Committee for Risk Assessment
RAC

Annex 1

Background Document

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

tetrahydrofuran

ECHA/RAC/DOC no CLH-O-0000000954-69-03/A1

TETRAHYDROFURAN
EC Number: 203-726-8
CAS Number: 109-99-9

Adopted
25 May 2010

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None

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: Tetrahydrofuran (THF)

EC Number: 203-726-8
CAS number: 109-99-9

Registration number (s): -

Purity: 99.5-99.9%

Impurities: Peroxide (as hydrogen peroxide) (unknown concentration) [JT Baker Chemical, 1986] can be formed when THF is exposed to air. Some grades may contain an inhibitor such as butyl hydroxy toluene (CAS No. 128-37-0), at less than 1%, to prevent peroxide formation (SIDS, 2000)

Proposed classification based on Directive 67/548/EEC criteria:

The Risk Assessment Committee has concluded that Carc Cat 3; R40 is an appropriate classification for THF. If added to the existing harmonised classification for this substance, this would give:

F; R11-19
Carc.Cat.3; R40
Xi; R36/37

Proposed classification based on GHS criteria:

The Risk Assessment Committee has concluded that Carc. 2; H351 is an appropriate classification for THF. If added to the existing harmonised classification for this substance, this would give:

Flam. Liq. 2; H225
Carc. 2; H351
Eye Irrit. 2; H319
STOT SE 3; H335

H019

Proposed labelling based on Directive 67/548/EEC criteria:

R-phrases: R11- 19 - 36/37-40
Symbol(s): F; Xn
S-phrases: S2- S16- S29- S33- S46

Proposed specific concentration limits (if any): as specified in Annex VI already:
Conc. >= 25% Xi; R36/37

Proposed notes (if any): none

JUSTIFICATION

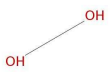
1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Chemical Name:	Tetrahydrofuran Synonyms: Butylene oxide, Cyclotetramethylene oxide, Diethylene oxide, 1,4-epoxybutane, Ethyl ethylene oxide, 2-Ethyloxirane, Furanidine, Oxacyclopentane, Oxolane, Tetramethylene oxide, THF
EC Name:	203-726-8
CAS Number:	109-99-9
IUPAC Name:	Tetrahydrofuran

1.2 Composition of the substance

Chemical Name:	Tetrahydrofuran
EC Number:	203-765-0
CAS Number:	7722-84-1
IUPAC Name:	Tetrahydrofuran
Molecular Formula:	C_4H_8O
Structural Formula:	
Molecular Weight:	72.12 g/mol
Typical concentration (% w/w):	-
Concentration range (% w/w):	99.5-99.9%

Chemical Name:	Hydrogen Peroxide
EC Number:	231-765-0
CAS Number:	7722-84-1
IUPAC Name:	Hydrogen peroxide
Molecular Formula:	H_2O_2
Structural Formula:	

Molecular Weight: 34 g/mol

Typical concentration (% w/w): -

Concentration range (% w/w): <0.5%

Classification

The following harmonised classification of hydrogen peroxide was agreed at the 19th ATP:

According to 67/548/CEE	*According to CLP*
R5 O; R8 Xn; R20/22 C; R35 with specific concentration limits: Xn; R20: C ≥ 50% Xn; R22: C ≥ 8% C; R35: C ≥ 70% C; R34: 50% ≤ C < 70% Xi; R37/38: 35% ≤ C < 50% Xi; R41: 8% ≤ C < 50% Xi; R36: 5% ≤ C < 8% O; R8: C ≥ 50% R5: C ≥ 70%	Ox. Liq. 1 – H271 Acute Tox. 4* – H332 Acute Tox. 4* – H302 Skin Corr. 1A – H314 with specific concentration limits: Ox. Liq. 1; H271: C ≥ 70%**** Ox. Liq. 2; H272: 50% ≤ C < 70%**** Skin Corr. 1A; H314: C ≥ 70% Skin Corr. 1B; H314: 50% ≤ C < 70% Skin Irrit. 2; H315: 35% ≤ C < 50% Eye Dam. 1; H318: 8% ≤ C < 50% Eye Irrit. 2; H319: 5% ≤ C < 8% STOT SE 3; H335; C ≥ 35%

Considering that H_2O_2 is hypothetically present in THF in concentration lower to 0.5% (based on THF minimal purity), no additional classification applies for THF due to this impurity.

Chemical Name: Butyl Hydroxy Toluene

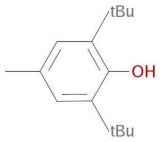
EC Number: 204-881-4

CAS Number: 128-37-0

IUPAC Name: 2,6-di-tert-butyl-p-cresol

Molecular Formula: $C_{15}H_{24}O$

Structural Formula:



Molecular Weight: 220.34 g/mol

Typical concentration (% w/w): -

Concentration range (% w/w): <1%

Classification: No harmonised classification

1.3 Physico-Chemical properties

The information provided in the proposal from France is reproduced in the following table, without further comment from the Risk Assessment Committee.

Table 1. Summary of physico-chemical properties

REACH Annex	Property	IUCLID section	Value or comment
VII, 7.1	Physical state at 20°C and 101.3 kPa	3.1	Colorless, mobile volatile liquid with a faintly fruity, ether-like odor (Odour threshold: 20-50 ppm [Hara, 1987]) and a pungent taste **(**Sax's Dangerous Properties of Industrial Materials, 2004)
VII, 7.2	Melting/freezing point	3.2	-108°C (Merck Index, 1996)
VII, 7.3	Boiling point	3.3	66°C (760 mm Hg) (Merck Index, 1996)
VII, 7.4	Relative density	3.4	0.89 (at 20°C) (BASF AG, 1993; IUCLID dataset, 2000)
VII, 7.5	Vapour pressure	3.6	17300 Pa (at 20°C) (BASF AG, 1993; IUCLID dataset, 2000)
VII, 7.6	Surface tension	3.10	No data
VII, 7.7	Water solubility	3.8	Miscible **(**Sax, 2004)
VII, 7.8	Partition coefficient n-octanol/water (log value)	3.7	Calculated and measured: 0.45 (BASF AG, 1993; IUCLID dataset, 2000)
VII, 7.9	Flash point	3.11	Close cup: -20°C (The German national institute PHYSIKALISCH-TECHNISCHE BUNDESANSTALT. (PTB), Data base PTB-Lab. 3.43, 2008/Chemsafe)
VII, 7.10	Flammability	3.13	THF is highly flammable Hazardous decomposition products: Toxic gases and vapors may be released in a fire involving THF. Vapour-air mixtures are explosive within flammable limits noted above: - upper flammable limit/ upper explosive limit (UFL): 46g/m^3 (±10%) or 1,5 Vol% (±10%): (Data base PTB-Lab. 3.43, 2008/Chemsafe) - lower flammable limit/lower explosive limit (LFL): 370g/m^3 (±5%) cr12,4 Vol% (±5%): (Data base PTB-Lab. 3.43, 2008/Chemsafe)
VII, 7.11	Explosive properties	3.14	Lower limit: 2% Upper limit: 11.8% **(**Sax, 2004) THF is thermally explosive when peroxides are formed (concentrations exceeding 1%). The substance has no explosive properties in the sense of EEC-Method A.14. May form explosive organic peroxides when exposed to air or light or with age.
VII, 7.13	Oxidising properties	3.15	No data
VII, 7.14	Granulometry	3.5	No data
XI, 7.15	Stability in organic solvents and identity of relevant degradation products	3.17	No data
XI, 7.16	Dissociation constant	3.21	No data
	Auto flammability	3.12	321°C (ARCO Chemical Company, 1994; IUCLID, 2000) 230 °C (DIN 51 794/IEC 60079-4), (Data base PTB-Lab. 3.43, 2008/Chemsafe) 215°C (Cefic)
	Reactivity towards container material	3.18	THF attacks some forms of plastics, rubber and coatings (ICSC, 1998)
	Thermal stability	3.19	No data
	Conversion factor		1 ppm = 0.00299 mg/L (1 ppm = 2.99 mg/m3) 1 mg/L = 334 ppm (1 mg/m3 = 0.334 ppm)

2 MANUFACTURE AND USES

2.1 Identified uses

Industrial
THF is used as a solvent for a variety of plastics, dyes, elastomers, etc., as a glue in joining plastics components (e.g. plumbing fittings), and for synthesis of motor fuels, pharmaceuticals, synthetic perfumes, organometallic compounds, and insecticides.

General public
THF is used as a solvent in aerosol paint concentrates, furniture polish and cleaners, laundry starch preparations, lubricating oils, paint and varnish removers, synthetic resin and rubber adhesives

3 CLASSIFICATION AND LABELLING

3.1 Classification in Annex VI of Regulation 1272/2008

Index number 603-025-00-0

According to 67/548/EEC	According to CLP
F; R11-19	Flam Liq. 2; H225
X; R36/37	Eye Irrit. 2; H319
	STOT SE 3; H335
Conc. >= 25% Xi; R36/37	Eye Irrit. 2; H319 C>= 25%
	STOT SE 3; H335 C>=25%

3.2 Self classification

Not applicable.

4 ENVIRONMENTAL FATE PROPERTIES

No evaluated in this dossier.

5 HUMAN HEALTH HAZARD ASSESSMENT

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

The following summary of toxicokinetic data relating to THF was provided by the French Competent Authority in their classification proposal. It is provided here without modification.

Absorption

Absorption is rapid and important by all routes (Bismuth, 2000), particularly through the lungs (alveolar membrane), the gastro-intestinal tract and the skin (Widstrom and Friis, 1989; Droz *et al.*, 1999; Cartigny *et al.*, 2001). THF can also readily penetrate the skin of rats and rabbits and can be lethal via this route (Wagner, 1972; Wagner, 1974; cited in IUCLID dataset, 2000).

Distribution

THF is widely distributed throughout the body of rats (Elovaara *et al.*, 1984; cited in IUCLID dataset, 2000). An administration of THF by gavage (200 mg/kg) in rats produced a peak in levels of THF in blood approximately 1 hour after exposure and a plateau during 1.5 to 2 hours after administration. The levels then gradually declined to negligible concentrations within 24 hours (Hara *et al.*, 1987; Nagata *et al.*, 1983). THF concentrations in adipose tissue and kidneys were ca. 1.3-3 fold higher than in blood and other tissues (Hara *et al.*, 1987; Nagata *et al.*, 1983, cited in IUCLID dataset, 2000). Exposure of 3000 ppm THF by inhalation conducted to higher concentrations in the thymus glands of rats immediately after exposure and remaining higher than in other tissues during elimination (Kawata and Ito, 1984). In humans exposed to 100 ppm for 20-minute periods, 60% of inhaled THF vapour is retained by the body (Wagner, 1972; Wagner, 1974).

