseous form may be considered as nonsignificant. TFE is not detectable in the polymerised products, but may be released (in particulate fumes) when e.g. coated pans are heated at very high temperatures. RAC agrees that the inhalation route should be considered as the most relevant route of exposure. Although TFE is a gas with high vapour pressure (32 395 hPa), other physicochemical properties such as slight water solubility (110 mg/L) and a $Log K_{ow}$ of 1.21 suggest that absorption via other routes of exposure cannot be excluded. Therefore, taking also into account the lack of data for the dermal/oral route, RAC in line with the DS's proposal and taking into account the CLP Annex I provisions (route of exposure is stated only where it is conclusively proven that no other routes of exposure cause the hazard) proposes that the classification should not be limited to the inhalation route.

RAC agrees with the DS that **classification as carcinogen, category 1B is warranted without any route specified.**

Specific concentration limit

The DS did not consider SCL setting. However, RAC considers appropriate to discuss a SCL for TFE.

Based on Dybing *et al.* (1997), estimates of potency defined as the daily dose (in mg/kg bw) inducing a tumour incidence of 25% upon lifetime exposure (T_{25}) values were established and compared to the guidance level given in EC, 1999. The lowest dose at which significant tumour responses were observed was 312 ppm TFE in the carcinogenicity studies on rat and mouse (NTP, 1997). Tumours with high spontaneous incidences were regarded as of less relevance for the SCL calculation. The following table shows the calculated T_{25} values and the resulting potency class for the remaining tumour types in order to identify the lowest T_{25} value.

Table*: SCL calculation*

Species/ sex	Tumour	Lowest dose with significant tumour response (ppm)	Net increase of incidence vs. control (%)	Dose corrected for 25% incidence (ppm)	Dose correction for 7 days of treatment (x(5/7))	Time correction (rat/none; mouse x(96/104 wks)	Conversion ppm to mg/m³ (1 ppm TFE = 4.088 mg/m³)(25 C)	6 hour respiratory volume (m³/kg bw	Corres ponding dose mg/kg bw for the SCL setting	Potency (EC, 1999)
Rat/ males	Liver/ combined adenoma and carcinoma	312	20	390	289	-	1139	0.29 (rat)	**330**	low
Rat/ females	Liver/ combined adenoma and carcinoma	312	14	557	398	-	1627	0.29 (rat)	472	low
Mouse/ males	Combined haemangioma or haemangio-sarcoma	312	26	300	214	198	809	0.5 (mouse)	404	low
Mouse/ females	Combined haemangioma or haemangio-sarcoma	312	31	252	180	166	678	0.5 (mouse)	339	low
Mouse/ males	Histiocytic sarcoma (all organs)	312	12	650	464	429	1.752	0.5 (mouse)	876	low
Mouse/ females	Histiocytic sarcoma (all organs)	312	20	390	279	257	1.051	0.5 (mouse)	526	low

The lowest T25 value (from combined adenoma and carcinoma of the liver of male rats) corresponds

to 330 mg/kg bw. This T 25 value is above 100 mg/kg bodyweight/day. This value could be indicative of a low potency of the substance. RAC takes the multiplicity of tumours and the short latency time (until first tumour occurrence) into account which contradicts a downgrading of the potency group. RAC concludes that the GCL should remain.

Supplemental information - In depth analyses by RAC

Analyses (Considerations in addition to factors considered by the DS)

Dose-dependency of tumours

Rat study

Dose-dependency was seen for kidney tumours (mainly due to adenomas) in high dose male and female rats. Significant increases of liver tumours were seen in mid dose male rats and all dose levels of female rats. Lack of dose dependency for liver tumours in high dose male rats could be related to lower survival rates. Adjustment for intercurrent mortalities (Table 10 of the NTP study) revealed a dose-dependent increase for liver tumours in all dose groups of male rats, the dose-dependency was less clear for high dose females when adjusted for the survival time.

Although survival rates were lower in female rats of all dose groups, body weight was only slightly lower in high dose female rats (after week 75) than in controls. As no other sign of clinical toxicity (except increased incidences of cataracts in high dose females) were seen and reduced survival rates was not linked to significantly lower body weights for the females, the reduced survival rates may be secondary to the degeneration of renal tubules (a target site relevant for the postulated mode of carcinogenic action) and/or tumour burden.

Dose dependency is less clear for the mononuclear cell leukaemia. Causal-relationship is weak for the male rats and cannot be excluded for the female rats taking into account the relatively high incidences in control groups and no clear dose-relationship, also after adjustment for survival.

Haemangiosarcomas found to be significantly increased in mid dose females only were considered as treatment-related by the DS. Despite a lack of further increase at the highest dose (only 1/50) the facts that this tumour is rarely found in controls and that there was a significant increase of angiectasis (a lesion that could be associated to hepatocellular tumours) in all dose groups of female rats are supporting the interpretation of a treatment-related effect.

Mouse study

Low survival rates in all dosed groups leading to termination of the study after week 96 set limitations on the analysis of dose-dependency. Adjustment of tumour rates for survival has only been done for the histiocytic sarcomas (Table 20 of the NTP report).

No clear treatment-related effect on the incidence of liver cell adenomas were seen in the dose groups of both sexes. In contrast, high incidence rates of hepatocellular carcinomas were seen in all dose groups of male and female mice.

Dose-related increased incidences of kidney adenomas were seen in mid and high dose groups of male mice and high dose group of female mice.

Histiocytic sarcomas (a malignant tumour with histiocyte-like cells, occurring in various organs) were significantly increased in all dose groups of male and female mice. Adjustment for survival (Table 20 of the NTP study) demonstrates high incidences at low and mid doses and peak incidences of 65.8

(male mice) and 78.2 % (female mice) at high dose while this tumour occurs rarely spontaneously.

