IARC MONOGRAPHS

TRICHLOROETHYLENE, TETRACHLOROETHYLENE, AND SOME OTHER CHLORINATED AGENTS

VOLUME 106

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 2-9 October 2012

LYON, FRANCE - 2014

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

TRICHLOROETHYLENE

Trichloroethylene was considered by previous IARC Working Groups in 1979, 1987, and 1995 (IARC, 1979, 1987, 1995). New data have since become available, and these have been 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.: 79-01-6

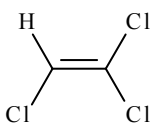
Deleted CAS Reg. No.: 52037-46-4

Chem. Abstr. Name: 1,1,2-Trichloroethene

IUPAC Systematic Name: Trichloroethylene

Synonyms: Ethinyl trichloride; ethylene trichloride; TCE; 1,1,2-trichloroethylene

1.1.2 Structural and molecular formulae and relative molecular mass



C_2HCl_3

Relative molecular mass: 131.39

1.1.3 Chemical and physical properties of the pure substance

Description: Nonflammable, mobile liquid. Characteristic odour resembling that of chloroform (O'Neil *et al.*, 2006)

Boiling-point: 86.9 °C (O'Neil *et al.*, 2006)

Melting-point: –84.8 °C (O'Neil *et al.*, 2006)

Density: 1.4642 at 20 °C/relative to H_2O at 4 °C (O'Neil *et al.*, 2006)

Spectroscopy data: Infrared (prism, 185; grating, 62), nuclear magnetic resonance (proton, 9266; C-13, 410) and mass (583) spectral data have been reported (Sadtler Research Laboratories, 1980; Weast & Astle, 1985).

Solubility: Slightly soluble in water (1.1 g/L at 25 °C); soluble in ethanol, diethyl ether, acetone and chloroform (O'Neil *et al.*, 2006); dissolves most fixed and volatile oils (O'Neil *et al.*, 2006)

Volatility: Vapour pressure, 100 Pa at 39 °C (Haynes, 2012); relative vapour density (air = 1.0), 4.53 (O'Neil *et al.*, 2006)

Stability: Photo-oxidized in air by sunlight (half-time, 5 days) giving phosgene and

dichloroacetyl chloride (EPA, 1985), which slowly decomposes with formation of hydrogen chloride by light in the presence of moisture (O'Neil *et al.*, 2006).

Reactivity: Incompatible with strong caustics and alkalis and with chemically active metals such as barium, lithium, sodium, magnesium, titanium and beryllium (NIOSH, 1994a)

Octanol/water partition coefficient (P): log P, 2.61 (Hansch *et al.*, 1995)

Conversion factor: $mg/m^3 = 5.37 \times ppm$

Calculated from: $mg/m^3 = (\text{relative molecular mass}/24.45) \times ppm$, assuming normal temperature (25 °C) and pressure (101 kPa).

1.1.4 Technical products and impurities

Trichloroethylene is available in the USA in vapour-degreasing, general-purpose and high-purity grades (DOW Chemical, 2012). In France, industrial-grade trichloroethylene is generally of high purity (> 99%). Depending on its intended use, it can contain one or more stabilizers, including amines, alcohols or epoxides (thymol, triethylamine, trimethyloxirane, epoxybutane, etc.) at low concentration (< 1%) (Table 1.1; Commission for Health and Safety at Work, 2012).

Possible impurities depend on the manufacturing route, the type and quality of feed stock used, the type of distillation equipment, and the technical specification being met. It is uncommon for any individual impurity to be present at a level in excess of 100 mg/kg and for the total impurities to exceed 1000 mg/kg (WHO, 1985).

Stabilizers, in the form of antioxidants or acid-receptors (such as phenolic, olephinic, pyrrolic, and/or oxiranic derivatives and aliphatic amines), are usually added in concentrations that normally range from 20 to 600 mg/kg; however, for limited quantities and special uses, concentrations as high as 5000 mg/kg may be used. The stabilizers used will depend on patent ownership and the technical specification being met (WHO, 1985).

Table 1.1 Some impurities and stabilizers found in commercial trichloroethylene

Impurities	Stabilizers
Carbon tetrachloride	Pentanol-2
Chloroform	Thymol
1,2-Dichloroethane	Triethanolamine
trans 1,2-Dichloroethylene	Triethylamine
cis 1,2-Dichloroethylene	2,2,4-Trimethylpentene
Pentachloroethane	Cyclohexene oxide
1,1,1,2-Tetrachloroethane	*n*-Propanol
1,1,2,2-Tetrachloroethane	Isobutanol
1,1,1-Trichloroethane	*N*-methylmorpholine
1,1,2-Trichloroethane	Diisopropylamine
1,1-Dichloroethylene	*N*-Methylpyrrole
Bromodichloroethylene	Methyl ethyl ketone
Tetrachloroethylene	Epichlorohydrin
Bromodichloromethane	
Benzene	

From WHO (1985)

Trade names for trichloroethylene include: Algylen, Anamenth, Benzinol, Cecolene, Chlorilen, Chlorylea, Chlorylen, CirCosolv, Crawhaspol, Densinfluat, Dukeron, Dow-Tri, Fleck-Flip, Flock Flip, Fluate, Germalgene, Lanadin, Lethurin, Narcogen, Narkosoid, Nialk, Perm-AChlor, Petzinol, Philex, Threthylen, Threthylene, Trethylene, Tri, Triad, Trial, Trichloran, Trichloren, Triclene, Trielene, Trielin, Trieline, Trilen, Trilene, Trimar, TRI-Plus M, Vestrol, Vitran, and Westrosol.

1.1.5 Analysis

Methods for the analysis of trichloroethylene have been reviewed by Delinsky *et al.* (2005) and Demeestere *et al.* (2007). Selected methods for the analysis of trichloroethylene in various matrices are identified in Table 1.2.

Table 1.2 Methods for the analysis of trichloroethylene

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Analyte collected on sorbent tube; thermally desorb to GC	GC/MS	NR	EPA (1999b)
	Air collected in specially prepared canister; desorb from cold trap to GC	GC/MS	NR	EPA (1999a)
Water	Purge with inert gas and trap; desorb to GC	GC/PID	0.02 µg/L	EPA (1988, 1995a)
		GC/HECD	0.01 µg/L	
	Purge with inert gas and trap; desorb to GC	GC/PID	0.01 µg/L	EPA (1988)
	Purge with inert gas and trap; desorb to GC	GC/MS	0.02 µg/L	EPA (1988, 1995b)
	Extract with methyl-*t*-butyl ether or pentane;	GC/EC	0.002 µg/L	EPA (1995c)
Liquid and solid wastes	Purge with inert gas and trap	GC/PID	0.02 µg/L	EPA (1996)
		GC/HECD	0.01 µg/L	
Blood	Purge with inert gas and trap on Tenax	GC/MS	0.004 ppb	Ashley *et al.* (1992)

EC, electron capture detection; FID, flame ionization detection; GC, gas chromatography; HECD, Hall electrolytic conductivity detection; MCD, microcoulometric detection; MS, mass spectrometry; PID, photoionization detection; ppb, parts per billion; NR, not reported

1.2 Production and use

1.2.1 Production process

(a) Manufacturing processes

Trichloroethylene was first prepared in 1864 by Emil Fischer in experiments on the reduction of hexachloroethane with hydrogen (Hardie, 1964). Commercial production of trichloroethylene began in Germany in 1920 and in the USA in 1925 (Mertens, 1993). Originally, the acetylene-based process consisted of two steps: first, acetylene was chlorinated with a chloride catalyst to produce 1,1,2,2-tetrachloroethane, and then this product was dehydrohalogenated to trichloroethylene (Mertens, 1993).

The current method of manufacture is from ethylene. Trichloroethylene can be produced using oxichlorination or noncatalytic chlorination of ethylene dichloride or other C2-chlorinated hydrocarbons. Another method involves using catalytic hydrogenation of tetrachloroethylene (ECSA, 2012). Trichloroethylene can also be produced by direct chlorination of ethylene in the absence of oxygen, giving a mixture of tetrachloroethane and pentachloroethane. The products are thermally cracked to produce a mixture of trichloroethylene, tetrachloroethylene and hydrochloric acid. This process was developed and is used in Japan (Linak *et al.*, 1992).

(b) Production volume

In general, there has been a continuing decline in demand for trichloroethylene over the years. Concern about the environmental and health and safety implications of chlorinated solvents has resulted in regulations and controls that have had an impact on the use and production of trichloroethylene. For example, in the USA the demand for trichloroethylene dropped from 244 939 tonnes (540 million pounds) in 1971 to only 68 038 tonnes (150 million pounds) in 1990 (NICNAS, 2000). In Sweden, 4564 tonnes were used in 1993, but only 91 tonnes by 2009 (KEMI, 2012). The production volume of trichloroethylene in the European Union in 1996 was thought to be between 51 000 and 225 000 tonnes per year (SCOEL/SUM, 2009). In 1995, this declining trend was reversed when the Montreal Protocol on Substances that Deplete the Ozone Layer was enacted: trichloroethylene was required in increasing amounts as a precursor in the manufacture of chlorofluorocarbon alternatives and as

a substitute for chemicals such as 1,1,1-trichloroethane (NICNAS, 2000).

In 2007, the USA was the largest consumer of trichloroethylene, followed by western Europe, China, and Japan (Glauser & Ishikawa, 2008).

1.2.2 Use

Trichloroethylene is best known for its use as a solvent for cleaning and degreasing metal parts. However, it has had numerous other uses, including as an anaesthetic, a heat-transfer medium, an extraction agent for fats and oils, as an intermediate in producing chlorofluorocarbons and other chemicals, and as an ingredient in many products for industrial and consumer use (Doherty, 2000).

Demand for trichloroethylene was generated mainly by the development of vapour degreasing after the 1920s and by the growth of the dry-cleaning industry in the 1930s. Trichloroethylene was replaced in dry-cleaning by tetrachloroethylene in the mid-1950s. By 1989, about 85% of the trichloroethylene produced in the USA was used in metal cleaning; the remaining 15% was equally divided between exports and miscellaneous applications. The pattern in Japan was similar to that in the USA, at 83% and 17%, respectively. In western Europe, 95% was used in vapour degreasing and 5% for other uses (Mertens, 1993). Similar use patterns have been reported for Canada (Moore *et al.*, 1991), and Finland (Mroueh, 1993). The use of trichloroethylene as solvent in Europe dropped by 85% from 1984 until 2006, with a further estimated decline of 60% from 2006 until 2010 (ECSA, 2012).

Currently the main use of trichloroethylene is as a feedstock material to produce other chemicals, such as fluorinated hydrocarbons and fluorinated polymers, which are being phased out under the Montreal Protocol. About 80% of current production in the European Union is used for this purpose (ECSA, 2012).

(a) Metal degreasing

Before the 1990s, the major use of trichloroethylene was in metal degreasing. Degreasing is important in all metalworking and maintenance operations to remove oils, greases, waxes, tars and moisture before final surface treatments, such as galvanizing, electroplating, painting, anodizing and application of conversion coatings. Trichloroethylene has been used in degreasing operations in five main industrial groups: furniture and fixtures, fabricated metal products, electric and electronic equipment, transport equipment and miscellaneous manufacturing industries. It has also been used in plastics, appliances, jewellery, automobile, plumbing fixtures, textiles, paper, glass and printing (Papdullo *et al.*, 1985; Linak *et al.*, 1992).

Metal degreasing operations using trichloroethylene are of two main types: cold degreasing and vapour degreasing. In cold degreasing, trichloroethylene is applied at room temperature; in vapour degreasing, the solvent vapours are condensed on the part to be cleaned. Cold degreasing by hand will result in higher exposures than vapour degreasing.

Cold degreasing refers to the process of degreasing by dipping or soaking articles in a degreasing liquid, or spraying, brushing, or wiping the cleaner onto articles at temperatures below boiling point. The cold process is frequently used in maintenance operations and on small parts. Cold degreasing activities include immersion in tanks, drums, or other containers, and spraying, brushing and wiping.

Vapour degreasing requires a tank with heating coils on the bottom and a condensing zone near the top. The solvent is heated to boiling, and the hot vapour fills the condensing zone near the top of the tank. Soiled objects are lowered into this zone, where the vapour condenses into a pure liquid solvent on the piece and dissolves and carries off dirt as it drains back into the tank. The part dries immediately (Papdullo *et al.*, 1985;

Linak *et al.*, 1992). Vapour degreasers can incorporate spraying as part of the degreasing process. In western Europe, in 1990, 120 000 tonnes of trichloroethylene were used in vapour degreasing and only 10 000 tonnes in cold degreasing (Linak *et al.*, 1992). Similarly, vapour degreasing was more common than cold degreasing in Australia in 1995 (NICNAS, 2000).

Ultrasonic agitation can be employed in hot or cold immersion degreasing, and is sometimes incorporated into vapour-degreasing systems. In ultrasonic degreasing, a transducer mounted on the bottom or side of a solvent-containing tank creates vibrations that cause the rapid expansion and contraction of microscopic bubbles in the solvent, resulting in a scrubbing action on parts that are immersed in the tank (NICNAS, 2000).

(b) Dry-cleaning industry

Trichloroethylene was used in the dry-cleaning industry in the 1930s, but was very harsh on clothes and was replaced by tetrachloroethylene in the mid-1950s. Trichloroethylene is still used in spotting agents to remove spots before cleaning the garments in the dry-cleaning machine or after the garments have been cleaned in the machine (Wolf & Morris, 2007).

(c) Other industrial applications

(i) Chemical intermediates

Trichloroethylene is used as a molecular-weight control agent in the manufacturer of polyvinyl chloride. About 10 million pounds [4500 tonnes] of trichloroethylene are used each year in the USA in the manufacture of polyvinyl chloride (IARC, 1995). As noted above, the largest use of trichloroethylene currently is as a feedstock for chlorofluorocarbons and hydrofluorocarbons (Doherty, 2000). Under the Montreal Protocol of the 1990s, chlorofluorocarbons are being phased-out due to their contribution to ozone depletion.

(ii) Textile industry

In the textile industry, trichloroethylene has been used as a carrier solvent for spotting fluids and as a solvent in dyeing and finishing (Fishbein, 1976; Linak *et al.*, 1992; Mertens, 1993). The main use of trichloroethylene in the textile industry is to clean cotton, wool and other fabrics. It is also used as a solvent for waterless dyeing (Doherty, 2000).

(iii) Consumer products

Some consumer products that have contained trichloroethylene include automotive products, wood finishes, typewriter correction fluids, cleaners and polishes, including for electronic equipment, treatments for leather and fabric, adhesives, paint-related products and lubricants (Sack *et al.*, 1992; ATSDR, 1997).

(iv) Other uses

Trichloroethylene has been used in miscellaneous chemical synthesis and solvent applications, including: as a synthesis feedstock for products such as paints, adhesives and cleaners; as a reactant to produce pesticide intermediates; in the chemical synthesis of flame-retardant chemicals; as a solvent in pharmaceutical manufacture; and as a carrier solvent in formulated consumer products such as insecticides, fungicides, paint removers, and paint strippers (EPA, 1989; Doherty, 2000).

(d) Miscellaneous

During the 20th century, trichloroethylene was used as an anaesthetic for dental and surgical procedures and in veterinary medicine (Doherty, 2000). It was also used as an extraction solvent for natural fats and oils (such as palm, coconut and soya bean oils), and for spices, hops, and the decaffeination of coffee (Linak *et al.*, 1992). The United States Food and Drug Administration (FDA, 1977) banned these uses of trichloroethylene because of its toxicity; its use in cosmetic and drug products was also discontinued (Mertens, 1993).

Table 1.3 Mean concentrations[a] of trichloroethylene in air in the USA, by year

Year	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998
Concentration (μg/m³)	1.4	1.39	1.68	4.87	1.69	1.84	2.86	1.37	1.12	0.95	0.78	0.65	0.74	0.88

[a] Representing about 1200 measurements in 25 states
From Wu & Schaum (2000)

1.3 Occurrence and exposure

1.3.1 Natural occurrence

Trichloroethylene has been reported in temperate, subtropical and tropical algae, and in one red microalga (Abrahamsson *et al.*, 1995).

1.3.2 Environmental occurrence

Trichloroethylene is widely distributed in the environment due to industrial emissions. Potential environmental exposure to trichloroethylene in the air, rainwater, surface waters, and drinking-water has been reviewed (ATSDR, 1997; Wu & Schaum, 2001). The partitioning tendency of trichloroethylene in the environment has been calculated as follows: air, 97.7%; water, 0.3%; soil, 0.004%; sediment, 0.004% (Boutonnet *et al.*, 1998).

A common method for disposal of trichloroethylene is incineration after mixing with a combustible fuel (Sittig, 1985).

(a) Air

Measurements of trichloroethylene in air in the USA, representing about 1200 measurements in 25 states (Table 1.3), suggest a general downward trend in mean concentrations of trichloroethylene in air, from about 1.5 μg/m³ in the late 1980s to 0.8 μg/m³ in the late 1990s. Concentrations in urban air were about three times higher than in rural areas, and mean concentrations of trichloroethylene were highest in commercial/industrial areas and lowest in forest areas (Wu & Schaum, 2000).

Table 1.4 presents some recent data on concentrations of trichloroethylene in air measured worldwide in remote, rural, suburban and urban sites. The data showed trends similar to those observed in the USA.

Concentrations of trichloroethylene in indoor air can increase when trichloroethylene-contaminated water is used domestically, for example, during showering (Andelman, 1985; Ömür-Özbek *et al.*, 2011).

(b) Soil

Trichloroethylene can be released into the soil through industrial discharges into surface waters and through leaching from landfill sites. It has been estimated that at least 9612 kg of trichloroethylene was released into soil from manufacturing and processing facilities in the USA in 1988 (ATSDR, 1997).

(c) Water

Owing to its widespread use, trichloroethylene occurs frequently at low concentrations in water supplies and in groundwater. Table 1.5 summarizes some recent concentrations of trichloroethylene reported in surface water, groundwater and drinking-water worldwide.

(d) Food

As a part of the Total Diet Program in the USA, 20 samples of 70 different foods purchased in supermarkets or restaurants in 1996–2000 were analysed for trichloroethylene. Trichloroethylene was found at a low frequency in 29 out of 70 food items at concentrations in the low microgram-per-kilogram range (Fleming-Jones & Smith, 2003).

In a survey of 35 whole milk samples in Las Vegas, NV, USA, trichloroethylene was found

Table 1.4 Concentrations of trichloroethylene in air

Location	Concentration (µg/m³)		Comments	Reference
	Mean	Range		
Outdoor air				
Remote				
Antarctica	NR	0.016–0.024	Five remote sites	Zoccolillo *et al.* (2009)
Atlantic Ocean	NR	[0.03–0.05]	Background tropospheric levels	Class & Ballschmiter (1986)
North America	< 0.02	NR	Remote background concentration	McCarthy *et al.* (2006)
Urban and rural				
Canada, Ottawa	0.08	0.01–1.49	Near 74 homes	Zhu *et al.* (2005)
Italy	NR	0.312–0.940	Four urban and suburban sites	Zoccolillo *et al.* (2009)
	NR	0.022–0.107	At 12 rural sites	
Japan, Shizuoka Prefecture	0.23[c]	0.11–0.80[a]	Near 25 homes	Ohura *et al.* (2006)
Japan, Hyogo Prefecture	NR	Range of medians, 0.053–0.22	At six sites over 5 years	Okada *et al.* (2012)
Spain, Tarragona	0.74	2.20[b]	Near large industrial complex	Ramírez *et al.* (2012)
USA, Georgia	[0.96]	4.59[b]	Near degreaser facility	Martin *et al.* (2005)
USA, five cities in NJ, NC, ND, CA	NR	0.083–1.78		Rappaport & Kupper (2004)
USA, Dallas, TX	8.5	1.1–327	Ambient air near gas wells	Rich (2011)
USA, Minnesota	0.43	< 0.04–25.31	At 25 sites in state (1991–1998)	Pratt *et al.* (2000)
USA, Seattle, WA	0.21	SD, 0.23	Ambient air at six sites	Wu *et al.* (2011)
Indoor air				
Canada, Ottawa, ON	0.06	0.01–0.87	75 homes	Zhu *et al.* (2005)
France	0.5[a]	< 0.4–4087	490 homes	Billionnet *et al.* (2011)
Japan, Shizuoka Prefecture	0.22[c]	0.10–0.78[a]	25 homes	Ohura *et al.* (2006)
USA, Endicott, NY	16[a]	0.18–140	70 housing blocks near contaminated site	Forand *et al.* (2012)
USA, Minneapolis, MN	0.7	0.2–1.4	284 households	Adgate *et al.* (2004)
USA, Missoula, MT	[0.02[c]][d]	[< 0.001–4.6][d]	80 homes	Ward *et al.* (2009)
USA, New Jersey	NR	< 1.1–13	100 urban and suburban homes	Weisel *et al.* (2008)

[a] 10th and 90th percentiles
[b] Maximum
[c] Median
[d] The Working Group noted that the units of concentration were not reported clearly in the original publication but assumed that these values were in µg/m³.
NR, not reported

at a mean concentration of 0.04 µg/L (range, < 0.01–0.27) (Hiatt & Pia, 2004).

The average concentration of trichloroethylene in 3 out of 17 samples of brown grease from food-preparation facility grease traps was 321.3 µg/L (range, 146–600) (Ward, 2012).

1.3.3 Occupational exposure

Degreasing is the main source of occupational exposure to trichloroethylene: cold degreasing by hand will result in higher exposures than vapour degreasing.

The United States National Institute for Occupational Safety and Health (NIOSH, 1994b)

Table 1.5 Concentrations of trichloroethylene in water

Country	Location	Concentration (µg/L)		Comments	Reference
		Mean	Range		
Ground-water					
China	Eastern China	NR	< 0.2–28	At five sites	Bi *et al.* (2012)
China, Taiwan	Country-wide	NR	1–231	Near eight contaminated sites	Fan *et al.* (2009)
China, Taiwan	Taoyuan City	253	0.1–1791	Near contaminated site	Lee *et al.* (2002)
Croatia	Sašnak	8.55	5.05–12.90	1995–1996	Vedrina-Dragojević & Dragojević (1997)
USA	Camp Lejeune, NC	NR	NR–57	Supply well near contaminated site	Sonnenfeld *et al.* (2001)
USA	Minnesota	NR	0.2–144	Near hazardous-waste disposal sites	Sabel & Clark (1984)
USA	Country-wide	NR	0.02–230	5000 samples, 1985–2002	Moran *et al.* (2007)
USA	Arizona	NR	1–239 ppb	Seven municipal wells near a contaminated site	Kioski *et al.* (1990)
Surface water					
Europe	Southern North Sea	0.049	< 0.012–0.27	10 locations, 1998–2000	Huybrechts *et al.* (2005)
Greece	Northern Greece	NR	< 0.02–40		Kostopoulou *et al.* (2000)
USA	Bush River, MD	NR	450–1600	Contaminated site near Aberdeen Proving Ground	Burton *et al.* (2002)
Drinking-water					
Malaysia	County-wide	NR	0.3–0.7		Soh & Abdullah (2007)
USA	Camp Lejeune, NC	399	1–1400		NRC (2009)
USA	Woburn, MA	267	NR		Costas *et al.* (2002)

NR, not reported

estimated that about 401 000 employees in 23 225 plants in the USA were potentially exposed to trichloroethylene. This estimate was based on a survey of products used in companies in 1981–83 and did not involve actual measurements.

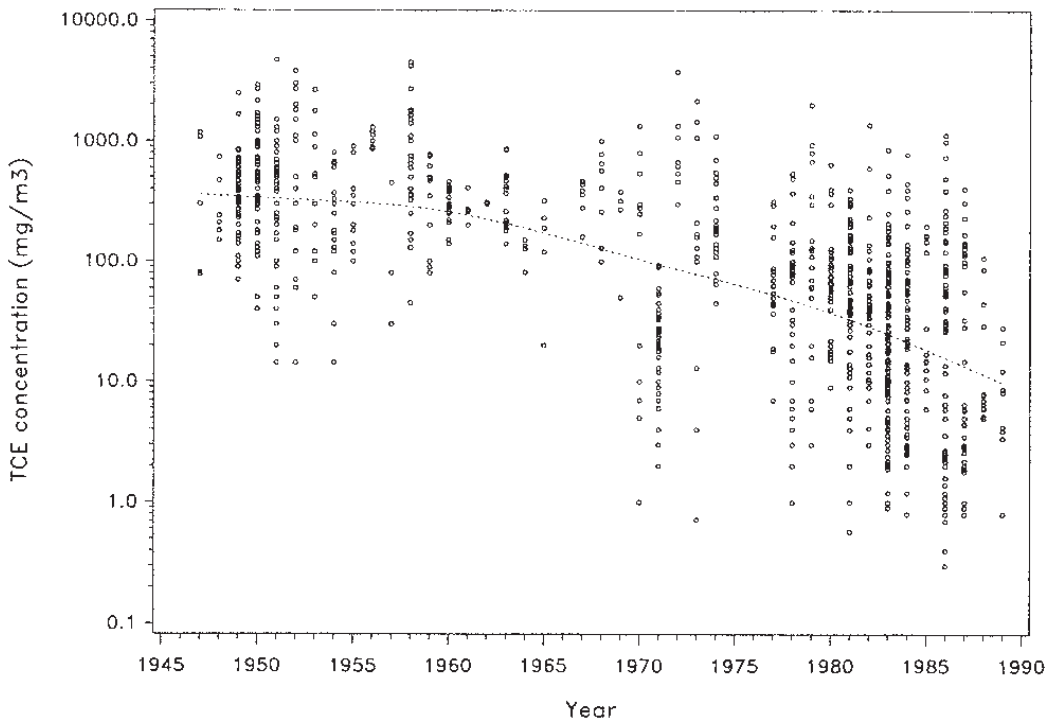
The European CAREX (CARcinogen EXposure) project estimated the number of exposed workers in 15 countries of the European Union (EU-15) to be approximately 276 000 in the early 1990s. The majority of the exposures occurred in industries producing metal products, machinery (including electrical machinery, apparatus and appliances) and in the manufacture of transport equipment. Considerable numbers of exposed workers were also found in construction, the wholesale and retail trades, restaurants and hotels, and in personal and household services, including dry-cleaning shops (Kauppinen *et al.*, 2000).

An update of CAREX for Italy by Mirabelli & Kauppinen (2005) showed a declining number of workers exposed from 42 000 to 34 500 for 1990–93 and 2001 respectively (excluding jobs considered as low-level or with low confidence in their assessment).

The total number of exposed individuals around metal vapour-degreasers in Germany was estimated at almost 11 000 in 1985, and this number decreased considerably to only about 400 in 1999 (von Grote *et al.*, 2003).

Results from CAREX Canada (2012) showed that approximately 13 000 workers were exposed

Fig. 1.1 Area and personal measurements (*n* = 1075) of trichloroethylene in occupational environments in Denmark, 1947–89



TCE, trichloroethylene
The curve shows a smoothing spline estimate of the average concentration
From Raaschou-Nielsen *et al.* (2002). Reprinted by permission of the publisher (Taylor & Francis Ltd, http://www.tandf.co.uk/journals/).

to trichloroethylene, 69% of these being men. Most exposures occur in metal manufacturing, printing and related support activities, textile-furnishing mills, other textile-product mills, and plastic-product manufacturing. Most of these jobs involve metal degreasing. With trichloroethylene no longer commonly in use as a solvent in dry-cleaning, it was estimated that only 200 workers are still exposed in this industry.

A cross-industry survey (2009–10) on use of organic solvents in almost 1500 workplaces in Japan revealed that use of trichloroethylene for degreasing, cleaning and wiping had ceased, and trichloroethylene has largely been replaced by isopropyl alcohol (Nagasawa *et al.*, 2011a). In a similar study in 1909 laboratories from four large research institutes, trichloroethylene was found infrequently in the air in biology laboratories (0.6%), medical laboratories (0.2%) and technology and engineering laboratories (0.9%), but not in agricultural and science laboratories (Nagasawa *et al.*, 2011b).

Table 1.6 summarizes the results of measurements of trichloroethylene in air for occupational exposures. For biomonitoring of its metabolite, trichloroacetate, in urine, the reader is referred to the *Monograph* on Trichloroacetic Acid in this Volume.