Biotransformation

The biotransformation of THF is not well known. It was hypothesized that THF undergoes an alpha-hydroxylation carried out by an inducible enzyme system (Elovaara *et al.*, 1984, cited in IUCLID dataset, 2000; ACGIH, 1991), followed by a subsequent ring opening similarly to dioxane (Droz *et al.*, 1999), which could give rise to a hepatotoxic aldehyde (butanal). A second pathway could be an oxidation of the hydroxyl group before the ring opening occurs, leading to the formation of a gamma-butyrolactone, a potential neurotoxic (convulsive action), and a gamma-hydroxibutyric acid (Bismuth, 2000).

Incubated in presence of rat S9 mix, THF is capable of inhibiting mixed function oxidases, such as cytochromes P450, which enhance toxicity of a number of compounds (in particular the 2E1 isoform catalysing the alcohol dehydrogenase in the metabolism of ethanol, so that an alcohol-conditioned increase of toxicity can result) and forming peroxides and formaldehyde (formaldehyde dehydrogenase) (Zeller *et al.*, 1964; Hofmann and Meinecke, 1964; Elovaara *et al.*, 1984; Moody, 1991; cited in IUCLID dataset, 2000). Repeated exposure of rats to THF could also increase specifically the activity of 7-ethoxycoumarin-O-deethylase in liver and kidneys, and lead to a decline of the concentration in tissues after 2 weeks (IUCLID dataset, 2000), by which means animals adapted by either improving the rate of metabolism or elimination of THF, which may also form possible toxic metabolites (BGIA Gestis).

Biochemical effects in the cerebellum were not detected, while gluteal muscle specimens showed increased succinate dehydrogenase activity in a dose-related manner. This points to effects on the energy metabolism. Muscle acetylcholine esterase activity was also increased showing possible effects on the myoneural junctions (Elovaara et al., 1984; cited in IUCLID dataset, 2000).

Elimination

A fraction of the absorbed THF is relatively rapidly eliminated in unchanged form via the kidneys or is exhaled (Bismuth, 2000; BGIA Gestis). In exposed rats to 15000 ppm of THF for 30 minutes, a rapid elimination from brain, thymus, lungs, heart, liver, kidneys, spleen and blood occurs during the first hour after the exposure and concentrations were ranged as following: blood > brain > kidneys > heart > liver > spleen > thymus > lung (200 mg/kg for the lung, 480-600 for the other organs, after 1 h when the concentrations fell 70-80%) (Kawata and Ito, 1984). Lower tissue concentrations were detected immediately after the last inhalation in rats exposed for seven days than rats given a single exposure. During the following 12-13 hours THF was almost completely eliminated. However, repeatedly exposed rats had higher concentrations 1 and 3 hours after the last

exposure. The authors suggested that repeated exposure by inhalation causes a decline in the rate of excretion through the lungs.

After an oral administration of 300 mg/kg body weight to rats, the biological half-life of THF in blood was 5 -7.5 hours (Nagata *et al.*, 1983; Hara *et al.*, 1987). A peak was detected 1.5-2 h after administration, then the levels declined gradually to negligible concentrations within 24 h.

In human, the highest excretion is by expiration. The same pattern than in rats was found in healthy volunteers exposed for three hours at 50 ppm. THF exposure resulted in 40% expiration of THF in males with normal breathing and 27% in males with deep breathing. The elimination half-life of THF was 30 minutes. In subjects exposed at 50 ppm THF in air for 6 hours, traces of THF were present at 3 hours after the end of exposure. In individuals exposed at 200 ppm THF for 3 hours, THF blood concentrations were higher at 1 hour after the end of exposure than immediately after cessation of exposure (ACGIH, 1991; Droz *et al.*, 1999).

Moreover a milk excretion of THF can occur since it was detected in mother's milk reported in 1 of 12 samples collected in four urban areas in New Jersey, Pennsylvania, and Louisiana (Pellizzari *et* al., 1982). The actual levels of these contaminants in the breast milk were not determined.

5.2 Acute toxicity

5.2.1 Acute toxicity: oral

Although data were presented for this endpoint in the original proposal submitted by the French Competent Authority, no case was made for an evaluation to be made by the Risk Assessment Committee. Consequently, the data have not been included in this background document.

5.2.2 Acute toxicity: inhalation

Not evaluated; no data were presented for this endpoint in the proposal that was submitted following the formal “Accordance check”.

5.2.3 Acute toxicity: dermal

Not evaluated; no data were presented for this endpoint in the proposal that was submitted following the formal “Accordance check”.

5.2.4 Acute toxicity: other routes

Not evaluated; no data were presented for this endpoint in the proposal that was submitted following the formal “Accordance check”.

5.2.5 Summary and discussion of acute toxicity

Not evaluated. No assessment of the acute toxicity of THF has been made by the Risk Assessment Committee. No opinion has been developed regarding classification for this endpoint.

5.2.6 Summary and Discussion of Specific Target Organ Toxicity – Single Exposure (STOT-SE)

Not evaluated; no data were presented in the proposal that was submitted following the formal "Accordance check". Consequently, the Risk Assessment Committee has not offered an opinion on the existing harmonised classification of tetrahydrofuran with STOT SE 3; H335. Similarly, no assessment is offered on the specific concentration limit of 25% that is associated with this classification.

5.3 Irritation

5.3.1 Skin

The proposal from France included data on the potential for THF to induce skin irritation, but did not propose classification for this endpoint. Also, the proposal did not include an argument to justify action on a community-wide basis. Consequently, the data have not been included in this background document and skin irritation remains an endpoint for which there is no harmonised classification of THF.

5.3.2 Eye

Not evaluated; no data were presented in the proposal that was submitted following the formal "Accordance check". Consequently, the Risk Assessment Committee has not offered an opinion on the existing harmonised classifications of tetrahydrofuran with Xi; R36 or Eye Irrit. 2; H319. Similarly, no assessment is offered on the specific concentration limit of 25% that is associated with this classification.

5.3.3 Respiratory tract

Not evaluated; no data were presented in the proposal that was submitted following the formal "Accordance check". Consequently, the Risk Assessment Committee has not offered an opinion on the existing harmonised classification of THF with Xi; R37. Similarly, no assessment is offered on the specific concentration limit of 25% that is associated with this classification.

5.3.4 Summary and discussion of irritation

Not evaluated. No assessment of the irritant potential of THF has been made by the Risk Assessment Committee. No opinion has been developed regarding classification for skin, eye and respiratory tract irritation.

5.4 Corrosivity

Not evaluated. No assessment of the corrosive potential of THF has been made by the Risk Assessment Committee. No opinion has been developed regarding classification for this endpoint.

5.5 Sensitisation

Not evaluated. No assessment of the sensitisation potential of THF has been made by the Risk Assessment Committee. No opinion has been developed regarding classification for this endpoint.

5.6 Repeated dose toxicity

These data are provided to facilitate an understanding of the general toxicity of THF to laboratory animals, as far as this may be of relevance to the opinion in relation to "Carcinogenicity".

5.6.1 Repeated dose toxicity: oral

Species	Dose mg/kg/body weight	Duration of treatment	Observations and Remarks	Method guideline	Ref.
Rat (Fischer 344)	0, 125 or 2000 Administered by gavage to male and female F344 rats	Insufficient information, but > 14 days	At 2000 mg/kg: increased mortality and body weight loss (20% in high-dose males and 13% in females). Histopathology in treated groups: acute inflammation of the trachea and serofibrious exudates in the tracheal lumen (result of THF aspiration), increased lung weights (result of repeated THF aspiration or viral infection), and hyperplasia, hyperkeratosis and inflammation of the epithelium and mucosa of the forestomach.	No (Study considered as inadequate by the NTP, terminated without complete processing or reporting)	Hetjmancik, 1983 ; cited in IUCLID dataset, 2000)
Rat	1780, 2225 & 2670 Administered to 5 rats/group in a 20% aq. solution by gavage (2.0, 2.5, 3.0 mL/kg)	2-4 weeks	There were no special macroscopic pathological changes, but frequent and significant dilated stomach, often containing THF. On histopathological study, 6 animals from the 3 groups examined showed liver lesions (vacuole-like changes of the liver cells, diffuse single or focal hepatic cells necrosis, proliferation of the Kupffer cells) and nephrosis.	No details	Jochmann, 1961, cited in IUCLID dataset, 2000
Mouse B6C3F1	63, 125, 250, 500, or 1000 mg/kg administered by gavage	Insufficient information, but > 49 days	One high-dose female died on day 49. The only significant effect reported was a decreased liver weight in all treatment groups, which was not dose-related but significant at 250 and 500 mg/kg.	No details	Hetjmancik, 1983, cited in IUCLID dataset, 2000

5.6.2 Repeated dose toxicity: inhalation

Species	Conc. mg/L	Exposure time (h/day)	Exposure duration	Observations and Remarks	Method guideline	Ref.
Rat (Wistar)	45	0.5 h/day 7 d/week to 29 males Control group: 5 males	7 days	Irritation symptoms of the skin and the mucosa (tears, mucus, bloody nasal secretions), no histopathological effect (brain, thymus, lung, heart, liver, kidneys, spleen). The dopamine content of the cerebrum was reduced 48 h after the last inhalation.	No details	Kawata and Ito, 1984,

Rat	10 - 193	2-6 h/day	1-30 days	Narcosis and irritation of the mucosa (severity of the symptoms concentration- and exposure time-dependant). No macro- or microscopic liver lesions; no clinical or histopathological indication of renal lesions.	No details	Hofmann and Oettel, 1954
Rat (Wistar)	9	1 h/day 5 days/week to 29 male rats Control group: 5 males	12 weeks	Irritation symptoms of the skin and the mucous membrane (tears, mucus, bloody nasal secretions). From the 4th week, a more significant decreased body weight was noted. Histopathology: lung bronchial epithelium showing papillar hyperplasia and partial catarrhal changes, renal lesions (albumin cylindrical structures in the renal tubules; hyaline-droplets degeneration); changes of the content noradrenalin and dopamine in the cerebrum. No changes in serum GOT, GPT, and AP were detected.	No details	Kawata and Ito, 1984, cited in IUCLID dataset, 2000
Rat (Sprague-Dawley)	0, 0.3, 0.6, 3, or 15	4 h/day 5 days/week to 10 males/group	12 weeks	0.3 mg/L: no significant effects compared to control, except for slight local irritation of the mucosa. 0.6 mg/L: nasal mucus: lesions of goblet and ciliar cells; vacuoles between the epithelial cells; reduced number of cilia tracheal mucosa: increased volume of mucus in the cilia; tumefaction of the cilia membrane, disorder of the ciliary movements 3 & 15 mg/L: affected liver function as indicated by serum chemistry tests (GOT, cholinesterase, and blood sugar values increased); effects on central nervous system. 15 mg/L: decreased body weight gain and significant changes of the relative organ weights; marked local irritation symptoms and morphological damage of the mucous membrane in the respiratory tract (nasal mucosa: partial destruction and pyknosis of the epithelial cells, damage of the goblet and ciliar cells; tracheal mucosa: disorder of the ciliary erection, occurrence of compound cells with tumefaction of the cellular walls), significant differences compared to control for the number of leukocyte, the glycaemia, and the liver values in haematological and clinical, chemical tests. NOAEL = 0.3 mg/L (100 ppm)	≈ OECD 413 Deficiencies: Only males studied Weight measurements for: brain, lung, heart, liver, spleen, and kidney Histological observation of lung tissues only, apparently	Katahira et al., 1982