Significant increases of angiectasis and haemangiomes/haemangiosarcomas in the liver and increased haematopoietic cell proliferation in the spleen were observed in male and female mice of all dose groups, thus supporting also the interpretation of a treatment-related genesis of haemangiosarcomas seen in the female rat. Haemangiomas/angiocarcomas are absent in male and female control groups of mice.

Interestingly, increased incidences of karyomegaly of renal tubular cells were observed in male mice of all dose groups and in female mice of mid and high dose groups. These lesions were characterised as enlarged, pleomorphic, vesicular, and had large hyperchromatic prominent nuclei and may indicate early loss of control of normal cell growth. It is rarely seen in control animals and could be associated to tumour development. However, although seen in mice after 13 weeks, 15 months and 96 weeks of treatment no kidney tumour developed.

Tumours were seen at doses without nonspecific toxicity or body weight effects

Rat study

Increased incidences of liver tumours were also seen at doses without any effect on the body weight (in mid dose males and in low and mid dose females).

10.10 Reproductive toxicity

Not evaluated in this dossier.

10.11 Specific target organ toxicity-single exposure

Not evaluated in this dossier.

10.12 Specific target organ toxicity-repeated exposure

No classification proposed.

The repeated dose toxicity studies reported below are provided only as supporting information for the carcinogenicity assessment.

Table19: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Non-guideline: 16-day repeated dose inhalation study. Fischer 344/N rats. 5 rats/sex/dose. Haematology, clinical biochemistry, gross necropsy: organ weights of	TFE (purity > 99 %). Whole body inhalation. 0, 312, 625, 1250, 2500 and 5000 ppm 6 hours/ day, 5 days/week.	↓ body weight in females at 5000 ppm. ↑ Relative liver weight in males at ≥ 312 ppm; ↑ absolute liver weight in males at 625 and 2500 ppm. ↑ Absolute kidney weights in males at ≥ 312 ppm and females at ≥ 1250 ppm; ↑ relative kidney weight in males at ≥ 312 ppm and females at ≥ 2500 ppm.	NTP, 1997.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
brain, heart, kidney, liver, lung, testes and thymus. Histopathological examination of animals at 0 and 5000 ppm. GLP compliant.		↑ incidence of renal tubule degeneration in males at ≥ 625 ppm and females at ≥ 1250 ppm.	
Non-guideline: 16-day repeated dose inhalation study. B6C3F1 mice. 5 mice/sex/dose. Haematology, clinical biochemistry, gross necropsy: organ weights of brain, heart, kidney, liver, lung, testes and thymus. Histopathological examination of animals at 0 and 5000 ppm. GLP compliant.	TFE (purity ≥ 99 %). Whole body inhalation. 0, 312, 625, 1250, 2500 and 5000 ppm 6 hour/ day, 5 days/week.	↑ Absolute and relative liver weights in females at 2500 ppm; ↑ absolute kidney weight in females at 5000 ppm: ↑ relative kidney weight in females at 2500 ppm. ↑ incidence of renal tubule karyomegaly in males at ≥ 250 ppm and in females at ≥ 2500 ppm.	NTP, 1997.
Non-guideline: Subacute inhalation toxicity. Crl:CD rats. 10/sex/dose. GLP compliant. Limited information reported in the registration dossier.	TFE (unknown purity). Whole body inhalation. 0, 101, 500, 991 and 2489 ppm. 6 hour/day, 5 days/week for 12 exposure days during a 2 week period. 14-day recovery period..	↑ Absolute and relative liver weight at ≥ 991 ppm; ↑ Absolute and relative kidney weight at ≥ 991ppm. Mild swelling of renal tubular epithelial cells, dilation of tubular lumen and cellular degeneration at 2489 ppm No significant observations at the end of the 14-day recovery period.	ECHA, 2018.
Similar to OECD 413: Subchronic inhalation toxicity. F344/N rats. 10/sex/dose. GLP compliant.	TFE (purity ≥ 99 %). Whole body inhalation. 0, 312, 625, 1250, 2500 and 5000 ppm 6 hour/ day, 5 days/week for 13 weeks.	↓ body weight in males and females at 5000 ppm. ↓ haematocrit, haemoglobin and erythrocyte count in males and females at 5000 ppm. ↑ incidence of proteinuria in males at ≥ 312 ppm and females at ≥ 2500 ppm. ↑ absolute and relative liver weight in males and females at 5000 ppm; ↑ absolute and relative kidney weight in males at ≥ 1250 ppm and females at ≥ 625 ppm; ↑ absolute and relative heart weight in males at ≥ 1250 ppm. ↑ incidence of renal tubule degeneration in males at ≥ 625 ppm and in females at ≥ 2500 ppm.	NTP, 1997.
Similar to OECD 413: 90-day (subchronic) inhalation toxicity study.	TFE (unknown purity). Whole body inhalation.	↓ body weight in males and females at 1989 ppm. ↑ glutamic-pyruvic transaminase activity in	ECHA, 2018.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Crl: CD rats. 15/sex/dose. GLP compliant. Limited details reported in the registration dossier. No tables of results.	0, 203, 606 and 1989 ppm for 6 hour/day, 5 days/week for 13 weeks.	females at 1989 ppm; ↓ serum albumin in females at ≥ 606 ppm. ↑ BUN in females at 1989 ppm; ↑ urinary pH in males at 1989 ppm; ↑ urinary volume in males and females at ≥ 606 ppm; ↓ urine osmolarity in males and females at 1989 ppm; ↓ urine creatinine in females at ≥ 203 ppm. ↑ relative kidney weight in males and females at 1989 ppm; ↑ absolute kidney weight in females at 1989 ppm; ↑ liver weight in females at ≥ 606 ppm. ↑ Incidence of nephrosis in males and females at ≥ 606 ppm.	
Similar to OECD 413: 90-day (subchronic) inhalation toxicity study. Lak:LVG(SUR) hamster. 15/sex/dose. GLP compliant. Limited details reported in the registration dossier. No tables of results.	TFE (unknown purity). Whole body inhalation. 0, 203, 606 and 1989 ppm for 6 hour/day, 5 days/week for 13 weeks.	↑ urinary fluoride in males and females at ≥ 203 ppm. ↑ incidence of testicular atrophy in males at 1989 ppm.	ECHA, 2018.
Similar to OECD 413: 90-day (subchronic) inhalation toxicity study B6C3F1 mice. 10/sex/dose. GLP compliant.	TFE (purity > 99 %). Whole body inhalation. 0, 312, 625, 1250, 2500 and 5000 ppm 6 hour/day, 5 days/week for 13 weeks.	↓ haematocrit, haemoglobin and erythrocyte count in males and females at 5000 ppm. ↑ incidence of polyuria in males and females at ≥ 2500 ppm. ↑ incidence of karyomegaly of the renal tubule epithelial cells in males and females at ≥ 1250 ppm.	NTP, 1997.

