

TETRAFLUOROETHYLENE

Tetrafluoroethylene was reviewed previously by the Working Group in 1979, 1987, and 1998 ([IARC, 1979, 1987, 1999](#)). New data have since become available, and these have been incorporated, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 116-14-3

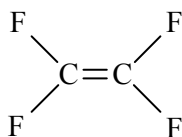
Chem. Abstr. Serv. Name: Tetrafluoroethylene

IUPAC Systematic Name:

1,1,2,2-Tetrafluoroethene

Synonyms: Perfluoroethylene, Perfluoroethene, Ethylene tetrafluoro-, tetrafluoroethene

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: C₂F₄

Relative molecular mass: 100.01

1.1.3 Chemical and physical properties of the pure substance

From [IFA \(2014\)](#), unless otherwise indicated

Description: Colourless gas, odourless or sometimes described as having a faint sweetish odour; extremely flammable

Boiling point: -75.63 °C

Melting point: -131.15 °C ([HSDB, 2014](#))

Density: 4216 kg/m³ at 15 °C at 1 bar

Solubility: Slightly soluble in water, 159 mg/L at 25 °C ([HSDB, 2014](#))

Vapour pressure: 2947 kPa at 20 °C

Stability: Decomposes into fluorine and fluorine compounds when heated ([HSDB, 2014](#))

Reactivity: A terpene inhibitor (limonene) is generally added to the monomer to prevent spontaneous polymerization.

Risk of explosion in contact with air or in the absence of air at elevated temperatures and/or pressures (> 600 °C and 100 kPa). The stabilized monomer is flammable in air if ignited (flammability limits: lower, 11%; upper, 60%) producing soot and carbon tetrafluoride ([Babenko et al., 1993](#); [HSDB, 2014](#)).

Incompatible with polymerization catalysts and peroxides. May react exothermically with

Table 1.1 Methods for the analysis of tetrafluoroethylene

Sample matrix	Sample preparation	Assay procedure	Limit of detection ^a	Reference
Air	Sample collected directly from the workplace	FTIR	0.17 ppm [$\approx$ 0.7 mg/m ³]	NIOSH (2003)
	Collection onto solid sorbents, such as activated charcoal, followed by solvent desorption	GC	0.18 ppm [$\approx$ 0.7 mg/m ³]	HSE (1997) ISO (2001)
	Air collected into a stainless steel container; sample analysed directly	GC/MS	NR	EPA (1999)

^a Detection limit reported by [ECETOC \(2003\)](#)

FTIR, Fourier transform infra-red spectrometry; GC, gas chromatography; MS, mass spectrometry; NR, not reported

chloroperoxytrifluoromethane, sulfur trioxide and several other substances ([HSDB, 2014](#)). May react if in contact with aluminium, copper and their alloys, resulting in an uncontrolled exothermic reaction ([ECHA, 2014](#)).

Octanol/water partition coefficient (P): log $P = 1.21$ (estimated) ([HSDB, 2014](#))

Conversion factor: Assuming normal temperature (25 °C) and pressure (101 kPa), 1 mg/m³ = 4.09 ppm, calculated from mg/m³ = (relative molecular mass/24.45) $\times$ ppm.

1.1.4 Technical products and impurities

Industrial-grade tetrafluoroethylene generally has a purity of > 99.7%. Impurities may include various chloro-fluoro compounds ([ECETOC, 2003](#)). Limonene may be added to prevent spontaneous polymerization ([HSDB, 2014](#)).

1.1.5 Analysis

A range of sampling and analytical methods can be used to measure exposure to tetrafluoroethylene, although there is only one validated method from the United States National Institute of Occupational Safety and Health (NIOSH), based on using a Fourier transform infra-red (FTIR) spectrometer to directly detect tetrafluoroethylene. Selected available methods are summarized in [Table 1.1](#).

Generic methods for the collection of volatile organic substances using solid sorbents such as activated charcoal, followed by analysis using gas chromatography (GC) have been used to measure occupational exposure. It is also possible to sample air contaminated with tetrafluoroethylene into a solid stainless steel container, and to then analyse the sample using gas chromatography-mass spectrometry (GC-MS).

1.2 Production and use

1.2.1 Production process

(a) Manufacturing processes

Tetrafluoroethylene is manufactured in a four-stage process involving the separate production of hydrogen fluoride and chloroform, which are subsequently reacted in the presence of antimony trifluoride to produce chlorodifluoromethane. The chlorodifluoromethane is pyrolysed at > 650 °C to produce tetrafluoroethylene ([ECETOC, 2003](#); [HSDB, 2014](#)).

(b) Production volumes

Worldwide production of tetrafluoroethylene in 1977 was estimated at 15 000–20 000 tonnes (cited in [IARC, 1999](#)), and market growth has since been 3–5% per annum ([Teng, 2012](#)). The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) has estimated that the annual world production of

tetrafluoroethylene in 2001 was 100 000 tonnes ([ECETOC, 2003](#)).

In 2000, an estimated 10 000–50 000 tonnes of tetrafluoroethylene was produced in the European Union ([European Chemicals Bureau, 2000](#)). The Toxic Substances Control Act Inventory Update Rule of the United States Environmental Protection Agency (EPA) indicated that annual production of tetrafluoroethylene and importation into the USA totalled 50–100 million pounds [22 000–45 000 tonnes] from 1998 to 2006 ([NTP, 2014](#)).

1.2.2 Uses

Tetrafluoroethylene is used in the manufacture of oligomers, fluoroelastomers and fluoropolymers. The main use of tetrafluoroethylene is in the manufacture of polytetrafluoroethylene that is used as nonstick coatings on cookware, membranes for clothing that are both waterproof and breathable, electrical-wire casing, fire- and chemical-resistant tubing, and plumbing thread seal tape. It reacts with perfluoronitrosoalkanes to produce nitroso rubbers. It is also used in the production of compounds and intermediates of low relative molecular mass, including for the manufacture of iodoperfluoroalkanes ([NTP, 2014](#)).

1.3 Occurrence and exposure

1.3.1 Environmental occurrence

(a) Natural occurrence

Tetrafluoroethylene has been detected in very low concentrations in natural gas, and in gaseous emissions from volcanic vents ([Gribble, 2010](#)). There are no other known natural sources.

(b) Air and water

Emission of tetrafluoroethylene to air or water may occur from primary production, or from use in the manufacture of other products.

Deliberate vent releases from industrial plants are generally destroyed by thermal oxidation ([ECETOC, 2003](#)).

Tetrafluoroethylene does not readily biodegrade in water, sediment, or soil, and has low potential to bioaccumulate in aquatic organisms ([ECHA, 2014](#)).

Gaseous tetrafluoroethylene degrades in the atmosphere by reaction with photochemically produced hydroxyl radicals, with a half-life of approximately 17 hours ([HSDB, 2014](#)). Modelling suggests that 99.99% of environmental emissions end in the air, with 0.008% in water ([ECHA, 2014](#)). An environmental survey realized by the government of Japan in 2012 detected tetrafluoroethylene in the air at 4 of the 10 sites tested, with concentrations up to 2.8 µg/m³. Tetrafluoroethylene was not detected in water ([Japanese Environmental Survey, 2012](#)).

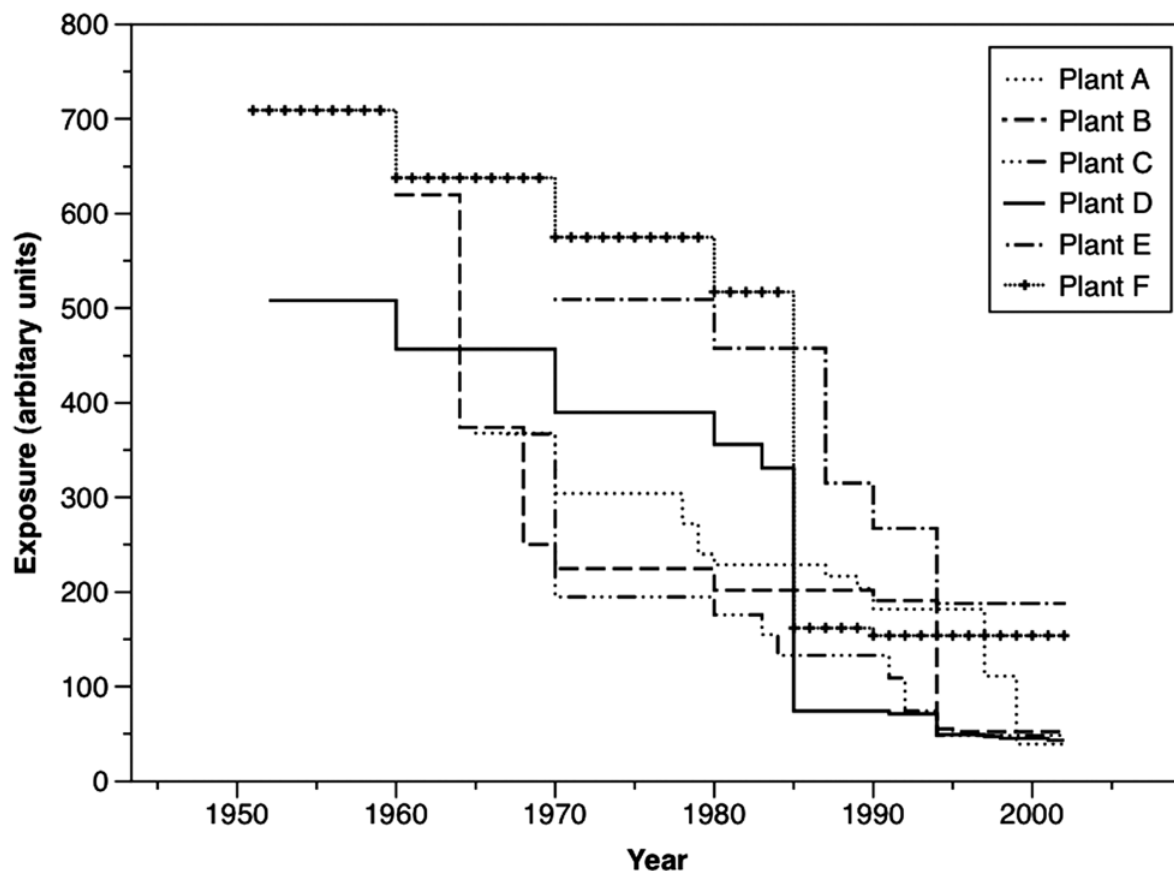
1.3.2 Occupational exposure

Occupational exposure occurs in the primary manufacture of tetrafluoroethylene and during the subsequent polymerization process.

Inhalation exposure has been measured in several European plants manufacturing tetrafluoroethylene. [ECETOC \(2003\)](#) reported levels of between 0.16 and 6 mg/m³ in one plant, and between < 0.4 and 6.1 mg/m³ (95% of samples, < 2 mg/m³) in a second plant, in both data sets as an 8-hour time-weighted average. No other published data were available for workplace exposures to tetrafluoroethylene.

As part of an international epidemiological study of workers in six plants manufacturing polytetrafluoroethylene in Germany, the Netherlands, Italy, the United Kingdom, and the USA (New Jersey and West Virginia), [Sleeuwenhoek & Cherrie \(2012\)](#) made estimates of exposure to tetrafluoroethylene by inhalation using modelling methodology. The exposure reconstructions were made using descriptive information about the workplace environment

Fig. 1.1 Change in levels of exposure to tetrafluoroethylene for operators working in polymerization areas of six plants manufacturing polytetrafluoroethylene



Reproduced from [Sleeuwenhoek & Cherrie \(2012\)](#), with permission of The Royal Society of Chemistry

Note: Plants A–F were located in Germany, the Netherlands, Italy, the United Kingdom, and the USA (New Jersey and West Virginia)

and work processes, including changes over time. The methodology allowed for key changes in exposure modifiers such as local ventilation, use of respiratory protective equipment, working in a confined space, outdoor work, cleanliness, and the level of involvement of the workers in the process (e.g. operator or supervisor). There were very few measurements of exposure available from the plants (all unpublished), and so the exposure estimates were expressed on an arbitrary dimensionless scale. Two assessors made assessments independently and the results were then combined ([Sleeuwenhoek & Cherrie, 2012](#)).

In each plant, the highest estimated exposures for tetrafluoroethylene occurred in the

polymerization area. The introduction of control measures, increasing process automation and other improvements, were judged to have resulted in exposures generally decreasing over time. In the polymerization area, the annual estimated decline in exposure to tetrafluoroethylene varied by plant from 3.8% to 5.7% (see [Fig 1.1](#)). The differences in the estimated exposure level for polymerization workers at any time were up to about fivefold. Part of these inter-plant differences can be explained by differences in technology and the work responsibilities of operators ([Sleeuwenhoek & Cherrie, 2012](#)). The biggest changes in exposure for polymerization workers were mainly due to the introduction of automatic

cleaning and automation at the autoclaves. Other improvements causing important declines in exposure levels were the introduction of localized ventilation and vacuum extraction at the end of the polymerization process (Sleeuwenhoek & Cherrie, 2012).