Two studies showed clear negative temporal trends in the concentrations of trichloroethylene measured in workplaces. In the USA, estimates of decline in concentration were 6.7% per year, indicating a halving of concentrations each decade (Hein *et al.*, 2010). In Denmark (Raaschou-Nielsen *et al.*, 2002), exposure to trichloroethylene declined at an similar rate from

Table 1.6 Occupational exposure to trichloroethylene

Country Time period	No. of plants	Job, task or industry	No. of samples[a]	Air concentration (mg/m³)		Reference
				Mean	Range	
Australia [1995]	1	Metal industry; degreasing; after replacement of FC113 with trichloroethylene [1995]	5	[47]	NR	Neghab *et al.* (1997)
Canada	1	Textile; spot removing	12	NR	5–118	Mirza *et al.* (2000)
China 2006	6	Degreasing processes in metal manufacturing (n = 4), Optical lenses manufacturing (n = 1), circuit boards manufacturing (n = 1)	235 (P) (80 workers)	[118.22]	-	Lan *et al.* (2010)
Denmark 1947–89	Many	Variety of industries	318 (A) 74 (A) 192 (A+P) 491 (A+P)	1947–1959: 586 1960–1969: 318 1970–1979: 198 1980–1989: 75	NR	Raaschou-Nielsen *et al.* (2002)
Finland 1982–85	11	Vapour degreasing	24 (A) 13 (P) TWA	[43.0] [37.6]	[< 5.4–20.9] [< 5.4–161]	Rantala *et al.* (1992)
	1	Rubber bonding	1 (A) TWA	NR	[32.2]	
	1	Museum textile restoration	2 (P) 1 hour	NR	[3303]	
Italy	1	Printing on glass industry	49 (P)	83.3	2.7–387	Imbriani *et al.* (2001)
	1	Printing industry, degreasing process	12 (P)	31–38	NR	Iavicoli *et al.* (2005)
Republic of Korea	90	Variety of industries	196	[18.5][b]	[ND–1719]	Moon *et al.* (2001)
Netherlands 1988	9	Rubber degreasing, cementing	137 (P)	4	NR	Kromhout *et al.* (1994)
Singapore	1	Metal degreasing	12 (P)	[159] TWA	[48–704]	Goh *et al.* (1998)
Sweden	14	Degreasing	336 (A)	[328]	[0–2230]	Ahlmark *et al.* (1963)
	570	Degreasing	35 000–40 000 (A)	[86]	3% [> 161]	
	19	Degreasing	29 (P)	27	3–144	Ulander *et al.* (1992)
Switzerland	10	Degreasing	96 (P)	[304]	[5.4–1799]	Grandjean *et al.* (1955)
United Kingdom	32	Degreasing	212 (P)	91% [< 161] 97% [< 269] 99% [< 537]	NR	Shipman & Whim (1980)
USA Period NR	60	Degreasing	433 (P)	[725]	[16–4833]	Morse & Goldberg (1943)
		Condenser, nonvented	187	[515]	[27–2110]	
		Condenser, vented	149	NR	NR	
Period NR	NR	Degreasing	146 (A)[b]	86% [< 537] 96% [< 1074]	NR	Hargarten *et al.* (1961)

Table 1.6 (continued)

Country Time period	No. of plants	Job, task or industry	No. of samples[a]	Air concentration (mg/m³)		Reference
				Mean	Range	
Period NR	1	Degreasing	11 (P)	[302]	[199–419]	Vandervort & Polakoff (1973)
Period NR	1	Degreasing ignition coils	(P)	NR	0–[537]	Bloom *et al.* (1974)
Period NR	1	Electronic cleaning	3 (P)	[446]	[408–483]	Gilles & Philbin (1976)
Period NR	1	Semi-conductor degreasing	10 (P)	16.1	2–57	Gunter (1977)
Period NR	1	Degreasing operator	20 (P)	[736]	[140–2024]	Kominsky (1976)
		Degreasing operator	7 (P)	[88.1]	[37.6–456]	
		Degreasing operator	6 (P)	[67.7]	[37.6–199]	
		Lathe operator next to degreaser	7 (P)	[52.1]	[37.6–129]	
Period NR	1	Aircraft degreasing	4 (P)	[21.5]	[5.4–37.6]	Okawa *et al.* (1978)
Period NR	1	Tank relining	8 (P)	[1.3]	ND–[5.4]	Burroughs (1980)
Period NR	1	Degreasing sheet metal	2 (P)	11	10–12	Johnson (1980)
			2 (A)	11	4–18	
Period NR	1	Degreasing, custom finishing	23 (P)	8.3	1–38	Ruhe & Donohue (1980)
			2 (A)	6	4–8	
Period NR	1	Vapour degreasing	14 (P)	333	26.9–1670	Burgess (1981)
Period NR	1	Degreasing, bus maintenance	3 (A)	3.0	ND–8.9	Love & Kern (1981)
Period NR	1	Degreasing	24 (STEL)	742	56–2000	Ruhe *et al.* (1981)
			9 (TWA)	145	37–357	
Period NR	1	Degreasing, plastics	2 (P)	4.8	2.7–7.0	Burroughs & Moody (1982)
Period NR	1	Degreasing, electronics	79 (P)	10.2	ND–209	Lee & Parkinson (1982)
Period NR	1	Degreasing, medical	5 (P)	5.4	1–16	Ruhe (1982)
			2 (A)	6.5	4–9	
Period NR	1	Degreasing, energy conservation products	2 (P)	36.5	22–51	Almaguer *et al.* (1984)
			10 (A)	1.1	0.54–3.2	
Period NR	1	Degreasing	9 (P)	716	39–2288	Belanger & Coye (1984)
		Degreasing	2 (A)	184	0.54–367	
		Silk screening	5 (P)	23.6	1.6–81.1	
Period NR	1	Degreasing aircraft	29 (TWA, P)	30.7	ND–208	Gorman *et al.* (1984)
			11 (TWA, A)	28.5	2–121	
			22 (STEL)	320	ND–1256	

Table 1.6 (continued)

Country Time period	No. of plants	Job, task or industry	No. of samples[a]	Air concentration (mg/m³)		Reference
				Mean	Range	
Period NR	1	Taxidermy	2 (A) 2 (P)	8.9 8.9	1.1–16.6 1.7–16	Kronoveter & Boiano (1984)
Period NR	1	Degreasing	(TWA) (STEL)	205 1084	117–357 413–2000	Landrigan *et al.* (1987)
Period NR	1	Metal insignia; degreasing	1 (TWA) 1 (Peak)	[478] [1558]	NR	Rosa (2003)
1940–98	Many	Variety of industries	484	[37.6][c]	[0.0011–5911]	Hein *et al.* (2010)[d]
11 countries	6	Paper and pulp industry, maintenance work	10 (A)	[715]	[ND–5406]	Teschke *et al.* (1999)

[a] P, personal air samples (breathing zone); A, area samples
[b] Geometric mean
[c] Median
[d] The study by Hein *et al.* (2010) overlaps with several of the studies in the USA presented above. Most measurements were taken after observation of operating deficiencies of degreasers between 1952 and 1957.
ND, not detected; NR, not reported; STEL, short-term exposure limit; TWA, time-weighted average

Table 1.7 Exposure to trichloroethylene in the general population

Country	Subjects	No. of subjects	Age (years)	Concentration in blood (µg/L)		Concentration in air (µg/m³)	Reference
				Mean	Range		
Germany	No known occupational exposure	39	23–52	< 0.1[a]	< 0.1–1.3	NR	Hajimiragha *et al.* (1986)
USA	NHANES 1994–96	677	20–59	0.017	NR	NR	Wu & Schaum (2000)
USA	SHIELD study, 2000–01	134	6–10	0.007[b]	NR	NR	Sexton *et al.* (2005)
USA	NHANES 1999–2000	290	20–59	0.013	0.007–0.332	NR	Jia *et al.* (2012)
USA	1981–87, adults	NR	NR	NR	NR	NR	Rappaport & Kupper (2004)
	Bayonne	139	NR	NR	NR	2.2	
	Elizabeth	191	NR	NR	NR	3.5	
	Greensboro	24	NR	NR	NR	1.1	
	Devils Lake	23	NR	NR	NR	0.5	
	Los Angeles	176	NR	NR	NR	1.6	
USA	MNCPES study	72	3–12	NR	NR	0.8	Adgate *et al.* (2004)

[a] Median
[b] Geometric mean, estimated

approximately 300 mg/m³ in the early 1950s to about 10 mg/m³ in 1990 (Fig. 1.1).

Numbers of exposed workers and reported concentrations have declined considerably in Europe and North America. Recently reported concentrations from Asia appeared to be somewhat higher than current exposures in Europe and North America (Table 1.6).

Trichloroethylene has been measured in the blood of 157 metal workers in the USA, who had an average concentration of 2.5 µg/L (range, 0–22 µg/L) (Pfaffenberger *et al.*, 1984).

1.3.4 Exposure of the general population

Several studies have examined blood concentrations of trichloroethylene in the general population (Table 1.7). The number of individuals with measurable concentrations of trichloroethylene is generally low and has declined in more recent years.

In a study of 134 children in the USA who wore charcoal-based passive air samplers for 2 days before blood collection, no association was seen between personal exposure to trichloroethylene and concentration in the blood, although few subjects had measurable blood concentrations (Sexton *et al.*, 2005).

In the National Health and Nutrition Examination Survey (NHANES) 1999–2000 in the USA, blood samples were taken from 290 subjects (Jia *et al.*, 2012). The mean concentration of trichloroethylene was 0.013 µg/L and 88% of samples were below the limit of detection. Samples of exhaled air were also taken for 361 subjects; for these samples the mean concentration of trichloroethylene was 3.48 µg/m³, and 76% of samples were below the limit of detection. Concentrations in air and blood were moderately associated. In the most recent survey in 2005–06, all 3178 subjects had concentrations below the limit of detection. Exposure of the general population from air, water and food was several orders of magnitude lower than occupational exposure (see Table 1.6).

Table 1.8 Occupational exposure limits for trichloroethylene worldwide

Country or region	Concentration (mg/m³)	Interpretation
Australia	54	TWA
Austria	3.3	TWA
Belgium	55	TWA
Canada (Quebec)	269	TWA
Denmark	55	TWA
France	405	TWA
Germany, Committee on Hazardous Substances	60	–
Hungary	270	–
New Zealand	269	TWA
Singapore	269	TWA
Sweden	50	–
Switzerland	260	TWA
USA, Occupational Safety and Health Administration	537	TWA
USA, American Conference of Governmental Industrial Hygienists	269	TWA
United Kingdom	550	TWA

TWA, 8-hour time-weighted average
From GESTIS (2012)

1.4 Regulations and guidelines

Concern began to arise in the end of the 1970s about the potential environmental and health effects of trichlororoethylene (Birkenfeld *et al.*, 2005). In the USA, several regulations at the county, state and national levels were passed to limit emissions of trichloroethylene. In Europe, directives were instituted to restrict marketing and sales to end-users (76/769/EC). During the 1980s, several European countries, and the European Union, began passing regulations to protect workers from exposure to trichloroethylene.

The classification of trichloroethylene as a carcinogen carrying an R45 risk phrase ("may cause cancer") in the European Union in the 1990s resulted in replacement of trichloroethylene in many processes. The National Toxicology Program (NTP) of the USA first listed trichloroethylene in 2000 (NTP, 2000), and it is currently classified as "reasonably anticipated to be a human carcinogen" (NTP, 2011).

International time-weighted averages (TWAs) for trichloroethylene vary considerably (Table 1.8) from less than 100 mg/m³ in Australia and many European countries to more than 500 mg/m³ in the United Kingdom and the USA. Risk phrases and evaluations are presented in Table 1.9.

2. Cancer in Humans

2.1 Introduction

There is substantial epidemiological literature on cancer and trichloroethylene, consisting of both cohort and case–control studies, as well as ecological studies of environmental exposures. These study designs cover exposure by dermal and inhalation routes in a variety of settings and are complementary regarding strengths and weaknesses. The cohort design typically provides a narrower range of occupations for exposure assessment than the case–control and also allows focus on heavily exposed workers,

Table 1.9 Regulations and evaluations for trichloroethylene worldwide, as of October 2012

Country	Organization	Classification	Significance
Europe	GHS	H350	May cause cancer (1B)
	European classification	R 45	May cause cancer
Germany	TRGS 905	K3	Substances that possibly are carcinogenic for humans and thus give cause for concern
	MAK Commission	1	Substances that cause cancer and make a considerable contribution to the risk of cancer
USA	EPA	Group C	Possible human carcinogen with threshold
	ACGIH	A2	Suspected human carcinogen
	NTP		Reasonably anticipated to be carcinogen

ACGIH, American Conference of Governmental Industrial Hygienists; EPA, Environmental Protection Agency; GHS, Global Harmonization System; MAK, Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area; NTP, National Toxicology Program; TRGS, Technical Regulations on Hazardous Substances.

while the case–control design allows control for some important potential confounders that may be difficult to capture from cohort studies. Since information on smoking was typically not available for cohort studies, results were reported for cancer of the lung as an indicator for tobacco smoking in the cohort, not because this cancer was of primary interest in relation to exposure to trichloroethylene.

Many cancers have been evaluated, but there has been a focus on tumours of the kidney, liver, and non-Hodgkin lymphoma. Exposure estimates have been based on a range of techniques, including simple job-title determinations, quantitative assessments, and biological monitoring. Trichloroethylene has been used in several industries and occupations, especially where metal degreasing is performed. Cohort studies have been conducted on workers in the aerospace and aircraft-repair industries, as well as in other industries. In many workplaces where trichloroethylene is used, other chlorinated solvents can also be found. There was some overlap of exposures to tetrachloroethylene and trichloroethylene in the studies evaluated in this *Monograph*, but this was likely to be small because of the different patterns of use in different industries. Overlap in exposures complicated the interpretation of study findings, but it should be remembered that workers are exposed to multiple agents in nearly all workplaces. Trichloroethylene and tetrachloroethylene have largely been used over different periods in different industries, therefore the exposure overlap was not likely to be great. For this evaluation, the Working Group included only studies that specifically mentioned exposure to trichloroethylene. Other studies on exposure to non-specified chlorinated solvents were reviewed, but were considered uninformative with regard to trichloroethylene.

2.2 Cohort studies

See Table 2.1 for an overview, and Table 2.2 for a detailed description of the studies.

2.2.1 Workers in the dry-cleaning industry

Trichloroethylene has been used in in dry-cleaning as the main solvent (from the 1930s to the 1950s) and is still used as a spotting agent to remove stains. From the 1940s, it began to be replaced by tetrachloroethylene, which is less damaging to fabric (Ludwig, 1981; IARC, 1995; Doherty, 2000). Dry-cleaning workers active in this early period may have been exposed to trichloroethylene, as well as to carbon tetrachloride, tetrachloroethylene and

other petroleum-derived solvents. Since use of these chlorinated solvents overlapped, and since all continued to be used as spotting agents, the literature contained few reports of cohort studies of health effects and cancer outcomes among dry-cleaning workers exposed exclusively to trichloroethylene (IARC, 1995). However, the main dry-cleaning solvent used from the 1960s onwards was tetrachloroethylene. [See Section 1.2.2 of this *Monograph* for a more detailed description of exposure to trichloroethylene in the dry-cleaning industry.]

No large studies of dry-cleaning workers exposed specifically to trichloroethylene were available to the Working Group. In a small study of 65 male dry-cleaning workers exposed heavily to trichloroethylene in Prague, Czech Republic, starting in the 1950s, the foIlow-up period was 5–50 years, all had 1–35 years of exposure to trichloroethylene, and 86% (n = 57) were traced until 1979 (Malek *et al.*, 1979). Nearly 60% of those tested had a urinary concentration of trichloroacetic acid in excess of 100 mg/L, with sporadic values in the region of 1000 mg/L. In total, six men were diagnosed with cancer: three had cancer of the lung, one had cancer of the tongue, one had cancer of the rectum, and one had tumours of the bladder and rectum. None were diagnosed with cancer of the kidney or liver. [There were not enough data to estimate relative risks. The Working Group believed this study was too small to be informative for the evaluation of trichloroethylene.]

[The Working Group did not consider that any other cohort studies on dry-cleaning workers were directly relevant to the evaluation of trichloroethylene, given the limited exposure to trichloroethylene and extensive exposure to tetrachloroethylene (see the *Monograph* on Tetrachloroethylene, Section 2.1, for a further discussion of cohort studies of dry-cleaning workers).]

2.2.2 Workers in the aircraft and aerospace industries

See Table 2.2

Several cohort studies of aircraft and aerospace workers potentially exposed to trichloroethylene in the USA have been published, almost all with cancer mortality as the outcome. Only two of these studies (Garabrant *et al.*, 1988; Spirtas *et al.*, 1991) were included in the previous evaluation (IARC, 1995). For some of these cohorts, including Spirtas *et al.* (1991), multiple follow-ups were published for a single study. In such situations, the Working Group evaluated only results from the most recent study, unless otherwise described. Since information on smoking was typically not available for cohort studies, results were reported for cancer of the lung as an indicator for tobacco smoking in the cohort, but not because lung cancer was of primary interest in relation to exposure to trichloroethylene.

Garabrant *et al.* conducted a retrospective cohort study of 2169 women and 11 898 men employed for at least 4 years at an aircraft-manufacturing company in San Diego county, USA, between 1958 and 1982 (Garabrant *et al.*, 1988). The cohort was established from company records. No information on individual exposures was available. Based on a small sample of 14 deaths and 56 controls with a total of 362 jobs held within the company, the estimated prevalence of exposure to trichloroethylene among the cohort was 37%. [It was unclear whether this was an opportunistic sample.] The cause of death was retrieved from the death certificate or from the California state death tapes, and coded by a trained nosologist. The national age-, sex-, race-, calendar-year-, and cause-specific mortality rates for the USA were used to calculate expected numbers of specific causes of death. Similar rates for San Diego County were also applied. In total, 12.8% of cohort members had died by the end of follow-up, and 95.3% of death certificates were obtained. The study included

Table 2.1 Selected results (estimated relative risks) of cohort studies on the association between cancer and exposure to trichloroethylene

Reference, study location	Cancer							
	Kidney	Non-Hodgkin lymphoma	Leukaemia	Combined haematological	Liver/biliary	Lung	Bladder	Cervix
Europe								
Axelson *et al.* (1994) Sweden	1.16 (0.42–2.52)	1.56 (0.51–3.64)	NR	NR	1.41 (0.38–3.60)	0.69 (0.31–1.30)	1.02 (0.44–2.00)	NR
Anttila *et al.* (1995a) Finland	0.87 (0.32–1.89)	1.81 (0.78–3.56)	1.08 (0.35–2.53)	1.51 (0.92–2.33)	2.27 (0.74–5.29)	0.92 (0.59–1.35)	0.82 (0.27–1.90)	2.42 (1.05–4.77)
Henschler *et al.* (1995) Germany	7.97 (2.59–18.59)	NR	NR	[1.10 (0.01–77.3)]	NR	NR	NR	NR
Hansen *et al.* (2001) Denmark	0.9 (0.2–2.6)	3.5 (1.5–6.9)	1.9 (0.6–4.4)	NR	2.6 (0.8–6.0)	0.8 (0.5–1.3)	1.1 (0.5–2.0)	3.8 (1.0–9.8)
Raaschou-Nielsen *et al.* (2001) Denmark	[1.21 (0.95–1.52)]	[1.26 (1.02–1.53)]	[1.15 (0.91–1.42)]	NR	[1.35 (1.02–1.75)]	[1.43 (1.32–1.55)]	[1.06 (0.92–1.21)]	1.9 (1.42–2.37)
USA								
Garabrant *et al.* (1988) San Diego, USA	0.93 (0.48–1.64)	NR	0.82 (0.47–1.34)	0.78 (0.56–1.08)	0.94 (0.40–1.86)	0.80 (0.68–0.95)	1.26 (0.74–2.03)	NR
Greenland *et al.* (1994) Massachusetts, USA	0.99 (0.30–3.32)	0.76 (0.24–2.42)	1.10 (0.46–2.66)	NR	0.54 (0.11–2.63)	1.01 (0.69–1.47)	0.85 (0.32–2.23)	NR
Morgan *et al.* (1998) Arizona, USA	1.32 (0.57–2.60)	NR	1.05 (0.50–1.93)	0.99 (0.64–1.47)	0.98 (0.36–2.13)	1.10 (0.89–1.34)	1.36 (0.59–2.68)	0
Ritz (1999) Ohio, USA	0.65 (0.21–1.51)	NR	1.09 (0.56–1.91)	1.28 (0.90–1.77)	1.66 (0.71–3.26)	1.03 (0.85–1.24)	1.17 (0.50–2.31)	NR
Zhao *et al.* (2005)[c] California, USA	7.40 (0.47–116)[a]	0.20 (0.03–1.46)[b]	0.20 (0.03–1.46)[b]	5.15 (1.20–22.2)[a]	NR	3.10 (1.09–8.79)[a]	3.68 (0.87–15.5)[a]	NR

Table 2.1 (continued)

Reference, study location	Cancer							
	Kidney	Non-Hodgkin lymphoma	Leukaemia	Combined haematological	Liver/biliary	Lung	Bladder	Cervix
Boice *et al.* (2006)[c] California, USA	2.22 (0.89–4.57)	0.21 (0.01–1.18)	1.08 (0.35–2.53)	0.74 (0.34–1.40)	1.28 (0.35–3.27)	1.24 (0.92–1.63)	1.66 (0.54–3.87)	NR
Lipworth *et al.* (2011) California, USA	0.66 (0.38–1.07)	1.31 (0.97–1.73)	0.88 (0.61–1.23)	NR	0.89 (0.57–1.33)	0.80 (0.71–0.90)	1.03 (0.72–1.43)	0
Radican *et al.* (2008) Utah, USA	1.18 (0.47–2.94)	1.36 (0.77–2.39)	0.64 (0.35–1.18)	1.06 (0.75–1.51)	1.25 (0.31–4.97)	0.83 (0.63–1.08)	0.80 (0.41–1.58)	1.67 (0.54–5.22)
Bahr *et al.* (2011) Kentucky, USA	0 cases	1.49 (1.02–2.10)	1.40 (0.46–4.24)	NR	0.43 (0.10–1.84)	0.75 (0.72–0.79)	NR	NR

[a] High exposure; 20-year lag; cancer incidence
[b] Non-Hodgkin lymphoma plus leukaemia combined
[c] Study populations overlap
NR, not reported

Table 2.2 Cohort studies of workers exposed to trichloroethylene

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Shindell & Ulrich (1985) Illinois, USA 1957–83	2646 employees	Not specific. Workers from a factory using trichloroethylene as a degreasing agent	All cancers		21	[0.85 (0.53–1.30)]	Age and calendar time Only SMR for overall cancer
			Respiratory cancer		9	[0.75 (0.34–1.42)]	
			Non-respiratory cancer		12	[0.49 (0.26–0.86)]	
Garabrant *et al.* (1988) San Diego, USA 1958–82	14 067 (11 898 men, 2169 women)	Not specific. Follow-up of aircraft-manufacturing workers employed ≥ 4 yr.	All causes mortality	Overall	1804	0.75 (0.72–0.79)	Age, sex, race, calendar year No information on individual exposure to trichloroethylene; 37% of jobs involved exposure to trichloroethylene. Expected numbers based on USA rates. Trend towards increasing mortality from cancer of the oesophagus, pancreas, and bladder with increasing duration of employment.
			All cancer deaths	Overall	453	0.84 (0.77–0.93)	
			Oesophagus	Overall	14	1.14 (0.62–1.92)	
			Liver and biliary tract	Overall	8	0.94 (0.40–1.86)	
			Breast	Overall	16	0.91 (0.52–1.48)	
			Cervix	Overall	ND	–	
			Kidney	Overall	12	0.93 (0.48–1.64)	
			Haematological	Overall	38	0.78 (0.56–1.08)	
			NHL	Overall	ND	–	
			Leukaemia	Overall	16	0.82 (0.47–1.34)	
			Lung	Overall	138	0.80 (0.68–0.95)	
			Bladder & urinary organs	Overall	17	1.26 (0.74–2.03)	
Axelson *et al.* (1994) Sweden 1958–87	1670 (1421 men, 249 women aged ≤ 79 yr)	Individual urinary measurement of TCA (trichloroethylene metabolite)	All cancers	Men	107	0.96 (0.80–1.16)	Age, calendar time SIR
			All cancers	Women	22	1.32 (0.85–1.99)	
			Liver	Men	4	1.41 (0.38–3.60)	
			Kidney	Men	6	1.16 (0.42–2.52)	
			NHL	Men	5	1.56 (0.51–3.64)	
			Lung	Men	9	0.69 (0.31–1.30)	
			Bladder	Men	8	1.02 (0.44–2.00)	

Table 2.2 (continued)

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Greenland *et al.* (1994) Massachusetts, USA 1969–84	512 cases, 1202 controls	JEM	Kidney		ND	0.99 (0.30–3.32)	Nested case–control study. White men only.
			Liver/biliary tract		ND	0.54 (0.11–2.63)	
			Lymphoma		ND	0.76 (0.24–2.42)	
			Leukaemia		ND	1.10 (0.46–2.66)	
			Lung		ND	1.01 (0.69–1.47)	
			Bladder		ND	0.85 (0.32–2.23)	
Anttila *et al.* (1995a) Finland 1967–92	1698 men, 1391 women	Individual measurement of urinary concentration of TCA (trichloroethylene metabolite)	All cancers	Both sexes	208	1.05 (0.92–1.20)	Age, calendar time SIR
				Years since first measurement:			
				0–9 yr	65	1.07 (0.82–1.36)	
				10–19 yr	83	0.84 (0.67–1.04)	
				≥ 20 yr	60	1.57 (1.20–2.02)	
				< 100 µmol/L	127	1.17 (0.98–1.38)	
				≥ 100 µmol/L	63	0.97 (0.74–1.23)	
			Liver	Both sexes	5	2.27 (0.74–5.29)	
				Years since first measurement:	0		
				0–9 yr	0		
				10–19 yr	2	1.74 (0.21–6.29)	
				≥ 20 yr	3	6.07 (1.25–17.7)	
				< 100 µmol/L	2	1.64 (0.20–5.92)	
				≥ 100 µmol/L	2	2.74 (0.33–9.88)	
			Cervix	Women	8	2.42 (1.05–4.77)	
				Years since first measurement:			
				0–9 yr	6	3.39 (1.24–7.38)	
				10–19 yr	1	0.84 (0.02–4.67)	
				≥ 20 yr	1	2.89 (0.07–16.1)	
				< 100 µmol/L	3	1.86 (0.38–5.45)	
				≥ 100 µmol/L	5	4.35 (1.41–10.1)	

Table 2.2 (continued)

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Anttila *et al.* (1995a) Finland 1967–92 (cont.)			Kidney	Both sexes	6	0.87 (0.32–1.89)	
				Years since first measurement:			
				0–9 yr	1	0.53 (0.01–2.95)	
				10–19 yr	5	1.39 (0.45–3.24)	
				≥ 20 yr	0		
			Haematological	Both sexes	20	1.51 (0.92–2.33)	
			NHL	Both sexes	8	1.81 (0.78–3.56)	
				Years since first measurement:			
				0–9 yr	1	0.83 (0.02–4.64)	
				10–19 yr	4	1.75 (0.48–4.47)	
				≥ 20 yr	3	3.24 (0.67–9.45)	
				< 100 µmol/L	5	2.01 (0.65–4.69)	
				≥ 100 µmol/L	2	1.40 (0.17–5.04)	
			Leukaemia	Both sexes	5	1.08 (0.35–2.53)	
				Years since first measurement:			
				0–9 yr	3	1.76 (0.36–5.16)	
				10–19 yr	0		
				≥ 20 yr	2	2.72 (0.33–9.83)	
				< 100 µmol/L	1	0.39 (0.01–2.19)	
				≥ 100 µmol/L	4	2.65 (0.72–6.78)	
			Lung		25	0.92 (0.59–1.35)	

Table 2.2 (continued)

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Henschler *et al.*(1995) 1956–92	169	Exposure to trichloroethylene during cleaning and degreasing at a cardboard factory. Exposure in the cardboard-machine area is considered high. Exposure was lower in the locksmiths' area and in the electrical workshop. Trichloroethylene was also regularly used to clean floors, work-clothes and hands.	All cancers		15	[0.87 (0.40–1.92)]	Adjusted for age. Further, age and calendar time rates from Denmark and East Germany were applied Probably cluster data Only data for kidney and haematological cancers
			Kidney		5	7.97 (2.59–18.59)	
			Haematological		1	[1.10 (0.01–77.3)]	
Morgan *et al.* (1998) Arizona, USA 1950–93	2555 men and 2178 women exposed to trichloroethylene	Company employees with ≥ 30 yr of experience rated exposure for each job, and a company industrial hygienist compiled ratings into a four-level JEM.	All cancers	Overall	270	0.92 (0.81–1.03)	Age, sex, calendar year SMR. Expected numbers based on national rates
				Low exposure	114	1.04 (0.86–1.25)	
				High exposure	156	0.84 (0.71–0.98)	
			Liver and biliary tract	Overall	6	0.98 (0.36–2.13)	
				Low exposure	1	0.49 (0.01–2.74)	
				High exposure	5	1.38 (0.45–3.21)	
			Liver		ND		
			Biliary tract		ND		
			Breast	Overall	16	0.75 (0.43–1.22)	
				Low exposure	11	1.03 (0.51–1.84)	
				High exposure	5	0.47 (0.015–1.11)	
			Cervix		0	0	
			Kidney	Overall	8	1.32 (0.57–2.60)	
				Low exposure	1	0.47 (0.01–2.62)	

Table 2.2 (continued)

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Morgan *et al.* (1998) Arizona, USA 1950–93 (cont.)				High exposure	7	1.78 (0.72–3.66)	
			Haematological	Overall	25	0.99 (0.64–1.47)	
				Low exposure	10	1.07 (0.51–1.96)	
				High exposure	15	0.95 (0.53–1.57)	
			Non-Hodgkin lymphoma		ND	–	
			Leukaemia	Overall	10	1.05 (0.50–1.93)	
				Low exposure	3	0.85 (0.17–2.47)	
				High exposure	7	1.17 (0.47–2.41)	
			Lung		97	1.10 (0.89–1.34)	
			Emphysema		15	1.36 (0.76–2.23)	
Ritz (1999) 1951–89 Ohio, USA	3814 white men	Job title and plant areas based historical exposure to chemical assessed by plant experts. Workers were classified into four categories of exposure, from none to heavy.	All cancers		328	1.10 (0.99–1.23)	Age, time since first hire, pay type, radiation dose Initiated to look at radiation effects.
			Liver		8	1.66 (0.71–3.26)	
				Light, > 5 yr	3	1.90 (0.35–10.3)	
				Medium, > 5 yr	1	8.82 (0.79–98.6)	
			Kidney		5	0.65 (0.21–1.51)	
			Haematological		37	1.28 (0.90–1.77)	
				Light, > 5 yr	15	1.85 (0.87–3.95)	
			NHL		ND	–	
			Leukaemia and aleukaemia		12	1.09 (0.56–1.91)	
			Lung		112	1.03 (0.85–1.24)	
			Emphysema		3	0.21 (0.04–0.61)	

Table 2.2 (continued)

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Hansen *et al.* (2001) Denmark 1968–96	658 men, 145 women	Individual measurement of urinary trichloroethylene metabolite	All cancers	Men	109	1.0 (0.9–1.3)	Age and calendar time SIR. For cervical cancer, an association was seen with earlier period of first employment. Overlapped with Raaschou-Nielsen *et al.* (2001, 2002).
			Oesophagus	Men	6	4.2 (1.5–9.2)	
			Liver & biliary tract	Men	5	2.6 (0.8–6.0)	
			Breast	Women	4	0.9 (0.2–2.3)	
			Cervix	Women	4	3.8 (1.0–9.8)	
			Kidney	Both sexes	[4]	[1.1 (0.3–2.8)]	
			NHL	Men	8	3.5 (1.5–6.9)	
			Leukaemia	Men	5	1.9 (0.6–4.4)	
			Lung	Men	16	0.8 (0.5–1.3)	
Zhao *et al.* (2005) California, USA 1950–2001	5049 for cancer incidence; 6044 men for total mortality	A JEM for potentially carcinogenic exposures, including job titles and periods was constructed based on extensive industrial-hygiene reviews	All cancers		ND	–	Considerable overlap with the study by Boice et al. (2006). RR for cancer incidence reported Time since first employment, SES, age at event
			Liver		ND	–	
			Kidney	Low	6	1.0 (ref)	
				Medium	6	1.87 (0.56–6.20)	
				High	4	4.90 (1.23–19.6)	
			P for trend			0.023	
			NHL and leukaemia	Low	28	1.0 (ref)	
				Medium	16	0.88 (0.47–1.65)	
				High	1	0.20 (0.03–1.46)	
			P for trend			0.097	
			Bladder	Low	20	1.0 (ref)	
				Medium	19	1.54 (0.81–2.92)	
				High	11	1.98 (0.93–4.22)	
			P for trend			0.069	
			Lung	High (mortality)	33	1.02 (0.68–1.53)	
				High (incidence)	14	1.11 (0.60–2.06)	

Table 2.2 (continued)

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Boice *et al.* (2006) California, USA 1948–99	8372 workers	Individual job titles were collapsed into categories and designated as administrative/ scientific or non-administrative. Test-stand mechanics and technicians were considered as having the greatest likelihood of exposure to trichloroethylene.	All cancers	Overall	121	1.00 (0.83–1.19)	SMR. Overlapped with Zhao et al. (2005). Use of trichloroethylene was discontinued in cleaning of (i) engines (middle to late 1960s); and (ii) small metal parts (1974). SMRs for < 5 yr or > 5 yr employment were above 1, but not statistically significant for kidney cancer.
			Oesophagus	Overall	3	0.88 (0.18–2.58)	
			Liver & biliary tract	Overall	4	1.28 (0.35–3.27)	
			Kidney	Overall	7	2.22 (0.89–4.57)	
			Haematological	Overall	9	0.74 (0.34–1.40)	
			NHL	Overall	1	0.21 (0.01–1.18)	
			Leukaemia	Overall	5	1.08 (0.35–2.53)	
			Lung	Overall	51	1.24 (0.92–1.63)	
			Emphysema	Overall	5	0.90 (0.29–2.11)	

Table 2.2 (continued)

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Radican *et al.* (2008) Utah, USA 1952–2000	14 455	Individual exposure to 21 solvents (including trichloroethylene) and chemicals was assessed based on different exposure surrogates (historical records, walk-through surveys and interviews of long-term employees). For trichloroethylene only, frequency and exposure patterns (intermittent and continuous) were assessed based on tasks and a cumulative exposure score was calculated	All cancer deaths	Overall	854	1.03 (0.91–1.17)	Age, race, sex HR reported. No significantly increased risks. Highest category of exposure generally had the highest risk.
			Emphysema		59	0.90 (0.56–1.44)	
			Lung		166	0.83 (0.63–1.08)	
			Oesophagus	Overall	17	1.88 (0.61–5.79)	
			Liver/biliary tract	Overall	31	1.12 (0.57–2.19)	
			Liver	Overall	8	1.25 (0.31–4.97)	
				Lowest tertile, men	4	3.28 (0.37–29.45)	
				Highest tertile, men	4	4.05 (0.45–36.41)	
			Breast	Overall	26	1.23 (0.73–2.06)	
			Cervix	Overall	6	1.67 (0.54–5.22)	
			Kidney	Overall	18	1.18 (0.47–2.94)	
				Lowest tertile, men	10	1.87 (0.59–5.97)	
				Second tertile, men	1	0.31 (0.03–2.75)	
				Highest tertile, men	5	1.16 (0.31–4.32)	
			Haematological	Overall	106	1.06 (0.75–1.51)	
				Lowest tertile, men	34	1.04 (0.63–1.74)	
				Second tertile, men	21	1.06 (0.59–1.88)	
				Highest tertile, men	33	1.25 (0.75–2.09)	
			NHL	Overall	46	1.36 (0.77–2.39)	
				Lowest tertile, men	18	1.83 (0.79–4.21)	
				Second tertile, men	7	1.17 (0.42–3.24)	
				Highest tertile, men	12	1.50 (0.61–3.69)	