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

A number of inhalation repeated dose toxicity studies with tetrafluoroethylene are available with exposure periods of between 14 and 90 days. In a 14- and 16-day studies in rats, increased kidney weights were observed in males from 312 ppm and females from 1250 ppm and renal tubule degeneration in males from 625 ppm and females from 1250 ppm. Liver weights were also increased in both sexes. In a 16-day study in mice, kidney weights were comparable with control. However, increased incidence of renal tubule epithelial cell karyomegaly, located in the inner renal cortex, was observed in males and females. Increased liver weights were reported in male rats and female mice from 5000 ppm, but this was not accompanied by histopathological findings.

In a 90-day study in rats, an increase in kidney weight was reported in male rats from 1250 ppm and female rats from 625 ppm. An increased incidence of renal tubule degeneration was observed in males from 625 ppm and in females from 2500 ppm, with the same etiology as observed in the 16-day study. A concentration-dependent proteinuria was observed in all exposed males and in females from 2500 ppm, which may be consistent with renal tubular degeneration. Alternation of haematocrit, haemoglobin and

erythrocyte count was observed in males and females which the study authors characterised as a normocytic, normochromic and non-responsive anaemia. In a 90-day study in mice, polyuria was observed in males and females at 2500 and 5000 ppm. An increased incidence of karyomegaly of the renal tubule epithelial cells was observed in males and females from 1250 ppm, with the same etiology as observed in the 16-day study. Similar to the 90-day rat study, a normocytic, normochromic and non-responsive anaemia was observed in both sexes. In a 90-day study in hamsters, no effects on kidney or liver were reported. An increased incidence of testicular atrophy was observed in males at 1989 ppm.

10.12.2 Comparison with the CLP criteria

Not evaluated as part of this dossier.

The information is provided as supportive information for the carcinogenicity assessment (see section 10.9).

10.12.3 Conclusion on classification and labelling for STOT RE

Not evaluated as part of this dossier.

The information is provided as supportive information for the carcinogenicity assessment (see section 10.9).

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS provided information on repeated dose toxicity studies as supportive information (without comparison with CLP criteria).

Table 19 of the CLH report summarises the results from a number of inhalation repeated dose toxicity studies with TFE with exposure periods of between 14 and 90 days. In the 14- and 16-day studies in rats, increased kidney weights were observed in males from 312 ppm and females from 1 250 ppm and renal tubule degeneration in males from 625 ppm and females from 1 250 ppm. Liver weights were also increased in both sexes. In a 16-day study in mice, kidney weights were comparable with control. However, increased incidence of renal tubule epithelial cell karyomegaly, located in the inner renal cortex, was observed in males and females. Increased liver weights were reported in male rats and female mice from 5 000 ppm, but this was not accompanied by histopathological findings.

In a 90-day study in rats, an increase in kidney weight was reported in male rats from 1 250 ppm and female rats from 625 ppm. An increased incidence of renal tubule degeneration was observed in males from 625 ppm and in females from 2 500 ppm, with the same etiology as observed in the 16-day study. A concentration-dependent proteinuria was observed in all exposed males and in females from 2 500 ppm, which may be consistent with renal tubular degeneration. Alteration of haematocrit, haemoglobin and erythrocyte count was observed in males and females which the study authors characterised as a normocytic, normochromic and non-responsive anaemia. In a 90-day study in mice, polyuria was observed in males and females at 2 500 and 5 000 ppm. An increased incidence of karyomegaly of the renal tubule epithelial cells was observed in males and females from 1 250 ppm, with the same etiology as observed in

the 16-day study. Similar to the 90-day rat study, a normocytic, normochromic and non-responsive anaemia was observed in both sexes. In a 90-day study in hamsters, no effects on kidney or liver were reported. An increased incidence of testicular atrophy was observed in males at 1 989 ppm.

10.13 Aspiration hazard

Not evaluated in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in this dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this dossier.

13 ADDITIONAL LABELLING

Not applicable.