Operators in the monomer area always wore breathing apparatus when undertaking tasks where exposure to tetrafluoroethylene was possible, and so inhalation exposure for these workers would have been very low. In this area of the plants there were small decreases in estimated exposure levels due to general environmental improvements, such as the use of more efficient pumps and gaskets (Sleeuwenhoek & Cherrie, 2012).

Tetrafluoroethylene exposure for workers in the finishing areas of the plants was consistently low over the history of the plant. The decline in exposure levels was generally smaller in finishing areas than in other areas, and the changes were primarily due to improved general ventilation (Sleeuwenhoek & Cherrie, 2012).

Historically, workers in polytetrafluoroethylene production were potentially exposed to both tetrafluoroethylene and the ammonium salt of perfluorooctanoic acid (PFOA), which is also the subject of a *Monograph* in the present volume). Only a small number of jobs with lower exposure to tetrafluoroethylene had no possible exposure to ammonium perfluorooctanoate. Workers in most jobs were exposed to both chemicals, and there was a strong positive correlation between estimated exposure to tetrafluoroethylene and ammonium perfluorooctanoate ($r = 0.72$, $P < 0.001$) (Sleeuwenhoek & Cherrie, 2012).

[The Working Group considered that the limited quantity of data on measured occupational exposure suggested that in about 2000 the highest tetrafluoroethylene exposure levels in manufacturing plants were about 6 mg/m^3 , and considering the temporal trends described above (average change over the history of production, about sixfold), it seems probable that the highest

occupational average exposures to tetrafluoroethylene in the polytetrafluoroethylene-manufacturing industry in the 1950s and 1960s would have been $< 40 \text{ mg/m}^3$.]

1.3.3 Exposure of the general population

No information was available about the levels of exposure to tetrafluoroethylene in the general population, although because of the necessity to contain the substance within an enclosed system due to its flammable nature, it is likely that any exposure is very low and localized around industrial facilities manufacturing or using tetrafluoroethylene. Tetrafluoroethylene is not detectable in its polymerized products, including polytetrafluoroethylene (analytical detection limit, $< 0.05\text{--}0.01 \text{ mg/kg}$) (ECETOC, 2003). When heated to temperatures above those normally used for cooking, polytetrafluoroethylene-coated pans may emit tetrafluoroethylene, although the major hazard in such circumstances is particulate fumes, which can cause serious acute effects (NIOSH, 1977).

1.4 Regulations and guidelines

Major national regulatory occupational exposure limits for tetrafluoroethylene are given in Table 1.2.

Tetrafluoroethylene has been registered under the Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) regulation of the European Union. All registered uses are under “PROC 1: Use in closed process, no likelihood of exposure” (ECHA, 2014).

The derived no-effect level (DNEL) under the REACH system for long-term exposure by inhalation based on systemic health effects is 6.4 mg/m^3 , from the registration entry of the manufacturer/importer in data from the European Chemicals Agency (IFA, 2014).

Tetrafluoroethylene is categorized in Europe in carcinogenic category 1B, with H350 “may

Table 1.2 Regulations and guidelines for occupational exposure to tetrafluoroethylene

Country or region	Long-term average concentration (mg/m ³)	Carcinogenicity
European Union (DNEL) ^a	6.4	Category 1B with H350 “may cause cancer”
USA (ACGIH) ^b	8.2	A3; confirmed animal carcinogen with unknown relevance to humans
USA (NTP) ^c	–	“Reasonably anticipated to be a human carcinogen”

^a DNEL, derived no-effect level; data from the GESTIS DNEL database ([IFA, 2014](#))

^b Eight-hour time-weighted average (8-hour TLV-TWA); data from American Conference of Governmental Industrial Hygienists. Note that for all long-term threshold limit values (TLVs), excursions in exposure level may not exceed three times the 8-hour TLV-TWA for more than a total of 30 minutes during a workday, and under no circumstances should these excursions exceed five times the 8-hour TLV-TWA, provided that the TLV-TWA is not exceeded ([ACGIH, 2013](#))

^c Data from the United States National Toxicology Program ([NTP, 2014](#))

Table 1.3 Acute exposure guideline levels (AEGs) for tetrafluoroethylene

Type of AEG	AEG in ppm (mg/m ³) for exposure duration				
	10 minutes	30 minutes	1 hour	4 hours	8 hours
AEG-1 ^a (non-disabling)	27 (110)	27 (110)	22 (89)	14 (56)	9 (37)
AEG-2 ^b (disabling)	69 (280)	6 (280)	55 (220)	34 (140)	23 (92)
AEG-3 ^c (lethal)	420 (1700)	420 (1700)	330 (1400)	210 (850)	100 (430)

^a AEG-1 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic non-sensory effects

^b AEG-2 is the concentration above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects, or have an impaired ability to escape

^c AEG-3 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death

From [NRC \(2015\)](#)

cause cancer”, under classification, labelling, and packaging Regulation (EC) No. 1272/2008) ([ECHA, 2015](#)).

In the USA, tetrafluoroethylene is classified as “reasonably anticipated to be a human carcinogen” by the National Toxicology Program (NTP) in its Report on Carcinogens ([NTP, 2014](#)).

Tetrafluoroethylene is included within the United States Toxics Release Inventory ([TRI, 2016](#)).

The Committee on Acute Exposure Guideline Levels of the United States National Research Council has set acute exposure guideline levels for tetrafluoroethylene (summarized in [Table 1.3](#); [NRC, 2015](#)). Acute exposure guideline levels represent threshold exposure limits for the general public, and are applicable to emergency

exposure periods ranging from 10 minutes to 8 hours. The American Industrial Hygiene Association has published emergency response planning guidelines for tetrafluoroethylene ([AIHA, 2013](#)).

2. Cancer in Humans

2.1 Cohort studies

See [Table 2.1](#) for study details

Only one cohort study analysing cancer risk in relation to exposure to tetrafluoroethylene was available to the Working Group. [Consonni et al. \(2013\)](#) studied mortality from cancer and from selected non-malignant diseases in a

Table 2.1 Cohort studies of cancer and occupational exposure to tetrafluoroethylene

Reference, location, follow-up period	Total subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	SMR (95% CI)	Covariates Comments
Consonni et al. (2013) , six plants in Europe and the USA, 1950/1970 – 2001/2008	4773 exposed workers (Germany, 690; Italy, 415; the Netherlands, 658; United Kingdom 756; USA, 2254)	JEM based on semiquantitative exposure estimates on an arbitrary scale	All cancers	Overall	187	0.77 (0.67–0.89)	Men only; all were ever exposed, national reference rates were used
			Oesophagus		11	1.23 (0.62–2.21)	
			Liver		8	1.27 (0.55–2.51)	
			Pancreas (157)		13	1.15 (0.61–1.97)	
			Lung		59	0.73 (0.56–0.95)	Categorized into tertiles based on number of deaths from all causes based on cumulative exposure
			Kidney and other urinary organs		10	1.44 (0.69–2.65)	
			Leukaemia		12	1.48 (0.77–2.59)	
			Liver	Cumulative exposure (unit-yrs)			
				Low (< 80.5)	1	0.59 (0.01–3.28)	<i>P</i> for trend, 0.24
				Medium (80.5–559)	2	0.95 (0.12–3.43)	
				High (≥ 560)	5	2.01 (0.65–4.70)	
			Kidney and other urinary organs	Cumulative exposure (unit-yrs)			
				Low (< 80.5)	2	0.93 (0.11–3.37)	<i>P</i> for trend, 0.87
				Medium (80.5–559)	6	2.58 (0.95–5.62)	
				High (≥ 560)	2	0.81 (0.10–2.93)	
			Leukaemia	Cumulative exposure (unit-yrs)			
				Low (< 80.5)	4	1.47 (0.40–3.76)	<i>P</i> for trend, 0.77
				Medium (80.5–559)	3	1.14 (0.24–3.34)	
				High (≥ 560)	5	1.83 (0.59–4.26)	

CI, confidence interval; ICD, International Classification of Disease; JEM, job-exposure matrix; SMR, standardized mortality ratio; yr, year

cohort including workers in six polytetrafluoroethylene-production sites in Europe (Germany, the Netherlands, Italy, England) and the USA (New Jersey, West Virginia) from 1950 to 2002. Production of polytetrafluoroethylene involves the use of ammonium perfluorooctanoate, exposure to which was also analysed. Follow-up was from start of production between 1950 and 1970, until 2008. Of the 5879 men identified, 4773 who were potentially exposed were included in the analysis.

Semiquantitative estimates of individual exposure to tetrafluoroethylene and ammonium perfluorooctanoate were reconstructed based on a specifically developed job-exposure matrix ([Sleeuwenhoek & Cherrie, 2012](#)). Standardized mortality ratios (SMRs) were calculated using national mortality rates as comparison. Plant-specific results were not presented.

In the overall analysis, elevated risks were seen for all cancer sites of a-priori interest: liver, 1.27 (95% CI, 0.55–2.51); kidney, 1.44 (95% CI, 0.69–2.65); and leukaemia, 1.48 (95% CI, 0.77–2.59). No significant trends in risk with increasing exposure were observed with cumulative exposure to tetrafluoroethylene, or with duration of exposure or time since exposure for any of the cancer sites of interest ([Table 2.1](#)). A significant downward trend in the risk of cancer of the lung was observed with increasing exposure duration, but not with other exposure metrics. Additional analyses using regional comparison rates did not materially change risk estimates. Eighty-eight percent of workers were exposed to ammonium perfluorooctanoate as well as to tetrafluoroethylene. Analysis of patterns of mortality with ammonium perfluorooctanoate or tetrafluoroethylene as the exposure of interest gave very similar results.

[The results suggested an elevated risk of cancer of the liver and kidney, and leukaemia. Direct control for possible non-occupational confounders was not possible; however, based on analysis of mortality patterns in the cohort

and general knowledge of exposures in the included plants, the Working Group judged that major confounding by alcohol, tobacco, hepatitis B virus, or vinyl chloride monomer was unlikely. The power of the study was, however, not sufficient to support a causal association with tetrafluoroethylene. The Working Group characterized this as a well-conducted study with thorough exposure assessment, which with a longer follow-up would be expected to have more deaths and hence more statistical power to detect any possible associations.]

2.2 Case-control studies

No case-control studies on cancer risk and exposure to tetrafluoroethylene were available to the Working Group.

3. Cancer in Experimental Animals

The carcinogenicity of tetrafluoroethylene in experimental animals was reviewed previously by the Working Group ([IARC, 1999](#)). The Working Group at this time identified two studies of carcinogenicity in rodents treated with tetrafluoroethylene by inhalation: one study in male and female mice, and one study in male and female rats.

3.1 Mouse

See [Table 3.1](#)

Groups of 48 male and 48 female B6C3F₁ mice (age, 7 weeks) were exposed to tetrafluoroethylene (purity, 98–99%) at a concentration of 0 (control), 312, 625, or 1250 ppm by inhalation for 6 hours per day, 5 days per week, for 95–96 weeks, with an observation period of 11 days after the final exposure. The study was terminated during week 96 because of reduced survival compared with controls. Mean body weights in

Table 3.1 Studies of carcinogenicity in mice exposed to tetrafluoroethylene by inhalation

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
B6C3F ₁ (M) 95–96 wk + 11 days recovery NTP (1997)	0 (control), 312, 625, 1250 ppm for 6 h/day, 5 days/wk 48 mice/group	Liver Haemangioma ^a : 0/48, 10/48 (21%)**, 5/48 (10%)*, 2/48 (4%) Haemangiosarcoma ^b : 0/48, 21/48 (44%)*, 27/48 (56%)*, 37/48 (77%)** Haemangioma or haemangiosarcoma (combined) ^c : 0/48, 26/48 (54%)*, 30/48 (63%)*, 38/48 (79%)** Hepatocellular adenoma: 17/48 (35%), 17/48 (35%), 12/48 (25%), 20/48 (42%) Hepatocellular carcinoma: 11/48 (23%), 20/48 (42%)*, 33/48 (69%)*, 26/48 (54%)* Hepatocellular adenoma or carcinoma (combined) ^c : 26/48 (54%), 34/48 (71%)*, 39/48 (81%)*, 35/48 (73%)* <i>Histiocytic sarcoma (all organs)^d</i> 0/48, 12/48 (25%)*, 7/48 (15%)*, 7/48 (15%)**	* $P < 0.05$ (Fisher exact test) ** $P < 0.01$ (Fisher exact test)	Purity, 98–99% Surviving animals: 38, 11, 2, 1 Statistical analysis adjusted for survival

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
B6C3F ₁ (F) 95–96 wk + 11 days recovery NTP (1997)	0 (control), 312, 625, 1250 ppm for 6 h/day, 5 days/wk 48 mice/group	<i>Liver</i> Haemangioma: 0/48, 5/48 (10%)*, 2/47 (4%), 1/47 (2%) Haemangiosarcoma: 0/48, 27/48 (57%)*, 27/47 (58%)*, 34/47 (72%)* Haemangioma or haemangiosarcoma (combined): 0/48, 31/48 (65%)*, 28/47 (60%)*, 35/47 (73%)* Hepatocellular adenoma: 15/48 (31%), 17/48 (35%), 20/47 (43%)*, 15/47 (32%) Hepatocellular adenoma (multiple): 1/48 (2%), 7/48 (15%)*, 9/47 (19%)*, 7/47 (15%)* Hepatocellular carcinoma: 4/48 (8%), 28/48 (58%)*, 22/47 (47%)*, 20/47 (43%)* Hepatocellular carcinoma (multiple): 0/48, 5/48 (10%), 7/47 (15%), 7/47 (15%) Hepatocellular adenoma or carcinoma (combined) ^g : 17/48 (35%), 33/48 (69%)*, 29/47 (62%)*, 28/47 (60%)* <i>Histiocytic sarcoma (all organs)^h</i> 1/48 (2%), 21/48 (44%)*, 19/47 (40%)*, 18/48 (38%)*	* <i>P</i> < 0.05 (Fisher exact test) ** <i>P</i> < 0.01 (Fisher exact test)	Purity, 98–99% Surviving animals: 36, 4, 6, 4

^a Historical incidence for 2-year NTP inhalation studies with chamber control groups at all laboratories (mean $\pm$ standard deviation): 2/947 (0.2% $\pm$ 0.7%); range, 0–2%. Historical

incidence for 2-year NTP inhalation studies with chamber control groups at Battelle Pacific North-western Laboratories: 1/448 (0.2% $\pm$ 0.7%); range 0–2%

^b Historical incidence at all laboratories: 12/947 (1.3% $\pm$ 1.7%); range, 0–6%. Historical incidence at Battelle Pacific North-western Laboratories: 2/448 (0.5% $\pm$ 0.9%); range, 0–2%

^c Historical incidence at all laboratories: 358/947 (37.8% $\pm$ 12.5%); range, 11–60%. Historical incidence at Battelle Pacific North-western Laboratories: 186/448 (41.5% $\pm$ 9.2%); range, 30–60%.