Rat	0.0002, 0.002, or 0.02	24 h/day 7 days/week to 15 rats/group	3 months	0.0002 mg/L: no effect. 0.002 mg/L: muscular chronaxy reduced; liver damage detected by the sulfobromein test. Histopathology: reversible, morphological changes in different organs. 0.02mg/L: muscular chronaxy significantly reduced after a 6-week exposure; coproporphyrin content in urine increased from the 7th week of exposure (indication on the state of CNS); reduced cholinesterase activity in blood, liver lesions by bromosulfur test. Histopathology revealed dystrophic changes in different tissues. NOAEL = 0.0002 mg/L (0.07 ppm)	No details	Popov, 1970, cited in IUCLID dataset, 2000
Rat (Fischer 344 / N)	0, 0.2, 0.6, 1.8, 5.4, or 15 99% purity	6 h/day 5 days/week 10 male & 10 females /group	13 weeks	≤ 5.4 mg/L: 1 animal with nasal and ocular secretions, without special results. 15 mg/L: symptoms of toxic effect on the CNS: ataxis (male and female), reduction with persistence of the period exposure (acclimatisation effect); significant reduced thymus and spleen weight (male and female), significant increased liver weight (female); increased number of erythrocytes and larger haematological changes; 5/20 males and 8/10 females showed acanthosis in (very) slight grades characterised by (multi)focal increase of the no-keratinised layer of the Schuppel epithelium; 2/10 males and 4/10 females had purulent inflammation of the forestomach. NOAEL = 5.4 mg/L (1800 ppm)	≈ OECD 413	Chhabra *et al*., 1990
Rat Sprague -Dawley	0, 1.5, 4.5, or 9	6 h/day 5 days/week	14 weeks	Only clinical and neurotoxicological findings were reported. 4.5 & 9 mg/L: rats showed diminished startle responses to an auditory alerting stimulus, transient and rapidly reversible supported by the lack of neurological effects, but no additional neurobehavioral or pathological effects. The demonstrated NOEL level of THF was 1.5 mg/L (500 ppm).	≈ OECD 413 .	Malley et al., 2001
Rat (Wistar)	0.6, 3, or 6	6 h/day 5 days/week 20 males per dose group	2-18 weeks	Sections were carried out at 2, 5, 13, and 18 weeks showed an increase in different enzymatic systems (oxidative) in the liver and the kidneys without induction of cytochrome P450, but no effect on the brain.	No details	Elovaara et al., 1984, cited in IUCLID dataset, 2000

Rat	9	8 h/day 5 days/week 50 males and 50 females per group	20 months	Significant increase of the relative liver weight in females compared to control (also found with the satellite group receiving 3000 ppm acetone). There were no toxicological symptoms, no macroscopic and histopathologic changes in the liver and the kidneys compared to controls.	No details	Zeller et al., 1964, cited in IUCLID dataset, 2000
Mouse B6C3F1	0, 0.2, 0.6, 1.8, 5.4, or 15 THF: 99% purity	6 h/day 5 days/week 10 males and 10 females per group	13 weeks	≤ 0.6 mg/L: one animal with nasal and ocular secretions, no other findings. 1.8 mg/L: significantly reduced thymus weight and significantly enhanced liver weight (male). 5.4 mg/L: toxic effect in the CNS (male/female), significantly reduced thymus weight (male) and significantly enhanced liver weight (male/ and female); minimal to mild centrilobular hepatocytomegaly (1 of 10 males). 15 mg/L: 3/10 males found dead within the exposure, 2/10 with inflammation of the urinary tract; toxic effect on the CNS (male/female); torpidity stopped up to 2 h after the end of exposure with an adaptation effect; significantly reduced weight gain and reduced thymus weight (male),reduced spleen weight (male and female) and enhanced liver weight (male and female); minimal to mild centrilobular hepatocytomegaly (7/10 males & 10/10 females); atrophy of the uterus and degeneration of the X-zone (inner cortex of the adrenal cortex): absence of fatty vacuolar change normally present in young female mice, X-zone markedly thinner and erythrocyte congestion in the capillaries. NOAEL = 0.2 mg/L	≈ OECD 413	Chhabra et al., 1990

5.6.3 Repeated dose toxicity: dermal

No data provided.

5.6.4 Other relevant information

Human data

Hathaway et al (1991) reported that THF is a central nervous system depressant in humans. They indicated that no human data were available on chronic effects.

Animal data via other routes

Species	Dose mg/kg/day	Exposure time (h/day) and duration of treatment	Observations and Remarks	Method guideline	Ref.

Rat	220, 1110 (0.25, 1.25 mL/kg) THF (20%) was suspended in soya oil	Daily i.p. injections For 4 months Groups of 11-15 animals per group	220 mg/kg: persistent narcotic symptoms, death after 2-4 months; in 5 of 6 animals examined: liver damage (vacuole-like transformation of the liver cells, diffuse necrosis of single and focal cells, proliferation of the Kupffer cells), nephrosis; all animals found dead during the study. 1110 mg/kg: narcotic symptoms; death after 1-2 months, all animals found dead during the study; similar but more severe effects than at 220 mg/kg. In the two dosed groups, 1-2 weeks before death, the authors observed marked weight loss, cachectic aspect, and in macroscopy: diffuse peritonitis, severe fibrinous purulent fusion of the loop of small intestine, compared to liver, spleen and kidneys; liver sometimes enlarged; haemorrhagia intestinal tissue.	No details	Jochmann, 1961

5.6.5 Summary and discussion of repeated dose toxicity

Limited data are available from repeated oral dose toxicity studies. Generally, increased mortality and bodyweight loss, with lesions to the stomach, liver and kidneys, have been observed in rats dosed from 125 mg/kg/day. From the limited data available, mice appear less sensitive than rats. These data do not impact on the proposal to classify THF for carcinogenicity.

In an inhalation study, Chhabra et al (1990) reported a NOAEL of 5.4 mg/L THF in Fischer 344 rats exposed 6 hours/day, 5 days/week for 13 weeks. At 15 mg/L there were a variety of toxic lesions observed, but none of these were in the kidneys which are the target organ for THF carcinogenicity in this species.

When B6C3F1 mice were similarly exposed, the NOEL found was 0.6 mg/L for inhalation to THF for 13 weeks up to 15 mg/L. At 15 mg/L there was increased mortality, inflammation of the urinary tract, toxic effect on the CNS (male/female); significantly reduced weight gain and reduced thymus weight (male), reduced spleen weight (male and female) and enhanced liver weight (male and female); minimal to mild centrilobular hepatocytomegaly (7 of 10 males and 10 of 10 females); atrophy of the uterus and degeneration of the X-zone (inner cortex of the adrenal cortex): absence of fatty vacuolar change normally present in young female mice, X-zone markedly thinner and erythrocyte congestion in the capillaries. Significantly reduced thymus weight and significantly enhanced liver weight are observed from 1.8 mg/L.

The Risk Assessment Committee concluded that none of the findings summarised in this section were particularly informative regarding the carcinogenic potential of THF. Further investigative studies addressing specific issues related to potential THF carcinogenicity are summarised in Section 5.8.

5.7 Mutagenicity

5.7.1 In vitro data

Tetrahydrofuran has been studied in bacterial gene mutation and mammalian cell cytogenetic assays, as well as a UDS test in rat hepatocytes. The key points of these studies are summarised in the following table.

<table>
<tr><th>Test</th><th>Cell type</th><th>Conc.</th><th>Metabolic activation</th><th>Results</th><th>Ref.</th></tr>
<tr><td>S typhimurium gene mutation

Non guideline, limited details available</td><td>Tester strains included

TA98
TA100
TA1535
TA1537
TA1538</td><td>2.5, 5, 10 & 20 mg/L</td><td>With and without rat or hamster S9</td><td>Negative</td><td>McMahon et al, 1979</td></tr>
<tr><td>S typhimurium gene mutation

OECD 471</td><td>Tester strains

TA98
TA100
TA1535
TA14537</td><td>Up to 10 mg/plate</td><td>With and without rat liver S9</td><td>Negative</td><td>Mortelmans et al, 1986</td></tr>
<tr><td>E coli reverse mutation assay

Similar to OECD 471 (preincubation)</td><td>WP2 uvrA</td><td>Up to 10 mg/ plate</td><td>With and without rat liver S9</td><td>Negative</td><td>Japan Chemical Industry, 1996, cited in IUCLID dataset 2000</td></tr>
<tr><td>E coli reverse mutation assay</td><td>WP2 uvrA</td><td>1 µmol/L</td><td>With and without S9 (rat liver S9?)</td><td>Very limited details available. Study authors claimed a positive result; response seen was 15% of that with the positive control, epibromohydrin.</td><td>Chemminke et al, 1982

(Russian article)</td></tr>
<tr><td>Cytogenetic assay:

Chromosome aberrations</td><td>CHO cells</td><td>500 to 5000 mg/L</td><td>With and without Aroclor-induced rat or hamster liver S9</td><td>Incubation times were 14 h (without S9) and 2h + 12h (with S9).