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15 ANNEX I

15.1 Detailed summary of NTP rat carcinogenicity study (NTP, 1997)

In a carcinogenicity study, comparable to OECD 451, groups of 60 Fischer 344/N rats were exposed via whole body inhalation to tetrafluoroethylene (purity > 98 %) for 6 hours per day, 5 days per week for 104 weeks. 10 males and 10 females were assigned to the 15 month interim evaluation. The target chamber concentrations were at 0, 156, 312 and 625 ppm for males and 0, 312, 625, 1250 ppm for females. Animals were observed twice daily and clinical findings were recorded monthly until 13 weeks before necropsy and twice monthly thereafter. Body weights were recorded weekly until week 13, monthly thereafter until thirteen weeks before necropsy, when they were recorded every two weeks. All animals were subject to necropsy. At the 15 month interim evaluation kidney, liver and lung were weighed and a haematological and clinical chemistry analysis and urinalysis were performed. Complete histopathological examination was performed on all animals at study termination. For the kidney, a single section of each kidney was initially prepared for each animal. However, a further six to ten sections at 1 mm intervals were then prepared and assessed for each animal.

Chamber concentrations were found to be within 10% of the acceptable range for the duration of the study.

At the 15 month interim assessment, no effect on haematological, clinical chemistry or urinalysis parameters was observed. There was an increase in kidney weight in high dose animals. A statistically significant increase in renal tubule degeneration was observed in males in all dose groups and in females in the mid and high dose groups. Renal tubule hyperplasia was also observed in 1/10 males in the mid and high dose groups and in 1/10 females in the mid dose group. An increase in liver weight was observed in females of the mid and high dose group. In the mid and high dose groups, there was also an increased incidence of clear cell foci in the liver in males and in mixed cell foci in the liver in females.

At study termination, the survival rates of the control, low, mid and high dose groups were 17/50, 12/50, 17/50 & 1/50 in males and 28/50, 16/50, 15/50 & 18/50 in females, respectively. There was a decrease in terminal body weight in high dose males and females. The terminal body weight as a percentage of the controls was 99 %, 99 % and 79 % for males and 97 %, 102 % and 91 % for females of the low, mid and high dose groups, respectively.

Table 20: Mean body weights and survival in rats at study termination (NTP, 1997)

	Males				**Females**			
Dose group (ppm)	**0**	**156**	**312**	**625**	**0**	**312**	**625**	**1250**
Number of animals	50	50	50	50	50	50	50	50
Average terminal body weight (g)	454	452	450	359	333	323	340	304
Number of animals surviving to study termination (week 103)	17	12	17	1	28	16	15	18

The only exposure related clinical finding was opacity of the eyes in females of the high dose group observed in 45/50 females (compared with 15/50 in the concurrent control), which the identified microscopically as cataracts.

A statistically significant increase in renal tubule adenomas was observed in males at the mid dose and a non-statistically significant increase in all treated females. The incidences were 0/50, 0/50, 6/50 and 3/50 in males and 0/50, 3/50, 1/50 and 3/50 in females of the control, low, mid and high dose groups, respectively. When renal adenomas and carcinomas were considered together, there was a statistically significant increase in high dose females. In order to further assess the renal lesions, additional step sections of the kidney were prepared and analysed. From this extended evaluation, a statistically significant increase in renal tubule adenoma was observed in high dose male and females. The incidences were 2/50, 4/50, 3/50 and 11/50 in males and 0/50, 0/50, 2/50 and 5/50 in females of the control, low, mid and high dose groups, respectively.

When the standard and extended sections were considered together, there was a statistically significant increase in renal tubule adenomas in mid (9/50) and high (13/50) dose males and high dose females (8/50) when compared with the concurrent control (2/50 in males and 0/50 in females). The incidence of renal adenomas in the historical control data from NTP 2-year inhalation studies was 0.9% (6/652) for males and 0.6% (1/650) for females. Therefore, the dose dependent increase in renal adenomas observed in this study is considered to be related to tetrafluoroethylene treatment.

Table 21: Incidence of neoplastic lesions in the kidney in rats (NTP, 1997)

	Males				**Females**			
Dose group (ppm)	**0**	**156**	**312**	**625**	**0**	**312**	**625**	**1250**
Number of animals examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Single Sections:								
Renal tubule adenoma	0	0	6*	3	0	3	1	3
Renal tubule carcinoma	1	0	2	0	0	0	0	2
Renal tubule adenoma or carcinoma	1	0	6	3	0	3	1	5**
Step Sections:								
Renal tubule adenoma	2	4	3	11**	0	0	2	5*
Renal tubule carcinoma	0	1	0	0	0	0	0	1
Renal tubule adenoma or carcinoma	2	5	3	11**	0	0	2	5*
Single and Step Sections:								
Renal tubule adenoma	2	4	9*	13**	0	3	3	8**
Renal tubule carcinoma	1	1	2	0	0	0	0	3
Renal tubule adenoma or carcinoma	3	5	9	13**	0	3	3	10*

** Significantly different from the control group (P≤0.05); ** significantly different from the control group (P≤0.01)*

An increased incidence of minimal to mild hyperplasia of the renal tubule was observed in high dose males and females. The study authors describe this hyperplasia as a focal lesion of minimal to mild severity, consisting of tubules which were dilated up to twice the normal diameter and lined with increased numbers of tubule epithelial cells. The cells within the hyperplastic lesions were reported to be generally similar to normal tubule cells but sometimes stained more basophilic than normal cells. The study authors distinguished this type of hyperplasia from regenerative epithelial changes often seen with nephropathy and thus considered it to be a pre-neoplastic lesion.