^a For the liver, lung, spleen, mesenteric lymph node, bone marrow, and kidney, historical incidence for 2-year NTP inhalation studies with chamber control groups at all laboratories (mean $\pm$ standard deviation): 6/950 (0.6% $\pm$ 1.2%); range, 0–4%. Historical incidence for 2-year NTP inhalation studies with chamber controls at Battelle Pacific North-western Laboratories: 2/450 (0.4% $\pm$ 0.9%); range, 0–2%.

^c Historical incidence at all laboratories: 1/937 (0.1% $\pm$ 0.5%); range, 0–2%. Historical incidence at Battelle Pacific North-western Laboratories: 0/446

Historical incidence at all laboratories: 5/937 (0.5% $\pm$ 1.0%); range, 0–3%. Historical incidence at Battelle Pacific North-western Laboratories: 2/446 (0.5% $\pm$ 0.9%); range, 0–2%

g. Historical incidence at all laboratories: 200/937 (21.3%)

^b Historical incidence at all laboratories: 26/941 (2.8% $\pm$ 3.1%); range, 0–10%. Historical incidence at Battelle Pacific North-western Laboratories: 14/447 (3.1% $\pm$ 3.0%); range, 0–8%.

h, hour; mo, month; wk, week

exposed groups were generally similar to those of the controls except at the end of the study, when body weight was decreased in mice at the highest dose. The survival rates of males in the group at 625 ppm (intermediate dose) and of all exposed groups of females were significantly less than those of the controls ([NTP, 1997](#)).

In male mice exposed to tetrafluoroethylene at a concentration of 0, 312, 625, or 1250 ppm, the incidence of liver haemangioma was significantly higher in the groups at the lowest and intermediate doses than in the control group. The incidences of haemangiosarcoma, and of haemangioma or haemangiosarcoma (combined), were significantly higher in all exposed groups than in the controls. The incidences of hepatocellular carcinoma, and of hepatocellular adenoma or carcinoma (combined), were significantly higher in all exposed groups. The incidence of eosinophilic foci in the liver was significantly higher in the groups at the intermediate and highest doses (1/48, 6/48, 7/48, 7/48).

The incidence of histiocytic sarcoma (in organs such as the liver, lung, spleen, mesenteric lymph node, bone marrow, and kidney) was significantly greater in all exposed groups than in the control group ([NTP, 1997](#)).

In female mice exposed to tetrafluoroethylene at a concentration of 0, 312, 625, or 1250 ppm, the incidence of liver haemangioma was significantly higher in the group at the lowest dose than in the controls. The incidences of haemangiosarcoma, and of haemangioma or haemangiosarcoma (combined), were significantly higher in all exposed groups. The incidence of hepatocellular adenoma was significantly higher in the group at the intermediate dose. The incidence of hepatocellular carcinoma, and of hepatocellular adenoma or carcinoma (combined), was significantly higher in all exposed groups. The incidence of eosinophilic foci of the liver was significantly higher in the groups at the lowest and intermediate dose (5/48, 13/48, 12/47, 7/47).

The incidence of histiocytic sarcoma (in organs such as liver, lung, spleen, mesenteric lymph node, bone marrow, and kidney) was significantly greater in all exposed groups than in the control group ([NTP, 1997](#)).

3.2 Rat

See [Table 3.2](#)

Groups of 50 male and 50 female F344/N rats (age, 7 weeks) were exposed to tetrafluoroethylene (purity, 98–99%) at a concentration of 0, 156 (males only), 312, 625, or 1250 (females only) ppm by inhalation for 6 hours per day, 5 days per week, for 104 weeks, with an observation period of 11 days after the final exposure. Mean body weights of exposed groups were generally similar to those of the controls except at the end of the study, when body weight was decreased in rats at the highest dose. The survival rates of males at 625 ppm (the highest dose) and of females in all exposed groups of were significantly less than those of the controls ([NTP, 1997](#)).

In male rats exposed to tetrafluoroethylene at a concentration of 0, 156, 312, or 625 ppm, the incidence of renal cell adenoma was significantly higher in the groups at the intermediate and highest dose than in the controls. The incidence of renal cell adenoma or carcinoma (combined) was significantly higher in the group at the highest dose. The incidence of hepatocellular carcinoma, and of hepatocellular adenoma or carcinoma (combined), was significantly higher in the group at the intermediate dose. The incidences of basophilic foci (22/50, 19/50, 33/50, 29/50), eosinophilic foci (3/50, 18/50, 22/50, 19/50) and mixed cell foci (5/50, 5/50, 16/50, 13/50) of the liver were significantly higher in the groups at the intermediate and highest dose ([NTP, 1997](#)).

The incidence of mononuclear cell leukaemia was significantly higher in males at the lowest and highest dose. There was a small but significant increase in the incidence of interstitial cell

Table 3.2 Studies of carcinogenicity in rats exposed to tetrafluoroethylene by inhalation

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance ^g	Comments
F344/N (M) 104 wk + 11 days NTP (1997)	0 (control), 156, 312, 625 ppm for 6 h/day, 5 days/wk, 104 wk 50 rats/group	<i>Kidney</i> Renal cell adenoma^{c,d}: 2/50 (4%), 4/50 (8%), 9/50 (18%)*, 13/50 (26%)** Renal cell carcinoma^c: 1/50 (2%), 1/50 (2%), 0/50, 0/50 Renal cell adenoma or carcinoma (combined)^c: 3/50 (6%), 5/50 (10%), 9/50 (18%), 13/50 (26%)** <i>Liver</i> Hepatocellular adenoma: 3/50 (6%), 6/50 (12%), 8/50 (16%), 5/50 (10%) Hepatocellular carcinoma: 1/50 (2%), 1/50 (2%), 10/50 (20%)*, 3/50 (6%) Hepatocellular adenoma or carcinoma (combined)^f: 4/50 (8%), 7/50 (14%), 15/50 (30%)*, 8/50 (16%) <i>Mononuclear cell leukaemia^a</i> 34/50 (68%), 43/50 (86%)*, 38/50 (76%), 31/50 (62%)** <i>Testis</i> Interstitial cell adenoma: 39/50 (78%), 40/50 (80%), 48/50 (96%)*, 47/50 (94%)**	*$P < 0.05$ (Fisher exact test) **$P < 0.01$ (Fisher exact test) *$P < 0.01$ (Fisher exact test) **$P = 0.005$ (Fisher exact test) *$P < 0.05$ (Fisher exact test) **$P < 0.05$ (Life table test) *$P < 0.007$ (Fisher exact test) **$P < 0.020$ (Fisher exact test)	Purity, 98–99% Surviving animals: 17, 12, 17, 1

Table 3.2 (continued)

[illegible]

^a Historical incidence for 2-year NTP inhalation studies with chamber control groups at all laboratories (mean $\pm$ SD): 356/655 (54.4% $\pm$ 8.8%); range, 34–66%; at Battelle Pacific Northwest Laboratories: 195/348 (56.0% $\pm$ 8.7%); range, 38–66%.

^b Historical incidence for 2-year NTP inhalation studies with chamber control groups at all laboratories (mean $\pm$ SD): 262/653 (40.1% $\pm$ 7.2%); range, 30–54%; at Battelle Pacific Northwest Laboratories: 199/248 (80.0% $\pm$ 6.7%); range, 58–100%.

^c Single and step sections combined

^d Historical incidence for 2-year NTP inhalation studies with chamber control groups at all laboratories (mean $\pm$ SD): 6/652; range, 0–4%; at Battelle Pacific North-western

Laboratories: 5/347; range, 0–4%

North-western Laboratories: 2/346; range, 0–2%
 f Historical incidence for 2-year NTP inhalation studies with chamber control groups at all laboratories (mean $\pm$ SD): 2/653; range, 0–2%; at Battelle Pacific North-western Laboratories: 11/347; range, 2–8%

^g The logistic regression test regards neoplasms in animals dying before terminal kill as nonfatal. The lifetable test regards neoplasms as being the cause of death

^h Historical incidence at all laboratories: 10/650, range, 0–6%; at Battelle Pacific North-western Laboratories: 7/346; range, 0–4%
ⁱ Historical incidence for all organs at all laboratories: 2/653, range, 0–2% (incidence in liver, 0/650)

h, hour; mo, month; SD, standard deviation; wk, week

adenoma of the testis in the groups at the intermediate and highest dose.

In female rats exposed to tetrafluoroethylene at a concentration of 0, 312, 625, or 1250 ppm, the incidence of renal cell adenoma or carcinoma (combined) was significantly higher in the group at the highest dose than in the controls. The incidence of haemangiosarcoma in the liver was significantly higher in the group at the intermediate dose. The incidence of hepatocellular adenoma was significantly higher in all exposed groups. The incidence of hepatocellular carcinoma was significantly higher in the groups at the lowest and intermediate dose. The incidence of hepatocellular adenoma or carcinoma (combined) was significantly higher in all exposed groups. The incidence of eosinophilic foci of the liver (1/50, 4/50, 5/50, 4/50) was significantly higher in the group at the intermediate dose, and the incidence of mixed cell foci (12/50, 14/50, 16/50, 18/50) was significantly higher in the group at the highest dose ([NTP, 1997](#)).

The incidence of mononuclear cell leukaemia was significantly higher in all exposed groups of females than in the controls.

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

Tetrafluoroethylene is a chemically unstable compound, and no studies on radioactively labelled tetrafluoroethylene were identified by the Working Group. Thus detailed, direct information on the degree of absorption, distribution and excretion of tetrafluoroethylene was not available. Tetrafluoroethylene is virtually insoluble in most solvents. Human exposures occur primarily through inhalation.

4.1.1 Absorption

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Indirect evidence for absorption of tetrafluoroethylene was available from several studies in experimental animals, including [Dilley et al. \(1974\)](#), who reported that in male Sprague-Dawley rats exposed to tetrafluoroethylene (3500 ppm) by inhalation for 30 minutes, fluoride excretion in the urine was significantly increased relative to controls.

Whole-body inhalational exposure to tetrafluoroethylene (“subacute”, short term, or long term) in male and female B6C3F₁ mice (up to 1250 ppm for up to 96 weeks), or male and female Fischer 344 rats (up to 625 ppm for 104 weeks) resulted in toxicity in multiple organs, indicating absorption of tetrafluoroethylene in the lung ([NTP, 1997](#)). Additional evidence of absorption via inhalation included the observation of toxicity after single and long-term inhalational exposures to tetrafluoroethylene in mice, hamsters, guinea-pigs, and rabbits, as summarized in a review by [Kennedy \(1990\)](#). However, because toxicity or lethality after a single dose by inhalation in rats was observed only at very high concentrations ([Clayton, 1967](#); [Odum & Green, 1984](#)), absorption via the lung is probably not very efficient, which is consistent with the very low solubility of tetrafluoroethylene. Low absorption in the lung was also confirmed by a study by [Ding et al. \(1980\)](#), who exposed rabbits to tetrafluoroethylene at 1000 ppm for 60 minutes via a face mask, and estimated alveolar absorption to be 6.8%.

No studies of oral or dermal exposure to tetrafluoroethylene were available to the Working Group.