Trial One:

(i) Without S9

<table>
<tr><th>Dose (µg/mL)</th><th>Total cells scored</th><th>No. of Abs</th><th>Cells with Abs (%)</th></tr>
<tr><td>Distilled water</td><td>100</td><td>7</td><td>7.0</td></tr>
<tr><td>MMC 0.15</td><td>50</td><td>23</td><td>30.0</td></tr>
<tr><td>THF 500</td><td>100</td><td>8</td><td>8.0</td></tr>
<tr><td>1600</td><td>100</td><td>17</td><td>16.0</td></tr>
<tr><td>5000</td><td>100</td><td>8</td><td>8.0</td></tr>
</table>
MMC: mitomycin C

(ii) With S9

<table>
<tr><th>Dose</th><th>Total</th><th>No.</th><th>Cells</th></tr>
</table></td><td>NTP, 1998</td></tr>
</table>

<table>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>
<table>
<tr><th>(µg/mL)</th><th>cells scored</th><th>of Abs</th><th>with Abs (%)</th></tr>
<tr><td>Distilled water</td><td>100</td><td>8</td><td>8.0</td></tr>
<tr><td>CP 15</td><td>100</td><td>28</td><td>23.0</td></tr>
<tr><td>THF 500</td><td>100</td><td>11</td><td>7.0</td></tr>
<tr><td>1600</td><td>100</td><td>11</td><td>10.0</td></tr>
<tr><td>5000</td><td>100</td><td>13</td><td>13.0</td></tr>
</table>
CP: cyclophosphamide

Trial Two

(i) Without S9
<table>
<tr><th>Dose (µg/mL)</th><th>Total cells scored</th><th>No. of Abs</th><th>Cells with Abs (%)</th></tr>
<tr><td>Distilled water</td><td>100</td><td>4</td><td>4.0</td></tr>
<tr><td>MMC 0.15</td><td>50</td><td>26</td><td>40.0</td></tr>
<tr><td>THF 3000</td><td>100</td><td>7</td><td>7.0</td></tr>
<tr><td>4000</td><td>100</td><td>9</td><td>7.0</td></tr>
<tr><td>5000</td><td>100</td><td>9</td><td>6.0</td></tr>
</table>
(ii) With S9
<table>
<tr><th>Dose (µg/mL)</th><th>Total cells scored</th><th>No. of Abs</th><th>Cells with Abs (%)</th></tr>
<tr><td>Distilled water</td><td>100</td><td>3</td><td>3.0</td></tr>
<tr><td>CP 15</td><td>50</td><td>21</td><td>18.0</td></tr>
<tr><td>THF 1000</td><td>100</td><td>4</td><td>4.0</td></tr>
<tr><td>2000</td><td>100</td><td>6</td><td>6.0</td></tr>
<tr><td>3000</td><td>100</td><td>12</td><td>12.0*</td></tr>
<tr><td>4000</td><td>100</td><td>8</td><td>7.0</td></tr>
<tr><td>5000</td><td>100</td><td>12</td><td>12.0</td></tr>
</table>
</td>
<td></td>
</tr>
<tr>
<td>Cytogenetic assay: sister chromatid exchange</td>
<td>CHO cells</td>
<td>500 to 5000 mg/L</td>
<td>With and without Aroclor-induced rat or hamster liver S9</td>
<td>Negative

NB: Incubation times were 26 h (without S9) and 2 h (with S9)</td>
<td>NTP, 1998</td>
</tr>
<tr>
<td>Unscheduled DNA synthesis</td>
<td colspan="2">Primary rat hepatocytes</td>
<td></td>
<td>Negative

Limited details available: abstract only</td>
<td>Mirsalis et al, 1983</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</table>

Additionally, two transformation assays are available. Although they are not strictly assays of genotoxicity, and were conducted without any available test guidelines being in place, they were provided in the original dossier submitted by the French Competent Authority and so are summarised in the following table.

Test	Cell type	Conc	Metabolic activity	Results	Reference
Mammalian cell transformation assay	Balb/c-3T3 cells	13.9 – 351 mM	No additional activation system	Negative NB: LD50 = 90.3 mM	Matthews et al, 1993
Viral DNA transformation assay	Syrian hamster embryo cells	No data	No data	Positive – but very limited details available Cultivated cells were exposed to THF in sealed chambers (2 or 20 hours); then determination of the survival rate and analysis for elevated sensitivity to SA7 virus transformation	Hatch et al, 1983 (abstract)

5.7.2 In vivo data

Test	Species & tissue	Exposure conditions	Results	Reference
Micronucleus OECD 474	B6C3F1 mouse Bone marrow	3 i.p. injections at 24 h intervals	Negative Groups of 5 or more animals; 2000 PCE per animal were scored.	Shelby & Witt, 1995
Micronucleus OECD 474	B6C3F1 mouse (10 male and 10 female per group) Peripheral blood	Inhalation 6h/day 4 day/week 14 weeks 0.2, 0.6, 1.8, 5.4 or 15 mg/L (600, 1800 or 5000 ppm)	Equivocal Females were negative and males displayed a marginal response in a trend test, but the highest effect observed was within historical control levels. Micronucleated cells/1000 cells PCEs NCEs Male Chamber control 1.50 1.18 THF 600 1.69 1.27 THF 1800 1.79 1.58* THF 5000 1.47 1.41 Female Chamber control 1.85 1.43 THF 600 1.01 1.16 THF 1800 1.34 1.15 THF 5000 1.29 1.18 (10 mice for erythrocyte scoring per group, except 7 mice in the 5000 ppm male mice group)	NTP, 1998
Chromosome aberration	Male B6C3F1 mouse 10/group Bone marrow	i.p. single injection 500, 1000 and 2000 mg/kg Harvest time 17 and 36 h post-exposure	Negative Signs of toxicity observed at the highest dose. 50 first division metaphase cells scored from each of 8 animals/dose	NTP, 1998

Sister chromatid exchange	Male B6C3F1 mouse 5/group Bone marrow	i.p. single injection 500, 1000 and 2000 mg/kg Harvest times 23 and 42 h MTD = 2000 mg/kg	Equivocal The initial test at 23 h was positive. A repeated test was negative and the results of the single trial performed with a 42-hour sample time were also negative. Dose Mean SCEs/cell (1) 23 h: MMC 0.5 7.04 THF 0 3.79 500 3.91 2000 6.27* (2) 23 h MMC 0.5 10.48 THF 0 5.70 500 6.02 2000 5.62 2500 (lethal) (3) 42 h: acrylamide: 160 9.37 THF 0 5.05 500 5.86 2000 4.05 2500 4.29	NTP, 1998
Unscheduled DNA synthesis	Male Fischer 344 rat Liver	In vivo gavage	Negative No other details (poster abstract)	Mirsalis et al, 1983

5.7.3 Other relevant information

Test	Species	Tissue	Exposure route & Harvest time	Observations and remarks	Method guideline	Ref
Drosophila SLRL test (sex-linked recessive lethal assay	Male *D. melanogaster* Wildtype Canton S	10 000, 40 000, & 125 000 ppm	Oral feed 72 h exposure (with regeneration food after 24 and 48 h)	Negative – no significant conclusions can be derived from this study.	OECD 477	NTP, 1998

5.7.4 Summary and discussion of mutagenicity

THF has given negative results in several well performed bacterial mutagenicity tests. As described above, there is one isolated positive result in the literature, from a study using E coli WP2 uvrA. However the study concerned appears not to have been conducted according to a standard guideline and so is afforded little weight. Negative results have been found in both an *in vitro* rat hepatocyte and an *in vivo* liver UDS test.

In a Chinese hamster ovary (CHO) cell chromosome aberration assay, a small increase in the frequency of cells with aberrations was seen in two individual experiments with exogenous metabolic activation (S9). No consistent findings were seen without S9. However, in a guideline study, no increase in chromosome aberrations was found in the bone marrow of male B6C3F1 mice treated the THF by intra-peritoneal (i.p.) injection. Furthermore, there was no evidence for a clear induction of micronuclei or sister chromatid exchanges in other mouse bone marrow or peripheral blood studies. Overall, the weight of evidence from these cytogenetic studies suggests that THF does not damage chromosomes.

In the absence of any clear evidence for mutagenicity either *in vivo* or in a number of *in vitro* tests, the Risk Assessment Committee agreed with the proposal of France that no classification is warranted for this endpoint.

5.8 Carcinogenicity

5.8.1 Carcinogenicity: oral

No data are available.

5.8.2 Carcinogenicity: inhalation

Species	Conc. mg/L	Exposure time (h/day) & duration of treatment	Observations and Remarks	Reference
Rat	9 mg/L	8 h/day 5 days/week 20 months Not a guideline method	50 males and 50 females per group were exposed to THF. No differences were observed compared to controls (liver function test, haematological test, clinical test, chemical test, relative liver and kidneys weight after a 12- and a 20-month exposure, histopathological analysis). THF was found not carcinogenic.	Zeller *et al.*, 1961, cited in IUCLID dataset, 2000
Rat (Fischer 344)	0, 0.6, 1.8, or 5.4 mg/L (0, 200, 600, or 1800 ppm) OECD 451	6 h/day 5 days/week 105 weeks	50 males and 50 females per group were exposed to THF. Survival, body weight, and clinical findings were similar to those of chamber controls (number of survivors in the controls: 12/50, and 5.4 mg/L group: 6/50) Males: Positive trend (P=0.037) for combined occurrence of renal tubule epithelial adenoma and carcinoma (relatively uncommon spontaneous renal neoplasms in two-year NTP studies), although pairwise comparisons between control and experimental groups were not significant. The incidence was as follows: historical control range: 0-4%; current control: 1/50 (2%); 200 ppm: 1/50 (2%); 600 ppm: 4/50 (8%); and 1800 ppm: 5/50 (10%). Tumours in the high-dose group appeared by day 668 (95 weeks), compared with day 733 (terminal sacrifice) in the controls. Slightly higher incidence of mammary gland fibroadenoma in high-dose males, but not considered significant: historical incidence: 4.2 ± 3.5%; current controls: 0/50 (0%); 200 ppm: 2/50 (4%); 600 ppm:	NTP, 1998