Table 2.2 (continued)

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Radican *et al.* (2008) Utah, USA 1952–2000 (cont.)			Leukaemia	Overall	27	0.64 (0.35–1.18)	
			Bladder	Overall	25	0.80 (0.41–1.58)	
Bahr *et al.* (2011) 1952–2003	6820 male workers	JEM based on discussions with current and past employees at the plant. Each job was ranked by likelihood of short-term exposure to trichloroethylene in four categories.	All cancer	Overall	146	1.08 (0.79–1.48)	Age and calendar time
			Oesophagus		ND	–	
			Liver and biliary tract	Overall	ND	0.43 (0.10–1.84)	
				Category 2	ND	0.34 (0.05–2.07)	
				Category 3	ND	0.39 (0.08–1.94)	
			Kidney		0	–	Surprisingly low number. Approximately same number as for NHL expected
			NHL	Overall	32	1.49 (1.02–2.10)	
				Category 2	ND	1.31 (0.47–3.65)	
				Category 3	ND	0.75 (0.27–2.12)	
			Leukaemia	Overall	24	1.40 (0.46–4.24)	
				Category 2	ND	0.73 (0.15–3.45)	
				Category 3	ND	1.89 (0.61–5.86)	
			Lung		146	0.75 (0.72–0.79)	

Table 2.2 (continued)

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Lipworth *et al.* (2011) California, USA 1960–2008	5443 workers	Job history from company records; 784 job codes were categorized into eight groups with similar factory-work activities, assessed by walk-through survey. On this basis, individuals were classified for exposure to trichloroethylene, tetrachloroethylene, mixed solvents and chromates (routine, intermittent, no likely exposure).	All cancers	Overall	986	0.92 (0.86–0.97)	Common exposure to other chemicals, including tetrachloroethylene Overlapped with Boice et al. (1999). Trend in increasing risk with increasing duration of exposure, this was statistically significant only for lung cancer ($P < 0.01$). Trichloroethylene was the primary organic solvent used for vapour degreasing until 1966, when it was replaced by tetrachloroethylene.
			Oesophagus	Overall	19	0.65 (0.39–1.01)	
				0	30	1.00 (ref)	
				< 1	7	0.53 (0.22–1.24)	
				1–4 yr	5	0.62 (0.23–1.63)	
				≥ 5 yr	7	0.77 (0.32–1.86)	
			Liver and biliary tract		24	0.89 (0.57–1.33)	
			Liver	Overall	ND	–	
				0	32	1.00 (ref)	
				< 1	10	0.67 (0.32–1.42)	
				1–4 yr	6	0.69 (0.28–1.71)	
				≥ 5 yr	8	0.83 (0.36–1.91)	
			Biliary tract	Overall	ND	–	
			Breast	Overall	12	1.03 (0.53–1.80)	
				0	61	1.00 (ref)	
				< 1	6	0.82 (0.34–1.98)	
				1–4 yr	1	0.31 (0.04–2.32)	
				≥ 5 yr	4	1.47 (0.50–4.32)	
			Cervix	Overall	0	[1.3 expected]	
			Kidney	Overall	16	0.66 (0.38–1.07)	
				0	33	1.00 (ref)	
				< 1	6	0.52 (0.21–1.30)	
				1–4 yr	3	0.42 (0.13–1.42)	
				≥ 5 yr	6	0.85 (0.33–2.19)	

Table 2.2 (continued)

Reference, location, follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Lipworth *et al.* (2011) California, USA 1960–2008 (cont.)			Haematological	Overall			
			NHL	Overall	50	1.31 (0.97–1.73)	
				0	50	1.00 (ref)	
				< 1	18	0.84 (0.48–1.47)	
				1–4 yr	14	1.10 (0.59–2.04)	
				≥ 5 yr	15	1.02 (0.55–1.90)	
			Leukaemia	Overall	23	0.88 (0.61–1.23)	
			Lung		267	0.80 (0.71–0.90)	

DCA, dichloroacetic acid; HR, hazard ratio; JEM, job-exposure matrix; ND, no data; NHL, non-Hodgkin lymphoma; ref., reference; RR, relative risk; SIR, standardized incidence ratio; SMR, standardized mortality ratio; TCA, trichloroacetate; yr, year

222 100 person-years of follow-up and 453 cancer deaths. The calculated standardized mortality ratio (SMR) for all causes and cancer overall was significantly decreased. No significant increase in standardized mortality ratio was seen for any cancer. The standardized mortality ratio for cancer of the lung was 0.80 (95% CI, 0.68–0.95) and there were significant deficits for cancers of the stomach and larynx. Deficits also appeared from diabetes mellitus, alcoholism, diseases of the nervous system, the circulatory system and the genitourinary system, as well as accidental and violent death. [The deficits indicated a strong healthy-worker effect and/or a strong selection of healthy workers for follow-up. The Working Group also noted that a relatively small proportion of workers was exposed to trichloroethylene in this study and therefore this study had a low impact on evaluate human carcinogenicity after exposure to trichloroethylene.]

Radican *et al*. extended follow-up of a cohort of civilian aircraft-maintenance workers from the Hill Air Force Base in Utah, USA, until 2000 (Spirtas *et al*., 1991; Blair *et al*., 1998; Radican *et al*., 2008). The cohort was composed of 14 444 people (10 730 men and 3725 women) employed for at least 1 year between 1952 and 1956 and identified from personnel records from the facility. Many different chemical exposures, including to other potential carcinogens, occurred at the facility. The methods for assessment of exposure within this cohort have been described previously (Stewart *et al*., 1991). Personal and area samples were available for some chemicals, including trichloroethylene (Stewart *et al*., 1991). Individual exposures for trichloroethylene, other solvents and chemicals were estimated based on job history, historical records, walk-through surveys and interviews of long-term employees and traditional monitoring results. Dichotomous exposure assessment was done for 21 solvents and chemicals. For trichloroethylene only, frequency and exposure patterns (intermittent and continuous) were assessed based on information on job tasks. Trichloroethylene levels in the degreasers was used until 1968 when trichloroethylene was replaced by 1,1,1-trichloroethane (Stewart *et al*., 1991). The National Death Index was used to assess the cause of death on the most recent follow-up. As of 31 December 2000, 8580 cohort members (68.1%) had died. The Cox model hazard ratio (HR) for all cancers was 1.03 (95% CI, 0.91–1.17; 854 deaths). No significantly increased hazard ratio appeared for any specific cancer in either men or women. Among the men potentially exposed to trichloroethylene compared with unexposed workers, the hazard ratios were 1.24 (95% CI, 0.41–3.71) for cancer of the kidney, 1.36 (95% CI, 0.59–3.11) for cancer of the biliary passage and liver, 1.12 (95% CI, 0.72–1.73) for lymphatic or haematopoietic cancer, and 1.66 (95% CI, 0.48–5.74) for cancer of the oesophagus. In women, the hazard ratios were 1.23 (95% CI, 0.73–2.06) for cancer of the breast and 1.67 (95% CI, 0.54–5.22) for cancer of the cervix. All the dose–response gradients for estimated exposure to trichloroethylene were relatively flat and based on small numbers. The hazard ratio for cancer of the lung was 0.83 (95% CI, 0.63–1.08). [The major strength of the study was the unusually long follow-up time, accounting for deaths of more than 68% of cohort members, and qualitative information on level of exposure to trichloroethylene].

Morgan *et al*. (1998) conducted a cohort study of mortality in 20 508 workers at Hughes Aircraft Manufacture, Arizona (Morgan *et al*., 1998) of whom 4733 (2555 men, 2178 women) were potentially exposed to trichloroethylene (1952–57). Study participants were identified from company records, and were eligible for inclusion in the study if they had been employed for at least 6 months between 1950 and 1985. Vital status was determined through the Social Security Administration and the National Death Index. Exposure to trichloroethylene was assessed by a four-level job-exposure matrix. In total, 4052 deaths occurred based on 105 852

person-years during 1950–93. The standardized mortality ratios for overall cancer were less than 1 for the entire cohort (SMR, 0.87; 95% CI, 0.82–0.92) and for the trichloroethylene-exposed subcohort (23% of workers) (SMR, 0.92; 95% CI, 0.81–1.03). The standardized mortality ratios were not significantly increased for any of the following cancers: kidney (SMR, 1.32; 95% CI, 0.57–2.6), lymphatic and haematological tissue (SMR, 0.99; 95% CI, 0.64–1.47), biliary passage and liver (SMR, 0.98; 95% CI, 0.36–2.13); breast (SMR, 0.75; 95% CI, 0.43–1.22), and lung (SMR, 1.10; 95% CI, 0.89–1.34). In an internal cohort analysis, the relative risk for cancer of the kidney was 1.89 (95% CI, 0.85–4.23; eight cancer deaths) for those workers with medium/high peak exposure versus those with none/low exposure. The internal comparison group minimizes concerns over the healthy-worker effect when an external comparison group is used. [The long-term follow-up was a strength of the study].

Zhao *et al.* reported a follow-up for mortality of 6044 selected male Rocketdyne workers from the aerospace division of the Santa Susana Field Laboratory in California, USA (Zhao *et al.*, 2005). The study was based on a previously established cohort in a study that focused on exposure to hydrazine and mortality (Ritz *et al.*, 1999). The source population was 55 000 Rockwell/Rocketdyne workers (now Boeing North America) at several facilities in the Los Angeles area, California, USA (Zhao *et al.*, 2005). Workers had been employed at least 2 years before 1980, and had never been monitored for exposure to radiation, indicating that they had never been employed at the nuclear facility. All employee records were matched with multiple sources to obtain information on vital status, and date of death. Underlying cause of death was coded by a licensed nosologist. Standardized mortality ratios for death during 1950–2001 were calculated. A subcohort of 5049 of these workers was followed up for cancer incidence. The cohort was matched against the Californian cancer registry and first primary incident cancer in 1988–2000 was identified. Cancer data were also retrieved from eight other state cancer registries to identify incident cancers for some of the cohort members who had left California during follow-up. A job-exposure matrix for potentially carcinogenic exposures, using job titles, was constructed based on extensive industrial hygiene reviews. Individual personnel records for all cohort members included job title and period engaged in each job. Each job was assigned to a category of cumulative exposure: none, low, medium, or high. Information on tobacco smoking was obtained from a small subset of 200 study participants. Proportional hazard modelling was used to estimate site-specific relative risks for cancer incidence and mortality. In the entire mortality cohort, 2117 (35%) had died by the end of 2001, and 600 people had died from cancer. Estimated medium or high cumulative exposure to trichloroethylene was positively associated with incidence of cancer of the kidney compared with low cumulative exposure (medium exposure: HR, 1.87; 95% CI, 0.56–6.20; high exposure: HR, 4.90; 95% CI, 1.23–19.6; *P* for trend, 0.023). Results were similar when further adjusted for all other carcinogens, including hydrazine (medium exposure: HR, 1.26; 95% CI, 0.26–1.14; high exposure: HR, 7.61; 95% CI, 0.65–9.14). For mortality from cancer of the kidney, the estimated relative risks were lower (medium exposure: HR, 1.43; 95% CI, 0.49–4.16; high exposure: HR, 2.03; 95% CI, 0.50–8.32). No significant associations were observed between estimated exposure to trichloroethylene and any other cancers. The relative risk of cancer of the lung was around 1, with the rate ratio for mortality being 1.02 (95% CI, 0.68–1.53) and for incidence, 1.11 (95% CI, 0.60–2.06). It was estimated that 40.7% of the trichloroethylene-exposed workers were smokers. [The Working Group noted that confounding by smoking was unlikely. The major strength of the study was the follow-up for mortality of more than 50 years,

and follow-up for cancer incidence since 1988. There was considerable overlap between this study and that of Boice *et al.* (2006), described below.]

The previous study by (Zhao *et al.*, 2005) overlapped considerably with a study of 8372 Rocketdyne workers (7083 men and 1289 women) employed for at least half a year in 1948–99; however, no information on cancer incidence was reported (Boice *et al.*, 2006). Study subjects were retrospectively identified from different overlapping sources, and information included name, date of birth, date of first employment, and termination, and job history. Information on death and vital status was retrieved from multiple sources. The cause of death was coded by a trained nosologist. The overall standard mortality ratio for any cancer in the trichloroethylene-exposed group was 1.00 (95% CI, 0.83–1.19). Similarily, the standard mortality ratio was 2.22 (95% CI, 0.89–4.57) for cancer of the kidney, 0.21 (95% CI, 0.01–1.18) for non-Hodgkin lymphoma, 1.28 (95% CI, 0.35–3.27) for cancer of the liver and biliary passage, and 1.24 (95% CI, 0.92–1.63) for cancer of the lung. The relative risk of kidney cancer associated with any potential exposure to trichloroethylene compared with never-exposed by duration of work as test-stand mechanic (considered to be the job with most intensive exposure) by internal comparison was 1.21 (95% CI, 0.33–4.35) for 0 test-years, 2.51 (95% CI, 0.27–23.5) for < 4 test-years and 3.13 (95% CI, 0.74–13.2) for ≥ 4 test-years.

Cancer mortality has been evaluated at the Lockeed Martin aircraft-manufacturing facility in Burbank, California, USA (Boice *et al.*, 1999; Lipworth *et al.*, 2011). In total, 77 943 workers employed on or after 1 January 1960 for at least 1 year were identified. Of these, 32 625 workers (41.9%) were employed in non-factory positions and had no chemical exposure. Each factory worker was classified as having routine, intermittent or no likely exposure to trichloroethylene, tetrachloroethylene, mixed solvents, or chromates, based on a procedure to estimate historical exposure (Marano *et al.*, 2000). Trichloroethylene was the primary organic solvent used for vapour degreasing until 1966, when it was replaced by tetrachloroethylene. [The proportion of workers involved in vapour degreasing was not available from the paper]. Deaths occurring between 1960 and 2008 (n = 34 298; 44%) were identified by several different methods, and coded by a trained nosologist. Expected numbers of deaths were based on race, age by calendar year, and sex-specific rates in the general population of California for white workers, and from the general population of the USA for non-white workers. In addition to calculation of standardized mortality ratios, relative risks of death from cancer were estimated for internal comparisons among factory workers' duration of potential exposure (0, < 1, 1–4, > 5 years) to trichloroethylene, tetrachloroethylene, mixed solvents, or chromates. The reference group for internal comparison consisted of 9520 factory workers considered to have no exposure to organic solvents or chromates. For factory workers potentially exposed to trichloroethylene, the standardized mortality ratio for overall cancer was 0.92 (95% CI, 0.86–0.97) based on 1 435 459 person-years. No significant increase was observed for any cancer. The standardized mortality ratio for cancer of bronchus, trachea and lung was significantly decreased (0.80; 95% CI, 0.71–0.90). The standardized mortality ratios for other cancers were: 0.66 (95% CI, 0.38–1.07) for kidney, 1.31 (95% CI, 0.97–1.73) for non-Hodgkin lymphoma, 0.89 (95% CI, 0.57–1.33) for biliary passage and liver, 0.65 (95% CI, 0.39–1.01) for oesophagus, 1.03 (95% CI, 0.53–1.80) for breast, and 0.00 (95% CI, 0.00–2.87) [1.3 expected] for cervix uteri,. No indication of increasing relative risk with increasing duration of potential exposure to trichloroethylene was seen in the internal cohort comparisons (P for trend, > 0.05), although the highest risk was seen in the highest exposure category for some cancer sites, including the kidney, liver, oesophagus

and prostate. [Overall, the results showed some weak but statistically significant decreases in standardized mortality ratios for cerebrovascular disease, nonmalignant respiratory disease, cirrhosis of the liver, all external causes of death, accidents, and suicides, indicating an healthy-worker effect. The indications of healthy-worker effect, and hence potential selection bias, limited the utility of this cohort. Furthermore, despite the long-term follow-up from a large cohort, the number of cases for most cancer diagnoses was relatively small. Finally, the period of exposure to trichloroethylene for vapour degreasers may have been relatively short for an unknown proportion of workers because exposure stopped in 1966].

2.2.3 Biological monitoring of trichloroacetic acid

In Sweden, Finland, and Denmark, routine urinary measurements of the main metabolite of trichloroethylene, trichloroacetic acid, have been conducted independently for several decades, and workers with such measurements have been linked to nationwide cancer registries (Axelson *et al.*, 1994; Anttila *et al.*, 1995a; Hansen *et al.*, 2001).

The study from Sweden is based on historical laboratory files from workers enrolled in a monitoring programme for trichloroacetic acid, offered to costume companies by the Swedish producer of trichloroethylene (Axelson *et al.*, 1994). The study was an update and expansion of a previous study (Axelson *et al.*, 1978). In total, 1727 exposed workers from 115 different companies, which had used the surveillance service at least once between 1955 and 1975, were identified from the laboratory files (Axelson *et al.*, 1978). Some files from the early period were not available. After exclusion of subjects who could not be unequivocally identified or who had emigrated, the final cohort included 1421 men and 249 women. The majority (81%) of the male cohort members had a urinary concentration of trichloroacetic acid of < 50 mg/L, roughly corresponding to an average concentration trichloroethylene of 20 ppm, or 110 mg/m^3. Study subjects were followed-up for mortality from 1955 until 1986, and for incident cancers from 1958 until 1987. Observed numbers of deaths and incident cancers were compared with the expected numbers, calculated from those for the standardized population. In the mortality analysis, the male cohort provided 22 446 person-years of observation with an overall standardized mortality ratio of 0.97 (95% CI, 0.86–1.10). In men, the standardized mortality ratio for overall cancer mortality was 0.65 (95% CI, 0.47–0.89) and the standardized incidence ratio (SIR) for all incident cancers was 0.96 (95% CI, 0.80–1.16; 107 observed cases; 23 516 person-years). In men, only the standardized incidence ratio for skin cancer was significantly elevated (SIR, 2.36; 95% CI, 1.02–4.65) while the SIRs for other cancers were 1.16 (95% CI, 0.42–2.52) for cancer of the kidney, 1.41 (95% CI, 0.38–3.60) for cancer of the liver, 1.56 (95% CI, 0.51–3.64) for non-Hodgkin lymphoma and 0.69 (95% CI, 0.31–1.30) for cancer of the lung. Overall mortality in women was significantly increased (SMR, 1.55; 95% CI, 1.02–2.31; n = 24). The standardized incidence ratio for overall cancer morbidity in women was 1.32 (95% CI, 0.85–1.99). Among the 22 observed cancers, 11 were cancers of the breast or the genital organs. There were no cases of cancer of the liver or lymphoma among the female members of the cohort. The standardized incidence ratio for cancer of the lung was not reported for women.

The study from Finland (Anttila *et al.*, 1995a, b), updated and expanded from a previous study (Tola *et al.*, 1980), was based on a database of workers who were monitored by the Finnish Institute of Occupational Health for urinary concentration of trichloroacetic acid after exposure to trichloroethylene between 1965 and 1982. The same database also included subjects monitored biologically for exposure to tetrachloroethylene and 1,1,1-trichloroethane, but for

more than 94% of the subjects, measurements were only recorded for one of the three organic solvents, usually trichloroethylene. The overall median concentration of urinary trichloroacetic acid was 10 mg/L for women and 8 mg/L for men, which indicated a low level of exposure, and lower than that in Sweden (Axelson *et al.*, 1994). There was a total of 11 534 measurements for all three organic solvents, and a single person could be identified for 3976 (> 93%) measurements. Of these, 3089 subjects (1698 men and 1391 women) had files with measurements of trichloroacetic acid in urine, with an average of 2.5 measurements per subject. Study subjects were followed up for mortality from 1965 until 1991 in the files of the Central Statistical Office of Finland, and for 1967 until 1992 in the files of the Finnish Cancer Registry. The standardized mortality ratios and incidence ratios were based on Finnish national rates. The overall mortality was close to the expected value. During the maximum of 26 years of follow-up for cancer among the entire cohort of workers exposed to organic solvents, a total of 31 552 person-years among men and 28 353 among women exposed to trichloroethylene contributed to the 237 observed cancers (112 men, 125 women). The standardized incidence ratio for entire cohort exposed to the three organic solvents (both sexes combined) was 1.04 (95% CI, 0.91–1.17), which was similar to that for workers exposed to only trichloroethylene (SIR, 1.05; 95% CI, 0.92–1.20). Only the standardized incidence ratio for cancer of the cervix was significantly increased (SIR, 2.42; 95% CI, 1.05–4.77). The standardized incidence ratios were 0.87 (95% CI, 0.32–1.89) for cancer of the kidney, 2.27 (95% CI, 0.74–5.29) for cancer of the liver, 1.81 (95% CI, 0.78–3.56) for non-Hodgkin lymphoma and 0.92 (95% CI, 0.59–1.35) for cancer of the lung.

The Danish cohort study (Hansen *et al.*, 2001; Raaschou-Nielsen *et al.*, 2001, 2002) was based primarily on information from files on measurements of urinary trichloroacetic acid performed by the Danish Institute of Occupational Health between 1947 and 1989 (Hansen *et al.*, 2001). A total of 2397 samples from workers at 275 companies were analysed, with an average of 2.2 measurements of urinary trichloroacetic acid available per worker. For the entire period, the mean and median values were 40 mg/L and 15 mg/L, respectively.These values were higher than the corresponding values in the study in Finland, and similar to those in the study in Sweden. Concentrations tended to decrease between 1947 and 1989. In addition, files for a total of 472 measurements of trichloroethylene in individual breathing-zone samples were available from 81 companies for 1974–89. The mean air trichloroacetic acid measurement level was 101 mg/m^3. A total of 658 men and 145 women, born between 1901 and 1979, were identified as exposed to trichloroethylene from the urinary trichloroacetic acid and the breathing-zone measurement files. Only persons alive as of 1 April 1968, when a unique personal identification number for each Danish resident was introduced, could be identified. Individuals could be identified for 64% of the trichloroacetic-acid files, and for 52% of the air-measurement files. These people were followed-up in the files of the Danish Cancer registry from 1968 until 1996, or from the first day of measurement, if later. A total of 109 men and 19 women with incident cancers were identified based on 13 796 and 2934 person-years, respectively. Standardized incidence ratios were based on reference data from the general Danish population. Standardized incidence ratios were reported and were 1.0 for all cancers combined for men and women, 1.1 (95% CI, 0.3–2.7) for cancer of the kidney for both sexes combined, 4.2 (95% CI, 1.5–9.2) for cancer of the oesophagus in men, 3.5 (95% CI, 1.5–6.9) for non-Hodgkin lymphoma, 2.6 (95% CI, 0.8–6.0) for cancer of the liver and biliary passages in men, and 3.8 (95% CI, 1.0–9.8; 4 observed cases) for cancer of the cervix. The standardized incidence ratio for cancer of lung was 0.9 (95% CI, 0.5–1.3) in men and 0.7 (95% CI, 0.01–3.8) in women.

[An overall strength of the three relatively similar Nordic cohort studies was the confirmed individual exposure to trichloroethylene as documented by measurement of a trichloroethylene metabolite, trichloroacetic acid, in the urine. The studies had a long-term follow-up for cancer incidence from reliable nationwide cancer registries established in the 1940s and 1950s. The limitations were the relatively small numbers, the lack of information on duration of exposure, and that measurement of biomarkers at a single point of time may not reflect quantitative exposure in the past or in the future. Finally, workers with high exposures may have been removed from tasks involving trichloroethylene exposure, or local levels of exposure may have been lowered, since the main aim of the monitoring programme was to avoid high exposures.]

2.2.4 Other cohorts of workers exposed to trichloroethylene

Shindell & Ulrich conducted a cohort study of 2646 employees (2216 white men and 430 women) at a manufacturing plant using trichloroethylene as a degreasing agent almost exclusively throughout the study period from 1957 to 1983 in Illinois, USA (Shindell & Ulrich, 1985). Workers who had been employed for at least 3 months were included. At the end of follow-up (1983), 618 of the workers (23.4%) were still working at the plant. National mortality rates were used to calculate the expected numbers of deaths. Nine deaths from respiratory cancer were identified versus 12 expected [SMR, 0.75; 95% CI, 0.34–1.42]. A significant decrease in mortality from non-respiratory cancer was found [SMR, 0.49; 95% CI, 0.26–0.86]. [The cohort was young, and few study participants were deceased.]

Greenland *et al.* (1994) performed a nested case–control study in workers (white men only) employed at a large transformer-manufacturing plant in Massachusetts, USA, to address earlier reports of excess mortality from cancer in this population. Only workers employed at the facility before the end of 1984, who died between 1969 and 1984, and with an available job history were included (512 cases and 1202 controls, which were primarily deaths from cardiovascular disease). Interviews with long-term management employees selected for their historical knowledge of the plant operations were used to identify chemicals used in the plant and to build a job-exposure matrix. The case–control study focused on specific exposures with potential carcinogenic effects: pyranol, benzene, trichloroethylene, other organic solvents, machine fluids, asbestos, resin systems. Trichloroethylene was used from 1930 to 1977 as a degreasing agent. No significant increase in relative risk of mortality was found for men who had any exposure to trichloroethylene. An odds ratio (OR) of 1.64 (95% CI, 0.82–3.29) was observed for cancer of the pancreas, and 1.26 (95% CI, 0.51–3.08) for the group of oral, laryngeal and pharyngeal cancers. The odds ratios for other sites were 0.99 (95% CI, 0.30–3.32) for cancer of the kidney, 0.76 (95% CI, 0.24–2.42) for lymphoma, 0.54 (95% CI, 0.11–2.63) for cancer of the liver and 1.01 (95% CI, 0.69–1.47) for cancer of the lung. [The Working Group noted that although this was a nested case–control study, results for the cohort had never been published. Furthermore, the Working Group agreed with the authors of this study that the potential for bias associated with loss to follow-up and exposure misclassification was large.]

Henschler *et al.* describe a cohort of 169 male trichloroethylene-exposed cardboard-factory workers from Germany (Henschler *et al.*, 1995). Although other organic solvents had been used at the factory, use of these solvents relative to trichloroethylene was considered small. An unexposed group of 190 male workers from the same factory, matched on age and physical job activity, was established. No information on air concentrations was available and no personal biological measurements of trichloroethylene had been conducted. Based on interviews with long-term

employees, and walk-through surveys, workers were considered to have high and long-term exposure to trichloroethylene. Of the 183 male cardboard factory workers exposed to trichloroethylene for at least 1 year between 1956 and 1975 who were identified from individual employee's records, 14 (7.6%) could not be contacted or refused to participate at closing day of the study, 31 December 1992. Information on dates of employment, work tasks and occupational exposures, intake of diuretics, and smoking habits was obtained by questionnaire. Vital status was identified by individual tracing. Physical examination included abdominal sonography. Fifty people in the trichloroethylene-exposed group and 52 people in the control group died during the study period. In cases of cancer, the date of diagnosis was the date of surgery. All renal cell tumours were verified by histopathological examination. The latency period between first exposure to trichloroethylene and date of diagnosis of cancer of the kidney was between 18 and 34 years. Five men exposed to trichloroethylene were diagnosed with cancer of the kidney (four renal cell tumours, and one urothelial cancer of the renal pelvis). An additional two men exposed to trichloroethylene were diagnosed in 1993, the year after closing the study. No cases of cancer of the kidney occurred in the group of people who were not exposed to trichloroethylene. The standardized incidence ratio for cancer of the kidney ($n = 5$), using reference rates for Denmark, was 7.97 (95% CI, 2.59–18.59). [The small size of the cohort and the use of abdominal sonography may indicate a cluster study.]

Ritz studied patterns of cancer mortality in 3814 white male uranium-processing workers employed for at least three months at the Fernald Feed Materials Production Center in Fernald, Ohio, USA (Ritz, 1999). The facility produced uranium metal products, and workers were potentially exposed to several non-radioactive chemicals, including trichloroethylene and cutting fluids. The facility operated from 1951 to 1989, which also was the period of follow-up for mortality. Workers were identified from company rosters and personal records, which included information on employment duration. Exposure to trichloroethylene and other chemicals, including crude levels (none, light, medium, high) was based on a job and plant-area exposure matrix developed by experts having been employed in the long term at the plant in the late 1970s and early 1980s. Vital status was determined from the social security system until 1979, and afterwards from the national death index. Death certificate information was available for 1045 workers. Persons not identified as deceased were assumed to be alive at the end of follow-up. Expected number of deaths was calculated from the national mortality rates for white men and with the NIOSH-CORPS cohort (Zahm, 1992). Smoking history was available for a subsample of approximately 20% of the workers. Overall mortality in the cohort was lower than among white American men (SMR, 0.84; 95% CI, 0.79–0.90), while cancer mortality was slightly increased (SMR, 1.10; 95% CI, 0.99–1.23). Non-significantly increased standardized mortality ratios for liver and biliary tract cancer were observed for workers with more than 5 years of exposure to trichloroethylene: 1.90 (95% CI, 0.35–10.3) for light exposure and 8.82 (95% CI, 0.79–98.6) for medium exposure (no workers were in the category of high exposure). The standardized mortality ratio for haematopoietic and lymphopoietic cancer and light exposure (the only category with exposed cases) was 1.85 (95% CI, 0.87–3.95), and some increases in ratios for these cancers were seen with increasing duration, and when exposure was lagged. Internal comparison showed a non-significantly elevated relative risk of haematopoietic and lymphopoietic cancers (combined) for workers with more than 10 years of exposure to trichloroethylene (RR, 2.17; 95% CI, 0.88–5.33). [Results not reported for other cancers of primary interest.] The standardized mortality ratio for lung cancer was 1.03 (95%

CI, 0.85–1.24). Based on the sample of tobacco smokers, there was no clear association between patterns of tobacco smoking and general level of exposure to chemicals. [The Working Group noticed that the study was initiated to look at the effects of radiation. Interpretation of the results was limited by the small number of cases. Tobacco smoking was not likely to be a confounder because the risk of cancer of the lung was not increased in the cohort.]

Raaschou-Nielsen *et al.* established a retrospective cohort of 40 049 "blue-collar" workers from Danish companies with documented use of trichloroethylene (Raaschou-Nielsen *et al.*, 2003). Information on 457 companies using trichloroethylene came from historical records (1947–89) of measurements of trichloroacetic acid in urine (Raaschou-Nielsen *et al.*, 2001) or air (Raaschou-Nielsen *et al.*, 2002) performed by the Danish National Institute of Occupational Health, the Danish Product Registry, the files of a dry-cleaning survey, and the archives of the company that for decades had been the main supplier of trichloroethylene to companies in Denmark. In total, 110 companies each with more than 200 employees were excluded due to the low proportion of employees expected to be exposed to trichloroethylene. Using the unique company identification number, all employees at the remaining 347 companies, including their unique national identification number, were retrieved from the records of a national pension fund with compulsory membership since its establishment in 1964. For all 152 726 employees identified, information on vital status, including date of death, emigration or disappearance, and job title was retrieved from the Central Population Registry. Based on the job title, 40 049 "blue-collar" workers who had been employed for at least 3 months at the companies using trichloroethylene were included in the study. Each worker was followed up in the Danish Cancer Registry from 1968 to 1997. National cancer rates were used to calculate standardized incidence rates. During follow-up, men contributed 588 047 person-years, while women contributed 118 270 person-years. The overall standardized incidence rate for cancer was 1.08 (95% CI, 1.04–1.12) in men and 1.23 (95% CI, 1.14–1.33) in women. The individual standardized incidence rates were calculated for the following cancers: renal cell carcinoma, 1.2 (95% CI, 0.93–1.51) for men and 1.2 (95% CI, 0.53–2.44) for women; non-Hodgkin lymphoma, 1.2 (95% CI, 0.98–1.52) for men and 1.4 (95% CI, 0.73–2.34) for women; primary cancer of the liver, 1.1 (95% CI, 0.74–1.64) for men and 2.8 (95% CI, 1.13–5.8) for women; and cancer of the gallbladder and biliary passages, 1.1 (95% CI, 0.61–1.87) for men, and 2.8 (95% CI, 1.28–5.34) for women. The standardized incidence rate for cancer of the cervix was significantly increased (SIR, 1.9; 95% CI, 1.42–2.37). For cancer of the oesophagus, the standardized incidence rate was 1.1 (95% CI, 0.81–1.53) in men, and 2.0 (95% CI, 0.54–5.16) in women. The standardized incidence ratio for oesophageal adenocarcinoma was 1.8 (95% CI, 1.2–2.7) in men, while there were no cases in women. The risk of cancer of the lung was significantly increased in both men and women, (SIR, 1.4; 95% CI, 1.28–1.51; and SIR, 1.9; 95% CI, 1.48–2.35, respectively). Standardized incidence rates for renal cell carcinoma and for non-Hodgkin lymphoma tended to increase with duration of employment and by employment before 1970 versus later. For cancer of the cervix, the highest risk was seen for women with initial potential exposure before 1970 (SIR, 2.4; 95% CI, 1.6–3.4) and with less than 1 year of potential exposure (SIR, 2.5; 95% CI, 1.7–3.5). [There may have been a bias in social selection because the risk for blue-collar workers was compared with that for the general population, which is a mixture of blue- and white-collar workers. Furthermore, uncontrolled confounding from tobacco smoking may have occurred, as indicated by the excess risk of cancer of the lung, particularly in women. This study population

partially overlapped with that of Hansen *et al.* (2001).]