4.1.2 Distribution

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

No data were available to the Working Group. Indirect evidence for distribution of tetrafluoroethylene to distal organs (kidney, liver, testes, etc.) after inhalation was available from several studies of toxicity after a single dose, or after long-term dosing, in experimental animals, as summarized above. In rats exposed by inhalation, metabolism of tetrafluoroethylene in the liver and kidney has been reported, suggesting distribution to these tissues ([Odum & Green, 1984](#)).

4.1.3 Metabolism

Unlike many other halogenated hydrocarbons, tetrafluoroethylene is not a substrate for cytochrome P450s ([Odum & Green, 1984](#)). However, tetrafluoroethylene is known to undergo metabolism, as shown by excretion of inorganic fluoride in the urine of male rats exposed to tetrafluoroethylene by inhalation ([Dilley et al., 1974](#)). [Odum & Green \(1984\)](#) have demonstrated that tetrafluoroethylene is metabolized to the glutathione conjugate S-(1,1,2,2-tetrafluoroethyl)glutathione (TFEG) in liver slices from Wistar rats.

Based on analogy with other halogenated compounds (e.g. trichloroethylene and tetrachloroethylene, also known as perchloroethylene; [Lash et al., 1988](#); [Lash & Parker, 2001](#); [Lash, 2005, 2007, 2011](#)), it can be postulated that metabolism of tetrafluoroethylene follows the classical mercapturate pathway, as shown in [Fig. 4.1](#) and [Fig. 4.2](#). Although most of the glutathione (GSH) conjugation occurs in the liver, as catalysed by the abundant glutathione S-transferase (GST) activity in both hepatic cytoplasm and microsomes, it can also occur in the kidneys. [Fig 4.1](#) details the chemical structures of three principal

tetrafluoroethylene metabolites that have been detected – TFEG, S-(1,1,2,2-tetrafluoroethyl)-L-cysteine (TFEC) and N-acetyl-S-(1,1,2,2-tetrafluoroethyl)-L-cysteine (NAcTFEC), as well as three putative metabolites thought to be reactive moieties formed from TFEC.

TFEG, whether formed in the liver or the kidney, can be sequentially degraded by gamma-glutamyltransferase (GGT) and cysteinylglycine dipeptidase on the external surface of the proximal tubular brush-border membrane of the kidney to yield the corresponding cysteine conjugate TFEC. TFEG formed in the liver can also be readily excreted into the bile, where it can undergo GGT- and dipeptidase-mediated degradation to form TFEC.

TFEC is a branching point in the tetrafluoroethylene metabolic pathway. TFEC may either be detoxified by the action of the cysteine conjugate N-acetyltransferase (NAT) to yield the mercapturate NAcTFEC, or may be bioactivated by one of the many enzymes with cysteine conjugate β -lyase (CCBL) activity to yield a reactive thiolate that ultimately produces nephrotoxicity ([Commandeur et al., 1996](#)). While TFEC, like many other cysteine S-conjugates of halogenated compounds ([Krause et al., 2003](#)), may also be a substrate for flavin-containing monooxygenases, generating a reactive sulfoxide, this possibility is not very likely because of the strength of the C–F bond relative to the C–Cl bond, and has never been tested.

The mercapturate NAcTFEC can be readily excreted in the urine, or may undergo deacetylation by aminoacylase III to regenerate the cysteine conjugate TFEC ([Commandeur et al., 1989](#); [Newman et al., 2007](#)). The potent nephrotoxicity of NAcTFEC in rats, and its low recovery in urine suggested that a high ratio of N-deacetylation/N-acetylation activity exists ([Commandeur et al., 1989](#)). TFEC is a substrate for one of the many enzymes that possess CCBL activity, whose

Fig. 4.1 Metabolism of tetrafluoroethylene by the glutathione-conjugation pathway



catalytic action leads to formation of a reactive and unstable thiolate (metabolite I, see [Fig. 4.1](#)).

The β -lyase reaction mechanism forming reactive, thioacetylating species from cysteine S-conjugates can occur by either a direct β -elimination reaction, or a transamination reaction. The former cleaves the C–S bond. The latter, with a suitable α -keto acid cosubstrate, yields either a thiolate directly, or an unstable propionic acid derivative that rearranges to release the thiolate ([Stevens et al., 1986](#); [Elfarrar et al., 1987](#)). Multiple mammalian enzymes are known to be capable of catalysing the CCBL reaction ([Cooper & Pinto, 2006](#)); however, the relative importance of each of these activities in TFEC bioactivation is presently unknown. Therefore, it is unclear whether TFEC is converted to the thiolate (metabolite I, see [Fig. 4.1](#)) by both mechanisms or only by a direct β -elimination reaction. The addition of α -keto- γ -methiolbutyrate, a keto acid shown to stimulate renal CCBL activity ([Elfarrar et al., 1987](#)), to incubations of purified cytosolic rat kidney CCBL with TFEC in the presence of pyridoxal-5'-phosphate did not stimulate activity ([Abraham et al., 1995](#)), suggesting that a direct β -elimination reaction may be more kinetically favourable for TFEC than for other substrates such as S-(1,2-dichlorovinyl)-L-cysteine (DCVC).

Regardless of how the thiolate is formed, it is believed to subsequently rearrange to form either difluorothionoacyl fluoride ([Fig. 4.1](#), metabolite II) or a thiirane ([Fig. 4.1](#), metabolite III). It is these two putative reactive intermediates that form covalent adducts with various renal cellular proteins, leading to nephrotoxicity.

Although tetrafluoroethylene conjugation with GSH occurs primarily in the liver, it may also occur in the kidney. Hepatic TFEG is readily excreted into the bile, where it undergoes GGT- and dipeptidase-mediated degradation to form TFEC. Renal TFEG undergoes degradation to TFEC on the luminal or brush-border plasma membrane of renal proximal tubules. Regardless of where the initial and degradation reactions

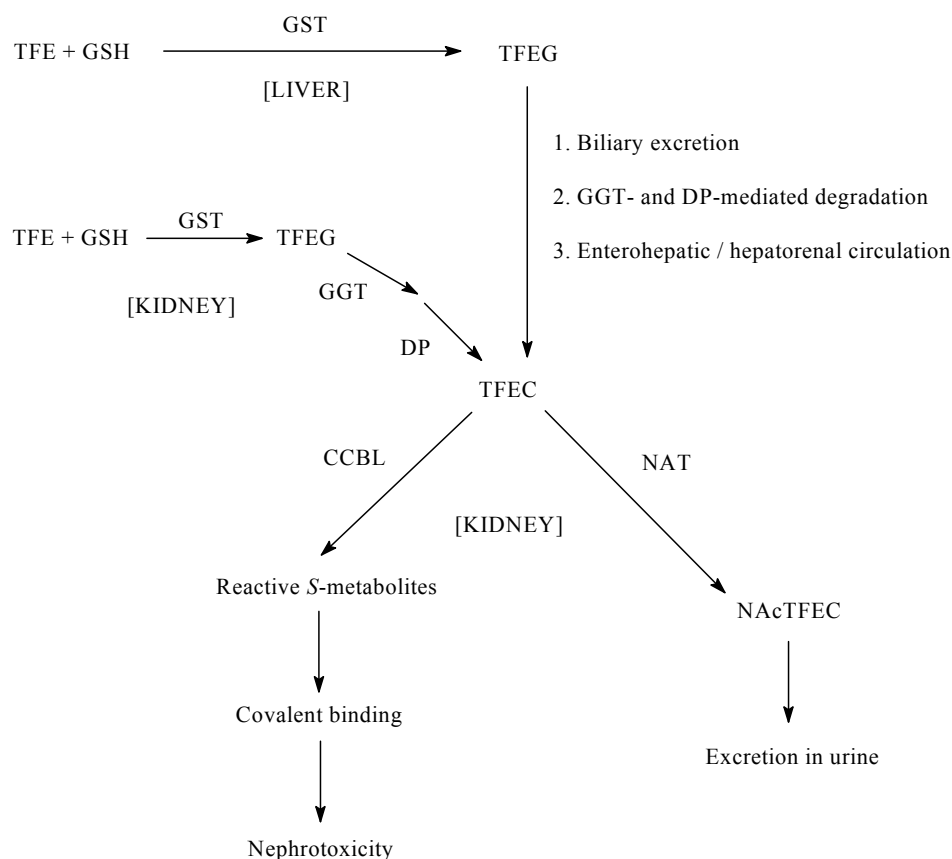
to form TFEC occur, all subsequent reactions leading to detoxification or bioactivation of TFEC occur in the kidney. These pathways of inter-organ metabolism and transport are summarized schematically in [Fig. 4.2](#).

(a) *Humans or human-derived tissues*

No direct evidence for tetrafluoroethylene metabolism in humans was available to the Working Group, but one published study quantified CCBL activity with TFEC in samples of human kidney ([McCarthy et al., 1994](#)). In this study, the authors compared cytosolic CCBL activity in cytosolic samples of human kidney cortex, measuring release of pyruvate on incubation with cysteine conjugates of several halogenated aliphatic and aromatic hydrocarbons. Highest activities were reported for TFEC and DCVC (the cysteine conjugate of trichloroethylene), which were metabolized at similar rates by human CCBL.

(b) *Rodents*

Metabolism of tetrafluoroethylene in vivo was demonstrated in rats by measurement of fluoride ion excretion in urine ([Dilley et al., 1974](#)). Among the several fluorocarbons tested, which included hexafluoropropene, trifluoroethylene, vinylidene fluoride, vinyl fluoride, hexafluoroethane, and tetrafluoroethylene, some of the highest rates of fluoride ion excretion were observed in rats exposed to tetrafluoroethylene. However, no studies are available that report rates of GSH conjugation of tetrafluoroethylene in experimental systems, nor are there published reports of rates of degradation of TFEG to TFEC. Activities of GGT and dipeptidase in renal proximal tubules are not rate-limiting for metabolism and are typically well in excess of what is necessary to catalyse GSH-conjugate degradation. For this reason, one does not see accumulation of GSH conjugates in renal tissue. Rather, it is the cysteine or *N*-acetylcysteine conjugates that can accumulate.

Fig. 4.2 Scheme for interorgan metabolism of glutathione-derived metabolites of tetrafluoroethylene

CCBL, cysteine conjugate β -lyase; DP, dipeptidase; GSH, glutathione; GGT, gamma-glutamyltransferase; GST, glutathione S-transferase; NAT, N-acetyltransferase; NAcTFEC, N-acetyl-S-(1,1,2,2-tetrafluoroethyl)-L-cysteine; TFE, 1,1,2,2-tetrafluoroethylene; TFEC, S-(1,1,2,2-tetrafluoroethyl)-L-cysteine; TFEG, S-(1,1,2,2-tetrafluoroethyl)glutathione
Compiled by the Working Group

[Green & Odum \(1985\)](#) compared metabolism of several cysteine conjugates of halogenated alkanes and alkenes by CCBL activity in rat kidney slices by measuring the release of pyruvate and ammonia. Among the conjugates tested as substrates, TFEC exhibited the fastest metabolism, with rates faster than those for well-established nephrotoxic and nephrocarcinogenic cysteine conjugates DCVC and S-(1,2,2-trichlorovinyl)-L-cysteine (TCVC; cysteine conjugate of tetrachloroethylene).

[MacFarlane et al. \(1989\)](#) purified cytosolic CCBL activity (also known as glutamine transaminase K) from rat kidney and assayed

activity during the course of purification with TFEC or DCVC (5 mM), and the non-nephrotoxic S-(2-benzothiazolyl)-L-cysteine (1 mM) as substrates. TFEC was by far the best CCBL substrate. [Abraham et al. \(1995\)](#) identified and partially purified a from rat kidney cytosol, and found that TFEC exhibited four- to fivefold higher activity than DCVC.

[Cooper et al. \(2001\)](#) co-purified mitochondrial heat shock protein 70 (HSP70) with a CCBL activity of high relative molecular mass, and demonstrated that TFEC was converted to a thioacylating species with associated release of pyruvate and ammonia. Three protein fractions

were identified that exhibited CCBL activity with TFEC as substrate. Thus multiple proteins in the rat kidney cortex are capable of activating TFEC to reactive species. In another study from the same group ([Cooper et al., 2002](#)), a mitochondrial aspartate aminotransferase was purified from rat liver and shown to catalyse CCBL activity with TFEC or DCVC as substrates. In this case, however, TFEC was a relatively poor substrate, exhibiting an apparent K_m of 25 mM and a V_{max} of 2 nmol/min per μ g protein. In contrast, DCVC exhibited K_m and V_{max} values of 2.5 mM and 3 nmol/min per μ g protein, respectively. In the same study, Cooper and colleagues also reported that TFEC underwent a β -elimination reaction to release pyruvate in the presence of cytosolic aspartate aminotransferase and alanine aminotransferase from pig heart ([Cooper et al., 2002](#)). These data emphasize that CCBL activity with TFEC as substrate is catalysed by multiple enzymes in multiple tissues. As explained above, it is the pattern of interorgan transport coupled with metabolism that determines the target-organ specificity of TFEC.