			3/50 (6%); and 1800 ppm: 4/50 (8%). Higher incidence of testes adenoma in treated rats (but well within the historical control range and not considered significant): historical control: 46-83%; current controls: 23/50 (46%); 200 ppm: 31/50 (62%); 600 ppm: 31/50 (62%); and 1800 ppm: 31/50 (68%) Increased incidence of epithelium hyperplasia in the prostate: current controls: 2/50 (4%); 200 ppm: 1/50 (2%); 600 ppm: 0/50 (0%); and 1800 ppm: 5/50 (10%). Females: Slightly greater incidence of mammary gland fibroadenoma in the high-dose group with a trend marginally significant (P=0.031), but the pairwise comparisons were not: historical control: 16-42%; controls: 23/50 (46%); 200 ppm: 22/50 (44%); 600 ppm: 29/50 (58%); and 1800 ppm: 31/50 (62%). The NTP concluded that THF exhibited some evidence of carcinogenic activity in male rats for renal tubule epithelial adenoma and carcinoma but no evidence of carcinogenic activity in females.	
Rat (Fischer 344) males	0, 0.6, 1.8 and 5.4 mg/L	6 h/day 5/days/week 5 days or 4 weeks or 4 weeks with a 21-d recovery period 6 rats/group	At 5.4 mg/L: modest increase in hyaline droplets, with increased staining for α 2u-globulin, observed in the cortical proximal tubules. The increases appeared to be associated with increases in cell proliferation (BrdU labelling) and apoptosis distributed in “hot spots” in the renal cortex. After a 21-day recovery period, the 5-day study produced recovery for proliferation but not for α 2u-globulin-stained cells. Also, there were no differences between control and dosed rats for the number of cells in hot spots (cortex).	Garner et al, 2002
Rat (Fischer 344)	0, 0.6, 1.8, or 5.4 mg/L (0, 200, 600, or 1800 ppm)	6 h/day 5 days/week 105 weeks	Review of selected histologic changes in the kidneys of male rats assigned to the NTP study (1998) Males: Incidence of tubular proliferative lesions similar among all exposure groups, when the incidence of all neoplastic (adenomas) and pre-neoplastic [atypical tubular hyperplasia (ATH) considered to represent a likely preneoplastic change] changes were combined: Adenomas: 10% (high-dose group) and 4% (control rats) ATH: 12% (high-dose group) and 10% (control rats) Females: No pre-neoplastic or neoplastic lesions (lowest severity grades for nephropathy). The two male rat carcinomas observed in the NTP study were diagnosed by Dr Hard as adenomas. However, several of the adenomas in the high-dose group, and one in the mid-dose group, were much larger than the two lesions diagnosed in the control males, which were both regarded as marginal tumours. Nephropathy: 44% of control (22/50) and 42% (21/50) of high-dose rats had the most severe grades of nephropathy (7-8). Twenty out of 22 ATH diagnosed by Dr Hard and 7/9 adenomas occurred in kidneys with	Hard, 2005

			grades 7-8 and the majority with grade 8 [females had less severe grades from 2 to 6 (mild to low-severe) with the exception of one control and one high-dose female with severity grade 7]. No evidence of cytoplasmic vacuolation, single cell death (e.g. apopotosis), simple tubule hyperplasia, or increased mitotic activity, and no mineralisation in the papilla, in tubule epithelium unaffected by CPN, particularly in the cortex and outer stripe of the outer medulla (OSOM) of high-dose male and female rats. Conclusion of Dr Hard: there was no evidence of a mode of action related to α2U-globulin nephropathy or cytotoxicity underlying renal tumor development. Advanced chronic progressive nephropathy (CPN) - a spontaneous renal disease commonly seen in ageing rats - appeared to play a dominant role in the incidence of atypical tubular hyperplasia (ATH), and perhaps the renal tubule tumors, across the dose groups. Remarks by French CA: no blind slide reading. No re-analysis of low-dose and mid-dose animals that were determined to have no renal lesions by NTP. Combining hyperplasia and neoplasia incidences for statistical analysis was not considered appropriate by the STP Hyperplasia Working group (STP Hyperplasia Working group, 2003).	
Rat (Fischer 344)	0, 0.6, 1.8, or 5.4 mg/L (0, 200, 600, or 1800 ppm)	6 h/day 5 days/week 105 weeks	Review of selected histologic changes in the kidneys of male rats from the NTP study (NTP, 1998). Incidence of tubular proliferative lesions similar among all exposure groups, when the incidence of all neoplastic (adenomas) and pre-neoplastic [atypical tubular hyperplasia (ATH) considered to represent a likely preneoplastic change] changes were combined: Adenomas: 14% (high-dose group) and 4% (control rats) ATH: 2% (high-dose group) and 8% (control rats). Essentially all tumours and hyperplasic changes occurred in rats with severe to “end-stage” chronic progressive nephropathy (CPN). However, exacerbation of CPN was not contributory because mean severity grades for CPN were very similar between the control and the high concentrations. Conclusion of the PWG: the observed proliferative changes (atypical tubular hyperplasias and adenomas) observed in the control and high-dose groups are likely due to regenerative processes associated with advanced CPN, and to proliferative changes resulting from either CPN and/or low-grade alpha 2U globulin nephropathy. Remarks from French CA: Combining hyperplasia and neoplasia for statistical analysis purpose was not considered appropriate by the STP Hyperplasia Working group (STP Hyperplasia Working group,	PWG, 2009

			2003),	
Mouse B6C3F1	0, 0.6, 1.8, or 5.4 mg/L (0, 200, 600, or 1800 ppm)	6 h/day 5 days/week 105 weeks 50 males and 50 females per group	Survival and lifespan of high-dose males were significantly lower than the survival and lifespan of controls (456 days versus 689 days for controls). Mean body weights of exposed male and female mice were not affected. The highest exposure concentration (1800 ppm) selected for male mice in this study exceeded the MTD (state of narcosis during and up to 1 h after the exposure periods). High-dose males had significantly greater incidences of nonneoplastic lesions of the urogenital tract than those in the chamber controls. The authors explained this by the inflammatory character of these lesions, which occurred primarily among the animals dying in the first 52 weeks of the study, suggested an ascending bacterial infection (due likely to prolonged wetting of the preputial fur). Females: 1800 ppm: increased incidence of hepatocellular neoplasms (adenoma and carcinoma) [85% versus 34% in controls (significant)], and of multiple hepatocellular neoplasms and liver necrosis. 200 & 600 ppm: increases in the incidences of liver neoplasms observed. They were not statistically significant, but the trend test was positive. Males: no indication of an increase in the incidences of hepatocellular neoplasms. The incidence of non-neoplasic effects in males was increased: iliac hyperplasia in the lymph node was 75% in low and mid-dose groups and 100 % in high dose group (0% in controls); hematopoietic cell proliferation in the spleen was increased with exposure (19%; 27%; 30%; 37%), and the incidence of thymus atrophy was enhanced in the higher exposure groups (6%; 5%; 12%; 25%). THF exhibited no evidence of carcinogenic activity in male mice but showed clear evidence of carcinogenic activity in female mice.	NTP, 1998
Mouse B6C3F1 (Female)	0, 0.6, 1.8, or 5.4 mg/L (0, 200, 600, or 1800 ppm) 10 animals /gp	6 h/day 5 days/week 5 days or 4 weeks or 4 weeks with a 21-d recovery period	Purpose: to investigate the possible non-genotoxic mode of action leading to an increase in liver tumours. Studied parameters: enzyme induction, cell proliferation (BrdU labelling index) and apoptosis, in female mouse liver. Results: No increased mortality, no clinical signs related to treatment, body weight not affected. <u>200 ppm:</u> No effects. <u>600 ppm:</u> Weak proliferative effects: no change in BrdU labelling index, increased mitotic index in mid-zonal region at 4 wk (reversible within 3 weeks) <u>1800 ppm:</u> Slightly increased liver weight (relative and absolute) after 4 weeks of exposure. Significant increase of CYP levels and EROD and PROD activities (5 days) (data not shown). Significant increased cell proliferation (at 5 days in mid-zonal and centrilobular regions; at 4 wk in centrilobular regions) (reversible within 3 weeks)	Gamer et al, 2002

			No morphological signs of cell degeneration or necrosis. No indication of cytotoxicity as the origin of the proliferative response, since no changes in the number of apoptotic cells (5 d or 4 wk), and no degenerative changes in cell organelles or subcellular compartments in midzonal or central hepatocytes after 4 wk (electronic microscopy). Remarks of French CA: Significant decrease of the number of apoptotic cells at 1800 ppm, after 21 recovery days. Increased CYP content and EROD and PROD activities at 1800 ppm were not reproduced by the same authors (Van Ravenzwaay *et al*., 2003)	
Mouse (B6C3F1) (Female)	0, 5.4 or 15 mg/L (0, 1800 or 5000 ppm) 18 animals /gp One_half pre-treated with 1-aminobenzatriazol (ABT) (CYP inhibitor)	6 h/day 5 days/week 5 days	Purpose: to investigate the effects of CYP inhibition on THF-induced hepatocellular proliferation in female mice. <u>1800 ppm</u>: no significant effects <u>5000 ppm:</u> deep narcosis resulting in 4 dead animals. Slightly decreased body weight. Increased microsomal enzymes: CYP content (x 98%), PROD (x 600%) and EROD (x 160%) activities. No effect on the subcellular morphology of hepatocytes. Increased cell proliferation in centrilobular region (indicated by increase of PCNA labelling index) <u>5000 ppm and pre-treatment with ABT</u>: ABT applied to inhibit THF oxidative metabolism. Microsomal enzyme induction was inhibited, but there was centrilobular fatty change and an increased proliferative response. Conclusion: The mitogenic effects seen in the livers of THF-treated mice are related to prevailing THF tissue concentration and not to the generation of THF oxidative metabolites. Remarks from the French CA: proliferation and induction of microsomal enzymes are observed at a very high and toxic concentration in female mice (5000 ppm) (2.8-fold higher than that one inducing liver tumors in the NTP study).	Van Ravenzwaay et al, 2003

5.8.3 Carcinogenicity: dermal

Species	Dose mg/kg/bw	Exposure time	Duration of treatment	Observations and Remarks	Guideline	Ref.
Mouse	89 mg/animal	25 weeks Post-observation period: 11.5 months	Twice a week	11 of 25 animals survived after 17.5 months. Mice showed four benign tumours (not specified).	No details	Mueller and Reichert, 1969, cited in IUCLID dataset,

						2000

5.8.4 Carcinogenicity: human data

Neither experiences in humans nor epidemiological studies are available.

5.8.5 Other relevant information

5.8.6 Summary and discussion of carcinogenicity

THF has been studied in a conventional 2-species bioassay by the US National Toxicology Program (NTP, 1998). Following long-term inhalation exposure, there was some evidence of carcinogenic activity in male rats for renal tubule epithelial adenoma and carcinoma, but no evidence of carcinogenic activity in females. A marginal increase in mammary gland fibroadenoma was also seen. In mice, there was no evidence of carcinogenic activity in males, but increased hepato-carcinogenicity was seen in females.

In order to determine whether these tumour findings justify classification of THF for carcinogenicity, it is necessary to consider the nature of their occurrence, including any mechanistic information and relevance to humans. Given that there is no significant evidence of THF being mutagenic (see Section 5.7), the focus of attention is on potential non-genotoxic mechanisms.

5.8.6.1 Kidney tumours in male rats

In the original analysis made by the NTP, the incidence of renal carcinoma was given as 0%, 0%, 0% and 4% in the male rats exposed to 0, 0.6, 1.8 and 5.4 mg/L THF, respectively. The incidences of renal adenoma were 2%, 2%, 8% and 6%. In a subsequent analysis, sponsored by industry (Hard, 2005), the 2 carcinomas were diagnosed as adenomas, but a dose-related increase in tumours was still evident. Hard also noted that several adenomas in the high dose group were much larger than the two seen in controls. Recently, in a further analysis of the pathology data, a "Pathology Working Group" (PWG), also concluded that the 2 carcinomas should have been recorded as adenomas, but again also confirmed the dose-related increase for renal tumours overall. The increases in adenomas in the highest dose group were clearly above the historical control range (range for adenoma and carcinoma combined 0-4%, rate 0.9% ± 1.3%).