Bahr *et al.* described a cohort of 6820 workers (90% white) from the Paducah gaseous-diffusion plant, Kentucky, USA, an uranium-enrichment plant that had been operating since 1952 (Bahr *et al.*, 2011). A job-exposure matrix with initially five levels of exposure to trichloroethylene was developed based on the experience of current and former workers. Each job held at the facility was applied to the matrix, including duration in that job. By the end of follow-up, 1638 workers (24.2%) had died out of the 6766 workers for whom usable data existed. The overall standardized mortality ratio for death among workers potentially exposed to trichloroethylene compared with the national population was 0.76 (95% CI, 0.72–0.79) based on 1340 deaths. Among men, the standardized mortality ratios were 0.75 (95% CI, 0.72–0.79) for cancer of the lung, trachea and bronchus, 1.49 (95% CI, 1.02–2.10) for non-Hodgkin lymphoma, and 1.15 (95% CI, 0.74–1.72) for leukaemia and aleukaemic leukaemia. The standardized mortality ratio for for cancer of the lung was 0.75 (95% CI, 0.72–0.79). Corresponding standardized rate ratios for the subcohort of white men were 0.72 (95% CI, 0.29–1.76) for cancer of the lung, 0.99 (95% CI, 0.40–2.46) for non-Hodgkin lymphoma, 1.40 (95% CI, 0.46–4.24) for leukaemia and aleukaemic leukaemia, and 0.43 (0.10–1.84) for cancer of the biliary passages and liver. No cancers of the kidney were reported. No clear dose–response patterns were seen for overall death, overall cancer, or for site-specific cancers. [Overall, this study was not well described, which limited interpretation. No numbers of incident cases, deaths, or rates were given for the SRR calculations. The number of people included in the different subcohorts was unclear. The source of information for vital status, date of death, and cause of death was not clearly described.]

2.2.5 Nonspecified chlorinated solvents

There were several studies of cohorts exposed to nonspecified chlorinated solvents (Chang *et al.*, 2003, 2005; Pukkala *et al.*, 2005; Sung *et al.*, 2007; Lindbohm *et al.*, 2009). Since exposures to trichloroethylene and tetrachloroethylene were not measured separately, and because of other confounding exposures, the Working Group considered that these studies were not informative for the evaluation of trichloroethylene.

2.3 Case–control studies

2.3.1 Cancer of the kidney

See Table 2.3

Seven case–control studies have specifically addressed the association between trichloroethylene and cancer of the kidney. Three of the studies were carried out in Germany.

In 1998, Vamvakas *et al.* published the first case–control study identifying the specific association between trichloroethylene and renal cell cancer (Vamvakas *et al.*, 1998). Cases were patients who underwent nephrectomy between 1987 and 1992 in a hospital in North Rhine-Westphalia, Germany: of the 73 patients treated during the study period, 58 patients were interviewed and enrolled. [None of the cases overlapped with those in the cohort study by Henschler *et al.* (1995) in the same area.] Controls were 84 patients from the accident wards of three other hospitals during 1993 (participation rate, 75%). Cases were histologically confirmed, and controls underwent abdominal echography to exclude cancer of the kidney. Information on occupational history, including exposure to other hazardous chemicals, and other risk factors was obtained by individual interview. The level of individual exposure to trichloroethylene was rated by applying a system that integrated total exposure time, as well as frequency and severity of acute pre-narcotic symptoms. Of the

Table 2.3 Case–control studies of renal cell cancer and exposure to trichloroethylene

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Vamvakas *et al.* (1998), Germany, 1987–93	58 84	Hospital	Interview and score including exposure time, frequency and severity of acute pre-narcotic symptoms	No exposure Ever Low Medium High	39 19 2 9 8	1 10.80 (3.36–34.75) 6.61 (0.50–87.76) 11.92 (2.55–55.60) 11.42 (1.96–66.79)	Age, sex, smoking, BMI, blood pressure, intake of diuretics Age, diastolic blood pressure Subjects may have had high exposure to trichloroethylene through cold degreasing and permanent high background exposures. Few subjects exposed to tetrachloroethylene.
Dosemeci *et al.* (1999) Minnesota, USA 1988–90	273 men and 165 women 462 men and 225 women	Cancer registry and general population	Occupational questionnaire and JEM	Trichloroethylene-exposed Men Women	55 33 22	1.30 (0.9–1.9) 1.04 (0.6–1.7) 1.96 (1.0–4.0)	Age, sex, smoking, BMI, hypertension and/or use of diuretics and/or anti-hypertension drugs Only current and usual jobs plus duration of employment in 13 specific occupations/industries and seven jobs were considered
Pesch *et al.* (2000a) Germany 1991–96	570 men and 365 women 2650 men and 1648 women	Population based, Cases from several hospitals, controls from local residency registries	Occupational questionnaire and JEM	*Men* No exposure Medium[a] High[a] Substantial[a] *Women* No exposure Medium[a] High[a] Substantial[a]	 68 59 22 11 7 5	 1 1.3 (1.0–1.8) 1.1 (0.8–1.5) 1.3 (0.8–2.1) 1 1.3 (0.7–2.6) 0.8 (0.4–1.9) 1.8 (0.6–5.0)	Smoking, age, region Not adjusted for BMI. No precision available on the types of jobs and tasks involving trichloroethylene exposure. [a] Exposure categories defined by 30th, 60th and 90th percentiles of exposure index in controls. Job-task matrix (JTEM)
Brüning *et al.* (2003) Germany 1999–2000	134 401	Hospital	Interview with patient or next-of-kin. Self-assessed exposure	No exposure < 10 yr 10- < 20 yr ≥ 20 yr	109 11 7 6	1 3.78 (1.54–9.28) 1.80 (0.67–4.79) 2.69 (0.84–8.66)	Smoking; Frequency-matched by sex and age Large difference in the results obtained from matrix assessment compared with subjects' self-assessment. No overlap with the cases in the Vamvakas *et al.* (1998) study

Table 2.3 (continued)

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Charbotel *et al.* (2006) France 1993–2003	87 316	Population	Occupational questionnaire and JTEM Cumulative dose	No exposure	49	1	Tobacco smoking, BMI; matched on sex and age
				Low	12	1.62 (0.75–3.47)	
				Medium	9	1.15 (0.47–2.77)	
				High	16	2.16 (1.02–4.60)	
				High	16	1.96 (0.71–5.37)	
				Cumulative dose, plus peaks			
				No exposure	49	1	
				Low/medium, no peaks	18	1.35 (0.69–2.63)	
				Low/medium, plus peaks	3	1.61 (0.36–7.30)	
				High, no peaks	8	1.76 (0.65–4.73)	
				High, plus peaks	8	2.73 (1.06–7.07)	
				High, plus peaks	8	2.63 (0.79–8.83)	
							The study was carried out in a geographical area with high prevalence of trichloroethylene exposure among the general population Trichloroethylene exposure was strongly associated with exposure to cutting fluids and petroleum oils. Tobacco smoking, BMI, cutting fluids, other petroleum oils; Matched on sex and age Tobacco smoking, BMI; matched on sex and age Tobacco smoking, BMI, cutting fluids, other petroleum oils; matched on sex and age
Charbotel *et al.* (2009) France	87 316	Population	Occupational questionnaire and JTEM	No exposure to trichloroethylene or cutting fluid	46	1	Tobacco smoking, BMI, other petroleum oils; matched on sex & age
				Exposure to cutting fluids but not trichloroethylene	3	2.39 (0.52–11.03)	
				Exposure to trichloroethylene but not to cutting fluids	15	1.62 (0.76–3.44)	
				Exposure to both and trichloroethylene < 50 ppm	12	1.14 (0.49–2.66)	
				Exposure to both and trichloroethylene ≥ 50 ppm	10	2.70 (1.02–7.17)	Cases diagnosed between 1993 and 2003, complementary analysis to Charbotel *et al.*, (2006)

Table 2.3 (continued)

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Moore *et al.* (2010) Central-eastern Europe 1999–2003	1097 1476	Hospital	Occupational questionnaire and JTEM	No exposure	777	1	Age, sex, centre Subjects with high confidence assessments only Increased risk in trichloroethylene-exposed subjects with at least one intact *GSTT1* allele, but not in subjects with two deleted alleles
				Any exposure	29	2.05 (1.13–3.73)	
				Below the median average intensity	31	1.73 (0.75–4.02)	
				Above the median average intensity	16	2.41 (1.05–5.56)	
				P for trend	0.02		
				Among patients with at least one intact GSTT1 allele:			
				Any exposure	32	1.88 (1.06–3.33)	
				Below the median average intensity	20	1.56 (0.79–3.10)	
				Above the median average intensity	12	2.77 (1.01–7.58)	
				Among subjects with two deleted alleles:			
				Any exposure	10	0.93 (0.35–2.44)	
				Below the median average intensity	6	0.81 (0.24–2.72)	
				Above the median average intensity	4	1.16 (0.27–5.04)	
Christensen *et al.* (2013) Montreal, Canada 1979–85	177 2532	533 population controls and 1999 cancer controls	Occupational questionnaire and expert assessment	Any level	5	0.9 (0.4–2.4)	Age, census tract median income, educational attainment (years), ethnicity (French-Canadian versus other), questionnaire respondent (self vs proxy) and smoking (cigarette-years)
				Substantial	2	0.6 (0.1–2.8)	

BMI, body-mass index; JEM, job-exposure matrix; JTEM, job-task exposure matrix; vs, versus

cases, most subjects had been engaged in cold-degreasing processes and there was an additional permanent background exposure from open tubs containing trichloroethylene, and evaporation from trichloroethylene-cleaned metal parts. Exposures to trichloroethylene and tetrachloroethylene were combined in the study, but only two controls and none of the cases had been exposed to tetrachloroethylene. The odds ratio for any exposure to trichloroethylene, adjusted for age, sex, smoking, body-mass index, blood pressure and intake of diuretics, was 10.80 (95% CI, 3.36–34.75). The analysis of intensity of exposure (including assessment from task description and pre-narcotic symptoms) compared with that of the unexposed individuals, adjusted for age and diastolic blood pressure, gave odds ratios of 6.61 (95% CI, 0.50–87.76) in the low-level category, 11.92 (95% CI, 2.55–55.60) in the medium -level and 11.42 (95% CI, 1.96–66.79) in the high-level category. [The Working Group noted that although the authors combined exposures to trichloroethylene and tetrachloroethylene "because of identical toxicological mechanisms," the reported associations for exposure to trichloroethylene were notable since no cases and only 2 out of 84 controls were exposed to tetrachloroethylene. Additionally, the authors reported permanent background exposure, and past exposure due to the use of trichloroethylene for all cleaning purposes in the plants, including cleaning floors, cloths, and also hands and arms. Narcotic symptoms, an indicator of substantially high peak exposures to solvents, were self-reported and therefore could be subject to reporting bias. There were several limitations to this study that might have biased the probability of exposure in the controls: cases and controls were not recruited in the same hospital; controls were younger than cases (mean age ± standard deviation: cases, 62 ± 9.7; and controls, 51 ± 13.0), but odds ratios were age-adjusted. Finally, the exposure assessment was self-reported, and not blinded to the outcome (case or control status). However, some of the cases were highly exposed to trichloroethylene according to their job tasks.]

Brüning *et al.* (2003) performed a case–control study in the same geographical area as Vamvakas *et al.* (1998). Histologically confirmed cases of renal cell cancer in people who had undergone a nephrectomy between June 1992 and April 2000 were included. [There was no overlap with the cases in the Vamvakas *et al.* (1998) study.] A total number of 162 incident eligible cases were identified, from which 134 cases (82.7%) were enrolled either with face-to-face interviews (n = 113) or with next-of-kin interviews (n = 21). Controls (n = 401) were recruited in the same hospital, in surgery departments or geriatric departments between 1999 and 2000 and interviewed face-to-face with the same structured questionnaire used in the previous study by Vamvakas *et al.* (1998). It included information on occupational history (job titles), tasks, and exposure to specific agents. The frequency and duration of exposure to trichloroethylene and tetrachloroethylene were self-assessed. Narcotic symptoms among subjects exposed to trichloroethylene and tetrachloroethylene were documented and considered to be indicators of substantially high peak exposures. Every job held for at least 1 year was classified according to a British job-exposure matrix (Pannett *et al.*, 1985). The job-exposure matrix provided an expert rating in terms of the probability and intensity of exposure to specified agents including organic solvents, but not specifically trichloroethylene. The products of duration, probability, and intensity of exposure were cumulated over all jobs held to obtain an estimate of lifetime exposure. The corresponding industries were classified according to the United Nations' International Standard Industrial Classification of all Economic Activities (ISIC), 1968. Data on exposure corresponding to these industries were obtained from the public database CAREX. Subjects' self-assessment of exposure to trichloroethylene and tetrachloroethylene (separately) was also used to allow a comparison

with the results of the study of Vamvakas *et al.* (1998).

Conditional logistic regression models were applied for risk estimation adjusted for sex, age and smoking. When considering employment in any industry with exposure to trichloroethylene or tetrachloroethylene using the CAREX database, an odds ratio of 1.80 (95% CI, 1.01–3.20) was observed. An odds ratio of 1.45 (95% CI, 0.59–3.58) was reported for exposure to organic solvents assessed using the British job-exposure matrix [exposure to trichloroethylene was not detailed in this specific assessment]. Finally when considering self-assessed exposure to trichloroethylene, compared with never exposed, the odds ratio was 3.78 (95% CI, 1.54–9.28) among subjects exposed for 1–10 years, 1.80 (95% CI, 0.67–4.79) exposed for 10–20 years and 2.69 (95% CI, 0.84–8.66) exposed for > 20 years. [The participation rate for controls was not provided. The use of proxy interviews to assess exposures may provide poor quality information, which would increase exposure misclassification. In this study there was a difference in the odds ratios associated with exposures obtained from matrix assessment (British job-exposure matrix) compared with subjects' self-assessment. Self-assessment of specific exposures may also lead to exposure misclassification. Other occupational exposures were found to increase the risk of renal cell cancer in this study but no model adjusted for these factors.]

Pesch *et al.* carried out a population-based case–control study in five different regions of Germany that differed from those in the studies by Vamvakas and Brüning (Pesch *et al.*, 2000a). Among the German nationals recruited, there were 935 confirmed cases of renal cell cancer (570 men and 365 women); the 4298 controls (2650 men and 1648 women) were frequency-matched to cases by region, sex and age (5-year groups). The response rate was 88% for cases and 71% for controls. Subjects were interviewed face-to-face with a structured questionnaire. The exposure assessment was based on the subject's occupational history (job titles) as well as on job-task descriptions for every job held for at least 1 year. A job-exposure matrix and a job-task exposure matrix were used to provide expert ratings in terms of the probability and the intensity of exposure to a specific agent. To obtain the subject's lifetime exposure, exposure indices were constructed using duration, probability and intensity of exposure over all job periods. For metal degreasing, the odds ratios were slightly, but insignificantly elevated for the majority of exposure categories in men and women. The odd ratios for exposure to solvents, especially to trichloroethylene, tetrachloroethylene and carbon tetrachloride, were slightly elevated in all exposure categories in males and females. Using the matrix specifically developed for the study to assess exposure to trichloroethylene (the job-task exposure matrix), the odds ratios in men adjusted for smoking, age and region were 1.3 (95% CI, 1.0–1.8), 1.1 (95% CI, 0.8–1.5) and 1.3 (95% CI, 0.8–2.1) for medium, high and substantial levels of exposure, respectively. In women, the corresponding odds ratios were 1.3 (95% CI, 0.7–2.6), 0.8 (95% CI, 0.4–1.9) and 1.8 (95% CI, 0.6–5.0), respectively. An overall estimate combining these category-specific odds ratios was provided by Pesch for a meta-analysis published in 2011 (Scott & Jinot, 2011); the odds ratio was 1.24 (95% CI, 1.03–1.49) for substantial exposure to trichloroethylene. [The Working Group noted several limitations of this study. The methods used for exposure assessment were not described in detail. The low prevalence of exposure observed for several occupations and specific exposures may reduce the power of the study. Prevalence of exposure and results differed according to which job-exposure matrix was used (job-exposure versus job-task exposure). No adjustment was performed for the other occupational exposures found to be significantly associated with risk of renal cell cancer in the study. The authors reported that no dose–response

trend was observed, but the results of statistical tests were not given. The strengths of the study were that cases and controls appeared to have been properly recruited and response rates were in accordance with those in the literature. No details were available on the types of jobs and tasks involving exposure to trichloroethylene.]

A case–control study was performed using the Minnesota cancer registry by Dosemeci *et al.* (1999). From 1988 to 1990, 796 white patients newly diagnosed with histologically confirmed renal cell cancer were identified. Population controls (n = 707) matched on race, age, and sex were recruited using random-digit dialling, or a listing of the Health Care Financing Administration for those aged > 65 years. A questionnaire, including demographic and ethnic variables, occupational and residential history, diet, smoking habits, medical history, and drug use, was administered face-to-face by trained interviewers. The participation rate for the occupational part was 64% for interviewed cases and 97% for interviewed controls. A previously developed job-exposure matrix was used to assess exposures to several chemicals, including trichloroethylene. After adjustment for age, sex, smoking, hypertension and/or use of diuretics and/or anti-hypertension drugs, and body-mass index, the risk of renal cell cancer associated with exposure to trichloroethylene was 1.30 (95% CI, 0.9–1.9) in the entire population, 1.04 (95% CI, 0.6–1.7) among men and 1.96 (95% CI, 1.0–4.0) among women. [The Working Group noted that only current and usual jobs were considered rather than a lifetime work history. Duration of employment in 13 specific occupations/industries and 7 jobs was ascertained, but no dose–response analysis was reported.]

A case–control study in Montreal, Canada, included cases of cancer in men occurring between 1979 and 1985 from 18 of the largest hospitals in the Montreal metropolitan area. Several cancer sites were considered (Christensen *et al.*, 2013). Only incident and histologically confirmed cancers were included. Of 4576 eligible patients with cancer, 3730 (participation rate, 82%) were recruited. For the population controls recruited from the general population, 533 were included out of the 740 eligible (participation rate, 72%). Cancer controls were also included in the analysis [but there was no description of which cancer sites or how many people were included in this group]. A panel of industrial hygienists reviewed each job history reported by study subjects and assessed exposure to 294 substances. Exposure assessment included degree of confidence that exposure had actually occurred, frequency of exposure during a normal working week, and concentration of the agent. Unconditional logistic regression was used to estimate odds ratios for risk of cancer at each site. An analysis was conducted including cancer controls and population controls, weighting the two groups equally. A total of 177 cases of cancer of the kidney were included. For exposure to trichloroethylene, the odds ratio was 0.9 (95% CI, 0.4–2.4) when considering any level of exposure, and 0.6 (95% CI, 0.1–2.8) for substantial exposure, after adjustment for age, income, education, ethnicity, questionnaire respondent, and smoking. [The Working Group noted the low precision and power of this study, which may not have been able to detect an effect due to the low prevalence of exposure to trichloroethylene in the controls (~3%).]

In France, Charbotel *et al.* (2006) carried out a case–control study in a region where trichloroethylene had been widely used as a degreasing agent in the screw-cutting industry. Cases were selected retrospectively from 1993, and prospectively for 1 year until the end of June 2003 from urology and oncology practices and hospitals. Deceased cases and controls were eligible, and for these, the next-of-kin were interviewed. Controls resident in the geographical study area at the time of diagnosis of the case's disease and matched on sex and year of birth were randomly selected from lists of patients at urology or

general practice clinics. Exclusion criteria for controls were: chronic kidney disease or cancer of the bladder, renal pelvis or ureter. Exposure to solvents (trichloroethylene, other chlorinated solvents) and other occupational exposures (oils, including cutting fluids and other oils; welding fumes, lead, cadmium, asbestos and ionizing radiation) was assessed by an industrial hygienist using information from the occupational questionnaires and a job task-exposure matrix (Fevotte *et al.*, 2006). The exposure assessment comprised semiquantitative estimates for trichloroethylene and qualitative estimates (low/medium/high) for the other occupational agents. The effect of cumulative and peak exposure was assessed. Conditional logistic regression analyses were performed to assess the association between trichloroethylene and risk of renal cell cancer. A total of 87 cases of renal cell cancer (participation rate, 74%) and 316 controls (participation rate, 78%) were included. Among general factors studied, only tobacco smoking and body-mass index were found to significantly increase the risk of renal cell cancer. An increased risk was identified for high cumulative exposure to trichloroethylene: the crude odds ratio was 2.23 (95% CI, 1.09–4.57) and the odds ratio adjusted for tobacco smoking and body-mass index was 2.16 (95% CI, 1.02–4.60). A dose–response relationship was identified (*P* for trend, 0.04). The odds ratios were even higher among the highest class of exposure (cumulative dose) plus peaks (crude OR, 2.70; 95% CI, 1.09–6.67; adjusted OR, 2.73; 95% CI, 1.06–7.07). When exposure to cutting fluids and to other petroleum oils were added to the conditional logistic regression model, the odds ratios for renal cell cancer were elevated, but not statistically significant for the highest class of cumulative exposure to trichloroethylene (OR, 1.96; 95% CI, 0.71–5.37) and in the high-exposure group with peaks (OR, 2.63; 95% CI, 0.79–8.83).

In a complementary analysis (Charbotel *et al.*, 2009) among the same subjects as reported in Charbotel *et al.* (2006), the odds ratios for semiquantitative estimates of 8-hour average exposure to trichloroethylene at the thresholds of 35 ppm, 50 ppm, and 75 ppm were respectively 1.62 (95% CI, 0.77–3.42), 2.80 (95% CI, 1.12–7.03), and 2.92 (95% CI, 0.85–10.09). The authors assessed the potential confounding effect of exposure to cutting fluids. In subjects exposed to trichloroethylene only, the odds ratio was 1.62 (95% CI, 0.76–3.44). In subjects exposed to cutting fluids and trichloroethylene at concentrations > 50 ppm, the odds ratio adjusted for body-mass index, tobacco smoking and exposure to other oils reached 2.70 (95% CI, 1.02–7.17). [The Working Group noted that exposure to trichloroethylene was not measured, but was estimated in ppm. This study is notable because it was carried out in a geographical area with a high prevalence of exposure among the general population. Risk factors for kidney cancer, and other occupational exposures were assessed and included in statistical models.]

A hospital-based case–control study on trichloroethylene and renal cell cancer was carried out between 1999 and 2003 in seven centres in four countries of central and eastern Europe (Moscow, the Russian Federation; Bucharest, Romania; Lodz, Poland; and Prague, Olomouc, Ceske-Budejovice and Brno, Czech Republic) (Moore *et al.*, 2010). This region is of interest for the study of occupational exposures because the prevalence and intensity of exposure have been greater than in other industrialized regions. This analysis assessed the interaction between exposure to trichloroethylene and *GSTT1* genotype because: (i) trichloroethylene-associated kidney damage occurs only after bioactivation through the reductive metabolic pathway that requires prior conjugation by hepatic and renal glutathione *S*-transferase (GSH); (ii) the GSTT1 enzyme conjugates small, halogenated compounds such as trichloroethylene; and (iii) GSTT1 is highly active in the kidney. Newly diagnosed and histologically confirmed cases

of cancer of the kidney (ICD-O2 code C.64) were included and controls were chosen among subjects admitted with non-tobacco-related conditions in the same hospital as the cases, and frequency matched with cases by sex, age, and study centre. The final study population included 1097 cases and 1476 controls. Face-to-face interviews were performed using standard questionnaires (tobacco consumption, anthropometric measures 1 year before diagnosis, personal and familial medical history). Information on each job held for at least 1 year was collected using a general questionnaire including a description of the tasks performed, machines used, working environment, location of tasks performed, and time spent on each task. In each centre, a team evaluated the frequency and intensity of exposure to various agents and groups of agents (including trichloroethylene), based on the questionnaires and their own experience and knowledge of historical working conditions at specific plants in their study area. An increased risk of renal cell cancer was observed among subjects ever exposed to trichloroethylene (OR, 1.63; 95% CI, 1.04–2.54). A trend was observed in relation to average intensity of exposure: below the median average intensity of exposure in controls (0.076 ppm), the odds ratio was 1.73 (95% CI, 0.75–4.02) while above the median the odds ratio was 2.41 (95% CI, 1.05–5.56; *P* for trend, 0.02). In subjects with at least one intact *GSTT1* allele, a significant association was found for those ever exposed to trichloroethylene (OR, 1.88; 95% CI, 1.06–3.33), and the odds ratios below and above the average exposure intensity were 1.56 (95% CI, 0.79–3.10) and 2.77 (95% CI, 1.01–7.58), respectively (*P* for trend, 0.02). In contrast, no increase in risk of renal cell cancer was observed among subjects with two deleted *GSTT1* alleles: the odds ratios were, respectively, 0.93 (95% CI, 0.35–2.44) in ever-exposed subjects, 0.81 (95% CI, 0.24–2.72) with below-average exposure, and 1.16 (95% CI, 0.27–5.04) with over-average exposure intensity. [The participation rate was stated to be high, but was not provided in the paper. Tobacco smoking, body-mass index and self-reported history of hypertension were evaluated as risk factors, but did not alter the odds ratios by > 10%, and therefore were not included in the final models. Despite being justified by the inclusion of hospital controls, the exclusion of tobacco-related conditions for control recruitment may have led to a selection effect for social class, i.e. controls may be less likely to be blue-collar workers and consequently less likely to be exposed to trichloroethylene. A selection bias due to the use of hospital controls may have occurred as suggested by a lack of association observed between tobacco smoking and risk of renal cell cancer. Studies *in vitro* and *in vivo* in humans have demonstrated that the GST pathway is active, but it is unclear which isoform is most active.]

2.3.2 Haematological malignancies

See Table 2.4

(a) Non-Hodgkin lymphoma

The association between non-Hodgkin lymphoma and exposure to trichloroethylene has been investigated in eight case–control studies in several countries.

In Sweden, a case–control study recruited all men with non-Hodgkin lymphoma from an oncology department between 1974 and 1978 (Hardell *et al.*, 1994). Controls were selected from the national population registry and matched according to sex, age, place of residence, and vital status. Deceased controls, drawn from the national registry for causes of death, were also matched for year of death. The self-administered questionnaire contained questions on job history and exposure to chemicals. The study included 105 patients with confirmed non-Hodgkin lymphoma and 335 controls. An increased risk of non-Hodgkin lymphoma was found in workers reporting exposure to trichloroethylene (crude OR, 7.2; 95% CI, 1.3–42.0). [Exposure assessment

was not described in detail in this publication. The odds ratio from this matched study did not appear to include only discordant pairs; the analysis was difficult to verify as there was no information on the non-exposed subjects.]

Data from two case–control studies with similar designs were pooled to investigate risk factors for non-Hodgkin lymphoma (Persson *et al.*, 1989, 1993; Persson & Fredrikson, 1999). In these studies, exposure assessment was based on information reported in a questionnaire posted to the subjects. A total of 199 cases and 479 controls were included. Among workers exposed to trichloroethylene, the odds ratio stratified by age and sex was 1.2 (95% CI, 0.5–2.4). [The Working Group noted that the exposure assessment was based on self-reported exposure.]

A population-based case–control study of lymphohaematopoietic tumours included all new cases of non-Hodgkin lymphoma diagnosed during 1991–93 in eight areas in Italy (Miligi *et al.*, 2006). The participation rate was 85% among cases of non-Hodgkin lymphoma (n = 1428) and 73% among controls (n = 1530). Cases were identified from hospital and pathology departments. Controls were randomly selected from residents in the general population. Face-to-face interviews were performed to collect information on occupational history, and exposure was assessed by an industrial hygienist. Odds ratios were adjusted for age, sex, education, and area. When considering all subtypes of non-Hodgkin lymphoma combined, the odds ratios associated with occupational exposure to trichloroethylene were 0.8 (95% CI, 0.5–1.3) for exposure at very low or low levels, or 1.2 (95% CI, 0.7–2.0) at medium or high levels. No association was found with duration of exposure. A non-statistically significant increased risk was observed for diffuse lymphoma (OR, 1.9; 95% CI, 0.9–3.7; 13 exposed cases), but not for other types of non-Hodgkin lymphoma. [The strengths of this study were that it included a large number of subjects and an expert assessment of exposure was performed.]

A case–control study in six regions in Germany included patients with malignant lymphoma (n = 710; participation rate, 87%) and controls (matched by sex, region, and age) recruited from population registers (n = 710; participation rate, 44%) (Seidler *et al.*, 2007). Interviewers collected a complete history of all jobs held for more than 1 year and specific job tasks. On the basis of job task-specific questionnaires, a trained occupational physician assessed exposure to chlorinated hydrocarbons, including trichloroethylene. Workers in the category of highest exposure to trichloroethylene (> 35 ppm-years) had an increased risk of malignant lymphoma (OR, 2.1; 95% CI, 1.0–4.8), but no significant dose–response relationship was observed (P for trend, 0.14). Estimated increased risks for workers in the category of highest exposure to trichloroethylene were also observed for B-cell non-Hodgkin lymphoma, T-cell non-Hodgkin lymphoma, Hodgkin lymphoma, diffuse large B-cell lymphoma, follicular lymphoma, and marginal zone lymphoma, but most precise for the B-NHL subtype (n = 554; OR, 2.3; 95% CI, 1.0–5.3). [The Working Group noted that the participation rate among controls was half that of cases and could represent a selection bias. In addition, the cumulative exposures appeared to have been very low.]