Although the putative reactive intermediates generated from TFEC by the catalytic action of CCBL ([Fig. 4.1](#), metabolites I, II, and III) have not been isolated, their structure has been deduced by the known chemistry of these types of halocarbons and by isolation and identification of protein adducts. [Hayden et al. \(1991\)](#) demonstrated the formation of an N^{α} -acetyl- N^{ϵ} -(difluorothionoacetyl)lysine adduct by ^{19}F and ^{13}C nuclear magnetic resonance spectroscopy and mass spectrometry.

[Commandeur et al. \(1989\)](#) showed that TFEC was readily converted to NAcTFEC in the presence of either rat liver or kidney supernatants when acetyl-CoA was added. The rate of N -acetylation in rat kidney was fivefold higher than in rat liver. These authors also showed that NAcTFEC was deacetylated to form TFEC in both rat liver and kidney supernatants. Deacetylation activity was again much faster in rat kidney than

in rat liver. This ability to readily deacetylate NAcTFEC in the target organ (i.e. the kidney) is likely a major factor in the potent cytotoxicity of NAcTFEC in vitro ([Commandeur et al., 1989](#)).

[Kraus et al. \(2000\)](#) purified NAT from porcine kidney microsomes and determined apparent kinetic parameters with several haloalkenyl cysteine conjugates. Among the conjugates tested as substrates, DCVC exhibited the lowest K_m (273 μM) and highest V_{max} (0.75 nmol/h). In contrast, TFEC was the poorest substrate, exhibiting a higher K_m (302 μM) and V_{max} (2.3 nmol/h) than DCVC. In agreement with the study by [Commandeur et al. \(1989\)](#), which showed a high ratio of deacetylation-to- N -acetylation activity in rat kidney, [Newman et al. \(2007\)](#) showed that NAcTFEC was a reasonably good substrate for mouse kidney aminoacylase III.

(c) Renal transport

As noted above, transport of S -conjugate metabolites across cellular membranes plays a critical role in the disposition of the various GSH-derived metabolites of tetrafluoroethylene. No direct evidence was available, however, on the membrane transport of either TFEG, TFEC, or NAcTFEC. Ample indirect evidence was available to conclude that several specific organic-anion and amino-acid carriers are likely involved. Pretreatment of rats with probenecid, the “classic” organic anion transport inhibitor, gave near complete protection from TFEC-induced nephrotoxicity ([Lock & Ishmael, 1998](#)). The presumption is that the presence of probenecid competitively inhibits the renal accumulation and subsequent bioactivation of TFEC.

Although there were no published studies on the transport of TFEG, TFEC, or NAcTFEC into renal proximal tubular cells, analogy with studies on the transport of the GSH-derived conjugates of trichloroethylene suggested that carrier proteins such as the organic anion transporter 1 and 3 (OAT1/3; soluble carrier *SLC22A6/8*) and possibly the sodium dicarboxylate carrier-3

Table 4.1 Studies of genotoxicity with tetrafluoroethylene and S-(1,1,2,2-tetrafluoroethyl)-L-cysteine

Test system	Results		Dose (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>Tetrafluoroethylene</i>				
Micronucleus test, B6C3F ₁ mouse peripheral erythrocytes, in vivo	–	NT	5000 ppm, inhalation, 6 h/day, 5 days/wk, 13 wk	NTP (1997)
<i>S-(1,1,2,2-tetrafluoroethyl)-L-cysteine</i>				
<i>Salmonella typhimurium</i> TA100, TA1535, TA1537, TA1538, TA98, or TA97, reverse mutation	–	–	250 mg/plate	Green & Odum (1985)

–, negative; HID, highest ineffective dose; h, hour; LED, lowest effective dose; NT, not tested; wk, week

(NaC3; *SLC13A3*) on the basolateral plasma membrane of renal proximal tubular cells may function ([Lash, 2005, 2011](#); [Lash et al., 2007](#)). These presumptions have not been validated by studies specifically testing the transport function of these carriers with tetrafluoroethylene conjugates are required.

4.1.4 Excretion

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In a study of male Sprague-Dawley rats exposed to tetrafluoroethylene (3500 ppm) by inhalation for 30 minutes ([Dilley et al., 1974](#)), excretion of fluoride ion in the urine was monitored for up to 14 days after exposure, and fluoride excretion was significantly higher than in controls in exposed rats in the apparent cyclic excretion of fluoride ion 6 days after exposure and again at 13–14 days. However, the overall extent of excretion could not be determined.

[Odum & Green \(1984\)](#) reported biliary excretion of the GSH-conjugation-derived tetrafluoroethylene metabolite TFEC after inhalational exposure in rats, suggesting that faecal elimination of the products of tetrafluoroethylene

metabolism is possible. However, the extent of reabsorption has not been determined, and no direct data on faecal elimination were available.

4.2 Genotoxicity and related effects

[Table 4.1](#) summarizes the studies carried out to investigate the genotoxic potential of tetrafluoroethylene and TFEC in mammalian systems in vivo and in bacterial systems.

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

(a) Mammalian systems

(i) Gene mutation

No results from standard studies of mutagenicity in vivo were available to the Working Group. In B6C3F₁ mice, mutations in codon 61 of the *H-ras* oncogene occurred at a significantly lower frequency (15%) in tetrafluoroethylene-induced hepatocellular tumours than in spontaneous liver tumours (56–59%) ([NTP, 1997](#)). [The Working Group noted that this finding suggested that tetrafluoroethylene causes tumours of the liver via a *ras*-independent pathway.]

(ii) Chromosomal aberration

No data were available to the Working Group.

(iii) Micronucleus formation

Tetrafluoroethylene did not induce micronucleus formation in vivo in peripheral erythrocytes of male and female mice treated for 13 weeks at a concentration of 5000 ppm given via inhalation ([NTP, 1997](#)).

(iv) DNA binding and other DNA damage

No data were available to the Working Group.

(b) Bacterial systems: gene mutations

Cysteine conjugates of tetrafluoroethylene were not mutagenic in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA97, with or without metabolic activation with S9 fraction of rat kidney ([Green & Odum, 1985](#)).

4.3 Biochemical and cellular effects

The available studies in humans and experimental animals provided limited data on the biochemical and cellular effects of tetrafluoroethylene. One postulated non-genotoxic mechanism through which tetrafluoroethylene may induce tumour formation is via a cytotoxic GSH conjugate ([Keller et al., 2000](#)). Organ-specific toxicity data are reviewed below.

4.4 Organ toxicity

4.4.1 Kidney

(a) Humans

In comparison with national rates, observed mortality rates for nephritis and nephrosis were 25% lower than expected, according to standardized mortality ratios (SMR, 0.75; 95% CI, 0.21–1.93) in a cohort study of tetrafluoroethylene-production workers in Germany, Italy, the Netherlands, and the USA ([Consonni et al., 2013](#)); this decreased risk was similar to that seen for

overall mortality (SMR, 0.77; 95% CI, 0.71–0.81). [The Working Group noted that because the number of deaths from nephritis or nephrosis (4 deaths) was a very small proportion of the total deaths observed (635 deaths; 0.63%), no conclusions about any association between nephritis or nephrosis and rates of mortality could be made.]

*(b) Experimental animals**(i) Rats*

In a 2-year study of carcinogenicity with tetrafluoroethylene, increases in the incidence of renal degeneration were observed in male Fischer F344/N rats exposed to tetrafluoroethylene at 156 ppm [640 mg/m³], and in female F344/N rats at 625 ppm [2560 mg/m³], and increases in the incidence of renal hyperplasia were observed in male and female rats at 625 ppm ([NTP, 1997](#)). Renal toxicity was also observed in 16-day and 13-week studies in F344/N rats treated with tetrafluoroethylene at concentrations of 625 ppm and higher; the damage was located predominantly at the corticomedullary junction. In addition, a review of data on the toxicity of tetrafluoroethylene indicated that rats exposed at 2500 ppm [10 250 mg/m³] for 6 hours per day, 5 days per week, for 2 weeks, or at 2000 ppm [8200 mg/m³] for 6 hours per day, 5 days per week, for 18 weeks, developed renal proximal tubule damage, which was more severe after 18 weeks than after 2 weeks ([Kennedy, 1990](#)). In study of toxicity in female F344 rats given tetrafluoroethylene by inhalation for up to 12 days, kidney weights were increased in rats exposed at 600 and 1200 ppm, and degeneration or necrosis of occasional tubule epithelial cells was reported in rats exposed at 1200 ppm ([Keller et al., 2000](#)). In male Alderley Park rats exposed to tetrafluoroethylene by inhalation at 6000 ppm [24 600 mg/m³] for 6 hours, there was marked renal necrosis involving the pars recta of the proximal tubules, and an increase in levels of blood and urine markers of nephrotoxicity, including plasma area, urine volume,

glucose, alanine transaminase, *N*-acetyl- β -D-glucosaminidase, GGT, and alanine aminopeptidase (Odum & Green, 1984).

Tetrafluoroethylene metabolites

Keller et al. (2000) exposed female F344 rats to TFEC at oral doses of 5, 20, or 50 mg/kg for 9 days; severe changes were observed in the pars recta of the outer stripe of the outer medulla. When given TFEC as an oral dose at 100 mg/kg, male Alderley Park rats had increased blood and urine markers of nephrotoxicity, including increases in plasma urea, urine volume, glucose, protein, alanine transaminase, *N*-acetyl- β -D-glucosaminidase, GGT, and alanine aminopeptidase (Odum & Green, 1984). Lock & Ishmael (1998) reported renal tubular necrosis in male Alderley Park rats given a single intraperitoneal injection of TFEC. Rats given TFEC at a dose of 25 or 50 mg/kg had renal necrosis that included extensive necrosis seen as a band of damage in the outer stripe of the outer medulla with occasional tubular casts (25 mg/kg), or severe necrosis with a diffuse band involving the outer medulla and the inner cortex with many tubular casts (50 mg/kg). Similarly exposed female Alderley Park rats had extensive necrosis seen as a band of damage in the outer stripe of the outer medulla with occasional tubular casts at 25 mg/kg, and severe necrosis at 50 mg/kg, as in male rats (Lock & Ishmael, 1998).

Commandeur et al. (1988) suggested that difluorothionoacetyl fluoride or difluorothioacetic acid, reactive intermediates of tetrafluoroethylene, induced nephrotoxicity specific to the proximal tubule, since necrosis in the region of the inner cortex was observed in male Wistar rats given a single intraperitoneal injection of NAcTFEC, the mercapturic acid of tetrafluoroethylene, at a dose of 112.5, 225, or 337.5 mg/kg.

(ii) *Mice*

In a 16-day study of toxicity preliminary to a study of carcinogenicity in B6C3F₁ mice, kidney weight increased in females exposed

to tetrafluoroethylene at a concentration of 5000 ppm [20 500 mg/m³] by inhalation (NTP, 1997). Renal tubule karyomegaly was observed, mainly in the inner cortex, of males and females exposed to 1250 ppm [5125 mg/m³] or more. Karyomegaly was observed in the same region in the subsequent 13-week study with tetrafluoroethylene at the same concentrations. In the succeeding 2-year study of carcinogenicity, renal tubule karyomegaly was increased at 625 ppm in male mice, and at 1250 ppm in female mice. In a 12-day study of toxicity of female B6C3F₁ mice, cell necrosis was reported in mice exposed to tetrafluoroethylene at 1200 ppm (Keller et al., 2000).

Tetrafluoroethylene metabolites

Keller et al. (2000) also exposed female B6C3F₁ mice to TFEC at an oral dose of 5, 20, or 50 mg/kg for 9 days by gavage; moderate to severe changes were observed in the pars recta of the outer stripe of the outer medulla.

(iii) *Other species*

According to a review by Kennedy (1990), Syrian hamsters exposed to tetrafluoroethylene at 2500 ppm [10 250 mg/m³] by inhalation for 6 hours per day, 5 days per week, for 2 weeks, or at 2000 ppm [8200 mg/m³] for 6 hours per day, 5 days per week, for 18 weeks, showed no signs of renal toxicity, but testicular atrophy was reported.

4.4.2 Liver

(a) *Humans*

Mortality rates for cirrhosis of the liver were similar to national rates (SMR, 1.03; 95% CI, 0.65–1.54) in a cohort study of tetrafluoroethylene-production workers at six plants in Europe and the USA (observed deaths, 23; expected deaths, 22.4) (Consonni et al., 2013). An excess risk of cirrhosis of the liver was observed at one of these plants (observed deaths, 12; expected deaths, 2.4); these cases were classified in the

group with low exposure. In the remaining five plants, there were 11 observed deaths, and 20 expected deaths from cirrhosis of the liver.

(b) *Experimental animals*

(i) *Rats*

In a 13-week study in Fischer 344/N rats, liver weights were increased in males and females exposed to tetrafluoroethylene at a concentration of 5000 ppm [20 500 mg/m³] by inhalation ([NTP, 1997](#)). In a 12-day study of toxicity in female F344 rats, liver weights were increased in rats exposed at 600 ppm [2460 mg/m³] by inhalation ([Keller et al., 2000](#)).