Renal tubule degeneration primarily located in the corticomedullary junction, was observed in all treated males and females at the mid and high dose groups. The incidences were 2/50, 20/50, 50/50 and 49/50 in males and 0/50, 0/50, 35/50 and 46/50 in females of the control, low, mid and high dose groups respectively. The study authors distinguished this from chronic progressive nephropathy due to its location at the corticomedullary junction and lack of thickened basement membranes, interstitial fibrosis, inflammatory infiltrates and protein cases in the tubules of the cortex and medullary which are typically observed with chronic progressive nephropathy. Therefore, the renal tubule degeneration observed was considered to be related to tetrafluoroethylene treatment.

Table 22: Incidence of non- neoplastic lesions in the kidney in rats (NTP, 1997)

	Males				Females			
Dose group (ppm)	**0**	**156**	**312**	**625**	**0**	**312**	**625**	**1250**
15 month interim evaluation								
Number of animals examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Nephropathy	10 (1.8)	10 (1.8)	10 (1.6)	10 (2.4)	8 (1.0)	9 (1.0)	9 (1.2)	10 (1.0)
Renal tubule degeneration	1 (1.0)	8** (1.0)	10** (2.0)	10** (3.0)	0	0	10** (2.0)	10** (2.6)
Renal tubule hyperplasia	0	0	1 (1.0)	1 (1.0	0	0	1 (1.0)	0
2-year study								
Number of animals examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Nephropathy	49 (2.3)	50 (1.9)	50 (2.7)	50 (3.5)	48 (1.7)	46 (1.5)	48 (1.7)	47 (2.0)
Renal tubule degeneration	2 (1.0)	20** (1.1)	50** (2.3)	49** (3.6)	0	0	35** (1.3)	46** (2.0)
Renal tubule hyperplasia	1 (1.0)	1 (1.0)	1 (4.0)	6*(1.3)	1 (2.0)	3 (1.7)	6 (1.2)	12 (1.8)

** Significantly different from the control group (P≤0.05); ** significantly different from the control group (P≤0.01)*

(#) Average severity grade of lesion: 1 = minimal; 2 = mild, 3 = moderate; 4 = marked

There was a statistically significant increase in the incidence of hepatocellular adenomas in females in all dose groups, the incidences being 0/50 (0 %), 4/50 (8 %), 5/50 (10 %) and 6/50 (12%) for the control, low, mid and high dose groups, respectively. The incidence of hepatocellular adenomas in historical control females in NTP 2-year inhalation studies is reported to be in the range of 0% - 6%. Therefore, the increase in hepatocellular adenomas observed in females in this study is considered to be treatment related. An increased incidence of hepatocellular adenomas in treated males was also observed although not statistically significant. However, it was noted that the incidence of hepatocellular adenomas in control males was 3/50 (6 %) which was above the incidence observed in the male historical control range (0 to 4 %) in NTP 2-year inhalation studies and so the biological significance of the increase in hepatocellular adenomas in treated males is unclear.

There was a statistically significant increase in hepatocellular carcinomas in mid dose males (10/50) and low (4/50 and mid (9/50) dose females. When hepatocellular adenoma and carcinomas were combined, there was a statistically significant increase in mid dose males (10/50) and all treated females (4/50, 9/50 and 2/50). The study authors commented that the reduced incidence of hepatocellular neoplasms in high dose groups could be due to the reduced survival rates in these animals.

The incidence of hepatic haemangiosarcomas was statistically significantly increased in mid dose females (5/50, 10 %) when compared with the concurrent control (0/50). The incidence of hepatic haemangiosarcomas observed in this study is above that observed in the historical control range for liver haemangiosarcomas (0/50) and haemangiosarcomas in all organs (2/653, 0.3 %) for NTP 2-year inhalation studies. Since haemangiosarcomas are rare malignant tumours in rats, the increased incidence observed in this study is considered related to tetrafluoroethylene exposure.

Table 23: Incidence of neoplastic lesions in the liver in rats (NTP, 1997)

	Males				Females			
Dose group (ppm)	**0**	**156**	**312**	**625**	**0**	**312**	**625**	**1250**
Number of animals examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Hepatocellular adenoma	3	6	8	5	0	4*	5**	6**
Hepatocellular carcinoma	1	1	10**	3	0	4*	9**	2
Hepatocellular adenoma or carcinoma	4	7	15**	8	0	7**	12**	8**
Haemangiosarcoma	0	0	0	0	0	0	5*	1

** Significantly different from the control group (P≤0.05); ** significantly different from the control group (P≤0.01)*

There was an increased incidence in hepatocellular foci. Eosinophilic foci were observed in all treated males and in females in the high dose group. Basophilic and mixed cell foci were observed in in males in the mid and high dose groups and mixed cell foci in females of the high dose group. While foci can occur spontaneously in rats, they are considered also to be potentially pre-neoplastic lesions (NTP, 2018).

The incidence of hepatic cystic degeneration was statistically significantly increased in males: 17/50, 39/50, 35/50 and 32/50 for the control, low, mid and high dose, respectively. The study authors report that while hepatic cystic degeneration occurs spontaneously at low incidences in aging rats, they are common following exposure to hepatocarcinogens. Therefore, it cannot be excluded that these lesions were as a result of tetrafluoroethylene exposure. In females the incidence of hepatic angiectasis was statistically significantly increased in all treated groups: 0/50, 9/50, 9/50 and 14/50 for the control, low, mid and high dose respectively and the study authors note that these lesions are occasionally associated with hepatocellular neoplasms.