In Connecticut, USA, a case–control study investigated the association between non-Hodgkin lymphoma and occupational exposure to solvents among women (Wang *et al.*, 2009). Subjects were recruited between 1996 and 2000 and included 601 cases (participation rate, 72%) and 717 controls selected by random-digit dialling (participation rate, 69%) or random selection from Medicare service files. Exposure assessment was based on occupational questionnaire and expert assessment using a job-exposure matrix. An increased risk of non-Hodgkin lymphoma in workers ever exposed to trichloroethylene was found (OR, 1.2; 95% CI, 0.9–1.8), after adjustment for age, family history of

Table 2.4 Case–control studies of non-Hodgkin lymphoma, and other haematological malignancies, and exposure to trichloroethylene

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Hardell *et al.* (1994) Sweden 1974–78	105 335	National population registry (alive) and national registry for causes of death (deceased)	Questionnaire soliciting working history and leisure-time activities	NHL	Trichloroethylene-exposed	4	7.2 (1.3–42)	Matched for sex, age, place of residence, and vital status NHL was associated with exposure to phenoxyacetic acids and chlorophenols, but trichloroethylene results were not adjusted for these exposures. Exposure assessment was not described in detail.
Persson & Fredrikson (1999) Sweden 1964–86	199 479	Population	Occupational agent exposure assessed by questionnaires mailed to participants	NHL	Any exposure to trichloroethylene	16	1.2 (0.5–2.4)	Stratified on age and sex
Miligi *et al.* (2006) Italy	1428 1530	Population	Face-to-face interviews, assessed by an industrial hygienist	All NHL subtypes	Very low/low	35	0.8 (0.5–1.3)	Sex, age, area, education
					Medium or high level	35	1.2 (0.7–2.0)	
					Test for trend		$P = 0.80$	
					> 15 year exposure duration	12	1.0 (0.5–2.6)	
				Small lymphocytic NHL	Any exposure	7	0.9 (0.4–2.1)	
				Diffuse NHL	Any exposure	13	1.9 (0.9–3.7)	
Seidler *et al.* (2007) Germany	710 710	Population	Using job-task-specific questionnaires	All malignant lymphoma	0	610	ref.	Age, sex, region, smoking, alcohol Increased risks were observed for workers in the highest category of exposure for malignant lymphoma nd several subtypes (B-cell NHL, T-cell NHL, Hodgkin lymphoma, DLBCL, FL, and marginal zone lymphoma)
					< 4.4 ppm-years	40	0.7 (0.4–1.1)	
					> 4.4–35 ppm-years	32	0.7 (0.5–1.2)	
					> 35 ppm-years	21	2.1 (1.0–4.8)	
					Test for trend		$P = 0.14$	
				B-NHL	0	47+	ref.	
					< 4.4 ppm-years	32	0.7 (0.5–1.2)	
					> 4.4–35 ppm-years	27	0.8 (0.5–1.3)	
					> 35 ppm-years	17	2.3 (1.0–5.3)	
					Test for trend		$P = 0.08$	

Table 2.4 (continued)

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Seidler *et al.* (2007) Germany (cont.)				T-NHL	0	27	Ref.	
					< 4.4 ppm-years	2	0.7 (0.2–3.3)	
					> 4.4–35 ppm-years	2	1.1 (0.2–5.1)	
					> 35 ppm-years	2	4.7 (0.8–26.1)	
					Test for trend		*P* = 0.09	
Wang *et al.* (2009) Conneticut, USA 1996–2000	601 717	Population	Linking coded occupational data with a JEM	Histopathologically verified NHL	Ever exposed	77	1.2 (0.9–1.8)	Age, family history, alcohol, race
					Low exposure intensity	64	1.1 (0.8–1.6)	
					Medium–high exposure intensity	13	2.2 (0.9–5.4)	Increased risk with exposure to other organic, solvents, benzene, formaldehyde
					Test for trend		*P* = 0.06	Overlapped with Deng *et al.* (2012)
Cocco *et al.* (2010) multicentre Europe 1998–2004	2348 2462	Population	In-person interviews on occupational history	B-NHL	All exposed	71	0.8 (0.6–1.1)	Age, sex, education, centre
					Low	26	0.9 [no CI]	
					Medium	16	0.5 [no CI]	
					High	29	1.0 [no CI]	
					Test for trend		*P* = 0.16	
				DLBCL	All exposed	17	0.7 (0.4–1.1)	
					Low	6	0.7 [no CI]	
					Medium	4	0.4 [no CI]	
					High	7	0.9 [no CI]	
					Test for trend		*P* = 0.16	
				FL	All exposed	11	1.2 (0.6–2.3)	
					Low	7	2.4 [no CI]	
					Medium	1	0.3 [no CI]	
					High	3	1.0 [no CI]	
					Test for trend		*P* = 0.65	
				CLL	All exposed	18	0.9 [0.5–1.5]	
					Low	6	1.0 [no CI]	
					Medium	3	0.4 [no CI]	
					High	9	1.2 [no CI]	
					Test for trend		*P* = 0.94	

Table 2.4 (continued)

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Cocco *et al.* (2010) multicentre Europe 1998–2004 (cont.)				MM	All exposed	9	0.6 [0.3–1.2]	
					Low	1	0.2 [no CI]	
					Medium	4	0.7 [no CI]	
					High	4	0.8 [no CI]	
					Test for trend		$P = 0.22$	
Purdue *et al.* (2011) USA; four SEER registries 1998–2000	1189 982	Population	Face-to-face interview for occupational exposures	NHL	Any exposure			Age, sex, study centre, race, education No assessment of exposure to other organic solvents
					Unexposed	599	Ref.	
					Possible exposure	545	1.1 (0.9–1.3)	
					Probable exposure	45	1.4 (0.8–2.4)	
					Test for trend		$P = 0.40$	
					Average weekly exposure (ppm-hours):			
					0	599	ref.	
					1–60	15	1.6 (0.7–3.8)	
					61–50	7	0.5 (0.2–1.4)	
					> 150	23	2.5 (1.1–6.1)	
					151–360	3	0.4 (0.1–1.8)	
					> 360	20	7.9 (1.8–34.3)	
					Per 90 estimated ppm-hours/week		1.11 (1.02–1.21)	
					Test for trend		$P = 0.02$	
					Years exposed:			
					0	599	Ref.	
					1–6 yr	22	2.1 (1.0–4.7)	
					7–16 yr	10	0.8 (0.3–2.1)	
					> 16 yr	13	1.3 (0.5–3.1)	
					17–24 yr	6	1.0 (0.3–3.4)	
					> 24 yr	7	1.7 (0.5–5.8)	
					Per 10 yr		1.13 (0.85–1.51)	
					Test for trend		$P = 0.40$	

Table 2.4 (continued)

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Purdue *et al.* (2011) USA; four SEER registries 1998–2000 (cont.)					Cumulative exposure (estimated ppm-hours):			
					0	599	Ref.	
					1–46 800	14	1.4 (0.6–3.3)	
					46 801–112 320	7	0.6 (0.2–1.7)	
					> 112 320	24	2.3 (1.0–5.0)	
					112 321–234 000	8	1.4 (0.5–4.4)	
					> 234 000	16	3.3 (1.1–10.1)	
					Per 65 520 estimated ppm-hours		1.10 (0.99–1.22)	
					Test for trend		$P = 0.08$	
					Average exposure intensity (estimated ppm):			
					0	599	ref	
					1–99	23	1.5 (0.8–2.9)	
					> 99	22	1.3 (0.7–2.7)	
					Per 99 estimated ppm		1.18 (0.80–1.76)	
					Test for trend		$P = 0.41$	

Table 2.4 (continued)

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Deng *et al.* (2012) Connecticut, USA 1996–2000	518 597	Population	Standardized questionnaire on job history	NHL	Trichloroethylene exposed and IL12A_07 genotype (rs582054)			Age, race Also, significantly increased risk with formaldehyde (NHL, DLBCL) and benzene (DLBCL); IL12A was only one of 30 SNPs selected from Th1/Th2 genes involved in immune function Overlapped with Wang *et al.* (2009)
					TT genotype	14	0.70 (0.34–1.42)	
					AT/AA genotype	51	2.09 (1.28–3.42)	
					Test for interaction		$P = 0.009$	
				DLBCL	TT genotype	4	0.59 (0.19–1.85)	
					AT/AA genotype	21	2.66 (1.42–4.96)	
					Test for interaction		$P = 0.0119$	
				FL	TT genotype	4	0.82 (0.25–2.72)	
					AT/AA genotype	10	1.71 (0.78–3.77)	
					Test for interaction		$P = 0.3498$	
Christensen *et al.* (2013) Montreal 1979–85	215 2341	Population plus controls with cancer at another site (excluding lung, and seven sites with low numbers)	Face-to-face interview on lifetime occupational history and assessment by industrial hygienists	NHL (ICD-9 200, 202)	Any trichloroethylene exposure	7	1.2 (0.5–2.9)	Age, income, education, ethnicity (French-Canadian versus other), questionnaire respondent (self versus proxy) and smoking (cigarette-years)
					Substantial exposure	3	1.0 (0.3–3.5)	

DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; JEM, job-exposure matrix; MM, multiple myeloma; NHL, non-Hodgkin lymphoma

haematopoietic cancers, alcohol consumption, and race. Compared with non-exposed subjects, the risk was elevated at a medium-to-high intensity of exposure (OR, 2.2; 95% CI, 0.9–5.4), but was still not statistically significant compared with that in the group with a low level of exposure (OR, 1.1; 95% CI, 0.8–1.6; *P* for trend, 0.06). [This study overlapped with that of Deng *et al.* (2012).]

Complementary analyses were carried out to determine whether the association between non-Hodgkin lymphoma and solvent exposure was modified by variation in genes of the immune system (Deng *et al.*, 2012). Samples of blood or buccal cells were collected from 518 patients and 597 controls, and the interaction between exposure to trichloroethylene and IL12A (rs582054) genotype was assessed. Among women with AT/AA genotypes who had been exposed occupationally to trichloroethylene, increased risks were observed for non-Hodgkin lymphoma overall (OR, 2.09; 95% CI, 1.28–3.42), diffuse large B-cell lymphoma (OR, 2.66; 95% CI, 1.42–4.96), and follicular lymphoma (OR, 1.71; 95% CI, 0.78–3.77). In contrast, among women who carried the IL12A (rs582054) TT genotype and who had been exposed occupationally to trichloroethylene, the risk estimates were below one and not statistically significant. [The Working Group noted that in this analysis, IL12A was only one of 30 single-nucleotide polymorphisms selected from Th1/Th2 genes involved in immune function. This study overlapped with that of Wang *et al.* (2009).]

A case–control study in six regions in Germany included patients with malignant lymphoma (*n* = 710; participation rate, 87%) and controls (matched by sex, region, and age) recruited from population registers (*n* = 710; participation rate, 44%) (Seidler *et al.*, 2007). Interviewers collected a complete history of all jobs held for more than 1 year and specific job tasks. On the basis of job task-specific questionnaires, a trained occupational physician assessed exposure to chlorinated hydrocarbons, including trichloroethylene. Workers in the category of highest exposure to trichloroethylene (> 35 ppm-years) had an increased risk of malignant lymphoma (OR, 2.1; 95% CI, 1.0–4.8), but no significant dose–response relationship was observed (*P* for trend, 0.14). Estimated increased risks for workers in the category of highest exposure to trichloroethylene were also observed for B-cell non-Hodgkin lymphoma, T-cell non-Hodgkin lymphoma, Hodgkin lymphoma, diffuse large B-cell lymphoma, follicular lymphoma, and marginal zone lymphoma, but most precise for the B-NHL subtype (*n* = 554; OR, 2.3; 95% CI, 1.0–5.3). [The Working Group noted that the participation rate among controls was half that of cases and could represent a selection bias. In addition, the cumulative exposures appeared to have been very low.]

A multicentric case–control study on occupational exposure to trichloroethylene and lymphoma, the Epilymph study, was conducted in the Czech Republic, France, Germany, Ireland, Italy, and Spain, from 1998 to 2004 (Cocco *et al.*, 2010). The study included 2348 cases of lymphoma and 2462 controls (hospital and population-based; matched by age, sex, residence in Germany and Italy). The overall participation rates were 88% for cases, 81% for hospital controls and 52% for population controls. Face-to-face interviews were carried out to collect data on occupational history, and exposures were assessed by an industrial hygienist. Odds ratios were adjusted for age, sex, education, and study centre. No association was found between occupational exposure to trichloroethylene and any subtype of non-Hodgkin lymphoma (B-cell non-Hodgkin lymphoma, diffuse large B-cell lymphoma, or follicular lymphoma). [This was a large study with expert assessment of exposure.]

Purdue *et al.* carried out a case–control study to analyse the association between non-Hodgkin lymphoma and exposure to trichloroethylene (Purdue *et al.*, 2011). Cases came from four

Surveillance, Epidemiology, and End Results (SEER) registry areas in the USA (Iowa, Los Angeles County, Seattle, Detroit) and were diagnosed between July 1998 and June 2000 (n = 1189; participation rate, 76%). Controls were recruited from the general population (n = 982; participation rate, 52%). Subjects were interviewed face-to-face to obtain a job history. Occupational exposure to trichloroethylene was assessed by an industrial hygienist. After adjustment for age, sex, study centre, race and education, workers who had an estimated average weekly exposure of > 150 ppm-hours had an odds ratio of 2.5 (95% CI, 1.1–6.1; P for trend, 0.02). An increased risk of non-Hodgkin lymphoma was also identified for cumulative exposure exceeding 112 320 estimated ppm-hours (OR, 2.3; 95% CI, 1.0–5.0; P for trend, 0.08). [The study included a large number of subjects and an expert assessment of exposure was performed. The Working Group noted the low participation rate among controls.]

A case–control study from Montreal (described in Section 2.3.1) included 215 men diagnosed with non-Hodgkin lymphoma and 2341 population and cancer controls (Christensen *et al.*, 2013). The criteria for inclusion of cancer controls were as follows: (i) contiguous sites were excluded as controls for the index cancer series; (ii) cancer of the lung was excluded; and (iii) subsamples were constituted such that no cancer site constituted more than 20% of any series of controls. The cancer controls were selected on the principle that for any solvent:site association, the odds ratio would not be greatly biased except in the implausible scenario that a particular solvent is a risk factor for most cancer sites, which should be detectable in the analysis using population controls. The odds ratio associated with exposure to trichloroethylene was 1.2 (95% CI, 0.5–2.9; seven exposed cases) for any exposure, and 1.0 (95% CI, 0.3–3.5; three exposed cases) for substantial exposure, after adjustment for age, income, education, ethnicity, questionnaire respondent, and smoking.

(b) Other haematological malignancies

Several studies reported associations between exposure to trichloroethylene and haematological malignancies other than non-Hodgkin lymphoma. Persson *et al.* reported a non-statistically significant increased risk of Hodgkin lymphoma (crude OR, 2.0; no P-value or confidence intervals provided) related to exposure to trichloroethylene in Sweden (Persson *et al.*, 1993). The study by Seidler *et al.* (2007) in Germany (described above) reported a non-significant increased risk of Hodgkin lymphoma (OR, 2.0; 95% CI, 0.4–10.5) in the category of highest exposure. The international Epilymph study (Cocco *et al.*, 2010) did not find any association between occupational exposure to trichloroethylene and Hodgkin lymphoma, chronic lymphocytic leukaemia, or multiple myeloma. Gold *et al.* (2011) found an association between (cumulative) occupational exposure to trichloroethylene and multiple myeloma. Seidler *et al.* (2007) found no association between occupational exposure to trichloroethylene and multiple myeloma or chronic lymphocytic leukaemia. Nordström *et al.* reported a non-significant association (OR, 1.5; 95% CI, 0.7–3.3) between exposure to trichloroethylene and hairy cell leukaemia in a study in Sweden (Nordström *et al.*, 1998).

Studies of childhood cancer, including leukaemia, have also evaluated exposure to trichloroethylene. Two of these studies identified an association between childhood leukaemia and paternal exposure to trichloroethylene (Lowengart *et al.*, 1987; McKinney *et al.*, 1991). In McKinney *et al.* (1991), the odds ratios for paternal exposure to trichloroethylene during the preconception, periconception and gestational, and postnatal periods were 2.27 (95% CI, 0.84–6.16), 4.40 (95% CI, 1.15–21.01), and 2.66 (95% CI, 0.82–9.19), respectively. In the study by Lowengart *et al.* (1987), a twofold non-significant increase in risk of leukaemia was found with paternal exposure to trichloroethylene 1 year

before pregnancy (OR, 2.0; P = 0.16), during pregnancy (OR, 2.0; P = 0.16), and after delivery (OR, 2.7; 95% CI, 0.64–15.60; P = 0.07). There were no associations with maternal exposure to trichloroethylene; few mothers were occupationally exposed to trichloroethylene.

Costas *et al.* (2002) conducted a case–control study evaluating 16 cases of childhood leukaemia occurring between 1969 and 1986, and 37 matched controls in Woborn, Massachussetts where, in 1979, two of the city's eight municipal drinking-water wells were closed when tests identified contamination with solvents including trichloroethylene. A personal interview gathered information specific to risk factors for leukaemia and use of public drinking-water in the home. An exposure value was assigned to each subject according to the addresses of residence. Non-significant increased risks were observed for different indices of exposure to contaminated municipal drinking-water wells before conception and during pregnancy (Costas *et al.*, 2002).

2.3.3 Cancer of the liver

A single case–control study investigated the association between occupational exposure to trichloroethylene and cancer of the liver (Christensen *et al.*, 2013) (described in Section 2.3.1). The analysis was based on 33 cases. Odds ratios were 1.1 (95% CI, 0.1–8.5; one exposed case) for any level of exposure and 2.1 (95% CI, 0.2–18; one exposed case) for substantial exposure.

2.3.4 Other sites

(a) Cervix

A case–control study to investigate the association between occupational exposure to trichloroethylene and cancer or dysplasia of the cervix was carried out in a region of France where trichloroethylene had been widely used (Charbotel *et al.*, 2013). Exposure assessment was the same as in the French renal cancer case–control study previously described (interview with specific questionnaire and assessment by an industrial hygienist using a job task-exposure matrix). Case and control subjects (67 subjects in each group) were recruited by gynaecologists on a voluntary basis. Interviews were performed to collect information on job history and tasks. Exposure assessment was conducted by an industrial hygienist. The crude odds ratio was 1.17 (95% CI, 0.54–2.52; 31 exposed cases). After adjustment for general risk factors that correlated significantly with cervical dysplasia or cervical cancer, the odds ratio was 1.51 (95% CI, 0.42–5.41; 17 exposed cases). [The Working Group noted that the number of subjects included was small.]

(b) Rectum and colon

Two case–control studies have reported on the association between cancers of the rectum and colon and exposure to trichloroethylene. The Montreal case–control study investigated risk for cancers of the rectum and colon (Christensen *et al.*, 2013). For rectal cancer, after adjustment for age, education, respondent status, cigarette smoking, beer consumption, and body-mass index, the odds ratio was 1.8 (95% CI, 0.9–3.6) for any exposure to trichloroethylene and 0.7 (95% CI, 0.2–2.6) for substantial exposure. For colon cancer, the odds ratios were 1.0 (95% CI, 0.5–2.0) and 1.2 (95% CI, 0.5–2.7), respectively.

A case–control study in Sweden showed a small non-significant increased risk of cancer of the colon (ICD-8 code, 153.01–153.89) in subjects who had been exposed to trichloroethylene (OR, 1.5; 95% CI, 0.4–5.7) (Fredriksson *et al.*, 1989). Among dry-cleaning workers reporting an exposure to trichloroethylene, the estimated risk of cancer of the colon increased sevenfold (OR, 7.4; 95% CI, 1.1–47.0, while the odds ratio for dry-cleaning workers was 2.0 (95% CI, 0.5–7.1; five cases and five controls). The odds ratios accounted for age, sex, physical activity. [The Working Group noted the sevenfold increased

risk in trichloroethylene-exposed dry-cleaning workers. The paper did not report when these workers were employed.]

(c) Brain

In northern New Jersey and Philadelphia, Pennsylvania, USA, a case–control study was carried out based on death certificates of 300 white men who died from astrocytic cancer of the brain between 1978 and 1980, and 320 controls who died in the same period (Heineman *et al.*, 1994). Information on occupational history was obtained from next of kin, and a job-exposure matrix was used to assess exposure to organic solvents. A statistically significant association was found between astrocytic cancer of the brain and exposure to some organic solvents, but not trichloroethylene (OR, 1.1; 95% CI, 0.8–1.6; 128 exposed cases).

A hospital-based case–control study was carried out in three hospitals in the USA to investigate the association between brain tumours and occupational exposures to several solvents (Neta *et al.*, 2012). Cases were recruited between 1994 and 1998. Controls were recruited from the same hospitals. An interview was carried out by a trained nurse, and occupational exposures were assessed by an industrial hygienist. Participation rates were near 90% for cases and controls. Proxy interviews were conducted for 16% of cases of glioma, 8% of cases of meningioma, and 3% of controls. No association was found between occupational exposure to trichloroethylene and risk of glioma or meningioma. Odds ratios were equal to or under one, and non-significant whatever the category of exposure to tricholoroethylene considered.

Ruder *et al.* (2013) evaluated risk of glioma from non-farm occupational exposure (ever/never and estimated cumulative exposure) to trichloroethylene among 798 cases and 1175 population-based controls aged 18–80 years, and non-metropolitan residents of Iowa, Michigan, Minnesota, and Wisconsin, USA [farmers were included, but exposures were "non-farm" because all farmers were considered to be exposed to all chlorinated solvents]. The methodology for the exposure assessment was the same as that used in Neta *et al.* (2012). Unconditional logistic regression was used to calculate odds ratios adjusted for frequency-matching variables, age group and sex, and age and education. Ever-exposure to trichloroethylene was associated with reduced risk of glioma (OR, 0.7; 95% CI, 0.6–0.9; 302 cases and 515 exposed controls). Mean estimated cumulative exposure was lower for cases (85.9 ppm-years) than controls (98.9 ppm-years); the odds ratio for glioma was 1.0 (95% CI, 0.9–1.1). In analyses limited to 904 participant blood donors (excluding controls reporting a previous cancer diagnosis) genotyped for glutathione-*S*-transferases *GSTP1, GSTM3*, and *GSTT1*, solvent-exposed individuals with functional *GST* genes (that might convert chlorinated solvents crossing the blood–brain barrier into cytotoxic metabolites) were not at increased risk of glioma. [The limitations of this study included the high percentage of proxy respondents and the lack of measurements of solvent levels at the workplace or in serum.]

Another study aimed at identifying paternal occupations associated with an increased risk of childhood cancer of the brain, with a focus on specific exposures using an assessment by an industrial hygienist (De Roos *et al.*, 2001). The analysis included 405 case fathers and 302 control fathers. A statistically non-significant increased risk was found with paternal exposure to trichloroethylene when considering self-assessed exposure (OR, 1.4; 95% CI, 0.7–2.9; 22 exposed cases; OR adjusted by age, maternal race, maternal age, and maternal education), but it was not confirmed when the analysis was restricted to exposure as assessed by an industrial hygienist (adjusted OR, 0.9; 95% CI, 0.3–2.5).

(d) Bladder

From 1991 to 1995, a population based case–control study was carried out in five regions of Germany (West Berlin, Bremen, Leverkusen, Halle, Jena) to evaluate the risk of urothelial cancer associated with several occupational exposures, including solvents (Pesch *et al.*, 2000b). The study included 1035 histologically confirmed cases of urothelial cancer, including urinary bladder (80% of cases in females, 90% in males), ureter and renal pelvis, and 4298 randomly selected controls recruited from local residency registries. The exposure assessment was based on the subject's history of jobs held for at least 1 year and job-task descriptions. Two job-exposure matrices adapted to the specific context of the study provided an expert rating in terms of the probability and the intensity of exposure to a specific agent. To characterize lifetime exposure, exposure indices were constructed using duration, probability and intensity of exposure over all periods of work. Conditional logistic-regression models were applied for risk estimation while adjusting for tobacco smoking as a confounder. A significantly increased risk was observed in males reporting the longest time for metal degreasing (OR, 2.3; 95% CI, 1.4–3.8). When considering exposures based on the job-task exposure matrix, a significantly increased risk was identified in men exposed to trichloroethylene at the highest (substantial) level (OR, 1.8; 95% CI, 1.2–2.7) or to tetrachloroethylene (OR, 1.8; 95% CI, 1.1–3.1). [Patients were probably exposed to both trichloroethylene and tetrachloroethylene, since 48 cases were exposed to substantial levels of chlorinated solvents, 22 to substantial level of tetrachloroethylene, and 36 to substantial levels of trichloroethylene. No dose–response relationships were reported and not all important potential confounders were accounted for.]

In a case–control study in Montreal, no association was found between cancer of the bladder and exposure to trichloroethylene (Christensen *et al.*, 2013). Adjusted odds ratios were 0.7 (95% CI, 0.3–1.4) for any level of exposure, and 0.6 (95% CI, 0.2–1.5) for substantial exposure.

(e) Other sites

A case–control study investigated the association between mortality from cancer of the pancreas and exposure to trichloroethylene (Kernan *et al.*, 1999). It was based on death certificates from 24 states in the USA (63 097 cases who died between 1984 and 1993). For each case, four controls were frequency-matched by state, race, sex, and age group. Occupations and industries were coded and a job-exposure matrix was used to assess occupational exposures. A significantly increased risk of death from cancer of the pancreas was found in black females with a low (OR, 1.1;95% CI, 1.0–1.3) or medium (OR, 2.3; 95% CI, 1.3–4.0) level of exposure to trichloroethylene, and in white females with a low (ORs 1.0; 95% CI, 1.0–1.1) or high (OR, 1.1; 95% CI, 1.0–1.3) level of exposure, and in white males with a medium level of exposure (OR, 1.1; 95% CI, 1.1–1.5). In the other categories of exposure, odds ratios ranged from 0.8 to 1.2 and were not statistically significant. [In this study, exposure assessment was based on job and industry information available on the death certificate. Only the most recent occupation and type of industry held by the decedent may be reported on death certificates, although usual occupation and industry were requested. This may have increased the possibility of misclassification of exposure.]

In the case–control study in Montreal, Canada, by Christensen *et al.* (2013), exposure to trichloroethylene was assessed in relation to risk of pancreatic cancer (n = 116; two exposed cases; OR, 0.8; 95% CI, 0.2–3.6), stomach cancer (n = 251; OR for any exposure, 0.6; 95% CI, 0.2–1.8; four exposed cases; OR for substantial exposure, 0.5; 95% CI, 0.1–2.4; two exposed cases), oesophageal cancer (OR, 0.9; 95% CI, 0.1–6.7; n = 99; one exposed case); melanoma (n = 103; eight exposed

cases; OR for any level of exposure, 3.0; 95% CI, 1.2–7.2; OR for substantial exposure, 3.2; 95% CI, 1.0–9.9; five exposed cases), and prostate cancer (n = 449; fourteen exposed cases; OR for any level of exposure, 1.3; 95% CI, 0.7–2.6; OR for substantial exposure, 1.2; 95% CI, 0.5–3.1; seven exposed cases).

In a separate publication concerning the case–control study in Montreal, solvent exposure was assessed in relation to cancer of the lung (Vizcaya *et al.*, 2013). Cancer of the lung was not included in the study by Christensen *et al.* (2013), but cases were recruited during two different periods, 1980–86 and 1995–2001. A pooled analysis of both studies (Vizcaya *et al.*, 2013; Siemiatycki, 1991) included 1313 male cases and 1225 male controls. Odds ratios were adjusted for age, smoking habits, education, socioeconomic status, ethnicity, exposure to eight known carcinogens and study period. Odds ratios were 1.7 (95% CI, 0.9–3.4) for any level of exposure to trichloroethylene and 1.1 (95% CI, 0.5–2.7) for substantial exposure. When stratified by histological subtype, a significantly increased risk of adenocarcinoma was reported for substantial exposure to trichloroethylene (OR, 2.7; 95% CI, 1.0–7.6).

2.4 Ecological studies

Several ecological studies have evaluated the risk of cancer associated with consumption of drinking-water contaminated by trichloroethylene and tetrachloroethylene; however, since these chemicals are volatile, exposure may occur through other routes, such as inhalation. One study evaluated indoor air pollution (ATSDR, 2006), and another study did not specify exposure route (Coyle *et al.*, 2005). Despite the different methodologies and cancer sites evaluated, these studies shared several limitations in the exposure assessments carried out, which suggested that the results should be interpreted with caution. First, no estimates of personal exposure were made; although measurements in the environment were taken in some studies, all exposure estimates were based on ecological approaches based on the address of residence. Second, the measurements available were mostly taken at the same time as the diagnosis or death, contributing to exposure misclassification. Third, most of the studies were based on residence at time of diagnosis or death, assuming that study subjects had been living in the same place for the relevant time-points of exposure. Fourthly, since exposure to trichloroethylene usually occurred simultaneously with exposure to tetrachloroethylene, other solvents and volatile chemicals, it was difficult to attribute the observed effects to a single chemical. Finally, the lack of covariates and adjustment for potential confounders in ecological studies may lead to confounding in risk estimates.

An ecological study was carried out in the Endicott area (Broome County, New York, USA), which had experienced contamination of groundwater by volatile organic compounds (VOCs), including trichloroethylene and tetrachloroethylene, originating from leaks, spills and runoff from local landfills (ATSDR, 2006, 2008). In some areas, groundwater pollution contaminated the adjacent soil vapour, which migrated through the soil into structures through cracks in building foundations (soil vapour intrusion). In the eastern study area, trichloroethylene was the most commonly found vapour intrusion-related contaminant in indoor air, at concentrations ranging from 0.18 to 140 mg/m^3. In the western study area, tetrachloroethylene was the most commonly found vapour intrusion-related contaminant in indoor air, at concentrations ranging from 0.1 to 3.5 mg/m^3. The study was conducted to evaluate the incidence of cancer in 1980–2001 and determine whether rates in the Endicott area differed from those in the rest of the state for the same years. Incidence data were obtained from the New York State cancer registry, and age-adjusted standardized incidence ratios

were calculated by dividing the observed number of cancer cases by the expected number. The total number of cancers for the study period (n = 347) was similar to that expected. The incidence of cancer of the testes was significantly elevated in the western study area (where concentrations of trichloroethylene were lower), while cancer of the kidney in men was significantly elevated in the eastern study area (where concentrations of trichloroethylene were higher). For the two geographical areas combined, the incidence of cancer of the testes, and incidence of cancer of the kidney in men and women combined, was significantly elevated (ATSDR, 2006). Childhood cancer (including ages 0–19 years) was evaluated separately. No significant increase in the incidence of leukaemia among children was noted in the study areas, nor was there any significant elevation in the incidence of overall or specific cancers among children during this period. The re-evaluation conducted in 2008 (ATSDR, 2008) limited the analysis to white individuals and showed little differences in overall cancer rates or standardized incidence ratios. The only difference was that cancer of the lung was borderline statistically significantly elevated. This re-evaluation showed also evidence for increased prevalence of smoking among those with cancer of the kidney, and some indication that several individuals diagnosed with testicular or kidney cancer may have been recent arrivals to the study area.

Coyle *et al.* (2005) conducted an ecological study in Texas, USA, to evaluate the influence of releases of some industrial chemicals, including trichloroethylene and tetrachloroethylene, on the incidence of cancer of the breast in 1995–2000. Assessment of exposure from air used data from the Toxic Release Inventory (a publicly accessible database compiled by the United States Environmental Protection Agency (EPA)). Counties were classified as exposed for a specific chemical if a release was reported to the Toxic Release Inventory. A total of 54 487 cases were identified from the Texas cancer registry. Counties reporting releases of trichloroethylene showed statistically significant increases in age-adjusted incidence of cancer of the breast compared with counties without reported releases (P = 0.010). Counties reporting releases of tetrachloroethylene also showed a higher incidence of cancer of the breast (P = 0.038). Specific exposure routes and levels of exposure were not provided (Coyle *et al.*, 2005).

Morgan & Cassady (2002) evaluated incident cases of cancer diagnosed between 1 January 1988 and 21 December 1998 in San Bernardino County, covering the greater Redlands area, USA. The drinking-water supply for the city of Redlands was contaminated by trichloroethylene and tetrachloroethylene, as confirmed by monitoring of wells from 1980, which detected trichloroethylene at concentrations of > 5 parts per billion (ppb). Concentrations of tetrachloroethylene in 2001 ranged from 5 to 98 ppb, although the city of Redlands had not delivered water containing tetrachloroethylene in excess of 18 ppb since testing began. The observed number of cases of cancer divided by the expected number defined the standardized incidence ratio, which was calculated for all cancer sites and 16 site-specific cancers. All cancer sites combined accounted for 3098 cases. More cases than expected were observed for cancer of the uterus (n = 124; SIR, 1.35 [95% CI, 1.06–1.70]) and skin melanoma (n = 137; SIR, 1.42 [95% CI, 1.13–1.77]). There were no significant differences between observed and expected numbers of cases for all cancers [combined], cancer of the thyroid (n = 40), or 11 other cancers. Significantly fewer than expected cases of cancer of the lung and bronchus were observed (n = 356; SIR, 0.71 [95% CI, 0.61–0.81]), and of cancer of the colon and rectum (n = 327; SIR, 0.86 [95% CI, 0.74–0.99]) (Morgan & Cassady, 2002).