(ii) *Mice*

In a long-term cancer bioassay, liver angiectasis was reported in male and female B6C3F₁ mice exposed to tetrafluoroethylene at concentrations at or above 312 ppm [1280 mg/m³] by inhalation; there was also increased liver and spleen haematopoietic cell proliferation in female mice at these dose levels ([NTP, 1997](#)). In the 16-day study of toxicity (preliminary to a study of carcinogenicity) in B6C3F₁ mice, there were increases in liver weights of female mice exposed to tetrafluoroethylene at concentrations of 2500 ppm [10 250 mg/m³] or more ([NTP, 1997](#)).

4.5 Susceptible populations

4.5.1 Polymorphisms

No data for tetrafluoroethylene specifically were available to the Working Group. Indirect evidence was available from data on other chemicals – methyl chloride and trichloroethylene – known to be metabolized through the same pathway. The predominant pathways for metabolism of tetrafluoroethylene are via GST in the liver, and via GGT and dipeptidase in the kidney ([Odum & Green, 1984](#); [Hayden et al., 1991](#); [Keller et al., 2000](#)); however, the GST isozyme(s) that may be involved in tetrafluoroethylene

conjugation reactions have not been identified. It is possible that humans may conjugate tetrafluoroethylene at different rates owing to known genetic polymorphisms in GST and other metabolizing enzymes. The following data concern tetrafluoroethylene-related chemicals that undergo GST-mediated conjugation.

For methyl chloride, one study classified humans into “fast,” “slow,” or non-conjugators (non-metabolizers) ([Nolan et al., 1985](#)). Fast metabolism may lead to rapid production of the toxic cysteine metabolite, making this population more susceptible to kidney damage. However, among conjugators, the rate of conjugation of tetrafluoroethylene with GSH is expected to fall within a threefold range ([Nolan et al., 1985](#); [Mulder et al., 1999](#)). In a study by [Löf et al. \(2000\)](#), glutathione S-transferase theta 1 (GSTT1) appeared to be the sole determinant of methyl chloride metabolism in humans; clearance of methyl chloride by metabolism, but not by exhalation, correlated well with GSTT1 activity.

For trichloroethylene, the role that polymorphisms in the genes encoding GST enzymes may play in cancer risk has been studied in several epidemiological studies. For example, [Brüning et al. \(1997\)](#) investigated the potential for an association between polymorphisms in glutathione S-transferase mu 1 (*GSTM1*) and *GSTT1* and risk of renal cell cancer in workers with high long-term occupational exposure to trichloroethylene. Among 45 patients with renal cell carcinoma, 27 carried at least one functional *GSTM1* gene, and 18 carried at least one functional *GSTT1* gene. The odds ratios for renal cell carcinoma were 2.7 for *GSTM1*+ individuals (95% CI, 1.18–6.33; *P* < 0.02), and 4.2 for *GSTT1*+ individuals (95% CI, 1.16–14.91; *P* < 0.05), respectively. The data from this cohort were re-evaluated by [Wiesenhütter et al. \(2007\)](#), who used data from additional control subjects to increase the size of the study population, finding that deletion polymorphisms in *GSTT1* and *GSTM1* had no

effect on the development of renal cell carcinoma attributable to trichloroethylene.

[Moore et al. \(2010\)](#) conducted a case-control study in central Europe (cases, 1097; controls, 1476) to assess the risk of renal cell carcinoma associated with occupational exposure to trichloroethylene (assessed from work history). Increased risk was observed among subjects who had ever been exposed to trichloroethylene [OR, 1.63; 95% CI, 1.04–2.54]. A significant association was found for trichloroethylene-exposed subjects with at least one intact *GSTT1* allele (active genotype; OR, 1.88; 95% CI, 1.06–3.33), but not for subjects with two deleted alleles (*GSTT1* null genotype; OR, 0.93; 95% CI, 0.35–2.44). Similar associations for all exposure metrics, including average intensity, were observed among *GSTT1*-active subjects (OR, 2.77; 95% CI, 1.01–7.58; $P_{\text{trend}} = 0.02$), but not among *GSTT1* null individuals (OR, 1.16; 95% CI, 0.27–5.04).

Among the transporter proteins known to be responsible for the uptake and cellular accumulation of tetrafluoroethylene conjugates, the influence of genetic polymorphisms has been best studied for OAT1 and OAT3 ([Erdman et al., 2006](#); [Lash et al., 2006](#); [Urban et al., 2006](#)). Expression and function of OATs and other organic-anion transporters have been shown to exhibit sex-dependent differences in humans and experimental animals ([Gotoh et al., 2002](#); [Kato et al., 2002](#); [Kobayashi et al., 2002](#); [Buist et al., 2003](#); [Buist & Klaassen, 2004](#); [Ljubojevic et al., 2004](#)), suggesting that transport differences are a contributing factor to sex-specific differences in susceptibility to toxicity caused by tetrafluoroethylene metabolites.

4.5.2 Lifestage

No data were available to the Working Group.

4.6 Mechanistic considerations

The mechanisms by which tetrafluoroethylene causes toxicity are largely unknown, and most of the available information on this compound concerns observational studies on effects in the target organs, and metabolism.

Based on knowledge of tetrafluoroethylene metabolism, it is likely that GSH conjugation in the liver, followed by CCBL-mediated formation of a reactive thiol, is the main route of metabolism of tetrafluoroethylene. The mercapturic acid pathway of bioactivation of tetrafluoroethylene is similar to that of several halogenated solvents such as trichloroethylene and tetrachloroethylene, hence nephrotoxicity is expected to be mediated by reactive metabolites derived from a cysteine conjugate. The proximal nephrotoxic reactive intermediate of the tetrafluoroethylene cysteine conjugate is difluorothionoacetyl fluoride, which formed by α -elimination of a fluoride anion from the initial thiolate (see [Fig. 4.1](#); [Commandeur et al., 1996](#)). In studies of acute and chronic effects of tetrafluoroethylene, kidney hypertrophy, proteinuria, renal tubular necrosis, and degeneration were observed in mice and rats ([Odum & Green, 1984](#); [NTP, 1997](#)), and karyomegaly in mice ([NTP, 1997](#); [Keller et al., 2000](#)). Tetrafluoroethylene caused increased proliferation and cellular hyperplasia in the rat kidney, and there was convincing evidence for kidney enlargement ([NTP, 1997](#); [Keller et al., 2000](#)). Dose-dependent normocytic, normochromic, nonresponsive anaemia observed in rats and mice exposed to tetrafluoroethylene in a 13-week study was attributed to possible alterations in erythropoietin metabolism in the kidney due to the presence of renal lesions ([NTP, 1997](#)). Together, these changes suggest that cytotoxicity followed by compensatory proliferation may be the main non-genotoxic mechanism of carcinogenesis in the kidney, although no data were available to the Working Group to confirm this possibility.

Tetrafluoroethylene was not found to be genotoxic in the few standard assays available; however, because traditional bacterial mutagenesis assays use liver-derived S9 fraction to test bioactivation, data obtained from such studies are less informative than experimental evidence obtained with kidney homogenates or purified enzymes responsible for biotransformation of nephrotoxic haloalkenes to GSH conjugation-derived reactive electrophiles ([Lash et al., 2014](#)). The cysteine conjugate of tetrafluoroethylene has been tested in some genotoxicity assays with no positive results reported; however, reactive metabolites formed through GSH conjugation of tetrafluoroethylene and TFEC metabolite may still contribute to the carcinogenicity of tetrafluoroethylene in the kidney via a genotoxicity mechanism.

Little is known about potential mechanisms in the liver. Tetrafluoroethylene is thought not to be metabolized through cytochrome P450-mediated oxidation ([Odum & Green, 1984](#)). However, hepatomegaly has been observed in rats ([NTP, 1997](#)) and mice ([Keller et al., 2000](#)), suggesting that either cytotoxicity followed by compensatory proliferation, or nuclear receptor-mediated hypertrophy, may be involved. No study has examined these mechanisms in detail, and it is not known whether tetrafluoroethylene is a ligand for nuclear receptors, such as peroxisome proliferator-activated receptor α (PPAR α). GSH conjugates of tetrafluoroethylene and other haloalkenes are not thought to be hepatotoxic or reactive, but no study tested potential hepatotoxicity of the GSH conjugate of tetrafluoroethylene, TFEG. Furthermore, it is not known what mechanism may lead to the formation of haemangiomas and haemangiosarcomas (very uncommon neoplasms in the mouse liver), which were observed in 2-year studies in mice ([NTP, 1997](#)).

The increased incidence of haematopoietic cell proliferation in female mice, and findings of mononuclear cell leukaemia in female rats have

not been attributed to a specific mechanism of toxicity ([NTP, 1997](#)).

The only available relevant mechanistic data in humans concerned indirect evidence for absorption of tetrafluoroethylene by inhalation. Some data were also available to suggest that metabolism of TFEC by human enzymes is comparable in efficiency to that of DCVC.

5. Summary of Data Reported

5.1 Exposure data

Tetrafluoroethylene is a fluorinated monomer that is produced by the pyrolysis of chlorodifluoromethane. Estimated annual world production of tetrafluoroethylene is more than 100 000 tonnes. It is used mainly as an intermediate in the production of the polymer polytetrafluoroethylene, which is used in a wide range of industrial and consumer products, e.g. non-stick coatings and waterproof clothing. The occupational setting is the main source of concern regarding exposure to tetrafluoroethylene, predominantly during its production and use in polymerization. Exposure levels have decreased (estimated from plants in the USA and in Europe at < 40 mg/m³ in the 1950s and 1960s, and now about < 6 mg/m³).

5.2 Human carcinogenicity data

Only one study evaluating the possible carcinogenic effect of tetrafluoroethylene has been reported. Moderately but not statistically significantly elevated standardized mortality ratios were observed for all sites of a-priori interest, i.e. cancers of the liver and kidney, and leukaemia, based on small numbers of cases. The study was well conducted in terms of completeness and follow-up of the cohort and exposure assessment, but study precision was low and

the possible confounding from ammonium perfluorooctanoate could not be ruled out due to the high correlation between the two exposures.

5.3 Animal carcinogenicity data

There were two well-conducted studies of carcinogenicity with tetrafluoroethylene: one inhalation study in mice (males and females), and one inhalation study in rats (males and females). Tetrafluoroethylene increased the incidence of liver haemangioma and/or haemangiosarcoma, hepatocellular adenoma and/or carcinoma, and histiocytic sarcoma in male and female mice. In male and female rats, tetrafluoroethylene increased the incidence of renal cell adenoma or carcinoma (combined), and of hepatocellular adenoma and/or carcinoma. In female rats, tetrafluoroethylene caused an increase in the incidence of haemangiosarcoma of the liver. In rats, tetrafluoroethylene also caused increases in the incidence of mononuclear cell leukaemia in males and females, and testicular interstitial cell (Leydig cell) adenoma in males.

5.4 Mechanistic and other relevant data

Tetrafluoroethylene is a volatile, chemically unstable compound with poor solubility. Humans are primarily exposed through inhalation. Tissue distribution of tetrafluoroethylene is poorly characterized, but there is evidence for toxic effects at various tissues after exposure by inhalation. Urinary and faecal excretion of tetrafluoroethylene and its metabolites is likely, but elimination has not been studied in detail.

Unlike other halogenated compounds, tetrafluoroethylene is not metabolized by cytochrome P450 enzymes. Metabolism of tetrafluoroethylene is thought to primarily occur through the glutathione-conjugation pathway in

the liver to the glutathione conjugate, which is further metabolized to the cysteine conjugate in the kidney. The resulting conjugate is an excellent substrate for cysteine conjugate β -lyase, which is known to form reactive electrophiles of cysteine conjugate metabolites of other halogenated compounds.

Limited data exist to characterize the potential genotoxicity of tetrafluoroethylene or its metabolites. No positive results were reported for either tetrafluoroethylene or its cysteine conjugate, but tests with kidney-derived metabolizing enzymes have not been performed.

Single, short-term, or long-term exposures to tetrafluoroethylene resulted in kidney toxicity in rats and mice. Both males and females were affected, although the effects in females occurred at a higher exposure level than in males. Liver enlargement and some evidence for liver toxicity have also been reported in studies with tetrafluoroethylene in rats and mice. Little is known about the mechanisms that may explain these adverse effects in the kidney and liver.

No study directly evaluated the potential role of genetic polymorphisms in the adverse health effects of tetrafluoroethylene. However, because of the major role that several glutathione S-transferase enzymes are likely to play in metabolism of tetrafluoroethylene, inter-individual variability in the formation of reactive electrophiles from the cysteine conjugate is plausible based on analogy to related chemicals. No studies were identified that explored whether lifestage susceptibility to tetrafluoroethylene exposure may exist.

Overall, the mechanistic data for tetrafluoroethylene are *weak* because the mechanistic events have not been directly established in humans or in experimental animals.

6. Evaluation

6.1 Cancer in Humans

There is *inadequate evidence* in humans for the carcinogenicity of tetrafluoroethylene.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of tetrafluoroethylene.