5.8.6.1.1 Relevance of the kidney tumours observed in male rats

Research into the aetiology of chemically-induced renal tumours in male rats has indicated that there are 2 principal modes of action for such species- and sex-specific toxicity: α 2u-globulin nephropathy and chronic progressive nephropathy (CPN).

α 2u-globulin nephropathy

Following a review by IARC in the late 1990s, substances that induce renal tumours in male rats as a result of α 2u-globulin nephropathy are no longer regarded as being of relevance to humans (Swenberg and Lehman-McKeeman, 1999). Six criteria were listed by IARC, and together they provide a stringent framework for establishing the role of α 2u-globulin nephropathy in male rat renal carcinogenesis:

IARC Criteria:

(i) Negative for genotoxicity in a battery of tests
(ii) Renal tumours occur only in male rats
(iii) Acute exposure exacerbates hyaline droplet formation
(iv) α 2u-Globulin accumulates in hyaline droplets
(v) Subchronic histopathological changes include granular cast formation and linear papillary mineralisation
(vi) Absence of hyaline droplets and characteristic histopathological changes in female rats and mice

In addition, IARC provided some additional criteria indicating additional supporting evidence that might also be available. These were: reversible binding of chemical (or metabolites) to α 2u-globulin; increased and sustained cell proliferation in P2 segment of proximal tubules in male rat kidneys; and dose-response relationship between hyaline droplet severity and renal tumour incidence.

The Risk Assessment Committee and the French CA that made the proposal to classify THF for carcinogenicity both considered the evidence available for THF renal toxicity and carcinogenicity against each of these criteria in turn.

(i) Negative for genotoxicity in a battery of tests

Although there are no data available for the possibility of THF genotoxicity in the kidney itself, the weight of available evidence from standard *in vitro* and *in vivo* tests suggests that THF does not possess genotoxic activity (see Section 5.7).

(ii) Renal tumours occur only in male rats

The renal tumours observed in the carcinogenicity study with THF were unique to the male rat, as no such tumours were seen in the female rats or in the mouse study. However, it appears doubtful that α 2u-globulin-associated renal nephropathy occurred in the sensitive rats. In the most relevant of the available repeated inhalation exposure studies, Chhabra et al (1990) found no renal toxicity in rats following 13 weeks exposure to THF at levels up to and including 15 mg/L. In the NTP carcinogenicity study itself, there was no demonstration of additional aspects of the pathological sequence of lesions associated with α 2u globulin nephropathy. Indeed, there was no substance-related increase in the incidence of:

- chronic progressive nephropathy in males (96%, 100%, 100%, 100%, at 0, 0.6, 1.8 and 5.4 mg/L) or in females (96%, 88%, 86%, 84%). These values demonstrate that the incidence of nephropathy was neither treatment-related nor limited to males.

- linear mineralization of papillary tubules [renal tubule mineralization in male rats: 16%, 14%, 4%, 10% (female rats: 94%, 92%, 100%, 92%].

- renal tubule hyperplasia (male rats: 14%, 10%, 12%, 14 %) (Lock and Hard, 2004).

Regenerative proliferation of epithelial cells in the P2 segment in response to the cell loss would be typical of a tumourigenic response in the male rat kidney via the α 2u-globulin mechanism (Melnick et al., 1996; Swenberg and McKeeman, 1999), but this was not found with THF.

The authors of the NTP study concluded that there was a lack of a chemical-related increase in the incidence and/or severity of age-related degenerative renal diseases (chronic progressive nephropathy) in THF-exposed male rats (Chhabra et al., 1998).

The lack of a clear, exposure-related increase in nephropathy in the THF exposed rats argues against the α 2u globulin mechanism being a significant factor in the development of the renal tumours that were observed in these animals.

(iii) Acute exposure exacerbates hyaline droplet formation

Gamer and coworkers demonstrated a slightly increased amount of hyaline droplets (but not granular casts) in proximal tubular cells in 5/6 male rats after 20 exposures at 5.4 mg/L (1800 ppm), 6 h per day, for 4 weeks (Gamer *et al.,* 2002) and Kawata and Ito (1984) observed hyaline-droplets in male rats exposed to 9 mg/L THF, one hour a day, for 12 weeks.

In contrast, Chhabra and co-workers found protein droplets in both control and exposed male rats in a 13-week study [included in the NTP Toxicity and Carcinogenesis study of THF (NTP, 1998)]. The findings in controls and treated rats differed: the protein droplets in controls were finer and more densely and diffusely distributed in the cytoplasm of tubular epithelial cells in the outer cortex, whereas in rats exposed to 5.4 mg/L THF the droplets were more coarse and concentrated in scattered foci in the outer cortex. However, the average severity grades (based on the amount of positively staining protein droplet (α 2u-globulin) accumulated in the cytoplasm of renal tubules and the character of the droplet aggregates for droplet accumulation did not differ significantly between the controls (2.6) and the 5.4 mg/L group (2.8) (Chhabra *et al*. 1998).

The presence of hyaline droplets is not mentioned in the 2-year NTP carcinogenesis study. Consequently no dose-response between hyaline droplet severity and renal tumour incidence can be established.

(iv) α 2u-Globulin accumulates in hyaline droplets

The study in male F344 rats demonstrated a modest accumulation of hyaline droplets containing α 2u-globulin in the renal cortex, as demonstrated by immunohistochemical evidence (Garner et al, 2002; Hard, 2005). This was seen at 5.4 mg/L THF after 5 or 20 exposures; the effect was related to THF exposure level but not to the period of exposure, and was associated with "hot spots" of increasing cell proliferation and apoptosis. It was suggested that tumour formation was consequently through induction of cell proliferation, but the absence of any recorded linear mineralisation in the papilla in the 2-year carcinogenicity study appears to weaken the argument for α 2u-globulin nephropathy being the pathway underlying the renal tubule tumours with this compound (Lock and Hard, 2004). Also, there were no differences between control and dosed rats for the number of cells in hot spots (cortex). After a 21-day recovery period, the 5-day study produced recovery for proliferation but not for α 2u-globulin-stained cells.

There is no evidence that THF could bind to α 2u-globulin from the available studies. Chemicals known to bind α 2u-globulin and produce both α 2u-globulin nephropathy and renal tubular tumours in male rats have a poor solubility in water (from non soluble to 80 g/L) and a high log Pow (from 1.67 to 4.2). In contrast, THF shows a high solubility in water (300 g/L) and a low log Pow (0.46). Also, hyaline droplet formation was not seen in the longer term inhalation studies (Hard, 2005).

(v) Subchronic histopathological changes include granular cast formation and linear papillary mineralisation

As discussed in (ii) (above), linear mineralisation of papillary tubules was seen in both male and female rats and was not related to THF exposures. This argues against a male rat specific α 2u-globulin mediated mechanism.

(vi) Absence of hyaline droplets and characteristic histopathological changes in female rats and mice

There was no robust evidence of THF exposure having produced increased hyaline droplets in female rats or mice. As discussed above, chronic progressive nephropathy and renal tubule mineralisation were evident in control and THF-exposed female rats.

Since the criteria established by the IARC Working Group (as reported by Swenberg and Lehman-McKeeman, 1999) are not fulfilled, the French CA concluded that the α 2u-globulin mechanism should not be deemed as an explanation for the renal tumour formation in male rats.

Chronic progressive nephropathy (CPN)

Hard and Kahn (2004) indicated that very specific criteria need to be met in order to conclude that a chemical increases the incidence of renal tubule tumour through an interaction with CPN:

(i) Slight but usually statistical increase in renal tubule tumours.

(ii) Exacerbation of CPN to very advanced stages of severity (especially end-stage CPN, a terminal condition resulting in renal failure because almost no normal parenchyma remains) at doses associated with tumour increase, in comparison to control rats in a 2-year carcinogenicity study.

(iii) Tumours are usually adenomas (typically basophilic) which are often of small size or borderline with ATH. Such tumours should be associated only with the highest grades of CPN severity (i.e., grades 7 (high-severe) and 8 (end-stage)). The tumours and any precursor foci of atypical hyperplasia must be restricted to CPN-affected parenchyma and they are usually observed only towards the end of the 2-year studies.

(iv) Careful microscopic examination of renal parenchyma not involved in the CPN process should reveal no evidence of compound-induced cellular injury or other changes that would suggest alternative modes of action.

The French CA and the Risk Assessment Committee both considered the rat carcinogenicity and other relevant findings against these criteria. The reports of Hard (2005) and PWG (2009) were taken into account.

Slight increase of the incidence of tumours

Although there has been some controversy over the diagnosis of the various neoplastic and non-neoplastic lesions in the control and THF exposed male rats (Hard, 2005; PWG, 2009), it appears that this criterion is met. THF produced a slight increase of renal tubule tumours in mid-dose and

high-dose male rats, not statistically different but higher than the historic control. In addition, the incidence of the renal tubule tumours occurred with a positive trend.

Although the PWG commented that there was no early tumour occurrence with THF, it was evident that renal tumours did appear *earlier* in the high-dose group compared to controls [days 668 and 733 (terminal sacrifice), respectively].

Both the PWG (2009) and Hard (2005) observed that when the incidence of all neoplastic (adenomas) and pre-neoplastic (atypical tubular hyperplasia (=ATH)) changes were combined; the incidence of tubular proliferative lesions was similar among all exposure groups, as shown in the following table.

Table 2: Summary of proliferative changes observed in male rats exposed to THF for 2 years, diagnosed by NTP, Hard and by the PWG.

Dose (ppm)		Number of ATH [a] (Hard, PWG) or hyperplasia (NTP)	Number of adenomas	Number of carcinomas	Total
0	NTP	7/50 [b]	1/50	0/50	6/50
	Hard 2005	5/50	2/50	0/50	7/50
	PWG 2009	4/50	2/50	0/50	6/50
200	NTP	5/50	1/50 [c]	0/50	5/50
	Hard 2005	3/50	1/50	0/50	3/50
	PWG 2009	2/50	0/50	0/50	2/50
600	NTP	6/50	4/50	0/50	9/50
	Hard 2005	5/50	3/50	0/50	8/50
	PWG 2009	4/50	2/50	0/50	6/50
1800	NTP	7/50	3/50	2/50 [d]	10/50
	Hard 2005	6/50	5/50	0/50	10/50
	PWG 2009	1/50	7/50	0/50	8/50

a. The PWG distinguished between "reactive" tubular hyperplasia (associated with CPN) and atypical tubular hyperplasia that was considered to represent a likely preneoplastic change[1]. Original NTP did not make a distinction between reactive and atypical hyperplasia, thus summation of overall proliferative changes that were likely to progress to neoplasia was not included in the initial NTP pathology report.

b. The table 2 in the Hard report cited 5/50 hyperplasia in control rats but the NTP reported 7/50.

c. The table 2 in the Hard report cited 0/50 adenoma in the low-dose group but the NTP reported 1/50.

d. These two carcinomas were diagnosed by Dr Hard and by the PWG as adenomas.