A study conducted in 1979–87 in New Jersey, USA, included 75 towns (Cohn *et al.*, 1994), of which 27 were included in a study reported by Fagliano *et al.* (1990). Concentrations of

trichloroethylene were measured in 1984–85, and an average concentration was assigned to each town. The highest concentration assigned was 67 µg/L. Co-existing chemicals included tetrachloroethylene (maximum, 14 ppb) and other volatile chemicals. The water supply of six towns contained trichloroethylene at concentrations of > 5 µg/L (average, 23.4 µg/L). The total incidence of leukaemia in women in these towns was significantly higher than in towns where the concentration of trichloroethylene in drinking-water was < 0.1 µg/L (RR, 1.4; 95% CI, 1.1–1.9); no such effect was seen for men (RR, 1.1; 95% CI, 0.84–1.4). The risk for women was particularly elevated for acute lymphocytic leukaemia, chronic lymphocytic leukaemia, and chronic myelogenous leukaemia. The risk of acute lymphocytic leukaemia in childhood was also significantly increased, in girls but not in boys. Increased risks of non-Hodgkin lymphoma were apparent in towns in the highest category of contamination with trichloroethylene (RR, 0.2; 0.94–1.5 for men; and RR, 1.4; 95% CI, 1.1–1.7 for women) and was particularly elevated for high-grade lymphoma.

Vartiainen *et al.* (1993) collected 24-hour urine samples from 95 and 21 inhabitants of two Finnish villages where the groundwater was contaminated with trichloroethylene (≤ 212 µg/L) and tetrachloroethylene (≤ 180 µg/L). The average excretion of trichloroethylene by inhabitants of the two villages was 0.55 and 0.45 µg/day, and that of two control groups was 0.36 and 0.32 µg/day; the corresponding figures for excretion of dichloroacetic acid were 0.78 and 1.3 µg/day versus 1.3 and 1.3 µg/day, and those for the excretion of trichloroacetic acid were 19 and 7.9 µg/day versus 2.0 and 4.0 µg/day. With the possible exception of non-Hodgkin lymphoma, which occurred in marginal excess in one of the villages (SIR, 1.4; 95% CI, 1.0–2.0; 31 cases), but not in the other (0.6; 95% CI, 0.3–1.1; 14 cases), neither overall cancer incidence nor the incidence of cancer of the liver or lymphohaematopoietic cancers was increased in the two villages compared with the control groups (Vartiainen *et al.*, 1993).

Studies were conducted in two counties in Arizona, USA, to address the possible association between consumption of drinking-water from trichloroethylene-contaminated wells and childhood leukaemia (Maricopa County, Flood *et al.*, 1990), or all childhood cancers and cancer of the testes (Pima County, Kioski *et al.*, 1990). In Maricopa County, two wells that were occasionally used to supplement the water supply were found to contain trichloroethylene at concentrations of 8.9 and 29.0 ppb [mg/L] in 1982; they were then taken out of service. The concentrations of trichloroethylene in contaminated municipal wells in Pima County were 1–239 ppb, with levels as high as 4600 ppb in wells at an Air Force facility in the area. No association was found between cancer at any of the sites examined and residence in the counties with contaminated wells, as opposed to residence in other areas of the county. The incidence rates in Maricopa and Pima counties were similar to those in other areas included in the United States SEER programme (Arizona Department of Health Services, 1995)

Mallin (1990) investigated incident cases of and deaths from cancer of the bladder among residents of eight north-western Illinois counties where a cluster of cases of cancer of the bladder had been observed in 1978–85. Incidence data from the Illinois State cancer registry (available from 1985) and medical records from hospitals in the study area were abstracted to identify incident cases. Expected numbers of cases were based on census data. Age-adjusted standard incidence ratios were calculated by county of residence and zip code (n = 97). Results revealed no excess risks by county, but there were two zip codes associated with significantly elevated risks, one of these had a significant excess in men (SIR, 1.5; 95% CI, 1.1–1.9) and women (SIR, 1.9; 95% CI, 1.2–2.8). This excess was primarily confined to one town in which standardized incidence ratios were

significantly elevated in men (SIR, 1.7; 95% CI, 1.1–2.6) and women (SIR, 2.6; 95% CI, 1.2–4.7). Further investigation revealed that one of four public drinking-water wells in this town had been closed due to contamination and that tests of two other wells revealed traces of trichloroethylene, tetrachloroethylene, and other solvents (Mallin, 1990).

Lagakos *et al.* (1986) studied childhood leukaemia in Woburn, a community in Massachusetts, USA, where water from two wells was contaminated with trichloroethylene and tetrachloroethylene, as well as other chemicals. Measurements made in 1979 showed that well-water contained trichloroethylene at a concentration of 267 ppb [µg/L], tetrachloroethylene at 21 ppb, and also arsenic at 2 ppb and chloroform at 11.8 ppb. [Exposure was estimated by algorithm.] Twenty cases of childhood leukaemia were diagnosed in the community in 1964–83, and these children had a significantly higher estimated cumulative exposure to water from the two contaminated wells than a random sample of children from the community (observed cumulative exposure, 21.1; expected cumulative exposure, 10.6; $P = 0.03$) (Lagakos *et al.* 1986).

Isacson *et al.* (1985) tabulated the average annual age-adjusted incidence rates for cancers of the bladder, breast, colon, lung, prostate or rectum per 100 000 population in towns in Iowa, USA, in 1969–81, by concentration of detectable VOCs in finished ground-water supplies. The concentrations of trichloroethylene were < 0.15 µg/L in one group of areas, and ≥ 0.15 µg/L in another. The concentrations of tetrachloroethylene were < 0.30 µg/L in one group of areas, and ≥ 0.30 µg/L in another. Other volatile chemicals present were 1,2-dichloroethane and 1,1,1-trichloroethane. There were virtually no differences in cancer incidence between these two groups for trichloroethylene and tetrachloroethylene (Isacson *et al.*, 1985).

2.5 Meta-analyses and pooled analyses

2.5.1 Cancer of the kidney and non-Hodgkin lymphoma

Several meta-analyses of the epidemiological literature on risk of cancer among persons exposed to trichloroethylene were available to the Working Group. The Working Group selected for discussion those meta-analyses that were recent and comprehensive, and assembled, presented, or analysed the literature in ways beyond the text of the individual publications, and that provided new information to the Working Group for their evaluation.

The EPA conducted a meta-analysis of epidemiological studies focusing on non-Hodgkin lymphoma and cancers of the kidney and liver as part of its evaluation of the carcinogenicity of trichloroethylene (Scott & Jinot, 2011). The meta-analysis followed an approach recommended by the National Research Council (2006). Criteria for inclusion in the meta-analysis included: (1) cohort or case–control design; (2) appropriate comparability of exposed and unexposed in cohort studies, and cases and controls in case–control studies; (3) potential for exposure to trichloroethylene and an actual estimate of exposure for individuals in the study; and (4) estimates of relative risk for non-Hodgkin lymphoma and cancers of the liver and kidney. Twenty-four studies met the inclusion criteria. Fixed- and random-effects models were fitted to data on overall exposure and on the highest exposure group. Sensitivity analyses examined the influence of individual studies and selection of alternative risk estimates from the publications. [It is important to point out that two studies with very high relative risks, (Henschler *et al.*, 1995, and Vamvakas *et al.*, 1998), were not included in this meta-analysis as they did not meet the inclusion criteria due to incomplete cohort identification or potential selection bias of study

controls.] Although information on smoking was not available in all the studies included, the meta-relative-risk (meta-RR) for cancer of the lung was 0.96 (95% CI, 0.76–1.21) indicating that, overall, smoking was not elevated among the individuals exposed to trichloroethylene in these studies. Fixed- and random-effects models were used in the primary analyses to calculate summary meta-relative-risk estimates for overall exposure to trichloroethylene and for the groups with highest exposure. Sensitivity analyses were conducted to evaluate the effect of including alternative risk estimates (when multiple estimates were available from a study) and to examine the impact of individual studies on the summary estimates. Heterogeneity among the studies was assessed using the Q-statistic and inconsistency between studies was assessed with the I^2 value. Publication bias was assessed in several ways, including funnel plots, the "trim and fill" procedure, forest plots of studies sorted by standard error, and cumulative meta-analyses of studies sorted by standard error. There was no major heterogeneity in overall exposure for any cancer. No single study was overly influential for any cancer. There was no evidence of publication bias for cancers of the kidney and liver, but there was a relationship between relative risk and study size for non-Hodgkin lymphoma. Overall meta-RRs for those exposed to trichloroethylene were 1.27 (95% CI, 1.13–1.43) for cancer of the kidney, 1.29 (95% CI, 0.07–1.56) for cancer of the liver and intrahepatic bile ducts, and 1.23 (95% CI, 1.07–1.42) for non-Hodgkin lymphoma. An adjustment technique to control for possible publication bias reduced the meta-RR for non-Hodgkin lymphoma to 1.15 (95% CI, 0.97–1.36). Meta-RRs for individuals in the groups with higher exposure for each study were higher than all-exposed combined. The meta-RRs in the categories of highest exposure were 1.58 (95% CI, 1.28–1.96) for cancer of the kidney, and 1.64 (95% CI, 1.31–2.04) when combining the 10 studies that reported results by exposure level), but similar for liver and intrahepatic bile ducts (1.28; 95% CI, 0.93–1.77), and 1.43 (95% CI, 1.13–1.82) for non-Hodgkin lymphoma.

Karami *et al.* (2012) conducted a meta-analysis of 15 cohort studies and 13 case–control studies evaluating the risk of cancer of the kidney associated with occupational exposure to trichloroethylene. [The Working Group noted that this meta-analysis largely overlapped with that by Scott & Jinot (2011), but included a slightly different set of studies due to updates or new publications]. Karami *et al.* (2012) included studies that specifically evaluated exposure from trichloroethylene, degreasing agents, or chlorinated solvents. Studies were classified by exposure to either trichloroethylene or chlorinated solvents. Studies of dry-cleaning workers were excluded, except for the one study that focused specifically on trichloroethylene. Also excluded were studies reporting proportionate mortality ratios only, and those not providing confidence intervals or numbers of observed and expected cases. Meta-RRs were estimated using a random-effects model. Heterogeneity across studies was evaluated by Higgin's I^2 statistic and Cochrane's Q test. No evidence for publication bias was found using Egger and Begg methods and funnel plots. The meta-RR for cancer of the kidney from cohort studies was 1.41 (95% CI, 0.98–2.05), and 1.26 (95% CI, 1.02–1.56) when the study by Henschler *et al.* (1995) was excluded. The meta-RR for case–control studies was 1.55 (95% CI, 1.18–2.05), and 1.35 (95% CI, 1.17–1.57) when the study by Vamvakas *et al.* (1998) was excluded. The combined relative risk for cohort and case–control studies was 1.41 (95% CI, 1.16–1.70). The meta-RR for exposure to chlorinated solvents was 1.13 (95% CI, 0.70–1.83). [The Working Group noted that exposure to other chlorinated solvents did not explain the association between trichloroethylene and cancer of the kidney in cohort studies, but it might in case–control studies.] The meta-RR from case–control studies was 1.96 (95% CI, 1.24–3.08)

for high-exposure groups and 1.55 (95% CI, 1.05–2.28) for low-exposure groups. Evaluation by year of publication found that meta-RRs were stronger when more recent publications were included, and it was suggested that this might reflect improved exposure assessment and less exposure misclassification. [The Working Group noted that studies with information on tobacco use were not selected for a separate analysis, although some case–control studies could adjust for smoking. The Working Group also noted that this meta-analysis included a subgroup of the exposed population from the Danish study.]

An early review by Wartenberg *et al.* (2000) found a meta-RR of 1.7 (95% CI, 1.1–2.7) for cancer of the kidney, and a meta-RR of 1.5 (95% CI, 0.9–2.3) for non-Hodgkin lymphoma. Kelsh *et al.* (2010) reviewed 23 studies on trichloroethylene and reported meta-RRs for cancer of the kidney of 1.24 (95% CI, 1.06–1.45) from all cohort studies with outlier data removed, 1.57 (95% CI, 1.06–2.30) for case–control studies, 1.34 (95% CI, 1.07–1.67) for cohort studies with higher-quality exposure data, and 0.88 (95% CI, 0.58–1.33) for cohort studies with lower-quality exposure data with outlier data removed. Mandel *et al.* (2006) in a meta-analysis of 18 studies (14 cohort and 4 case–control) of non-Hodgkin lymphoma reported meta-RRs of 2.33 (95% CI, 1.39 to 3.91) for non-Hodgkin lymphoma from studies with higher-quality exposure data, 0.84 (95% CI, 0.73–0.98) from studies with lower-quality exposure data, and 1.39 (95% CI, 0.62–3.10) from case–control studies.

2.5.2 Other cancers

In the meta-analysis by Wartenberg *et al.* (2000) (cited above), studies were classified into tier I (studies in which exposure to trichloroethylene is best characterized and exposure inferred for individual study subjects). For tier I studies, meta-RRs were 1.5 (95% CI, 0.6–3.7) for Hodgkin lymphoma, 1.0 (95% CI, 0.5–2.1) for leukaemia, 1.5 (95% CI, 0.7–3.3) for multiple myeloma, 1.9 (95% CI, 1.0–3.4) for cancer of the liver, 1.0 (95% CI, 0.6–1.6) for cancer of the bladder, 2.4 (95% CI, 1.2–4.8) for cancer of the cervix, and 0.8 (95% CI, 0.6–1.1) for cancer of the lung. For tier II studies (studies in which there is putative exposure to trichloroethylene, but individuals are not identified as uniquely exposed to trichloroethylene), meta-RRs were 1.0 (95% CI, 0.5–2.1) for cancer of the bladder, and 0.6 (95% CI, 0.3–1.3) for cancer of the lung.

Alexander *et al.* (2007) reported meta-RRs for higher quality studies of 1.30 (95% CI, 1.09–1.55) for cancers of the liver and biliary tract combined, and 1.41 (95% CI, 1.06–1.87) for primary cancer of the liver. The meta-RR for lower-quality studies was 0.87 (95% CI, 0.55–1.38) for cancers of the liver and biliary tract. Alexander *et al.* (2006) reported a meta-RR of 1.05 (95% CI, 0.80–1.38) for multiple myeloma, and 1.11 (95% CI, 0.93–1.32) for leukaemia.

[The Working Group noted that the lack of an excess for cancers of the lung and bladder indicated that smoking did not explain the excesses observed for other cancers; if individuals exposed to trichloroethylene were heavier smokers than the comparison populations, an excess of cancer of the lung would be observed.]

3. Cancer in Experimental Animals

Several recent reports have summarized the evidence for carcinogenicity associated with exposure to trichloroethylene (EPA, 2011a, b; NTP, 2011a). In most studies in rodents (i.e. rats and mice, with one study in hamsters), trichloroethylene has been administered by inhalation, or orally by gavage rather than in drinking-water, because of its volatility.

Studies of exposure to trichloroethylene in rats and mice are summarized in Table 3.1 and Table 3.2, respectively. Information is provided

on the experimental paradigm, tumour incidence, statistical significance of findings, and comments. These studies were carried out over 26 years and varied with regard to completeness of reporting, statistics performed, and nomenclature used to describe tumour pathology. In some earlier studies, the number of individual tumour diagnoses was presented for each exposure group (rather than the incidence of tumour-bearing animals). The presence of stabilizers is indicated due to concerns regarding their potential contribution to carcinogenicity.

3.1 Mouse

3.1.1 Oral administration

Groups of 50 male and 50 female B6C3F$_1$ mice (age, 8 weeks) were given epichlorohydrin-free trichloroethylene at a dose of 0 or 1000 mg/kg bw per day by gavage in corn oil, 5 days per week, for 103 weeks (NTP, 1990). Survival was poor in males and considered to have resulted from "toxic nephropathy." [The Working Group noted the limited power of the study due to the use of a single dose and the premature mortality observed.]

Groups of male and female B6C3F$_1$ mice (age, 35 days) were given commercial-grade trichloroethylene (containing epichlorohydrin as a stabilizer) by gavage in corn oil at time-weighted average doses of 0 (n = 20), 1169 (n = 50), or 2339 (n = 50) mg/kg bw per day for males, and 0 (n = 20), 869 (n = 50), or 1739 (n = 50) mg/kg bw per day for females, 5 days per week; the mice were exposed non-continuously at variable concentrations for 78 weeks, and surviving animals were killed at 90 weeks (NCI, 1976). Survival was lower in males than in females, and to a greater extent, in treated versus vehicle controls (male mortality before termination: 12 out of 20 controls, 15 out of 50 at the lower dose, and 27 out of 50 treated with the higher dose; female mortality: 0 out of 20 controls, 8 out of 50 at the lower dose, and 8 out of 47 at the higher dose). The decreased survival in male mice treated with trichloroethylene at the higher dose was attributed to the presence of hepatocellular tumours. [The Working Group noted that "toxic nephrosis" may have also contributed to premature mortality]. An increased incidence of hepatocellular carcinoma was reported in males and females with decreased latency of tumour development also reported for males at the higher dose. Increased compound-related "toxic nephrosis" was reported in both sexes (> 90% at both doses in both sexes), but without further definition by the study authors. While control males were noted to have specific diagnoses of hydronephrosis, chronic inflammation, pyelonephritis, and amyloidosis, none were diagnosed with "toxic nephrosis." No kidney pathology was noted for female controls. A single male treated with the higher dose was diagnosed with a rare renal tubule adenoma. The incidence of malignant lymphoid tissue tumours and bronchiolo-alveolar tumours was elevated in both sexes, although not significantly. [The Working Group noted the low number of controls (n = 20), some reporting deficiencies, and early mortality in trichloroethylene-treated groups that decreased the sensitivity of the assay to detect a response. In addition, study animals were housed in rooms together with animals exposed to volatile agents, but the Working Group did not find an apparent effect on the tumour response.]

Groups of male B6C3F$_1$ mice (age, 8 weeks) were given commercial-grade trichloroethylene at doses of 0 (n = 10) or 800 (n = 75) mg/kg bw per day by gavage in corn oil, 5 days per week, for 76 weeks (Anna *et al.*, 1994). [No indication was given regarding purity or presence of stabilizers in the trichloroethylene administered, but commercial-source information was provided.] Only liver was examined for histopathology. Treatment with trichloroethylene increased the incidence of hepatocellular adenoma and hepatocellular carcinoma (P < 0.05). [The Working

Table 3.1 Studies of carcinogenicity with trichloroethylene in mice

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours (number or tumour bearing animals/total number of animals)	Significance	Comments
Mouse, B6C3F$_1$ (M, F) 103 wk NTP (1990)	Gavage 0, 1000 mg/kg bw in corn oil 1/day, 5 days/wk, 50/group per sex	*Males*		Epichlorohydrin-free trichloroethylene (purity, 99.9%) Significantly reduced survival of treated males ($P = 0.004$) (toxic nephropathy). Decreased latency of hepatocellular tumours in exposed males; hepatocellular carcinomas observed after 57 wk in treated group and in controls after 75 wk. Statistical tests: compared with controls: [a]life table, [b]incidental tumour test, [c]Fisher exact test
		Hepatocellular adenoma: 7/48, 14/50[a,b]	[a]$P = 0.002$, [b]$P = 0.048$	
		Hepatocellular carcinoma: 8/48, 31/50[a,b,c]	[a]$P < 0.001$, [b]$P < 0.001$, [c]$P < 0.001$	
		Hepatocellular adenoma or carcinoma (combined): 14/48, 39/50[a,b,c]	[a]$P < 0.001$, [b]$P < 0.001$, [c]$P < 0.001$	
		Harderian gland adenoma: 0/48, 4/48[a]	[a]$P = 0.044$	
		Renal tubule adenoma: 1/49, 0/50	NS	
		Renal tubule carcinoma: 0/49, 1/40	NS	
		Females		
		Hepatocellular adenoma: 4/48, 16/49[a,b,c]	[a]$P < 0.001$, [b]$P < 0.001$, [c]$P < 0.003$	
		Hepatocellular carcinoma: 2/48, 13/49[a,b,c,]	[a]$P < 0.001$, [b]$P < 0.002$, [c]$P < 0.002$	
		Hepatocellular adenoma or carcinoma (combined): 6/48, 22/49[a,b,c]	[a]$P < 0.001$, [b]$P < 0.001$, [c]$P < 0.001$	
		Malignant lymphoma: 7/48, 13/49[a]	[a]$P = 0.047$	
		Lymphoma or leukaemia (combined): 7/48, 14/49[a]	[a]$P = 0.032$	
		Leukaemia: 0/48, 1/49	NS	
		Bronchioloalveolar adenoma: 0/48, 4/48[a]	[a]$P = 0.040$	
		Bronchioloalveolar carcinoma: 1/48, 0/48	NS	
		Harderian gland adenoma: 0/48, 3/49	NS	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours (number or tumour bearing animals/total number of animals)	Significance	Comments
Mouse, B6C3F$_1$ (M, F) 90 wk NCI (1976)	Gavage 0, 1169, 2339 mg/kg bw in corn oil (M) 0, 869, 1739 mg/kg bw in corn oil (F) 1/d, 5 d/wk for 78 wk 20, 50, 50	*Male*		Commercial-grade trichloroethylene (purity > 99%), stabilized with 0.09% epichlorohydrin and 0.19% 1,2-epoxybutane. Initial doses of 1000 and 2000 mg/kg bw for males and 700 and 1400 mg/kg bw for females were increased after 12 wk (TWA doses, 1169 and 2339 mg/kg bw for males and 869 and 1739 mg/kg bw for females). Study animals were housed with animals exposed to volatile agents. For males, 12/20 controls, 15/50 low-dose, and 27/50 high-dose died before scheduled termination. For females, 0/20 controls, 8/50 low-dose, and 8/47 high-dose died before scheduled termination. Increased mortality in treated males was attributed to the hepatocellular tumours. In highest-dose male mice, the first hepatocellular carcinomas were seen at 27 wk; nine others were found in males dying by wk 78. Early occurrence was not observed in low-dose males or in females. Agent-related increase in the incidence of "toxic nephrosis" (> 90% at both doses in both sexes). Elevated incidences of malignant lymphoid tissue tumours and bronchioloalveolar tumours in both sexes, although not significant. Statistical tests: Trend: [d]age-adjusted tests (Tarone) for linear trend Compared with control: [e]differences between treated and matched control: modifications of Cox and Tarone tests.
		Renal tubule adenoma: 0/20, 0/50, 1/48	NS	
		Hepatocellular carcinoma: 1/20[d], 26/50[e], 31/48[e]	[d]$P < 0.001$, [e]$P = 0.04$, [e]$P = 0.001$	
		Malignant lymphoma, reticulum-cell sarcoma or lymphosarcoma [malignant lymphoid tissue tumours]: 1/20, 4/50, 2/48	NS	
		Bronchioloalveolar adenoma: 0/20, 5/50, 1/48	NS	
		Bronchioloalveolar carcinoma: 0/20, 0/50, 1/48	NS	
		Bronchioloalveolar adenoma or carcinoma (combined): 0/20, 5/50, 2/48	NS	
		Female		
		Hepatocellular carcinoma: 0/20[d], 4/50, 11/47[e]	[d]$P = 0.002$, [e]$P = 0.008$	
		Malignant lymphoma, reticulum-cell sarcoma or lymphosarcoma [malignant lymphoid tissue tumours]: 1/20, 5/50, 6/47	NS	
		Bronchioloalveolar adenoma: 1/20, 2/50, 5/47	NS	
		Bronchioloalveolar carcinoma: 0/20, 2/50, 2/47	NS	
		Bronchiolo alveolar adenoma or carcinoma (combined): 1/20, 4/50, 7/47	NS	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours (number or tumour bearing animals/total number of animals)	Significance	Comments
Mouse, B6C3F$_1$ (M) 76–77 wk Anna *et al.* (1994)	Gavage 0, 800 mg/kg bw in corn oil, 1/d, 5 d/wk for 76 wk 10, 75	Hepatocellular adenoma: 2/10, 50/75* Hepatocellular adenoma (average number of tumours/mouse): 0.2/mouse, 1.27/mouse Hepatocellular carcinoma: 0/10, 30/75* Hepatocellular carcinoma (average number of tumours/mouse): 0/mouse, 0.57/mouse	*[$P < 0.05$] NR *[$P < 0.05$] NR	Trichloroethylene, commercial grade. No indication of stabilizer presence or purity. Anna *et al.* (1994): text reports 800 mg/kg bw as daily exposure level, but Table 1 reports 1700 mg/kg bw. Only liver tumours were examined histopathologically. All treated mice were alive at wk 76.
Mouse, B6C3F$_1$ (M) 79 wk Bull *et al.* (2002)	Gavage 0, 1000 mg/kg bw in 5% aqueous solution of Alkamuls, 1/d, 7 d/wk 15, 40	Liver, gross lesions identified as "tumours": 3/15, 33/40* Hepatocellular adenoma: 2/15, 23/36 Hepatocellular carcinoma: 1/15, 7/36	*$P < 0.05$ NR NR	Purity and source, NR. Only liver tumours were examined. Statistics: Tumour incidence compared using Fisher exact test.
Mouse, B6C3F$_1$ (M) 61 wk Herren-Freund *et al.* (1987)	Drinking-water 0 (NaCl control), 22–40 mg/l [volatilization reduced concentration from 40 to 22 mg/L after 3 d] 22, 32	Hepatocellular adenoma: 2/22, 3/32 Hepatocellular carcinoma: 0/22, 3/32	NS NS	Purity, 99%. No indication of stabilizer addition. Only liver tumours were examined histopathologically.
Mouse, Swiss Ha/ICR (M, F) 89 wk Van Duuren *et al.* (1979)	Gavage 1 d/wk 0 (control), 0.5 mg/mouse in 0.1 mL trioctanoin 30/group	Forestomach papilloma: 0/30, 1/30 (M) 0/30, 2/30 (F)	 NS NS	Industrial grade. Purified, with no indication of stabilizer addition. Only lung, liver and stomach were examined histopathologically; only data for forestomach tumours were presented.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours (number or tumour bearing animals/total number of animals)	Significance	Comments
Mouse, B6C3F$_1$ (M, F) Lifespan Maltoni *et al.* (1986, 1988) Male (Exp. BT306 bis) Female (Exp. BT306)	Inhalation 0, 100, 300, 600 ppm, 7 h/d, 5 d/wk for 78 wk 90 M and 90 F/group	Hepatoma [malignant] (M): 17/90, 20/90, 27/90, 21/90 Hepatoma [malignant] (F): 3/90, 4/90, 4/90, 9/90 Pulmonary tumours [hyperplasia, adenoma and adenocarcinoma] (F): 4/90, 6/90, 7/90, 15/90* Pulmonary adenocarcinoma (M): 0/90, 1/90, 0/90, 0/90	NS NS *$P < 0.05$ (Fisher exact test) NS	Highly purified and epoxide-free trichloroethylene was used with butyl-hydroxy-toluene added as stabilizer. Experiment [using mice from NCI source] (BT306) was repeated with males [from Charles River Laboratory] (BT306 bis) due to excessive mortality in males from fighting. The incidence of hepatoma increased in males (BT306 bis) and females (BT306). Incidence of pulmonary tumours increased in females primarily from later-stage adenomas. The previous IARC Working Group (IARC, 1995) found dose-related increases in the incidence of pulmonary tumours in females (Cochran-Armitage linear trend text).
Mouse, Swiss (M, F) Lifespan Maltoni *et al.* (1986, 1988) (Exp. BT305)	Inhalation 0, 100, 300, 600 ppm, 7 h/d, 5 d/wk for 78 wk 90/group/sex	Hepatoma [malignant] (M): 4/90, 2/90, 8/90, 13/90* Pulmonary tumours [hyperplasia, adenoma and adenocarcinoma] (M): 10/90, 11/90, 23/90*, 27/90** Pulmonary adenocarcinoma (M): 0/90, 0/90, 0/90, 1/90 Hepatoma [malignant] (F): 0/90, 0/90, 0/90, 1/90 Pulmonary tumours [hyperplasia, adenoma and adenocarcinoma] (F): 15/90, 15/90, 13/90, 20/90	*$P < 0.05$ (Fisher exact test) *$P < 0.05$, **$P < 0.01$ (Fisher exact test) NS NS NS	Highly purified (purity, 99.9%) and epoxide-free trichloroethylene was used with butyl-hydroxy-toluene added as stabilizer. The previous IARC Working Group (IARC, 1995) found dose-related increases in the incidences of lung and liver tumours in males (Cochran-Armitage linear trend test).

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours (number or tumour bearing animals/total number of animals)	Significance	Comments
Mouse, Swiss (M, F) Lifespan Maltoni *et al.* (1986, 1988) (Exp. BT303)	Inhalation 0, 100, 600 ppm, 7 h/d, 5 d/wk for 8 wk 100, 60, 72	Hepatoma [malignant] (M): 1/100, 3/60, 4/72	NS	Highly purified (purity, 99.9%) and epoxide-free trichloroethylene, with butyl-hydroxy-toluene added as stabilizer.
Mouse, Crj:CD-1 (ICR) (F) 107 wk Fukuda *et al.* (1983)	Inhalation 0, 50, 150, 450 ppm, 7 h/d, 5 d/wk, for 104 wk 50/group	Lung adenocarcinoma: 1/49, 3/50, 8/50*, 7/46*	*$P < 0.05$	Reagent-grade trichloroethylene (purity, 99.8%) containing 0.13% carbon tetrachloride, 0.02% benzene and 0.02% epichlorohydrin. Only one hepatocellular adenoma at highest dose, none in other dose groups).Treatment-related increase in lung tumours observed. The previous IARC Working Group (IARC, 1995) found a significant dose–response trend: $P = 0.034$, Cochran-Mantel-Haenszel test.

d, day; mo, month; NR, not reported; NS, not significant; wk, week; yr, year.

Table 3.2 Studies of carcinogenicity with trichloroethylene in rats

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours (number or tumour bearing animals/total number of animals)	Significance	Comments
Rat, F344/N (M, F) 103 wk NTP (1990)	Gavage 0, 500, 1000 mg/kg bw in corn oil, 1/d, 5 d/wk 50/group per sex	*Males* Kidney carcinoma: 0/48[d,e,f], 0/49, 3/49[a,b] Kidney adenoma or carcinoma (combined): 0/48[d,e], 2/49, 3/49[a,b] Peritoneum, malignant mesothelioma: 1/50, 5/50[a], 0/49 Peritoneum, all mesothelioma: 1/50, 5/50[a], 1/49	[d]$P = 0.009$, [e]$P = 0.009$, [f]$P = 0.038$, [a]$P = 0.028$, [b]$P = 0.028$ [d]$P = 0.019$, [e]$P = 0.030$, [a]$P = 0.028$, [b]$P = 0.028$ [a]$P = 0.042$ [a]$P = 0.042$	Epichlorohydrin-free trichloroethylene (purity, 99.9%). Significantly reduced survival in trichloroethylene-treated groups compared with vehicle controls due to chronic "toxic nephropathy". High mortality due to gavage error: 1 male control, 3 low-dose males, 10 high-dose males, 2 female controls, 5 low-dose females, and 5 high-dose female rats were killed. Renal tubular adenocarcinoma occurred in a single female at the highest dose. Statistical tests: Compared with controls: [a]life table, [b]incidental tumour test, [c]Fisher exact test Trend tests: [d]life table, [e]incidental tumour test, [f]Cochran-Armitage trend test.
Rat, Osborne-Mendel (M, F) 110 wk NCI (1976)	Gavage 0, 549, 1097 mg/kg bw in corn oil, 1/d, 5 d/wk for 78 wk Non-continuous exposure (cycle of 1 wk untreated followed by 4 wk of treatment) 20, 50, 50	*Males* Kidney carcinoma: 0/20, 1/50, 0/50 *Females* Mammary gland fibroadenoma (multiple): 0/20, 1/45, 3/48	NS NS	Commercial-grade trichloroethylene (purity, > 99%), stabilized with 0.09% epichlorohydrin and 0.19% 1,2-epoxybutane. Doses were changed after 7 and 16 weeks of treatment based on monitoring of body-weight changes and survival. Study animals were housed with animals exposed to volatile agents. High incidence of chronic respiratory disease. Chronic "toxic nephropathy" observed in treated rats of both sexes. For males, 17/20 control, 42/50 low-dose, and 47/50 high-dose animals died before scheduled termination. For females, 12/20 control, 35/48 low-dose, and 37/50 high-dose animals died before scheduled termination. Reporting was inconsistent. [The Working Group considered that the slight increase in the incidence of multiple mammary fibroadenoma was associated with exposure.]