6.3 Overall evaluation

Tetrafluoroethylene is *probably carcinogenic to humans (Group 2A)*.

6.4 Rationale

In the absence of adequate data on cancer in humans and adequate mechanistic data, the overall evaluation for the carcinogenicity of tetrafluoroethylene was upgraded from *Group 2B* to *Group 2A* based on unusual results in studies of cancer in experimental animals. Tetrafluoroethylene induced neoplasms at multiple sites, affecting cells of differing embryological origin, and were present in rats (renal cell adenoma or carcinoma combined, hepatocellular carcinoma, and mononuclear cell leukaemia) and mice (liver haemangiosarcoma, hepatocellular carcinoma, and histiocytic sarcoma) of both sexes. There was also a significant increase in the incidence of the rare liver haemangiosarcoma in female rats. Also, the tumour incidences are very high, especially liver haemangiosarcoma in mice, even at the lowest doses tested. This indicates that tetrafluoroethylene is a potent carcinogen.

References

- Abraham DG, Patel PP, Cooper AJL (1995). Isolation from rat kidney of a cytosolic high molecular weight cysteine-S-conjugate β -lyase with activity toward leukotriene E4. *J Biol Chem*, 270(1):180–8. doi:[10.1074/jbc.270.1.180](https://doi.org/10.1074/jbc.270.1.180) PMID:[7814371](https://pubmed.ncbi.nlm.nih.gov/7814371/)
- ACGIH (2013). TLVs and BEIs. Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati (OH): American Conference of Governmental Industrial Hygienists.
- AIHA (2013). Emergency response planning guidelines. Falls Church (VA): American Industrial Hygiene Association Available from: <https://www.aiha.org/get-involved/AIHAGuidelineFoundation/EmergencyResponsePlanningGuidelines/Documents/2013ERPValues.pdf>, accessed 2 February 2014.
- Babenko YI, Lisochkin YA, Poznyak VI (1993). Explosion of tetrafluoroethylene during nonisothermal polymerization. *Combust Explos Shock Waves*, 29(5):603–9. doi:[10.1007/BF00783714](https://doi.org/10.1007/BF00783714)
- Brüning T, Lammert M, Kempkes M, Thier R, Golka K, Bolt HM (1997). Influence of polymorphisms of GSTM1 and GSTT1 for risk of renal cell cancer in workers with long-term high occupational exposure to trichloroethene. *Arch Toxicol*, 71(9):596–9. doi:[10.1007/s002040050432](https://doi.org/10.1007/s002040050432) PMID:[9285043](https://pubmed.ncbi.nlm.nih.gov/9285043/)
- Buist SCN, Cherrington NJ, Klaassen CD (2003). Endocrine regulation of rat organic anion transporters. *Drug Metab Dispos*, 31(5):559–64. doi:[10.1124/dmd.31.5.559](https://doi.org/10.1124/dmd.31.5.559) PMID:[12695343](https://pubmed.ncbi.nlm.nih.gov/12695343/)
- Buist SCN, Klaassen CD (2004). Rat and mouse differences in gender-predominant expression of organic anion transporter (OAT1–3; SLC22A6–8) mRNA levels. *Drug Metab Dispos*, 32(6):620–5. doi:[10.1124/dmd.32.6.620](https://doi.org/10.1124/dmd.32.6.620) PMID:[15155553](https://pubmed.ncbi.nlm.nih.gov/15155553/)
- Clayton JW (1967). Fluorocarbon toxicity and biological action. *Fluor Chem Rev*, 1(2):197–252.
- Commandeur JNM, Brakenhoff JPG, De Kanter FJJ, Vermeulen NPE (1988). Nephrotoxicity of mercapturic acids of three structurally related 2,2-difluoroethylenes in the rat. Indications for different bioactivation mechanisms. *Biochem Pharmacol*, 37(23):4495–504. doi:[10.1016/0006-2952\(88\)90665-X](https://doi.org/10.1016/0006-2952(88)90665-X) PMID:[3202890](https://pubmed.ncbi.nlm.nih.gov/3202890/)
- Commandeur JNM, De Kanter FJJ, Vermeulen NPE (1989). Bioactivation of the cysteine-S-conjugate and mercapturic acid of tetrafluoroethylene to acylating reactive intermediates in the rat: dependence of activation and deactivation activities on acetyl coenzyme A availability. *Mol Pharmacol*, 36(4):654–63. PMID:[2811861](https://pubmed.ncbi.nlm.nih.gov/2811861/)
- Commandeur JNM, King LJ, Koymans L, Vermeulen NPE (1996). Bioactivation of S-(2,2-dihalo-1,1-difluoroethyl)-L-cysteines and S-(trihalo-1,1-difluoroethyl)-L-cysteines by cysteine S-conjugate β -lyase: indications

- for formation of both thionoacylating species and thiiranes as reactive intermediates. *Chem Res Toxicol*, 9(7):1092–102. doi:[10.1021/tx960049b](https://doi.org/10.1021/tx960049b) PMID:[8902263](https://pubmed.ncbi.nlm.nih.gov/8902263/)
- Consonni D, Straif K, Symons JM, Tomenson JA, van Amelsvoort LG, Slegers A, et al. (2013). Cancer risk among tetrafluoroethylene synthesis and polymerization workers. *Am J Epidemiol*, 178(3):350–8. doi:[10.1093/aje/kws588](https://doi.org/10.1093/aje/kws588) PMID:[23828249](https://pubmed.ncbi.nlm.nih.gov/23828249/)
- Cooper AJL, Bruschi SA, Iriarte A, Martinez-Carrion M (2002). Mitochondrial aspartate aminotransferase catalyses cysteine S-conjugate β -lyase reactions. *Biochem J*, 368(Pt 1):253–61. doi:[10.1042/BJ20020531](https://doi.org/10.1042/BJ20020531) PMID:[12137566](https://pubmed.ncbi.nlm.nih.gov/12137566/)
- Cooper AJL, Pinto JT (2006). Cysteine S-conjugate β -lyases. *Amino Acids*, 30(1):1–15. doi:[10.1007/s00726-005-0243-4](https://doi.org/10.1007/s00726-005-0243-4) PMID:[16463021](https://pubmed.ncbi.nlm.nih.gov/16463021/)
- Cooper AJL, Wang J, Gartner CA, Bruschi SA (2001). Co-purification of mitochondrial HSP70 and mature protein disulfide isomerase with a functional rat kidney high-M(r) cysteine S-conjugate β -lyase. *Biochem Pharmacol*, 62(10):1345–53. doi:[10.1016/S0006-2952\(01\)00802-4](https://doi.org/10.1016/S0006-2952(01)00802-4) PMID:[11709194](https://pubmed.ncbi.nlm.nih.gov/11709194/)
- Dilley JV, Carter VL Jr, Harris ES (1974). Fluoride ion excretion by male rats after inhalation of one of several fluoroethylenes or hexafluoropropene. *Toxicol Appl Pharmacol*, 27(3):582–90. doi:[10.1016/0041-008X\(74\)90037-4](https://doi.org/10.1016/0041-008X(74)90037-4) PMID:[4852067](https://pubmed.ncbi.nlm.nih.gov/4852067/)
- Ding XC, Yu HT, Hu M, Liu CF, Ko FZ (1980). [Study of the absorption, distribution and elimination of the four organic fluorides in rabbits (author's transl)]. *Zhonghua Yu Fang Yi Xue Za Zhi*, 14(1):39–42. [Chinese, English abstract] PMID:[7227081](https://pubmed.ncbi.nlm.nih.gov/7227081/)
- ECETOC (2003). Tetrafluoroethylene. (CAS No. 116–14–3). JACC No 42. Brussels: European Centre for Ecotoxicology and Toxicology of Chemicals.
- European Chemicals Bureau (2000). Substance ID: 116-14-3. IUCLID Dataset. International Uniform Chemical Information Database. European Chemicals Bureau, European Commission. Available from: http://esis.jrc.ec.europa.eu/doc/IUCLID/data_sheets/116143.pdf, accessed 2 February 2014.
- ECHA (2014) Tetrafluoroethylene. General information. Helsinki: European Chemicals Agency. Available from: <http://echa.europa.eu/registration-dossier/-/registered-dossier/15453/1>, accessed 16 September 2014.
- ECHA (2015) Tetrafluoroethylene. Brief profile. Helsinki: European Chemicals Agency. Available from: <http://echa.europa.eu/registration-dossier/-/registered-dossier/15453/1>, accessed 21 July 2016.
- Elfarra AA, Lash LH, Anders MW (1987). α -Ketoadic acids stimulate rat renal cysteine conjugate β -lyase activity and potentiate the cytotoxicity of S-(1,2-dichlorovinyl)-L-cysteine. *Mol Pharmacol*, 31(2):208–12. PMID:[3807895](https://pubmed.ncbi.nlm.nih.gov/3807895/)
- EPA (1999). Compendium method TO-15. Determination of volatile organic compounds (VOCs) in air collected in specially-prepared canisters and analysed by gas chromatography/mass spectrometry (GC/MS). Cincinnati (OH): United States Environmental Protection Agency.
- Erdman AR, Mangravite LM, Urban TJ, Lagpacan LL, Castro RA, de la Cruz M, et al. (2006). The human organic anion transporter 3 (OAT3; SLC22A8): genetic variation and functional genomics. *Am J Physiol Renal Physiol*, 290(4):F905–12. doi:[10.1152/ajprenal.00272.2005](https://doi.org/10.1152/ajprenal.00272.2005) PMID:[16291576](https://pubmed.ncbi.nlm.nih.gov/16291576/)
- Gotoh Y, Kato Y, Stieger B, Meier PJ, Sugiyama Y (2002). Gender difference in the Oatp1-mediated tubular reabsorption of estradiol 17 β -D-glucuronide in rats. *Am J Physiol Endocrinol Metab*, 282:E1245–54.
- Green T, Odum J (1985). Structure/activity studies of the nephrotoxic and mutagenic action of cysteine conjugates of chloro- and fluoroalkenes. *Chem Biol Interact*, 54(1):15–31. doi:[10.1016/S0009-2797\(85\)80149-6](https://doi.org/10.1016/S0009-2797(85)80149-6) PMID:[4017103](https://pubmed.ncbi.nlm.nih.gov/4017103/)
- Gribble GW (2010). Naturally occurring organohalogen compounds - a comprehensive update. New York (NY): Springer WeinNewYork. doi:[10.1007/978-3-211-99323-1](https://doi.org/10.1007/978-3-211-99323-1)
- Hayden PJ, Yang Y, Ward AJ, Dulik DM, McCann DJ, Stevens JL (1991). Formation of difluorothionoacetyl-protein adducts by S-(1,1,2,2-tetrafluoroethyl)-L-cysteine metabolites: nucleophilic catalysis of stable lysyl adduct formation by histidine and tyrosine. *Biochemistry*, 30(24):5935–43. doi:[10.1021/bi00238a018](https://doi.org/10.1021/bi00238a018) PMID:[1904276](https://pubmed.ncbi.nlm.nih.gov/1904276/)
- HSDB (2014). Tetrafluoroethylene. Hazardous substance database (HSDB). Available from: <https://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm>, accessed 1 February 2014.
- HSE (1997). Methods for the determination of hazardous substances: volatile organic compounds in air. Bootle, England: Health and Safety Laboratory, Health and Safety Executive. Available from: <http://www.hse.gov.uk/pubns/mdhs/pdfs/mdhs88.pdf>, accessed 16 September 2014.
- IARC (1979). Some monomers, plastics and synthetic elastomers, and acrolein. *IARC Monogr Eval Carcinog Risk Chem Hum*, 19:1–513. Available from: <http://monographs.iarc.fr/ENG/Monographs/vol1-42/mono19.pdf> PMID:[285915](https://pubmed.ncbi.nlm.nih.gov/285915/)
- IARC (1987). Overall evaluations of carcinogenicity: an updating of IARC Monographs volumes 1 to 42. *IARC Monogr Eval Carcinog Risks Hum*, Suppl 7:1–440. Available from: <http://monographs.iarc.fr/ENG/Monographs/suppl7/index.php> PMID:[3482203](https://pubmed.ncbi.nlm.nih.gov/3482203/)
- IARC (1999). Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide. *IARC Monogr Eval Carcinog Risks Hum*, 71:1–315. Available from: <http://monographs.iarc.fr/ENG/Monographs/vol71/index.php> PMID:[10507919](https://pubmed.ncbi.nlm.nih.gov/10507919/)