Table 24: Incidence of non- neoplastic lesions in the liver in rats (NTP, 1997)

	Males				Females			
Dose group (ppm)	**0**	**156**	**312**	**625**	**0**	**312**	**625**	**1250**
2-year study								
Number of animals examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Basophilic Focus	22	19	33*	29**	41	38	41	37
Clear Cell Focus	7	8	11	3	10	3	12	9
Eosinophilic Focus	3	18**	22**	19**	1	4	5*	4
Cystic Degeneration	17	39**	35**	32**	1	4	1	3
Mixed Cell Focus	5	5	16**	13**	12	14	16	18*
Angiectasis	0	3	2	3	0	9**	9**	14**

** Significantly different from the control group (P≤0.05); ** significantly different from the control group (P≤0.01)*

The incidence of mononuclear cell leukaemia was statistically significantly increased in males of the low dose group. The incidence in the control, low, mid and high dose groups was 34/50, 43/50, 38/50 and 31/50, respectively. The incidence of this lesion in control males (68 %) exceeded the historical control range for all types of leukaemia (34 – 66 %) in NTP 2-year inhalation studies. Also, the reduced survival rate in the high dose group makes the interpretation of the increase at the low dose group difficult. Therefore, the biological significance of the increase in low dose males is unclear. In females, there was an increased incidence of mononuclear cell leukaemia in all treated females, which was statistically significant at the low and high dose. The incidences in the control, low, mid and high dose groups were 16/50, 31/50, 23/50 and 36/50,

respectively. The incidence of mononuclear cell leukaemia in control females was within the historical control range observed in female rats in 2-year NTP inhalation studies (30 % - 54 %) and therefore the statistically significant increased incidence of this lesion in low and high dose females is considered to be related to tetrafluoroethylene exposure.

A significant increase in the incidence of testicular interstitial cell adenomas was observed in males of the mid (96 %) and high (94 %) dose when compared with both the concurrent (78 %) and historical control range for NTP 2-year inhalation studies (54 – 83 %). It is noted that this type of tumour is common in aging F334/N rats and therefore the biological significant of the increase in exposed males is unclear.

The incidence of fibroadenoma in the mammary gland was decreased in all treated females when compared with the concurrent control. The incidence in the control, low, mid and high dose groups were 22/50, 11/50, 9/50 and 7/50, respectively. It is noted that fibroadenomas are common benign tumours in mammary gland of female F344/N rats and therefore the biological significance decreased incidence in this study is unclear.

Table 25: Incidence of other neoplastic lesions in rats (NTP, 1997)

	Males				**Females**			
Dose group (ppm)	**0**	**156**	**312**	**625**	**0**	**312**	**625**	**1250**
Number of animals examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Haemangiosarcoma	0	0	0	0	0	0	5*	1
Mononuclear Cell Leukaemia	34	43*	38	31	16	31**	23	36**
Testes: Interstitial cell adenoma	39	40	48**	47*	-	-	-	-
Mammary gland: Fibroadenoma	-	-	-	-	22	11*	9**	7**

** Significantly different from the control group (P≤0.05); ** significantly different from the control group (P≤0.01)*

Table 26 Incidences of neoplastic lesions in historical control rats in 2-year NTP inhalation studies as reported in NTP, 1997.

	Males			**Females**		
Neoplastic lesion	**Total**	**Mean ± SD**	**Range**	**Total**	**Mean ± SD**	**Range**
Kidney						
Renal tubule adenoma	6/652	0.9% ± 1.3%	0% - 4%	1/650	0.2% ± 0.6%	0% - 2%
Renal tubule carcinoma	0/652	-	-	1/650	0.2% ± 0.6%	0% - 2%
Renal tubule adenoma or carcinoma	6/652	0.9% ± 1.3%	0% - 4%	2/650	0.3% ± 0.8%	0% - 2%
Liver						
Hepatocellular adenoma	20/653	3.1% ± 2.8%	0% - 8%	9/650	1.4% ± 2.1%	0% - 6%
Hepatocellular carcinoma	8/653	1.2% ± 1.5%	0% - 4%	1/650	0.2% ± 0.6%	0% - 2%
Hepatocellular adenoma or carcinoma	28/653	4.3% ± 2.9%	2% - 9%	10/650	1.5% ± 2.0%	0% - 6%
Mononuclear Cell Leukaemia	356/655	54.4% ± 8.8%	34% - 66%	262/653	40.1% ± 7.2%	30% -54%
Haemangiosarcoma (all organs)	-	-	-	2/653	0.3% ± 0.8%	0% - 2%
Testes: Interstitial cell adenoma	450/655	68.7% ± 8.7%	54% - 83%	-	-	-

15.2 Detailed summary of NTP mouse carcinogenicity study (NTP, 1997)

In a carcinogenicity study, comparable to OECD 451, groups of 58 B6C3F1 mice were exposed via whole body inhalation to tetrafluoroethylene (purity > 98%) for 6 hours per day, 5 days per week for 95-96 weeks. 10 males and 10 females were assigned to the 15 month interim evaluation. The target chamber concentrations were 0, 312, 625, 1250 ppm. Animals were observed twice daily and clinical findings were recorded monthly until five weeks before necropsy and twice monthly thereafter. Body weights were recorded weekly until week 13, monthly thereafter until five weeks before necropsy, when they were recorded every two weeks. All animals were subject to necropsy. At the 15 month interim evaluation, kidney, liver and lung were weighed and a haematological and clinical chemistry analysis and urinalysis was performed. Complete histopathological examination was performed on all animals at study termination.

Chamber concentrations were found to be within 10 % of the acceptable range for the duration of the study.