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours (number or tumour bearing animals/total number of animals)	Significance	Comments
Rat, Osborne-Mendel (M, F) 103 wk NTP (1988)	Gavage 0, 500, 1000 mg/kg bw in corn oil, 1/d, 5 d/wk 50/group/sex	*Males*		trichloroethylene (99.9%) stabilized with diisopropylamine. The Working Group noted the chemically-induced toxicity, reduced survival, and incomplete documentation of experimental data. Chronic "toxic nephropathy" observed in treated rats of both sexes, fatal gavage error increased in treated males. Statistical tests: Compared with controls: [a]Life Table, [b]incidental tumour test, [c]Fisher exact test Trend tests: [d]life table, [e]incidental tumour test, [f]Cochran-Armitage trend test.
		Kidney adenoma: 0/50, 6/50[a,b,c], 1/50	[a]$P = 0.007$, [b]$P = 0.007$, [c]$P = 0.013$	
		Kidney carcinoma: 0/50, 0/50, 1/50	NS	
		Kidney adenoma or carcinoma (combined): 0/50, 6/50[a,b,c], 2/50	[a]$P = 0.007$, [b]$P = 0.007$, [c]$P = 0.013$	
		Females		
		Kidney adenoma: 0/50, 0/50, 1/49	NS	
		Bronchioloalveolar adenoma: 0/50, 0/50, 1/50	NS	
		Bronchioloalveolar carcinoma: 0/50, 1/50, 0/50	NS	
		Adrenal cortex adenoma: 16/50[d], 13/50, 19/49[a]	[d]$P = 0.008$, [a]$P = 0.011$	
Rat, ACI (M, F) 2 yr NTP (1988)	Gavage 0, 500, 1000 mg/kg bw in corn oil, 1/d, 5 d/wk 50/group per sex	*Males*		Trichloroethylene (purity, 99.9%) stabilized with diisopropylamine. The Working Group noted the chemically-induced toxicity, reduced survival, and incomplete documentation of experimental data. Chronic "toxic nephropathy" and fatal gavage error were increased in treated rats of both sexes. Statistical tests: Compared with controls: [a]life table Trend test: [d]life table, [e]incidental tumour test
		Kidney carcinoma: 0/50, 1/49, 0/49	NS	
		Bronchioloalveolar adenoma: 0/50, 2/49, 0/49	NS	
		Testis, benign interstitial cell tumours: 36/49[d,e], 23/49[a], 17/49[a]	[d]$P < 0.001$, [e]$P = 0.019$, [a]$P = 0.024$, [a]$P < 0.001$	
		Females		
		Kidney adenoma: 0/48, 2/47, 0/43	NS	
		Kidney, tubular cell adenocarcinoma [kidney carcinoma] or NOS adenocarcinoma: 0/48, 1/47 (NOS), 1/43	NS	
		Kidney, tubular cell adenocarcinoma [kidney carcinoma] or NOS adenocarcinoma, or adenoma: 0/48, 3/47[a], 1/43	[a]$P = 0.044$	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours (number or tumour bearing animals/total number of animals)	Significance	Comments
Rat, August (M, F) 2 yr NTP (1988)	Gavage 0, 500, 1000 mg/kg bw in corn oil, 1/d, 5 d/wk 50/group/sex	*Males* Kidney adenoma: 0/50, 1/50, 1/49	NS	Trichloroethylene (purity, 99.9%) stabilized with diisopropylamine. The Working Group noted the chemically-induced toxicity, reduced survival, and incomplete documentation of experimental data. Chronic "toxic nephropathology" and fatal gavage error were increased in treated rats of both sexes. Statistical tests: Compared with controls: [a] life table, [b] incidental tumour test, Trend tests: [d] life table, [e] incidental tumour test, [f] Cochran-Armitage trend test.
		Kidney carcinoma: 0/50, 1/50, 0/49	NS	
		Subcutaneous tissue, sarcoma: 0/50[d,e], 1/50, 3/49[b]	[d]$P = 0.033$, [e]$P = 0.032$, [b]$P = 0.050$	
		Testis, benign interstitial cell tumours: 34/50, 30/50[a], 26/49	[a]$P = 0.049$	
		Females		
		Kidney adenoma: 1/49, 2/48, 0/50	NS	
		Kidney carcinoma: 0/49, 2/48, 0/50	NS	
		Kidney adenoma or carcinoma (combined): 1/49, 4/48, 0/50	NS	
		Leukaemia (monocytic type or NOS): 1/50[d,e,f], 0/50, 5/50	[d]$P = 0.027$, [e]$P = 0.020$, [f]$P = 0.037$	
		Thyroid, C-cell adenoma or carcinoma (combined): 0/49, 4/49, 1/50	NS	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours (number or tumour bearing animals/total number of animals)	Significance	Comments
Rat, Marshall (M, F) 2 yr NTP (1988)	Gavage 0, 500, 1000 mg/kg bw in corn oil, 1/d, 5 d/wk 50/group/sex	*Males* Kidney adenoma: 0/49, 1/50, 0/47 Kidney carcinoma: 0/49, 0/50, 1/47 Testis, benign or malignant interstitial cell tumours: 17/46[d,e,f], 21/48[a,b], 32/48[a,b,c] In one high-dose treated rat, a malignant interstitial cell tumour was diagnosed. All sites, mesothelioma: 2/50, 2/50, 3/50 *Females* Kidney adenoma: 1/50, 1/48, 0/44 Kidney carcinoma: 0/50, 1/48, 1/44 Kidney adenoma or carcinoma (combined): 1/50, 2/48, 1/44	NS NS [d]$P < 0.001$, [e]$P < 0.001$, [f]$P = 0.003$, [a]$P < 0.001$, [b]$P < 0.001$, [a]$P < 0.001$, [b]$P < 0.001$, [c]$P = 0.004$ NS NS NS NS	Trichloroethylene (purity, 99.9%) stabilized with diisopropylamine was used. The Working Group noted the chemically-induced toxicity, reduced survival, and incomplete documentation of experimental data. Chronic "toxic nephropathology" and fatal gavage error were increased in treated rats of both sexes. Statistical tests: Compared with controls: [a] life table, [b] incidental tumour test, [c] Fisher exact test Trend tests: [d] life table, [e] incidental tumour test, [f] Cochran-Armitage trend test
Rat, Sprague-Dawley (M, F) Lifespan Maltoni *et al.* (1986) (Exp. BT301)	Gavage 0, 50, 250 mg/kg bw in olive oil, 1/d, 4–5 d/wk for 52 wk 30/group/sex	*Males* Leukaemia (malignant neoplasms of the hemolymphoreticular system): 0/30, 2/30, 3/30	NS	Trichloroethylene was highly purified (purity, 99.9%) and epoxide-free with butyl-hydroxy-toluene (20 ppm) added as stabilizer.

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours (number or tumour bearing animals/total number of animals)	Significance	Comments
Rat, Sprague-Dawley (M, F) Lifespan Maltoni *et al.* (1986, 1988) (Exp. BT304 and Exp. BT304 bis)	Inhalation 0, 100, 300, 600 ppm, 7 h/d, 5 d/wk for 2 yr 135, 130, 130, 130 (M) 145, 130, 130, 130 (F)	*Male*		Trichloroethylene was highly purified (purity, 99.9%) and epoxide-free with butyl-hydroxy-toluene (20 ppm) added as stabilizer. Adjusted incidences (EPA, 2011a) reflect the number of rats alive at 47 wk. Rare kidney carcinomas reported for males (4/130) and females (1/130) at the highest dose (600 ppm). One testicular tumour was malignant at the highest dose. Slight dose-related increase in incidences of pituitary adenoma in female rats in BT 304 but not BT304 bis (data not shown). The previous IARC Working Group (IARC, 1995) reported significant, dose-related increase in the incidence of Leydig cell interstitial tumours of the testis ($P < 0.001$; Cochran-Mantel-Haenszel test)
		Kidney carcinoma – overall (corrected): 0/135 (0/122), 0/130 (0/121), 0/130 (0/116), 4/130 (4/124)	[NS]	
		Leukaemia (malignant neoplasms of the haemolymphoreticular system): 9/135, 13/130, 14/130, 15/130	NS	
		Immunoblastic lymphosarcoma [lymphoma]: 1/135, 5/130, 4/130, 2/130	[NS]	
		Testis, interstitial cell tumours (mainly benign): 6/135, 16/130*, 30/130**, 31/130**	*$P < 0.05$, **$P < 0.01$	
		Female		
		Kidney carcinoma – overall (corrected): 0/145 (0/141), 0/130 (0/128), 0/130 (0/127), 1/130 (1/127)	[NS]	
		Leukaemia (malignant neoplasms of the haemolymphoreticular system): 7/145, 9/130, 2/130, 11/130	NS	
		Immunoblastic lymphosarcoma [lymphoma]: 0/145, 4/130*, 1/130, 1/130	*$P < 0.05$	

d, day; mo, month; NOS, not otherwise specified; NS, not significant; wk, week; yr, year

Group noted the limitations in reporting and design of the study.]

Groups of male B6C3F$_1$ mice (age, 6 weeks) were given trichloroethylene (in a 5% aqueous solution of Alkamuls) by gavage at doses of 0 (n = 15), or 1000 (n = 40) mg/kg bw for 79 weeks (Bull *et al.*, 2002). Trichloroethylene purity, source, and presence of stabilizers were not reported. Only liver was examined for treatment-related induction of tumours. The incidence of gross lesions (also identified as liver "tumours") was higher in treated mice ($P < 0.05$) than in controls. Not all liver "tumours" (nodules, hepatocellular adenoma, or hepatocellular carcinoma) were confirmed by histopathological examination of the 40 trichloroethylene-treated mice. However, 1 out of 15 controls and 23 out of 36 treated mice developed hepatocellular adenoma, and 1 out of 15 controls and 7 out of 36 treated mice developed hepatocellular carcinoma. [The Working Group noted the limitations in reporting and design of the study (e.g. differences in the number of animals in control versus treatment groups, low numbers of animals studied, and limited focus on liver lesions).]

Groups of male B6C3F$_1$ mice (age, 4 weeks) were given drinking-water containing sodium chloride at a concentration of 2 g/L (n = 22), or trichloroethylene (purity, 99%) at a concentration of 40 mg/L (n = 32) for 61 weeks (Herren-Freund *et al.*, 1987). Because of volatilization, the concentration of trichloroethylene was reduced to 22 mg/L after 3 days (drinking-water was changed twice per week). Only liver was examined for treatment-related induction of tumours. A few hepatocellular carcinomas were observed in three treated mice, but not in control mice. [The Working Group noted the low number of animals, limited pathology examination, shortened exposure period, and variable concentrations of trichloroethylene, which limited the power of the study to detect a carcinogenic response.]

Groups of 50 male and 50 female Swiss (ICR/HA) mice (age, 5 weeks) were exposed to trichloroethylene in corn oil by daily gavage five times per week for 18 months, and were observed for an additional 6 months (Henschler *et al.*, 1984). Four groups were exposed to purified trichloroethylene (purity, > 99.9%) alone, or with either epichlorohydrin, 1,2-epoxybutane, or a combination of both epichlorohydrin and 1,2-epoxybutane as stabilizers. A fifth group was exposed to industrial-grade trichloroethylene (purity, 99.4% with 0.11% epichlorohydrin, 0.20% 1,2-epoxybutane, 0.05% carbon tetrachloride, and 0.01% chloroform). A control group was exposed to corn oil only. Exposure doses were 2.4 g/kg bw for males and 1.8 g/kg bw for females, but exposure was stopped for all groups during weeks 35–40, 65, and 69–78, and all doses were reduced by a factor of two from the fortieth week onwards because of toxicity attributable to gavage. Survival for females was higher than for males in all groups. The number of tumours diagnosed in all mice for each exposure group was reported, rather than incidence of tumours.

No significant differences between treatment groups were reported, except for increases in the incidence of forestomach tumours in several groups exposed to trichloroethylene and stabilizers. A single renal cystadenoma was diagnosed in an untreated male and none in untreated females, while there was one renal cystadenoma in males and four in females treated with purified trichloroethylene only. There was a slight, not statistically significant, increase in the incidence of lung bronchioloalveolar adenoma in the four groups of females treated with trichloroethylene versus the control groups. The previous Working Group (IARC, 1995) had noted that the incidence of hepatocellular tumours (adenoma and carcinoma combined) in male mice was: 3 out of 50 controls; 6 out of 50 mice treated with purified trichloroethylene; and 9 out of 50 mice treated with industrial-grade trichloroethylene, and that no survival-adjusted analysis of tumour

incidence was performed. The Working Group noted the reporting deficiencies in this study, the non-continuous exposure, uncertainty in diagnostic terminology for kidney tumours, and the attribution of carcinogenic responses to trichloroethylene rather than stabilizers.]

Groups of 30 male and 30 female Swiss (ICR/HA) mice (age, 6–8 weeks) were given purified trichloroethylene at a dose of 0 or 0.5 mg by gavage in 0.1 mL of trioctanoin as a vehicle, once per week, for 79 weeks [no indication of stabilizers, purity not reported] (Van Duuren *et al.*, 1979). Only sections of lung, liver, and stomach were taken for histopathological examination. No excess incidence of tumours was reported. [The Working Group noted that the dose was ~400 times lower than in other gavage studies. The Working Group also noted limitations in the design (e.g. short exposure duration, few animals, and histopathology of only lung, liver, and stomach), and reporting of the study.]

3.1.2 Inhalation

Groups of 90 male and 90 female B6C3F$_1$ mice (age, 12 weeks) were exposed by inhalation to highly purified epoxide-free trichloroethylene (containing butyl-hydroxy-toluene as a stabilizer) at a concentration of 0, 100, 300, or 600 ppm for 7 hours per day, 5 days per week, for 78 weeks, and observed for their lifespans (Maltoni *et al.*, 1986, 1988). Due to excessive fighting and mortality, experiments in males were repeated using a different animal source. "Hepatoma" described all malignant tumours of hepatic cells of different histological subtypes, and of various degrees of malignancy. Diagnoses of pulmonary tumour included adenomatous hyperplasia–early adenoma, adenoma, and adenocarcinoma. Statistically significant increases in the incidence of pulmonary tumours were observed in treated females at the highest dose; increases were reported to be primarily in later-stage adenoma. An increased incidence of hepatoma was observed in males and females, with statistical significance achieved as a combination of tumours between both sexes. [The previous IARC Working Group (IARC, 1995) had found a dose-related increase in the incidence of pulmonary tumours in female B6C3F$_1$ mice (Cochran-Armitage linear trend test). The Working Group noted the poor presentation of data, and that combining descriptors of pulmonary hyperplasia with early adenoma decreased the ability to discern emergence of less common but more malignant neoplastic states.]

In a second experiment in the same report, groups of 90 male and 90 female Swiss mice of unspecified strain (age, 11 weeks) were exposed by inhalation to highly purified epoxide-free trichloroethylene (containing butyl-hydroxy-toluene as a stabilizer) at a concentration of 0, 100, 300, or 600 ppm for 7 hours per day, 5 days per week, for 78 weeks, and observed for their lifespans (Maltoni *et al.*, 1986, 1988). Using the same pathological descriptions as above, an increased incidence of pulmonary tumours was reported in males (statistically significant at the two higher doses; primarily early-stage tumours). Increased incidence of hepatoma was noted for males at the two higher concentrations (statistically significant only at the highest concentration). Overall, a lower background rate and increase in incidence of hepatoma by trichloroethylene was seen in male Swiss mice than in male B6C3F$_1$ mice using the same experimental paradigm. [The previous Working Group (IARC, 1995) had found dose-related increases in the incidence of pulmonary tumours and hepatoma in male Swiss mice (Cochran–Armitage linear trend test). The Working Group noted similar issues concerning data presentation and pathological descriptors as for the studies in B6C3F$_1$ mice discussed above.] In a third experiment in the same report, groups of male and female Han:NMRI Swiss mice (age, 11 weeks) were similarly exposed to epoxide-free trichloroethylene (containing butyl-hydroxy-toluene as a stabilizer) at a concentration of 0 (n = 100), 100 (n = 60) or

600 (n = 72) ppm for a shorter time (8 weeks). A non-significant higher incidence of hepatoma was observed in males, but not females.

Groups of 30 male and 30 female Han:NMRI mice [age not reported] were exposed by inhalation to highly purified trichloroethylene (containing triethanolamine as a stabilizer) at a concentration of 0, 100, or 500 ppm for 6 hours per day, 5 days per week, for 18 months, and observed for 12 additional months (Henschler *et al.*, 1980). The number of tumours diagnosed in all mice for each exposure group, rather than the tumour incidence, was reported. A separate diagnosis was used for renal cystadenoma and renal adenoma. A single renal adenoma was reported in males at the lower dose, and a single renal adenocarcinoma [renal tubule carcinoma] was diagnosed in the control group. [The Working Group noted that the low frequency of renal tumours in this strain during this time period (one renal adenocarcinoma and one anaplastic carcinoma out of 614 males, with no kidney tumours observed in 591 females) (Bomhard & Mohr, 1989).] Statistically significant increases in age-adjusted incidence ($P < 0.01$) and trend in the incidence ($P < 0.05$; EPA, 2011a) of malignant lymphoma (9 out of 29, 17 out of 30, and 18 out of 28 for groups at 0, 100, or 500 ppm, respectively) were reported in treated females. [The Working Group noted the low number of animals studied and limited reporting.]

Groups of 50 female Crj:CD-1(ICR) mice (age, 7 weeks) were exposed by inhalation to reagent-grade trichloroethylene (containing epichlorohydrin as a stabilizer) at a concentration of 0, 50, 150, or 450 ppm for 7 hours per day, 5 days per week, for 104 weeks (Fukuda *et al.*, 1983). The experiment was terminated after 107 weeks. Only malignant tumours were counted when both malignant and benign tumours were observed in the same organ. The number of tumours diagnosed in all animals for each exposure group was reported, rather than tumour incidence, with the exception of lung adenocarcinoma. A low number of liver tumours was reported (only one hepatocellular adenoma in the group at the highest dose, but none in other groups). [The Working Group noted that the background rate for hepatocellular adenoma was low for this strain and sex in contemporary studies (Maita *et al.*, 1988).] The increased incidence of lung adenocarcinoma was statistically significant [$P < 0.05$] in the groups at the two higher doses compared with controls. [The Working Group noted the limited reporting of this study.]

3.1.3 Skin application

In a study of two-stage carcinogenesis in mouse skin, groups of 30 female ICR/Ha Swiss mice (age, 6–8 weeks) were treated with single doses of 1.0 mg of trichloroethylene [no indication of stabilizers, purity not reported] in 0.1 mL of acetone by topical application to the shaved dorsal skin; after 14 days, 12-*O*-tetradecanoylphorbol 13-acetate (TPA; 2.5 µg in 0.1 mL of acetone) was applied topically, three times per week for at least 49 weeks (Van Duuren *et al.*, 1979). A total of 9 skin papillomas were found in 4 out of 30 trichloroethylene-treated mice, and 10 papillomas were found in 9 out of 120 TPA-treated controls. Groups of 30 female ICR/Ha Swiss mice (age, 6–8 weeks) were also treated with trichloroethylene by repeated topical application, at a dose of 1.0 mg per mouse, three times per week, for 83 weeks. No tumours were observed at the site of application. [The Working Group noted the small number of animals studied and the low dose used. The volatility of the compound was also noted as a limitation to this study protocol.]

3.1.4 Subcutaneous injection

Groups of 30 female ICR/Ha Swiss mice (age, 6–8 weeks) were treated with purified trichloroethylene [no indication of stabilizers, purity not reported] by subcutaneous injection at a dose of 0 or 0.5 mg in 0.05 mL of trioctanoin, once per

week, for at least 74 weeks (Van Duuren *et al.*, 1979). No tumours were observed at the injection site in either group. [The Working Group noted the limited number of animals studied and the low dose used.]

3.2 Rat

3.2.1 Oral administration

Groups of 50 male and 50 female F344/N rats (age, 8 weeks) were treated with epichlorohydrin-free trichloroethylene by gavage in corn oil at a dose of 0, 500, or 1000 mg/kg bw per day for 103 weeks (NTP, 1990). Significantly reduced survival in trichloroethylene-treated groups compared with vehicle controls was due to gavage error and chronic "toxic nephropathy." Rarely seen in controls (background incidence in male rats, 0.4%), toxic nephropathy was distinct from the spontaneous chronic progressive nephropathy commonly observed in aged rats. Cytomegaly and karyomegaly of tubular epithelial cells of the inner renal cortex were observed in > 98% of all trichloroethylene-treated rats. Although decreased survival was noted in trichloroethylene-treated groups, an increased incidence, and positive trend in the incidence of rare kidney adenoma or carcinoma (combined), and of kidney carcinoma, were observed in males at the higher dose (carcinomas in 3 out of 49 rats at the higher dose versus 0 out of 48 controls). One case of kidney carcinoma was also reported in females at the higher dose, but no statistically significant increases in tumour incidence were reported. [The Working Group noted the rarity of kidney adenoma and carcinoma in this rat strain, with the background incidence in males reported as 0.7% (10 out of 1352) for adenoma and 0.2% (3 out of 1352) for carcinoma; in females, the background incidence has been reported as 0% (0 out of 1348) for adenoma and 0.1% (1 out of 1348) for carcinoma (Haseman *et al.*, 1998). A low background incidence of kidney adenoma or carcinoma was also reported in F344/N rats treated with trichloroethylene in corn oil by gavage, in studies conducted by the NTP. The EPA (2011a) cited the incidence of kidney carcinoma in unexposed rats fed an NIH-07 diet (conditions used before 1995, when the NTP studies on trichloroethylene were conducted), and treated with corn oil by gavage, as 0.5% (2 out of 400) for males and 0% (0 out of 400) for females. For F344/N rats fed an NIH-07 diet, the historical control rate for kidney adenoma or carcinoma (combined) was 1.3% (5 out of 400) for males and 0.3% (1 out of 400) for females.] A statistically significant increase in the incidence of peritoneal malignant mesothelioma was also observed in males at the lower dose (5 out of 50 treated rats versus 1 out of 50 controls). [The Working Group noted that mesothelioma (at all sites) is an uncommon tumour in untreated male F344/N rats, with the background incidence reported as 3.0% (40 out of 1354 rats in feeding studies and 28 out of 905 rats in inhalation studies) (Haseman *et al.*, 1998).]

Groups of male and female Osborne-Mendel rats (age, about 45 days) were treated with commercial-grade trichloroethylene (containing epichlorohydrin as stabilizer) at time-weighted average doses of 0 (n = 20), 549 (n = 50), or 1097 (n = 50) mg/kg bw in corn oil by gavage for 78 weeks (non-continuous exposure at concentrations that were varied due to toxicity and mortality) and killed after 110 weeks (NCI, 1976). Premature mortality was high (84–94% in males, and 50–74% in females). A high incidence of chronic respiratory disease was noted among the rats without differences in type, severity, or morbidity by sex or group, and chronic "toxic nephropathy" was observed only in treated rats of both sexes. A few malignant mixed tumours and hamartomas of the kidney were observed in controls, with one observed in a male at the lower dose, which were considered not related to chemical treatment. A single trichloroethylene-treated male had a rare kidney carcinoma.

No statistically significant increases in tumour incidence were reported in either sex. [The Working Group considered that a slight increase in the incidence of multiple mammary fibroadenoma was associated with exposure to trichloroethylene in females (3 out of 48 at the highest dose versus 0 out of 20 untreated); the incidence of single fibroadenoma was not increased with treatment (3 out of 20, 5 out of 45, and 7 out of 48 for controls, and rats at the lower and higher doses, respectively). The Working Group noted the high rate of early mortality in control and treated rats, limited duration of treatment, and lower numbers of animals studied in control groups. In addition, study animals were housed in the same rooms as animals exposed to volatile agents, but the Working Group did not observe an effect on the tumour response.]

Groups of 50 male and 50 female Osborne-Mendel rats (age, 8 weeks) were treated with trichloroethylene (containing diisopropylamine as a stabilizer) at a dose of 0, 500, or 1000 mg/kg bw in corn oil by gavage for 103 weeks (NTP, 1988). More trichloroethylene-treated male rats than controls were killed by gavage error (controls, 1 out of 50; lower dose, 6 out of 50; and higher dose, 7 out of 50), while similar rates of accidental death occurred in all groups of females (six to eight per group). One bronchioloalveolar adenoma and one bronchioloalveolar carcinoma were diagnosed in two trichloroethylene-treated females, but no such tumours were observed in those receiving the vehicle only. After adjustment for survival, a positive trend and an increase (for the group at the higher dose) in the incidence of adrenal cortex adenoma, was observed in females. Treatment with trichloroethylene also caused cytomegaly and karyomegaly of proximal tubule cells, and chronic "toxic nephropathy" [see description above in NTP (1990)], but not in controls. Tubular hyperplasia [distinct from chronic progressive nephropathy] was noted to occur at low rates in trichloroethylene-treated rats, and less frequently in controls. All animals exposed to trichloroethylene for up to 2 years had renal tubular cell cytomegaly, and most rats (80%) had chronic "toxic nephropathy." Incidences of kidney adenoma, and kidney adenoma or adenocarcinoma (combined) were increased in trichloroethylene-treated males (0 out of 50 controls, 6 out of 50 at the lower dose, and 2 out of 50 at the higher dose), and were statistically significant in animals at the lower dose. A rare kidney adenoma was diagnosed in a single trichloroethylene-treated female. [The Working Group noted that the background incidence in this strain for kidney adenoma and carcinoma was reported to be 0% (0 out of 100 in males and females; 50 untreated and 50 with corn oil by gavage) (Solleveld & Boorman, 1986). After inclusion of the data for 70 control rats from the NCI (1976) and NTP (1988) studies, the average background rate was 0% (0 out of 170) for male and female rats of this strain.] [Chemical-induced toxicity, reduced survival, and incomplete documentation of experimental data were noted by the Working Group.]

In the same publication, groups of male and female ACI rats (age, 6.5 weeks) were treated with trichloroethylene using the same experimental paradigm (NTP, 1988). Higher numbers of trichloroethylene-treated male and female rats were killed by gavage error, than controls (males: 0 out of 50 controls, 11 out of 49 at the lower dose, and 18 out of 49 at the higher dose; females: 2 out of 48 controls, 14 out of 47 at the lower dose, and 12 out of 43 at the higher dose). Renal tubular cell cytomegaly was noted in 80–98% of treated males and 90% of treated females exposed for up to 2 years, while approximately 40% of treated males and females had chronic "toxic nephropathy;" these pathologies were not seen in controls. A single male treated with trichloroethylene at the lower dosewas diagnosed with a kidney carcinoma. While no kidney neoplasms were reported in females in the control group, females treated with trichloroethylene at the lower dose had an incidence of 2 out of 47 for kidney

adenoma, and 3 out of 47 for tubular cell adenocarcinoma [kidney carcinoma] or adenocarcinoma, NOS (not otherwise specified) or kidney adenoma (statistically significant); the group treated with the higher dose had an incidence of one out of 43 for tubular cell adenocarcinoma [kidney carcinoma] or adenocarcinoma, NOS. [The Working Group noted that these tumours are rare in this strain of rat (0 out of 98 males and 0 out of 97 females for kidney adenoma or carcinoma) (Solleveld & Boorman, 1986). After inclusion of the control data from this study, the average number of control rats with kidney tumours was 0% (0 out of 148 for males and 0 out of 147 for females).] An increased incidence of testicular interstitial cell adenoma was diagnosed in surviving trichloroethylene-treated males (a positive trend and a higher incidence in both groups treated with trichloroethylene). Although the incidence of interstitial cell tumours was not significantly higher than that in vehicle controls (using the incidental tumour test), there was a positive trend when the incidence of interstitial cell tumours was based on survival of animals until the week in which the first tumour appeared (i.e. at week 75, incidence in males was 36 out of 43, 23 out of 26, and 17 out of 17 for rats in the control group, at the lower dose and higher dose, respectively). Bronchioloalveolar adenoma was diagnosed in one trichloroethylene-treated female, but not in females receiving vehicle only.

The same publication also provided results for male and female August rats (age, 8 weeks) using the same experimental paradigm (NTP, 1988). Higher numbers of trichloroethylene-treated male and female rats were killed by gavage error than controls (males: 6 out of 50 controls, 12 out of 50 at the lower dose, and 11 out of 49 at the higher dose; females: 1 out of 49 controls, 6 out of 48 at the lower dose, and 13 out of 50 at the higher dose). More than 90% of treated males and females exposed for up to 2 years had renal tubular cell cytomegaly, and about 20–60% of males and females had chronic "toxic nephropathy." Tubular cell hyperplasia, distinguishable from that observed for chronic progressive nephropathy, was also noted in a few individuals in trichloroethylene-treated groups only. In males, the incidence of kidney adenoma was zero out of 50 controls, one out of 50 at the lower dose, and one out of 49 at the higher dose, with one male treated with trichloroethylene at the lower dose also diagnosed with kidney carcinoma. In females, the incidence of kidney adenoma or adenocarcinoma (combined) was one out of 49 controls, four out of 48 at the lower dose, and zero out of 50 at the higher dose, with two females treated with trichloroethylene at the lower dose diagnosed with rare kidney carcinoma. [The Working Group noted that, although not statistically significant, these kidney tumours are rare in this strain (0% incidence of kidney adenoma in males (0 out of 100 rats) and 1% incidence in females (1 out of 99 rats), and 0% incidence of kidney carcinoma in either sex) (Solleveld & Boorman, 1986). With the addition of the controls from this study, the number of unexposed rats with kidney tumours was 0 out of 150 (0%) for each sex of this strain.] There was a statistically significant trend in increasing incidence of subcutaneous tissue sarcoma (the incidence was significantly increased for rats at the higher dose) in trichloroethylene-treated males. After adjustment for survival, there was also a small increase in the incidence of testicular interstitial cell tumours at the lower dose. A positive trend in the incidence of leukaemia was observed in females. After taking survival into account, the incidence of leukaemia was slightly increased in the group at the higher dose (1 out of 50 controls versus 5 out of 50 females at the higher dose). The incidence of thyroid C-cell adenoma or carcinoma (combined) was slightly increased in females at the lower dose (0 out of 49 controls versus 4 out of 49 females at the lower dose).