[1] Simple tubule hyperplasia = increase in the number of epithelial cells without altering the single-cell-layer aspect of the tubule lining.
Atypical tubule hyperplasia (ATH) = complex internal proliferation of the epithelial lining of a tubule beyond the normal single cell layer into, and usually obliterating, the lumen, but essentially retaining the integrity of the tubule outline. Tubule proliferation transcends from atypical hyperplasia to adenoma when it exceeds the integrity of a single tubule, and/or when vascular ingrowth into the proliferative focus can be discerned. The distinction between adenoma and carcinoma is the presence of multiple areas of necrosis/hemorrhage in the latter, usually accompanied by a trend towards increased cellular pleomorphism. It is widely accepted that ATH in the rat kidney is a preneoplastic lesion, that is, ATH, adenoma and carcinoma represent sequential stages in the continuum from preneoplastic to malignant proliferation

In response, the The French CA argued that it is not appropriate to simply undertake a statistical analysis for combined hyperplastic and neoplastic lesions (STP Hyperplasia Working group, 2003). Hyperplasia should be considered in a weight-of-evidence approach. In those cases in which hyperplastic lesions are believed to be relevant for assessment of carcinogenicity, qualitative evaluation of hyperplastic lesions is more appropriate than statistical analysis. Although THF exposure induced adenomas, the ATH frequency was not treatment-related. However, as the tumours develop preferentially on ATH, they might mask the true incidence of ATH in exposed animals. The French CA concluded that the low incidence of ATH in high-dose rats may have been caused by either a progression or a rapid change to neoplasia caused by THF.

Exacerbation of CPN

An association between CPN and the presence of atypical tubular hyperplasia (ATH) and renal tubule tumours has been observed from several carcinogenic studies in which chemical administration exacerbated the severity of CPN. The underlying factors associated with this relationship are not known but, most likely, are multi-factorial and complex (Seely and Hard, 2008). Seely et al (2002) observed that "nearly 100% of male rats that live for 2 years have some degree of CPN, therefore chemically exacerbated CPN is generally detected by an increase in severity rather than increases in incidence".

The PWG observed that essentially all tumours and hyperplasic changes occurred in rats with severe to end-stage CPN (Table 3) (PWG, 2009). As mean severity grades for CPN were very similar between the control and the high concentrations, it appears that exposure to THF had no effect on CPN.

The French CA agreed with the PWG that CPN was not exacerbated by THF since the same incidence and severity were observed in control and high-dose groups of male rats. Indeed, almost all male rats displayed nephropathy (Table 4) and 44% of control (22/50) and 42% (21/50) of high-dose rats had severe grades of nephropathy (7-8) (Table 3). Twenty out of 22 ATH were diagnosed by Hard (2005), and 7/9 adenomas occurred in kidneys with grades 7-8 (the most severe grades) and the majority with grade 8.

The incidence of tubular adenomas was 3.5-fold higher in the high exposure group of rats than in controls, whereas CPN was of the same incidence and severity in both groups. Therefore, the French CA concluded that it was a consequence of exposure and not CPN that an increase in adenoma incidence was observed in the exposed animals. THF appears to increase renal adenoma incidence based on a mechanism independent of CPN.

The French CA also commented that THF does not exacerbate CPN, but THF carcinogenic potency is exacerbated by CPN.

Table 3: Group incidence and severity of chronic progressive nephropathy (CPN) in the 2-year carcinogenicity study (from Hard, 2005)

Dose group (ppm)	Rats in group	Rats assessed for CPN	Rats with severity grade*:								
			0	1	2	3	4	5	6	7	8
Males											
0	50	48	0	2	0	2	4	4	**14**	9	13
200	50										
600	50										
1800	50	48	0	0	0	0	1	8	**18**	8	13
Females											
0	50	47	0	0	1	8	**18**	13	6	1	0
200	50										
600	50										
1800	50	49	0	1	2	8	**19**	11	7	1	0

0, *no lesions*; 1, *minimal*; 2, *mild*; 3, *low-moderate*; 4, *mid-moderate*; 5, *high-moderate*; 6, *low-severe*; 7, *high-severe*; 8, *end-stage*.

Table 4: Non-neoplasic changes in the urinary system from male rats exposed to THF for 2 years (NTP, 1998)

Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	3 (6%)	3 (6%)	2 (4%)	5 (10%)
Fibrosis	1 (2%)			
Inflammation, suppurative				1 (2%)
Metaplasia, osseous				1 (2%)
Nephropathy, chronic	48 (96%)	50 (100%)	50 (100%)	50 (100%)
Thrombosis	1 (2%)		1 (2%)	3 (6%)
Cortex, necrosis			1 (2%)	3 (6%)
Pelvis, dilatation	2 (4%)	1 (2%)		
Pelvis, transitional epithelium, hyperplasia	16 (32%)	13 (26%)	16 (32%)	18 (36%)
Pelvis, transitional epithelium, inflammation, suppurative		1 (2%)		1 (2%)
Renal tubule, degeneration	1 (2%)			
Renal tubule, hyperplasia	7 (14%)	5 (10%)	6 (12%)	7 (14%)
Renal tubule, inflammation, suppurative	1 (2%)			
Renal tubule, mineralization	8 (16%)	7 (14%)	2 (4%)	5 (10%)
Renal tubule, pigmentation, hemosiderin				1 (2%)
Urinary bladder	(50)	(50)	(49)	(50)
Hemorrhage	2 (4%)	1 (2%)		2 (4%)
Inflammation, suppurative	2 (4%)	1 (2%)		
Transitional epithelium, hyperplasia	2 (4%)	1 (2%)		3 (6%)

Basophilic adenomas of small size

In the NTP study, Chhabra and coworkers diagnosed renal tubule adenomas and carcinomas (Chhabra *et al*., 1998). The adenomas were composed of multiple solid nests of polygonal basophilic cells separated by a delicate vascular stroma. The authors stated that the neoplastic cells showed mild cellular and nuclear pleomorphism and atypia, and occasional mitotic cells were evident. Renal tubule carcinomas were well-demarcated nodular masses generally larger than adenomas. Carcinomas were composed of karyomegalic atypical cells and invariably contained areas in which there was loss of the tubular architecture and/or large sheets or nests of atypical cells containing large vacuoles.

In his follow up analysis of the histology slides from the NTP study, Hard (2005) diagnosed the two carcinomas observed in the high-dose males as adenomas. However, he also noted that "several of the adenomas in the high-dose group, and one in the mid-dose group, were much larger than the two lesions diagnosed in the control males, which were both marginal tumours" (Hard, 2005).

Therefore, it appears that the adenomas described are basophilic but *not* of small size.

Absence of alternative modes of action

In tubular epithelium unaffected by CPN, particularly in the cortex and outer stripe of the outer medulla (OSOM) of high-dose male and female rats, Hard (2005) found "no evidence of cytoplasmic vacuolation, single cell death (e.g. apoptosis), simple tubule hyperplasia, or increased mitotic activity, and no mineralisation in the papilla". This argues against sustained cytotoxicity and cell regeneration as a mode of action involved in the aetiology of the THF-induced renal tumours.

In addition, Lock and Hard (2004) reported that the small lesions they observed were not restricted in their distribution to either cortex or OSOM, and that this was contrary to the situation when tumours are associated with a specific site of renal tubule injury.

In conclusion, regarding CPN, it was the view of the French CA that the criteria of Hard and Khan (2005) were not all met. They argued that the highest incidence of tubular adenomas, seen in the high-dose group of male rats, is not correlated with an increase in CPN. It appears therefore that different mechanisms are probably linked to the two phenomena. The highest incidence of tubular adenoma and the highest grade of CPN both occurred in THF-exposed male rats (top dose group), whereas only the highest grade of CPN was seen in the controls. The French CA therefore concluded that there was not sufficient evidence to conclude definitively that the increased adenomas seen in THF-exposed rats had been a result of CPN induction only.

5.8.1.2 Overall conclusion of the Risk Assessment Committee on the renal tumours in male rats

As discussed in detail in the preceding sections of this document, the available evidence points to THF being a non-genotoxic carcinogen in the male rat kidney. The carcinogenic response seen on repeated exposure to THF was small but, a positive exposure-related trend was seen for adenomas and/or carcinomas. When judged against rigorous criteria, the available data do not allow the Committee to conclude that either a THF-mediated mechanism involving α 2u-globulin nephropathy or chronic progressive nephropathy (CPN) could account for these findings. Therefore there remains uncertainty about the mechanism(s) involved in the aetiology of these tumours.

5.8.6.2 Mammary gland fibradenoma in female rats

The French CA did not place any weight on the findings in the mammary glands of THF-exposed rats. Mammary gland fibroadenoma is a relatively common benign tumour finding in female Fischer 344 rats (NTP historical control range relevant to the THF study was 16- 42%). There was a marginally positive treatment-related trend for this tumour type with female rat exposure to THF (NTP, 1998). However, pair wise comparisons were not statistically significant. In addition, the concurrent control group also gave a tumour frequency above the historical control range. It seems doubtful, therefore, that the findings in the mammary gland were toxicologically significant.

5.8.6.3 Liver tumours in female mice

In high-dose female mice, the incidence of liver tumours were significantly greater than those of the controls. The increases in the incidences of liver neoplasms in the low-and mid-dose groups were not statistically significant, but the trend test was positive. The incidence of hepatocellular adenoma in the control, low-, mid-, and high-dose group was 12/50 (24%), 17/50 (34%), 18/50 (36%), and

31/48 (65%), respectively. For hepatocellular carcinoma, the respective incidences were: 6/50 (12%), 10/50 (20%), 10/50 (20%), and 16/48 (33%). The incidence of hepatocellular adenoma and carcinoma (combined) in the control, low-, mid-, and high-dose group was 17/50 (34%), 24/50 (48%), 26/50 (52%), and 41/48 (85%), respectively.

Although there was no exposure-related increased incidence of hepatocellular tumours in male mice, this could be explained by a very high incidence of such tumours in the controls (70%) and to the lower survival rate of high-dose mice. Alternatively, there may be an inherent sex difference in THF-induced liver neoplasms in mice (Chhabra *et al*., 1998).

It has been well documented that B6C3F1 mice are exceptionally sensitive for developing liver tumours, as seen in the control animals in the THF carcinogenicity study.