At the 15 month interim assessment, no effect on haematological, clinical chemistry or urinalysis parameters was observed. A statistically significant increase in the incidence of renal tubule dilation was observed in males at the mid and high dose and in renal tubule karyomegaly in both sexes in the mid and high dose groups, which occurred in the absence of a change in kidney weight. In the liver, there was an increased incidence of angiectasis in all treated animals, which was statistically significant in mid dose males and low dose females. There was a statistically significant increase in eosinophilic foci in mid and high dose females. There was an increased incidence of hepatic haemangiosarcomas in males in the high dose group (3/10) and in females of the low dose (1/10) when compared with the concurrent controls (0/10). There was also an increased incidence of hepatocellular adenoma in females.

Table 27: Incidence of neoplastic lesions in mice at 15 month interim assessment (NTP, 1997)

	Males				**Females**			
Dose group (ppm)	**0**	**312**	**625**	**1250**	**0**	**312**	**625**	**1250**
Number of animals examined	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Haemangiosarcoma	0	0	0	3	0	1	0	0
Hepatocellular adenoma	6	2	4	1	0	2	3	2
Hepatocellular carcinoma	2	4	2	2	0	3	1	3

At study termination, the survival rates of the control, low, mid and high dose groups were 38/48, 11/48, 2/48 and 1/48 for males and 36/48, 4/48, 6/48 and 4/48 for females. Due to the reduced survival the study was terminated during week 96. The study authors concluded that the reduced survival was related to the increased incidence in hepatic neoplasms. There was a decrease in body weight in the treatment groups at study termination however the number of surviving mice in the treatment groups was significantly less than the control and therefore no conclusion can be drawn regarding the effect of tetrafluoroethylene treatment on body weight.

Table 28: Mean body weights and survival in mice at study termination (NTP, 1997)

	Males				**Females**			
Dose group (ppm)	**0**	**156**	**312**	**625**	**0**	**312**	**625**	**1250**
Number of animals	48	48	48	48	48	48	48	48
Average terminal body weight (g)	49.2[(a)]	44.7[(a)]	46.5[(b)]	44.6[(c)]	50.5[(a)]	43.8[(b)]	50.8[(a)]	40.1[(a)]
Number of animals surviving to study termination	38	11	2	1	36	4	6	4

Final weights on (a) week 94; (b) week 92 and (c) week 90

There was increase in the incidence of hepatic haemangiomas in both sexes, which was statistically significant at the low dose and in males only at the mid dose. The incidences in control, low, mid and high dose groups were 0/48 (0 %), 10/48 (21 %), 5/48 (10 %) and 2/48 (4 %) for males and 0/48 (0 %), 5/48 (10 %), 2/47 (4 %) and 1/47 (2 %) for females, respectively. Multiple haemangiomas were found all treated males and females with the exception of high dose females. The incidences of hepatic haemangiomas observed in all treatment groups in this study are above that observed the in historical control male (2/947, 0.2 %) and female (1/937, 0.1 %) mice from NTP 2-year inhalation studies. Although no clear dose response was observed, as the background incidence of hepatic haemangiomas in mice is low, the increased incidence observed in this study is considered related to tetrafluoroethylene exposure.

The incidence of hepatic haemangiosarcomas was statistically significantly increased in all treated groups when compared with the concurrent control. The incidence in the control, low, mid and high dose groups were 0/48 (0 %), 21/48 (44 %), 27/48 (56 %) and 37/48 (77 %) in males and 0/48 (0 %), 27/48 (56 %), 27/47 (57 %) and 34/47 (72 %) in females, respectively. The incidence of multiple haemangiosarcomas was also increased in all treated groups. The incidence of hepatic haemangiosarcomas observed in this study is also above that observed the in historical control male (12/947, 1.3 %) and female (5/937, 0.5 %) mice from NTP 2-year inhalation studies. Therefore, due to the clear dose response and the low background incidence of hepatic haemangiosarcomas in mice, the increase is considered to be treatment related.

The incidence of haemangiosarcomas in other organs was reported to be low but a few animals with hepatic haemangiosarcomas also displayed haemangiosarcomas in lung, mesentery, pancreas, ovary, bone marrow and subcutaneous tissues. However, no dose response relationship was evident and it was not clear whether these non-hepatic haemangiosarcomas were metastases or developed concurrently. No haemangiosarcomas in any organ were observed in the control animals. Therefore, the biological significance of the occurrence of haemangiosarcomas in other organs in these animals is unclear.

There was a statistically significant increase in hepatocellular carcinomas in males and females of all dose groups when compared with the concurrent control. The incidence in the control, low, mid and high dose groups were 11/48 (23 %), 20/48 (42 %), 33/48 (69 %) and 26/48 (54 %) in males and 4/48 (8 %), 28/48 (58 %), 22/47 (47 %) and 20/47 (43 %) in females, respectively. There was also a statistically significant increase in multiple hepatocellular carcinomas in males and females in all dose groups. The incidence was 4/48 (8 %), 9/48 (19 %), 9/48 (19 %) and 6/48 (13 %) in males and 0/48 (0 %), 5/48 (10 %), 7/47 (15 %) and 7/47 (15 %) in females of the control, low, mid and high dose groups, respectively. The incidence of hepatocellular adenomas was significantly increased in mid dose females (20/47. 45 %) compared with the concurrent control (15/48, 31 %)). The combined incidence of hepatocellular adenomas and carcinomas were also significantly increased in all treated males and females. As there is a clear dose response in both sexes, it is considered that the increase incidence of these tumours was treatment related.