- IFA (2014). Tetrafluoroethylene. GESTIS DNEL Database. Hazardous substance information system of the German social accident insurance. IFA [Institute for Occupational Safety & Health of the German Social Accident Insurance]. Available from: <http://dnel-en.itrust.de/>, accessed 27 October 2015.
- ISO (2001). Workplace air quality. Sampling and analysis of volatile organic compounds by solvent desorption/gas chromatography pumped sampling method. International Organization for Standardization, ISO, 16200-1:2001. Available from: <https://www.iso.org/obp/ui/#iso:std:iso:16200:-1:ed-1:vl:en>.
- Japanese Environmental Survey (2012). Results of the initial environmental survey in fiscal year 2012. Available from: http://www.env.go.jp/chemi/kurohon/en/http2013e/pdf/03_Chapter1.pdf, accessed 29 October 2015.
- Kato Y, Kuge K, Kusuhara H, Meier PJ, Sugiyama Y (2002). Gender difference in the urinary excretion of organic anions in rats. *J Pharmacol Exp Ther*, 302(2):483–9. doi:[10.1124/jpet.102.033878](https://doi.org/10.1124/jpet.102.033878) PMID:[12130705](https://pubmed.ncbi.nlm.nih.gov/12130705/)
- Keller DA, Kennedy GL Jr, Ross PE, Kelly DP, Elliott GS (2000). Toxicity of tetrafluoroethylene and S-(1,1,2,2-tetrafluoroethyl)-L-cysteine in rats and mice. *Toxicol Sci*, 56(2):414–23. doi:[10.1093/toxsci/56.2.414](https://doi.org/10.1093/toxsci/56.2.414) PMID:[10911001](https://pubmed.ncbi.nlm.nih.gov/10911001/)
- Kennedy GL Jr (1990). Toxicology of fluorine-containing monomers. *Crit Rev Toxicol*, 21(2):149–70. doi:[10.3109/10408449009089877](https://doi.org/10.3109/10408449009089877) PMID:[2083033](https://pubmed.ncbi.nlm.nih.gov/2083033/)
- Kobayashi Y, Hirokawa N, Ohshiro N, Sekine T, Sasaki T, Tokuyama S, et al. (2002). Differential gene expression of organic anion transporters in male and female rats. *Biochem Biophys Res Commun*, 290(1):482–7. doi:[10.1006/bbrc.2001.6180](https://doi.org/10.1006/bbrc.2001.6180) PMID:[11779196](https://pubmed.ncbi.nlm.nih.gov/11779196/)
- Kraus T, Uttamsingh V, Anders MW, Wolf S (2000). Porcine kidney microsomal cysteine S-conjugate N-acetyltransferase-catalyzed N-acetylation of haloalkene-derived cysteine S-conjugates. *Drug Metab Dispos*, 28(4):440–5. PMID:[10725313](https://pubmed.ncbi.nlm.nih.gov/10725313/)
- Krause RJ, Lash LH, Elfarra AA (2003). Human kidney flavin-containing monooxygenases and their potential roles in cysteine s-conjugate metabolism and nephrotoxicity. *J Pharmacol Exp Ther*, 304:185–191. doi:[10.1124/jpet.102.042911](https://doi.org/10.1124/jpet.102.042911) PMID:[12490590](https://pubmed.ncbi.nlm.nih.gov/12490590/)
- Lash LH, Chiu WA, Guyton KZ, Rusyn I (2014). Trichloroethylene biotransformation and its role in mutagenicity, carcinogenicity and target organ toxicity. *Mutat Res*, 762:22–36 doi:[10.1016/j.mrrev.2014.04.003](https://doi.org/10.1016/j.mrrev.2014.04.003)
- Lash LH (2005). Role of glutathione transport processes in kidney function. *Toxicol Appl Pharmacol*, 204(3):329–42. doi:[10.1016/j.taap.2004.10.004](https://doi.org/10.1016/j.taap.2004.10.004) PMID:[15845422](https://pubmed.ncbi.nlm.nih.gov/15845422/)
- Lash LH (2007). UNIT 6.12 Glutathione-dependent bioactivation. *Curr Protoc Toxicol*, Chapt6:6.12.1–6.12.16. doi:[10.1002/0471140856.tx0612s34](https://doi.org/10.1002/0471140856.tx0612s34)
- Lash LH (2011). Renal membrane transport of glutathione in toxicology and disease. *Vet Pathol*, 48(2):408–19. doi:[10.1177/0300985810375811](https://doi.org/10.1177/0300985810375811) PMID:[20656901](https://pubmed.ncbi.nlm.nih.gov/20656901/)
- Lash LH, Jones DP, Anders MW (1988). Glutathione homeostasis and glutathione S-conjugate toxicity in kidney. *Rev Biochem Toxicol*, 9:29–67.
- Lash LH, Parker JC (2001). Hepatic and renal toxicities associated with perchloroethylene. *Pharmacol Rev*, 53:177–208. PMID:[11356983](https://pubmed.ncbi.nlm.nih.gov/11356983/)
- Lash LH, Putt DA, Cai H (2006). Membrane transport function in primary cultures of human proximal tubular cells. *Toxicology*, 228(2–3):200–18. doi:[10.1016/j.tox.2006.08.035](https://doi.org/10.1016/j.tox.2006.08.035) PMID:[16997449](https://pubmed.ncbi.nlm.nih.gov/16997449/)
- Lash LH, Putt DA, Xu F, Matherly LH (2007). Role of rat organic anion transporter 3 (Oat3) in the renal basolateral transport of glutathione. *Chem Biol Interact*, 170(2):124–34. doi:[10.1016/j.cbi.2007.07.004](https://doi.org/10.1016/j.cbi.2007.07.004) PMID:[17719021](https://pubmed.ncbi.nlm.nih.gov/17719021/)
- Ljubojevic M, Herak-Kramberger CM, Hagos Y, Bahn A, Endou H, Burckhardt G, et al. (2004). Rat renal cortical OAT1 and OAT3 exhibit gender differences determined by both androgen stimulation and estrogen inhibition. *Am J Physiol Renal Physiol*, 287(1):F124–38. doi:[10.1152/ajprenal.00029.2004](https://doi.org/10.1152/ajprenal.00029.2004) PMID:[15010355](https://pubmed.ncbi.nlm.nih.gov/15010355/)
- Lock EA, Ishmael J (1998). The nephrotoxicity and hepatotoxicity of 1,1,2,2-tetrafluoroethyl-L-cysteine in the rat. *Arch Toxicol*, 72(6):347–54. doi:[10.1007/s002040050513](https://doi.org/10.1007/s002040050513) PMID:[9657282](https://pubmed.ncbi.nlm.nih.gov/9657282/)
- Löf A, Johanson G, Rannug A, Warholm M (2000). Glutathione transferase T1 phenotype affects the toxicokinetics of inhaled methyl chloride in human volunteers. *Pharmacogenetics*, 10(7):645–53. doi:[10.1097/00008571-200010000-00007](https://doi.org/10.1097/00008571-200010000-00007) PMID:[11037805](https://pubmed.ncbi.nlm.nih.gov/11037805/)
- MacFarlane M, Foster JR, Gibson GG, King LJ, Lock EA (1989). Cysteine conjugate β -lyase of rat kidney cytosol: characterization, immunocytochemical localization, and correlation with hexachlorobutadiene nephrotoxicity. *Toxicol Appl Pharmacol*, 98(2):185–97. doi:[10.1016/0041-008X\(89\)90224-X](https://doi.org/10.1016/0041-008X(89)90224-X) PMID:[2711386](https://pubmed.ncbi.nlm.nih.gov/2711386/)
- McCarthy RI, Lock EA, Hawksworth GM (1994). Cytosolic C-S lyase activity in human kidney samples-relevance for the nephrotoxicity of halogenated alkenes in man. *Toxicol Ind Health*, 10(1–2):103–12. doi:[10.1177/074823379401000107](https://doi.org/10.1177/074823379401000107) PMID:[7570611](https://pubmed.ncbi.nlm.nih.gov/7570611/)
- Moore LE, Boffetta P, Karami S, Brennan P, Stewart PS, Hung R, et al. (2010). Occupational trichloroethylene exposure and renal carcinoma risk: evidence of genetic susceptibility by reductive metabolism gene variants. *Cancer Res*, 70(16):6527–36. doi:[10.1158/0008-5472.CAN-09-4167](https://doi.org/10.1158/0008-5472.CAN-09-4167) PMID:[20663906](https://pubmed.ncbi.nlm.nih.gov/20663906/)
- Mulder TP, Court DA, Peters WH (1999). Variability of glutathione S-transferase alpha in human liver and plasma. *Clin Chem*, 45(3):355–9. PMID:[10053036](https://pubmed.ncbi.nlm.nih.gov/10053036/)
- Newman D, Abuladze N, Scholz K, Dekant W, Tsuprun V, Ryazantsev S, et al. (2007). Specificity of aminoacylase III-mediated deacetylation of mercapturic

- acids. *Drug Metab Dispos*, 35(1):43–50. doi:[10.1124/dmd.106.012062](https://doi.org/10.1124/dmd.106.012062) PMID:[17012540](https://pubmed.ncbi.nlm.nih.gov/17012540/)
- NIOSH (1977). Criteria for a recommended standard. Occupational exposure to decomposition products of fluorocarbon polymers. DHEW (NIOSH) Publication No. 77-193. Washington (DC): United States department of Health, Education, and Welfare, National Institute for Occupational Safety and Hygiene. Available from: <http://www.cdc.gov/niosh/pdfs/77-193a.pdf>, accessed November 2015.
- NIOSH (2003). Organic and inorganic gases by extractive FTIR spectrometry. NIOSH method 3800. In: NIOSH manual of analytical methods, fourth ed. Cincinnati (OH): National Institute for Occupational Safety and Hygiene. Available from: <http://www.cdc.gov/niosh/pdfs/3800.pdf>, accessed 16 September 2014.
- Nolan RJ, Rick DL, Landry TD, McCarty LP, Agin GL, Saunders JH (1985). Pharmacokinetics of inhaled methyl chloride (CH₃Cl) in male volunteers. *Fundam Appl Toxicol*, 5(2):361–9. doi:[10.1016/0272-0590\(85\)90084-3](https://doi.org/10.1016/0272-0590(85)90084-3) PMID:[3988005](https://pubmed.ncbi.nlm.nih.gov/3988005/)
- NRC (2015). Twenty-second interim report of the Committee on Acute Exposure Guideline Levels for Selected Airborne Chemicals. Vol. 19. Washington (DC): National Research Council, The National Academies Press. Available from: http://www.nap.edu/catalog.php?record_id=18432, accessed 16 September 2014.
- NTP (1997). NTP toxicology and carcinogenesis studies of tetrafluoroethylene (CAS No. 116-14-3) in F344 rats and B6C3F1 mice (inhalation studies). *Natl Toxicol Program Tech Rep Ser*, 450:1–321. PMID:[12594525](https://pubmed.ncbi.nlm.nih.gov/12594525/)
- NTP (2014). Tetrafluoroethylene. Report on carcinogens, thirteenth edition. 12: 401–402. Research Triangle Park (NC): National Toxicology Program. Available from: <http://ntp.niehs.nih.gov/ntp/roc/content/profiles/tetrafluoroethylene.pdf>, accessed 19 July 2016.
- Odum J, Green T (1984). The metabolism and nephrotoxicity of tetrafluoroethylene in the rat. *Toxicol Appl Pharmacol*, 76(2):306–18. doi:[10.1016/0041-008X\(84\)90012-7](https://doi.org/10.1016/0041-008X(84)90012-7) PMID:[6495336](https://pubmed.ncbi.nlm.nih.gov/6495336/)
- Sleeuwenhoek A, Cherrie JW (2012). Exposure assessment of tetrafluoroethylene and ammonium perfluorooctanoate 1951–2002. *J Environ Monit*, 14(3):775–81. doi:[10.1039/c2em10930a](https://doi.org/10.1039/c2em10930a) PMID:[22322341](https://pubmed.ncbi.nlm.nih.gov/22322341/)
- Stevens JL, Robbins JD, Byrd RA (1986). A purified cysteine conjugate β -lyase from rat kidney cytosol. Requirement for an α -keto acid or an amino acid oxidase for activity and identity with soluble glutamine transaminase K. *J Biol Chem*, 261(33):15529–37. PMID:[3782077](https://pubmed.ncbi.nlm.nih.gov/3782077/)
- Teng H (2012). Overview of the development of the fluoropolymer industry. *Applied Sciences*, 2:496–512. doi:[10.3390/app2020496](https://doi.org/10.3390/app2020496)
- TRI (2016). TRI2014: Tetrafluoroethylene. Toxics Release Inventory. Bethesda: United States Library of Medicine, Toxicology Data Network (TOXNET). Available from: <https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~tkhRne:l>, accessed 21 July 2016.
- Urban TJ, Sebro R, Hurowitz EH, Leabman MK, Badagnani I, Lagpacan LL, et al. (2006). Functional genomics of membrane transporters in human populations. *Genome Res*, 16(2):223–30. doi:[10.1101/gr.4356206](https://doi.org/10.1101/gr.4356206) PMID:[16354753](https://pubmed.ncbi.nlm.nih.gov/16354753/)
- Wiesenhütter B, Selinski S, Golka K, Brüning T, Bolt HM (2007). Re-assessment of the influence of polymorphisms of phase-II metabolic enzymes on renal cell cancer risk of trichloroethylene-exposed workers. *Int Arch Occup Environ Health*, 81(2):247–51. doi:[10.1007/s00420-007-0200-5](https://doi.org/10.1007/s00420-007-0200-5) PMID:[17479278](https://pubmed.ncbi.nlm.nih.gov/17479278/)