In their proposals, the French CA commented that species-specific liver tumours in mice are particularly evident under conditions of induced (chronic) liver injury. In this context, they observed that only a slight increase in liver necrosis was observed in female mice exposed to 5.4 mg/L THF in the NTP study (Table 5). In addition, no effects on survival or clinical observations were noted in female mice.

Table 5: Summary of the incidence of non-neoplastic lesions in female mice in the 2-year inhalation study of tetrahydrofuran (from NTP, 1998)

	Chamber Control	200 ppm	600 ppm	1,800 ppm
Liver	(50)	(50)	(50)	(48)
Angiectasis	1 (2%)	1 (2%)	1 (2%)	
Basophilic focus	1 (2%)			1 (2%)
Clear cell focus		1 (2%)		1 (2%)
Eosinophilic focus	7 (14%)	9 (18%)	7 (14%)	11 (23%)
Hematopoietic cell proliferation		1 (2%)	2 (4%)	3 (6%)
Hemorrhage	1 (2%)			
Hepatodiaphragmatic nodule	1 (2%)			1 (2%)
Inflammation, chronic	1 (2%)	1 (2%)	1 (2%)	
Mixed cell focus		3 (6%)		1 (2%)
Necrosis	3 (6%)			7 (15%)
Pigmentation	1 (2%)			
Thrombosis				1 (2%)
Vacuolization cytoplasmic, focal		1 (2%)		
Bile duct, cyst	1 (2%)	1 (2%)		

The French CA commented that the induction of a cell proliferation response in the rodent liver by chemicals is one biologically plausible mode of action for the carcinogenesis in this tissue. Sustained increase in cell proliferation may lead to the promotion of growth of pr-initiated cells and subsequently to tumour formation. In a 14-week inhalation study with THF, Gamer *et al* (2002) observed a proliferation of centrilobular hepatocytes in B6C3F1 mice but no morphological signs of cell degeneration or necrosis, at concentrations corresponding to those that cause tumours in the NTP study. However, this effect was reversible, demonstrating that it was likely a transient adaptative effect. In addition, the French CA noted, there was no evidence of increased cell proliferation in the NTP study. Consequently, it appeared that increased cell proliferation could not be the explanation for the highest incidence of liver tumours seen in female mice.

Moreover, Van Ravenzwaay et al (2005) mentioned that non genotoxic chemicals that induce hepatic metabolic enzyme systems and increase liver weights have been frequently observed to increase liver tumors formation in mouse strains, which have a high spontaneous background of

liver tumors such as the B6C3F1 mouse. They observed that THF induced an increase of CYP content and of EROD and PROD activities together with an increase of the weight of liver. But these effects were observed at a very high and toxic concentration, i.e. 5000 ppm, but not at 1800 ppm (5/4 mg/L) THF, which is the concentration at which liver tumours were induced.

Based on these data, the French CA concluded that the mode of action for liver tumour induction by THF was not clearly understood. Although they recognised that the tumours were seen in a sensitive mouse strain, they further concluded that it could not be excluded that the liver tumour findings "might be relevant for humans".

5.8.7 Summary and conclusion of the Risk Assessment Committee on carcinogenicity classification

THF does not appear to be a genotoxic substance and there are no epidemiological reports available to suggest that it may cause cancer in humans. However, the current concern about its potential carcinogenicity stems from the results of a standard 2-species carcinogenicity study reported by the NTP 12 years ago. The major tumour findings reported by the NTP are summarised in Table 6 below.

Table 6: Summary of the main tumour findings in the NTP inhalation carcinogenicity studies

	Dose (mg/L)	0	0.6	1.8	5.4
F344 Rats					
Males	Renal adenoma	2%	2%	8%	6%
	Renal carcinoma	0%	0%	0%	4%
	Combined adenoma/carcinoma	2%	2%	8%	10%
	Historical control range (combined) = 0-4%				
Females	Mammary gland fibroadenoma	46%	44%	58%	62%
	Historical control range = 16-42%				
B6C3F1 Mice					
Females	Hepatocellular adenoma	24%	34%	36%	65%
	Hepatocellular carcinoma	12%	20%	20%	33%
	Combined adenoma/carcinoma	34%	48%	52%	85%

The Risk Assessment Committee noted the re-analyses of the rat kidney histopathology reported by Hard (2005) and the PWG (2009), as discussed in the preceding sections of this document.

In the rat study, survival at the end of the 2-year exposure period was generally low, with that in the high-dose group (6/50) being lower than in the control group (12/50 controls). The clinical signs and body weights of these two groups were reported to be similar, and so 5.4 mg/L did not appear to be an excessively toxic concentration.

Although the THF exposure-related increase seen in male rat kidney tumours was small, it is judged to have been indicative of a carcinogenic effect. The PWG (2009), in their recent analysis of the key data, focussed on the possibility that regenerative processes associated with severe chronic progressive nephropathy or low-grade α 2u-globulin nephropathy likely contributed to the formation of renal tumours in the exposed animals. The PWG (2009) concluded that neither of these mechanisms would pose a "risk" to humans. The Risk Assessment Committee, however, found that definitive evidence for either of these 2 non-genotoxic mechanisms being involved was lacking. It

was therefore not possible to dismiss this carcinogenic hazard in considering the classification of THF.

The trend towards a greater incidence of mammary gland fibroadenoma in female rats following exposure to THF was statistically significant, whereas a similar trend in males was not. However, the incidence of this tumour in the female control group was high, and pair wise statistical comparisons with exposure groups were not significant. This concurrent control incidence of mammary gland fibroadenoma was outside the NTP's historical control range for the same strain and species. Taking into account all these factors, the Risk Assessment Committee did not find the evidence for a significant carcinogenic effect of THF in the mammary gland of rats to be convincing.

The only tissue that showed evidence of increased tumour incidence in mice was the liver, in which there was a trend towards an increased incidence of hepatocellular adenoma/carcinoma with increasing exposure in females. These tumours occurred in the absence of an obvious hepatotoxic effect and at a concentration that did not result in clinical signs of toxicity. However, although the mode of action for induction of the tumours has not been clarified, the tumours occurred in the highly sensitive B6C3F1 strain of mouse. As THF is non-genotoxic, and no increases in liver tumours were seen in exposed rats, the Risk Assessment Committee concluded that the findings were most likely to have been specific to the strain and species tested.

As there is no epidemiological evidence regarding the carcinogenicity of THF to humans, a classification in Category 1 (Directive 67/548/EEC) or Category 1a (CLP Regulation) is not appropriate.

Although evidence for carcinogenic responses was found in both rats and mice, the tumour types found were largely benign in nature, sex-specific and occurred at a low incidence rate. There are significant doubts about the relevance to humans of all the experimental tumour findings and, given that THF is non genotoxic, classification in Category 2 (Directive 67/548/EEC) or Category 1b (CLP Regulation) is also judged inappropriate.

Looking specifically at the criteria for deciding between Category 3 (Directive 67/548/EEC)/ Category 2 (CLP Regulation) and no classification, the Risk Assessment Committee concluded that the findings in relation to kidney tumours in THF-exposed male rats were sufficient to justify classification. The possible mechanism(s) of kidney tumour formation had not been identified clearly, and so there remained uncertainty about extrapolation to humans. Also, such neoplasms are not well known to occur in male Fischer 344 rats spontaneously with a high incidence.

The B6C3F1 strain of mouse has been well established as sensitive to liver tumour induction, and no other tumour type was detected in this species. Consequently, in accordance with the criteria, the liver tumour findings would not in themselves justify classification of THF. However, the absence of increased kidney tumours in the exposed B6C3F1 mice does not detract from the findings in male rats.

Overall, therefore, it was concluded by the Risk Assessment Committee that THF meets the criteria for carcinogenicity classification in Category 3 (Directive 67/548/EEC) and Category 2 (CLP Regulation).

In consideration of label to accompany the classification, although the carcinogenic findings were in inhalation studies, there are no significant grounds to indicate the concern is limited to this route of exposure.

Directive 67/548/EEC: Carc Cat 3; R40

CLP Regulation: Carc 2; H351

5.8.8 Other information relating to carcinogenicity of THF

The NTP considers THF as reasonably anticipated a carcinogen (NTP, 1998). The ACGIH classified THF as A3 Confirmed Animal Carcinogen with Unknown Relevance to Humans (ACGIH, 2004). In addition, the DFG placed THF in category 4 (i.e. Substances with carcinogenic potential for which genotoxicity plays no, or at most a minor role) (DFG website).

5.9 Toxicity to reproduction

Not evaluated. No assessment of the potential reproductive toxicity of THF has been made by the Risk Assessment Committee. No opinion has been developed regarding classification for this endpoint.

Justification that action is required on a Community-wide basis

In accordance with Article 115 of REACH and Article 36 (i) of the CLP Regulation, the Risk Assessment Committee formed an opinion on the harmonised classification of THF relating to the endpoints of mutagenicity and carcinogenicity only. For reproductive toxicity and respiratory sensitisation no data were available and therefore the Risk Assessment Committee did not evaluate these endpoints.

The original classification proposal submitted by France included the following rationale for assessing other endpoints.

“Relevant acute and repeated toxicity, and mutagenicity data were also reported in this dossier to allow a better understanding of the toxicological profile of THF in relationship with the assessment of its CMR properties. When relevant, potential classifications for endpoints other than CMR are discussed in the proposal to take advantage of having the information available to the competent expert group”.

Since this did not provide a justification for additional action on a Community-wide bases, the Risk Assessment Committee did not evaluate any further endpoints.

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ANNEX – LIST OF ABBREVIATIONS

ACGIH	American Conference of Industrial Hygienists
AP	alkaline phosphatase
BAT	Biologische Arbeitsstofftoleranzwerte (Biological Tolerance Value)
BGIA	Berufsgenossenschaftliche Institut für Arbeitsschutz
BRDU	BromoDeoxyUridine
CHO	Cell Hamster Ovary
DFG	Deutsche Forschungsgemeinschaft
GLP	Good Laboratory Practice
GOT	glutamic-oxalacetic transaminase
GPT	glutamic-pyruvate transaminase
IARC	International Agency for Research on Cancer
IUCLID	International Uniform Chemical Information Database
i.p	intra-parenteral
MAK	Maximale Arbeitsplatzkonzentrationen (Maximum Allowable Concentration)
MTD	Maximum Tolerated Dose
NOHSC	National Occupational Health Safety Commission
NTP	National Toxicology Program
PCE	Polychromatic Erythrocyte
s.c	subcutaneous
SCE	Sister Chromatide Exchange
SHE	Syrian Hamster Embryo
STEL	Short Term Exposure Limit
THF	Tetrahydrofuran
TLV	Threshold Limit Value
TWA	Time Weighted Average