Table 29: Incidences of neoplastic lesions in the liver in mice (NTP, 1997)

	Males				Females			
Dose group (ppm)	**0**	**312**	**625**	**1250**	**0**	**312**	**625**	**1250**
Number of animals examined	**48**	**48**	**48**	**48**	**48**	**48**	**47**	**47**
Liver								
Hepatocellular adenoma	17	17	12	20	15	17	20*	15
Hepatocellular carcinoma	11	20**	33**	26**	4	28**	22**	20**
Hepatocellular carcinoma, multiple	4	9**	9**	6*	0	5**	7**	7**
Combined hepatocellular adenoma or carcinoma	26	34**	39**	35**	17	33**	29**	28**
Haemangioma	0	10**	5*	2	0	5*	2	1
Haemangioma, multiple	0	7**	2	1	0	1	1	0
Haemangiosarcoma	0	21**	27**	37**	0	27**	27**	34**
Haemangiosarcoma, multiple	0	16**	17**	18**	0	8**	12**	15**
Combined haemangioma or haemangiosarcoma	0	26**	30**	38**	0	31**	28**	35**

** Significantly different from the control group (P≤0.05); ** significantly different from the control group (P≤0.01)*

In the liver, there was an increased incidence in hepatocellular eosinophilic foci in females at the low dose and in both sexes at the mid and high dose. As discussed above, foci are considered to be a potential pre-neoplastic lesion (NTP, 2018).

There was also a statistically significant increase in coagulative multifocal necrosis of the liver in males of the mid and high dose groups. Haematopoietic cell proliferation in the liver was also increased in all treated females: 3/48 (6 %), 19/48 (40 %), 13/47 (28 %) and 15/47 (32 %) for the control, low, mid and high dose females, respectively. The study authors note that both lesions are often observed with malignant hepatic neoplasm in mice and therefore are considered to be treatment related.

Table 30: Incidence of non- neoplastic lesions in the liver in mice (NTP, 1997)

	Males				Females			
Dose group (ppm)	**0**	**156**	**312**	**625**	**0**	**312**	**625**	**1250**
2-year study								
Number of animals examined	**48**	**48**	**48**	**48**	**48**	**48**	**47**	**47**
Necrosis, Coagulative, Multifocal	4	3	13*	11*	4	11	8	8
Eosinophilic Focus	1	6	7*	7*	5	13*	12**	7
Haematopoietic Cell Proliferation	1	2	4	0	3	19**	13	15**
Angiectasis	0	6**	10**	13**	1	9	6	4

** Significantly different from the control group (P≤0.05); ** significantly different from the control group (P≤0.01)*

There was a statistically significant increase in histiocytic sarcoma in all organs in all treated males and females. The incidence was 0/48 (0 %), 12/48 (25 %), 7/48 (15 %) and 7/48 (15 %) for males and 1/48 (2 %), 21/48 (44 %), 19/47 (40 %) and 18/47 (38 %) for females of the control, low, mid and high dose groups, respectively. The incidence of histiocytic sarcoma in all organs observed in this study is above that observed in historical control males (6/950, 0.6 %) and females (26/941, 2.7 %) from NTP 2-year inhalation studies. The highest incidences were observed in liver and lung, with lower incidences in the spleen, lymph nodes,

bone marrow and kidney. Therefore, due to the clear dose response and the low background incidence of this tumour in mice, it is considered to be treatment related.

Table 31: Incidences of histiocytic sarcoma in mice (NTP, 1997)

	Males				Females			
Dose group (ppm)	**0**	**312**	**625**	**1250**	**0**	**312**	**625**	**1250**
Liver	0/48	12/48	7/48	7/48	1/48	21/48	19/47	18/47
Lung	0/48	7/48	4/48	3/48	1/48	16/48	13/47	13/47
Spleen	0/48	2/48	1/46	2/46	1/48	7/48	8/46	9/47
Mesenteric lymph node	0/47	4/42	1/41	2/40	1/43	6/40	7/41	6/43
Bone marrow	0/48	1/48	1/47	2/47	0/48	6/48	5/46	4/47
Kidney	0/48	3/48	3/48	2/48	1/48	7/48	4/47	3/47
Uterus					1/48	0/48	0/45	0/47
All organs	0/48	12/48**	7/48**	7/48**	1/48	21/48**	19/47**	18/47**

** Significantly different from the control group (P≤0.05); ** significantly different from the control group (P≤0.01) Statistical significance only calculated for "all organs".*

The incidence of haematopoietic cell proliferation in the spleen was significantly increased in all treated animals and the severity increased with increasing dose of tetrafluoroethylene. The study authors note that this lesion is often associated with the presence of malignant neoplasms in livers in mice.

Table 32: Incidences of neoplastic lesions in historical control mice in 2-year NTP inhalation studies as reported in NTP, 1997.

	Males			Females		
Neoplastic lesion	**Total**	**Mean ± SD**	**Range**	**Total**	**Mean ± SD**	**Range**
Liver						
Hepatocellular adenoma	200/947	21.1% ± 11.6%	4% - 46%	114/937	12.2% ± 9.7%	0% - 40%
Hepatocellular carcinoma	184/947	19.4% ± 5.8%	9% - 29%	103/937	11% ± 6.7%	0% - 30%
Hepatocellular adenoma or carcinoma	358/947	37.8% ± 12.5%	11% - 60%	200/937	21.3% ± 11.9%	3% - 54%
Haemangioma	2/947	0.2% ± 0.7%	0% - 2%	1/937	0.1% ± 0.5%	0% - 2%
Haemangiosarcoma	12/947	1.3% ± 1.7%	0% - 6%	5/937	0.5% ± 1.0%	0% - 3%
Haematopoietic system						
Histiocytic sarcoma	6/950	0.6% ± 1.2%	0% - 4%	26/941	2.8% ± 3.1%	0% - 10%