In a fourth study reported in the same publication (NTP, 1988), groups of 50 male and 50

female Marshall rats (age, 7 weeks) were exposed to trichloroethylene using the same experimental paradigm. Higher numbers of trichloroethylene-treated rats were killed by gavage error than controls (males: 2 out of 49 controls, 12 out of 50 at the lower dose, and 25 out of 47 at the higher dose; females: 3 out of 50 controls, 14 out of 48 at the lower dose, and 18 out of 44 at the higher dose). More than 90% of treated males and females, exposed for up to 2 years, had renal tubular cell cytomegaly, and 36–49% of males and 63% of females had chronic "toxic nephropathy." Tubular cell hyperplasia, distinguishable from that observed for chronic progressive nephropathy, was also noted in a few individuals in trichloroethylene-treated groups only. With regard to kidney tumours, results were similar to the other strains tested by this paradigm, with a few treated male and female rats exhibiting rare kidney tumours. In males, the incidence of kidney carcinoma was 1 out of 47 rats at the higher dose compared with 0 out of 49 controls. In females, the incidence of kidney adenoma or adenocarcinoma (combined) was 1 out of 50 controls, 2 out of 48 rats at the lower dose, and 1 out of 44 rats at the higher dose, with an incidence of kidney carcinoma of 0 out of 50 controls, 1 out of 48 rats at the lower dose, and 1 out of 44 rats at the higher dose. For males, a much higher trichloroethylene treatment-related increase in the incidence of testicular interstitial cell tumours was observed compared with the other strains. The increased incidence of interstitial cell tumours had statistically significant trends, with and without survival adjustment; the increased incidence was also statistically significant compared with controls in both treatment groups after survival adjustment, and in the group treated with the higher dose, without survival adjustment. One trichloroethylene-treated male at the higher dose was diagnosed with a rare malignant interstitial cell tumour. In addition, there was a slight increase in the total number of mesotheliomas (peritoneal and tunica vaginalis) in males at the lower dose, after adjustment for survival.

[The Working Group noted the early mortality described in the four studies encompassed in the NTP (1988) publication, as well as the observation of rare tumours and increased incidence of more common tumours after treatment with trichloroethylene. Comparison of tumour incidence with historical background rates is difficult for the strains employed in these studies, especially for sites other than kidney. The Working Group also noted that the early mortality reduced the statistical power of the bioassays.]

Groups of 30 male and 30 female Sprague-Dawley rats (age, 13 weeks) were exposed to highly purified epoxide-free trichloroethylene (containing butyl-hydroxy-toluene as stabilizer) at a dose of 0, 50, or 250 mg/kg bw per day in olive oil by gavage, 4–5 days per week, for 52 weeks, and observed for their lifespans (Maltoni *et al.*, 1986). As in F344/N rats (NTP, 1990), an increased incidence of renal tubule meganucleocytosis (cytokaryomegaly) was observed in males at the higher dose. Slight increases in the incidence, or trend in the incidence of leukaemia were observed in trichloroethylene-treated males. Leukaemia was reported to be primarily immunoblastic lymphosarcoma [lymphoma]. [The Working Group noted that although deaths attributable to gavage error were not reported, the power of the study was limited by the small number of animals in each group and the short duration of exposure in older animals (see additional comments from the Working Group below regarding the diagnosis of lymphoma in rats from this laboratory).]

3.2.2 Inhalation

Groups of 130–145 male and female Sprague-Dawley rats (age, 12 weeks) were exposed by inhalation to highly purified epoxide-free trichloroethylene (containing butyl-hydroxy-toluene as stabilizer) at a concentration of 0, 100,

300, or 600 ppm, 7 hours per day, 5 days per week, for 104 weeks, and observed for their lifespans (Maltoni *et al.*, 1986, 1988). Tumour results were reported separately, and as a combination of two experiments that were conducted at the same time; both experiments used the same protocol and "were divided by litter distribution within the two experiments." The EPA (2011a) adjusted the incidence of kidney tumours to reflect the number of animals alive at 47 weeks (i.e. the date at which the first diagnosis of renal tubular meganucleocytosis was made). There was an increase in the incidence of testicular interstitial cell tumours (mainly benign) in all groups of trichloroethylene-exposed males. As with Marshall rats exposed by gavage (see Section 3.2.1; NTP, 1988), one male rat at the higher dose was diagnosed with a rare malignant testicular cell tumour. [The Working Group noted that the previous IARC Working Group (IARC, 1995) had reported a significant, dose-related increase in the incidence of Leydig cell interstitial tumour of the testis ($P < 0.001$; Cochran–Mantel–Haenszel test).] A slight dose-related increase in the incidence of pituitary adenoma in female rats in one of the two studies was noted, but the data were not shown. There was also an increase in the incidence of immunoblastic lymphosarcoma [lymphoma] in females at the lower dose. [The Working Group noted the increased power of this study to detect a response from the large number of animals in the combined exposure groups, and the use of four, rather than three, exposure groups. The Working Group also noted the limited reporting of findings for individual animals, and that the diagnoses of rat lymphoma in this laboratory (especially those in the lung) have been questioned in other studies; however, other diagnoses of solid tumour, such as testicular interstitial cell adenoma, were confirmed in the reviews carried out by those other studies (NTP, 2011b, c).] As for observations in studies in other rat strains (NTP, 1988, 1990), and the gavage study from the same laboratory (see Section 3.2.1; Maltoni *et al.*, 1986), an increased incidence of renal tubule meganucleocytosis (cytokaryomegaly) was reported in male rats exposed to trichloroethylene by inhalation at the two higher doses. Rare kidney carcinomas, originating from tubular cells, were reported in 4 out of 130 males at the highest dose versus 0 out of 135 controls; one kidney carcinoma, identified as cortical, was reported in a female at the highest dose. No kidney carcinomas "of the same pattern [originating from tubular cells] has ever been observed in nearly 50 000 Sprague-Dawley rats (untreated, vehicle treated, or treated with different chemicals) examined" in their laboratory. [The Working Group noted that variations of this strain have a low background incidence of kidney tumours (McMartin *et al.*, 1992; Keenan *et al.*, 1995; Brix *et al.*, 2005; Dinse *et al.*, 2010); for kidney carcinoma, background incidence has been reported as 0% (0 out of 350 males and 0 out of 350 females from various dietary groups) (Keenan *et al.*, 1995), 0.51% (3 out of 585 males) and 0% (0 out of 584 females) (McMartin *et al.*, 1992), 0.21% (1 out of 472 females) (Dinse *et al.*, 2010) and 0.27% (1 out of 370 females) (Brix *et al.*, 2005). Overall, the average background incidence of kidney carcinoma was 0.11% in females and 0.32% in males.]

Groups of 30 male and 30 female Han:WIST rats [age not reported] were exposed by inhalation to purified trichloroethylene (containing triethanolamine as a stabilizer) at a concentration of 0, 100, or 500 ppm for 6 hours per day, 5 days per week, for 18 months, and killed after 36 months (Henschler *et al.*, 1980). The number of tumours diagnosed in all animals for each exposure group, but not tumour incidence, was reported. Survival did not differ among groups, and no statistically significant treatment-related increase in the number of tumours was seen. [For this study as a whole, the Working Group noted the small number of animals studied in each group, and the inability to compare tumour findings from this study with those of other

studies and historical databases, given that only the number of tumours per exposure group was reported and not tumour incidence. The Working Group noted that use of the term "cystadenoma" has only been used by these authors among the studies included in this review and does not appear in other historical databases (Solleveld & Boorman, 1986; Haseman *et al.*, 1998; Harlan, 2011).]

Groups of 50 female CrJ:CD(SD) rats (age, 7 weeks) were exposed by inhalation to reagent-grade trichloroethylene (containing epichlorohydrin as a stabilizer) at a concentration of 0, 50, 150, or 450 ppm for 7 hours per day, 5 days per week, for 107 weeks (Fukuda *et al.*, 1983). Increased mortality was reported in the control group compared with treated groups (i.e. about 75% of the rats in the three treated groups and 50% of controls were alive at 100 weeks). Only malignant tumours were counted when both malignant and benign tumours were observed in the same organ. The number of tumours per total number of animals in each group was reported, but not the tumour incidence. No statistically significant increases in number of tumours per exposure group were reported, but rare tumours were reported in trichloroethylene-treated groups.

A renal clear cell carcinoma [kidney carcinoma] was reported in the group treated with the highest dose. A single bronchioloalveolar adenoma was reported in each of the groups at the intermediate and highest doses, but not in controls. [The Working Group noted that bronchioloalveolar adenoma is rare in Harlan Sprague-Dawley female rats (McMartin *et al.*, 1992; Keenan *et al.*, 1995; Brix *et al.*, 2005; Dinse *et al.*, 2010).] A single mammary gland carcinosarcoma was diagnosed in in each of the groups at the intermediate and highest doses. A rare hepatocellular carcinoma was observed in the group at the highest dose, and a cystic cholangioma observed in the group at the lowest dose, but not in controls. [The Working Group noted that hepatocellular carcinomas and bile duct tumours are rare in Harlan Sprague-Dawley female rats (McMartin *et al.*, 1992; Keenan *et al.*, 1995; Brix *et al.*, 2005; Dinse *et al.*, 2010.] [The Working Group noted the increased mortality in the control group, the limited reporting, and the rarity of some tumour findings in trichloroethylene-treated groups. The Working Group also noted the inability to compare tumour incidence with other studies, given that only the number of tumours per exposure group was reported.]

3.3 Hamster

Groups of 30 male and 30 female Syrian hamsters [age unspecified] were treated by inhalation with trichloroethylene (purity, > 99.9%; containing 0.0015% triethanolamine as a stabilizer) at a concentration of 0, 100, or 500 ppm for 6 hours per day, 5 days per week, for 18 months, and observed for 30 months (Henschler *et al.*, 1980). The number of tumours diagnosed in all hamsters for each exposure group was reported, but not the incidence. Survival was reported to be similar in treated and control groups. No significant increase in number of tumours was reported. [The Working Group noted that the low number of animals studied in each group and the abbreviated exposure period limited the power of the study to detect a response.]

3.4 Administration with known carcinogens or other modifying factors

Seven groups of 23–33 male B6C3F$_1$ mice (age, 15 days) were given a single intraperitoneal injection of *N*-ethyl-*N*-nitrosourea (ENU) in 0.1 mol/L sodium acetate at a dose of 0, 2.5, or 10 mg/kg bw; from age 4 weeks, the mice were given drinking-water containing trichloroethylene at a dose of 0, 3, or 40 mg/L for 61 weeks (Herren-Freund *et al.*, 1987). [Trichloroethylene was reported to

have a purity of more than 99% and the presence of stabilizers was not addressed.] Because of volatilization, the concentration of the 40 mg/L dose was reduced to 22 mg/L after 3 days. [The previous Working Group (IARC, 1995) noted that the highest concentration of trichloroethylene given was equivalent to a daily dose of 6 mg/kg bw, which was low.] Only livers were examined for treatment-related induction of tumours. The incidence of hepatocellular adenoma or hepatocellular carcinoma was not increased in mice that received trichloroethylene only, in comparison with vehicle controls, and trichloroethylene did not promote liver tumours in mice initiated with ENU. [The Working Group noted the low levels of exposure to trichloroethylene, the small number of animals examined, and the abbreviated exposure duration, all of which limited the interpretation of results.]

3.5 Effects of stabilizers

Although some studies used trichloroethylene containing two carcinogenic compounds as stabilizers (epichlorohydrin [IARC Group 2A] and 1,2-epoxybutane [IARC Group 2B]), consistent patterns of tumour induction were observed in studies using trichloroethylene with or without small amounts (< 1%) of these carcinogenic stabilizers.

The study in mice by Henschler *et al.* (1984) was designed to specifically analyse the effect of stabilizers on tumour induction by trichloroethylene and reported no significant differences in systemic tumorigenesis between groups treated with pure trichloroethylene, industrial trichloroethylene, or trichloroethylene containing very small amounts of stabilizers. The relatively low susceptibility to liver tumours in the strain tested (Swiss HA:ICR) decreased the ability of the study to detect differences in liver tumorigenesis, but also decreased the probability of premature mortality before termination of the study. The NTP (1990) study was a better example of the lack of a confounding effect of stabilizers on the results of trichloroethylene carcinogenicity studies; rats and mice exhibited patterns of tumour induction without stabilizers that were similar to those reported for similar paradigms using stabilizer-containing trichloroethylene). Thus, the evidence suggested that concentrations of these stabilizers were too low to be the cause of tumours.

3.6 Carcinogenicity of metabolites

See also individual *Monographs* on Dichloroacetic acid, Trichloroacetic acid, and Chloral Hydrate in this Volume.

Groups of female ICR/Ha Swiss mice (age, 6–8 weeks) were treated with trichloroethylene oxide in 0.1 mL of acetone by skin application at a dose of 0 (n = 30) or 2.5 mg (n = 100) three times per week, for their lifespans. In a separate experiment, groups of female ICR/Ha Swiss mice (age, 6–8 weeks) were treated by injection in the flank with trichloroethylene oxide (purity, 90%, with 10% dichloroacetyl chloride) in 0.05 mL of trioctanoin at a dose of 0 (n = 30) or 500 µg (n = 100) once per week (Van Duuren *et al.*, 1983). No skin papillomas were reported in the skin-application experiment and only one fibrosarcoma was reported in the subcutaneous-injection experiment. Liver, stomach, and kidney tissue were routinely sectioned, but results were not reported. Trichloroethylene epoxide had a rapid rate of hydrolytic decomposition (half-life,1.5 minute). [The Working Group noted the low number of animals tested and raised questions regarding the stability of the compound tested.]

In a study of two-stage carcinogenesis in mouse skin, groups of 30 female ICR/Ha Swiss mice (age, 6–8 weeks) were treated with purified trichloroethylene oxide at a dose of 1.0 mg in 0.1 mL of acetone by application to the shaved dorsal skin; after 14 days, 12-*O*-tetradecanoylphorbol 13-acetate (TPA; 2.5 µg in 0.1 mL of acetone)

was applied topically, three times per week for at least 49 weeks (Van Duuren *et al.*, 1979). A total of three skin papillomas were found in 3 out of 30 trichloroethylene oxide-treated mice, and 10 papillomas were found in 9 out of 120 TPA-treated controls. [The Working Group noted the low number of animals tested and raised questions regarding the stability of the compound tested.]

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

4.1.1 Absorption

(a) Humans

Trichloroethylene is a lipophilic solvent of low relative molecular mass, and can readily cross biological membranes. It is taken up in the lungs, with pulmonary absorption approaching steady-state within a few hours after the start of exposure (Monster *et al.*, 1976; Vesterberg & Astrand, 1976; Vesterberg *et al.*, 1976; Fernández *et al.*, 1977). Measured values of pulmonary retention range from 35% to 70% in individuals, with generally greater values at rest and lower values associated with physical activity (Soucek & Vlachova, 1960; Bartonicek, 1962; Astrand & Ovrum, 1976; Monster *et al.*, 1976; Jakubowski & Wieczorek, 1988). One factor in pulmonary absorption is the equilibrium ratio between blood and air concentrations of trichloroethylene (blood:air partition coefficient), which has been measured *in vitro* using vial equilibrium methods. Mean values reported for human blood range from 8 to 12 (Sato *et al.*, 1977; Sato & Nakajima, 1979; Fiserova-Bergerova *et al.*, 1984; Gargas *et al.*, 1989; Koizumi, 1989; Fisher *et al.*, 1998).

Data on oral absorption were derived from case reports of accidental occupational or intentional (suicidal) ingestion, and suggested that trichloroethylene is also readily absorbed by this route. Trichloroethylene and its metabolites were reported in blood and/or urine at the first available sampling times after exposure, the earliest of which was 13 hours, with peak amounts in blood within the first 24 hours (Brüning *et al.*, 1998; Perbellini *et al.*, 1991; Yoshida *et al.*, 1996). However, quantitative estimates of oral bioavailability in humans were not available because the ingested amounts were not known precisely, and because all cases underwent gastric intubation and/or lavage.

Dermal absorption of vapour and liquid in humans has been shown to be rapid on contact, with peak values of trichloroethylene in exhaled breath reported within 5–30 minutes after exposure (Sato & Nakajima, 1978; Kezic *et al.*, 2000, 2001). In addition, Kezic *et al.* (2000) reported high inter-individual variability in dermal flux, as well as varying degrees of skin irritation that may have increased absorption (Kezic *et al.*, 2001).

(b) Experimental systems

Dallas *et al.* (1991), using a nose-only face-mask exposure system in rats, reported retention of about 70% during the second hour of a 2-hour exposure by inhalation. Data on exposure by whole-body inhalation in rats and mice were also consistent with considerable pulmonary absorption (Fisher *et al.*, 1991; Greenberg *et al.*, 1999; Simmons *et al.*, 2002).

Mean values for the blood:air partition coefficient in rats and mice, measured *in vitro* using vial equilibrium methods, ranged from 13 to 26 (Sato *et al.*, 1977; Gargas *et al.*, 1989; Koizumi, 1989; Fisher *et al.*, 1991; Barton *et al.*, 1995; Abbas & Fisher, 1997; Simmons *et al.*, 2002; Mahle *et al.*, 2007). [The Working Group noted that these values were higher than those measured in humans, but the higher partition coefficient in rodents only partially explained their greater retention by inhalation, as pulmonary absorption

also depends on other factors such as alveolar ventilation, hepatic blood flow, and metabolism.]

Most of the data from experimental systems *in vivo* involved oral exposures, and were consistent with rapid and extensive absorption after ingestion. Radiolabelling studies have reported that up to 98% of the administered dose was excreted in the urine and about 1–4% was eliminated unchanged in expired air, consistent with virtually complete absorption (Prout *et al.*, 1985; Dekant *et al.*, 1986a). The rate of absorption is affected by stomach contents and vehicle. Fasted animals exhibited greater bioavailability, higher peak blood levels, and shorter terminal half-lives compared with non-fasted animals (D'Souza *et al.*, 1985). Withey *et al.* (1983) and Staats *et al.* (1991) examined the effect of vehicle on gastrointestinal absorption of several compounds in rats, including trichloroethylene. Withey *et al.* (1983) noted faster and more extensive uptake with an aqueous vehicle than with oil, with significant differences in peak concentration and time to reach peak concentration. Staats *et al.* (1991) showed similar differences between aqueous and oil vehicles in rats, and also estimated gastrointestinal absorption and transfer coefficients by fitting their data to a physiologically based pharmacokinetic model. Stomach absorption coefficients were estimated to be three to four times greater for aqueous vehicle than for oil, but estimated coefficients for stomach–duodenal transfer and duodenal absorption were not affected by the vehicle.

4.1.2 Distribution and body burden

(a) Humans

Once absorbed, trichloroethylene enters the blood circulation and undergoes rapid systemic distribution to tissues. Data in humans on tissue distribution *in vivo* were limited to tissues taken from autopsies after accidental poisonings, or from surgical patients exposed environmentally, so the level of exposure was typically unknown. Tissue levels reported in autopsies after accidental poisonings showed wide systemic distribution across all tested tissues, including the brain, muscle, heart, kidney, lung, and liver (Ford *et al.*, 1995; De Baere *et al.*, 1997; Dehon *et al.*, 2000; Coopman *et al.*, 2003). Human populations exposed environmentally show detectable levels of trichloroethylene in various tissues, including the liver, brain, kidney, and adipose, and also in maternal milk (McConnell *et al.*, 1975; Pellizzari *et al.*, 1982; Kroneld, 1989). In addition, trichloroethylene has been shown to cross the human placenta during childbirth (Laham, 1970).

Because of its lipophilicity, trichloroethylene preferentially partitions to tissues with a high lipid content, such as fat. The equilibrium ratio of tissue:blood concentrations (the partition coefficient) has been measured *in vitro* using vial equilibrium methods in human fat, brain, kidney, liver, lung, and muscle (Sato *et al.*, 1977; Fiserova-Bergerova *et al.*, 1984; Fisher *et al.*, 1998). The reported human tissue: blood partition coefficients were highest for fat (52–64), with those for the remaining tissues ranging from 0.5 to 6.0.

(b) Experimental systems

Reports of detailed tissue-distribution experiments in rats and mice exposed to trichloroethylene orally or by inhalation (Savolainen *et al.*, 1977; Pfaffenberger *et al.*, 1980; Abbas & Fisher, 1997; Greenberg *et al.*, 1999; Simmons *et al.*, 2002; Keys *et al.*, 2003) suggested that trichloroethylene is distributed to all tissues examined, including the kidney, liver, lung, brain, fat, muscle, gastrointestinal tract, heart, and spleen, with the highest concentrations measured in fat.

Partition coefficients have been measured *in vitro* for a large array of tissues in rats and mice, including the brain, fat, heart, kidney, liver, lung, muscle, spleen and testis (Sato *et al.*, 1977; Fisher *et al.*, 1989, 1991; Gargas *et al.*, 1989; Koizumi, 1989; Barton *et al.*, 1995; Abbas & Fisher, 1997;

Simmons *et al.*, 2002; Rodriguez *et al.*, 2007). The reported rodent tissue: blood partition coefficients were highest for fat (23–36), with those for the remaining tissues ranging from 0.5 to 3.

4.1.3 Metabolism

(a) Overview

Metabolism is critical to the various adverse effects of trichloroethylene in biological systems. Moreover, with the exception of solvent effects that occur at extremely high exposures to trichloroethylene, all the adverse effects can be attributed to specific metabolites of trichloroethylene. The basic metabolic pathways for trichloroethylene have been deciphered over many years and have been summarized in several relatively recent reviews and published documents (e.g. IARC, 1995; Lash *et al.*, 2000a; Chiu *et al.*, 2006; EPA, 2011a). This section summarizes the two major pathways by which trichloroethylene is metabolized in humans and experimental animals: the cytochrome P450 (CYP)-dependent oxidative pathway and the glutathione (GSH)-conjugation pathway. Key urinary metabolites are identified that are often used to estimate exposure in environmental or occupational settings.

Quantitatively, flux through the CYP-dependent oxidative pathway far exceeds that through the GSH-conjugation pathway in all species studied, including humans. The metabolites generated by the CYP-dependent oxidative pathway are mostly chemically stable, while several of those generated by the GSH-conjugation pathway are highly reactive. [The Working Group noted that the high flux and chemical stability of most of the oxidative metabolites do not infer that these metabolites are not linked to adverse effects. Also, some highly reactive metabolites generated by the GSH-conjugation pathway may be difficult to detect, thus suggesting that interpretations regarding toxicological importance that are based on quantitative differences in estimated flux must be made with caution.]

(i) CYP-dependent oxidation

The metabolism of trichloroethylene by the oxidative pathway is initiated by the action of several CYP enzymes. While this step occurs predominantly in the liver, it can also occur in a large number of other tissues, including the kidney (Cummings *et al.*, 2000a, 2001), lungs (Odum *et al.*, 1992; Green *et al.*, 1997a; Forkert *et al.*, 2005, 2006), and male reproductive tissues (Forkert *et al.*, 2002, 2003). The overall scheme of oxidative metabolism of trichloroethylene is shown in Fig. 4.1. Several of the metabolites are chemically stable and have been detected in urine; these are highlighted in the scheme.

The initial step in the metabolism of trichloroethylene is catalysed by one of several CYP enzymes and results in formation of an enzyme-bound intermediate (trichloroethylene-epoxide-CYP), which is chemically unstable and, therefore, shown in parentheses. This trichloroethylene-epoxide-CYP can have one of three fates: Conversion to (i) trichloroethylene epoxide; (ii) *N*-hydroxy-acetyl-aminoethanol; or (iii) chloral, which is in equilibrium with chloral hydrate. The majority of the flux is towards chloral hydate. In fact, chloral hydrate is typically the first stable metabolite recovered in incubations of tissues, cells, or microsomes with trichloroethylene.

Trichloroethylene epoxide spontaneously generates either dichloroacetyl chloride (another chemically unstable and reactive species), or oxalic acid, which is readily excreted in the urine. Dichloroacetyl chloride undergoes spontaneous dechlorination to produce dichloroacetate.

Chloral hydrate/chloral undergoes either reduction by alcohol dehydrogenase or CYP to generate trichloroethanol, or oxidation by aldehyde dehydrogenase to form trichloroacetate. Trichloroacetate is typically the major urinary metabolite of trichloroethylene that is recovered, although trichloroethanol is also a significant urinary metabolite. Trichloroethanol can be oxidized by CYPs to yield trichloroacetate,

or can undergo glucuronidation by uridine diphosphate (UDP)-glucuronosyltransferases (UGTs) to produce trichloroethanol glucuronide. Both trichloroethanol and its glucuronide are recovered in the urine, although it is typically trichloroethanol that is mostly recovered due to hydrolytic removal of the glucuronide moiety during sample preparation. Although trichloroacetate is generally poorly metabolized and readily excreted in urine, it may undergo dechlorination to yield dichloroacetate (see *Monograph* on Trichloroacetic Acid in this Volume). Hence, there are two sources of dichloroacetate, dichloroacetyl chloride derived from trichloroethylene epoxide, and trichloroacetate derived from chloral/chloral hydrate.

Although dichloroacetate is a urinary metabolite of trichloroethylene, it can also undergo further metabolism: dichloroacetate can be dechlorinated to form monochloroacetate, which is excreted in the urine; or dichloroacetate is metabolized by a fairly unique GST isoform GST-zeta to yield glyoxylic acid, which is eventually broken down to carbon dioxide (see *Monograph* on Dichloroacetic Acid in this Volume).

Thus, the major CYP-derived, oxidative metabolites of trichloroethylene that are found in urine of exposed animals or humans include dichloroacetate, trichloroacetate, trichloroethanol and its glucuronide, monochloroacetate, and oxalic acid.

A summary of the major oxidative metabolites formed from trichloroethylene, site of formation, the data source (animals, humans, or both), and whether the metabolite is systemically available, is presented in Table 4.1. Systemic availability is based on the chemical stability of the metabolite; those that are relatively stable may be transported from their site of formation into the blood stream and be delivered to other potential target organs, while those that are chemically unstable and reactive tend to remain near their site of formation and react with cellular molecules, including DNA, proteins, and lipids.

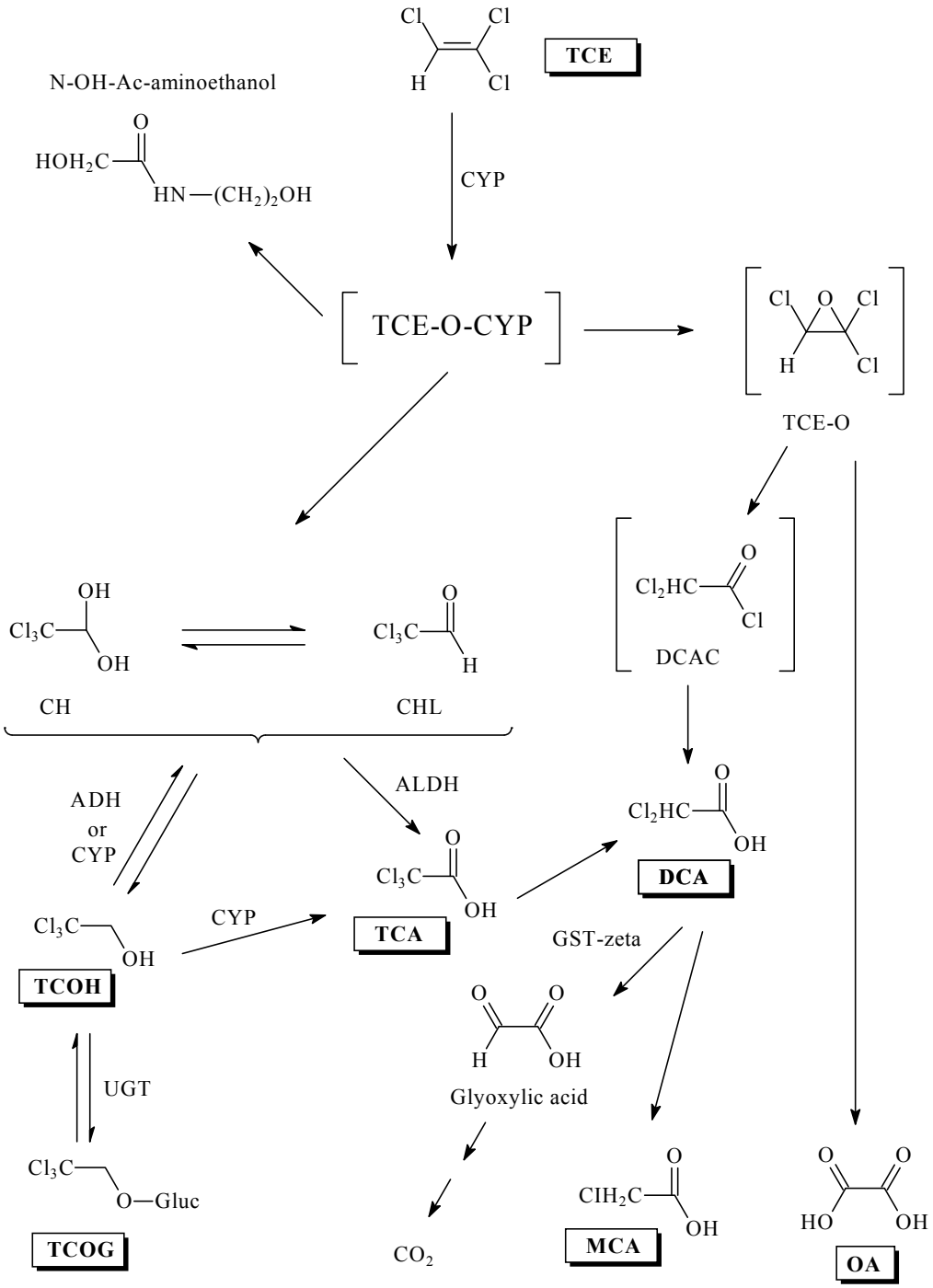
(ii) GSH conjugation

As shown in Fig. 4.2, trichloroethylene undergoes a substitution nucleophilic S_N2 displacement reaction with GSH, releasing Cl^- ion and *S*-(1,2-dichlorovinyl)glutathione (DCVG) as products. Although this initial GSH-conjugation step can occur in many tissues, it occurs primarily in the liver owing to first-pass metabolism and the high content of GSTs in the liver (the various GST isoforms can account for as much as 5% of total cytosolic protein in rat or human liver).

DCVG, whether it is formed within the kidneys or in the liver, is then processed predominantly in the kidneys by a sequence of two hydrolytic enzymes on the proximal tubular brush-border membrane, γ-glutamyltransferase (GGT) and cysteinylglycine dipeptidase, to yield the corresponding cysteine conjugate, *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC).

DCVC can be viewed as a major branching point in this metabolic pathway, as it can have three potential fates. First, DCVC can be *N*-acetylated by the microsomal cysteine conjugate *N*-acetyltransferase to form the mercapturate *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine (NAcDCVC). Second, DCVC can be a substrate for cysteine-conjugate *β*-lyase (CCBL) activities to generate the reactive thiolate *S*-(1,2-dichlorovinyl)-thiol (DCVT). DCVT spontaneously rearranges to form either chlorothioketene or chlorothionoacetyl chloride (Dekant *et al.*, 1988; Völkel & Dekant, 1998), both of which are chemically unstable and reactive and are believed to be the molecular species responsible for formation of covalent adducts derived from DCVC with nucleic acids (Müller *et al.*, 1998a, b), proteins (Hayden *et al.*, 1991), and phospholipids (Hayden *et al.*, 1992). Third, DCVC can be a substrate for the flavin-containing monooxygenase (FMO), yielding a reactive sulfoxide, *S*-(1,2-dichlorovinyl)-L-cysteine

Fig. 4.1 Scheme for oxidative metabolism of trichloroethylene



Trichloroethylene undergoes cytochrome P450 (CYP)-dependent oxidation to form either a trichloroethylene–CYP intermediate or an epoxide intermediate. Further processing through either non-enzymatic rearrangements or the actions of aldehyde dehydrogenase (ALDH), alcohol dehydrogenase (ADH), CYPs, or glutathione-*S*-transferase zeta (GSTZ) yield a variety of metabolites, including chloral (CHL) and chloral hydrate (CH), dichloroacetate (DCA), trichloroacetate (TCA), trichloroethanol (TCOH) and its glucuronide (TCOG), monochloroacetate (MCA), and oxalate (OA). Names of metabolites that are recovered in urine are shown in boxes and those that are chemically unstable or reactive are shown in brackets. Other abbreviations: DCAC, dichloroacetyl chloride; GSH, glutathione; N-OH-Ac-aminoethanol, *N*-hydroxyacetyl aminoethanol; trichloroethylene-O, trichloroethylene epoxide; UGT, UDP-glucuronosyltransferase.

Table 4.1 Formation and systemic availability of trichloroethylene and its metabolites

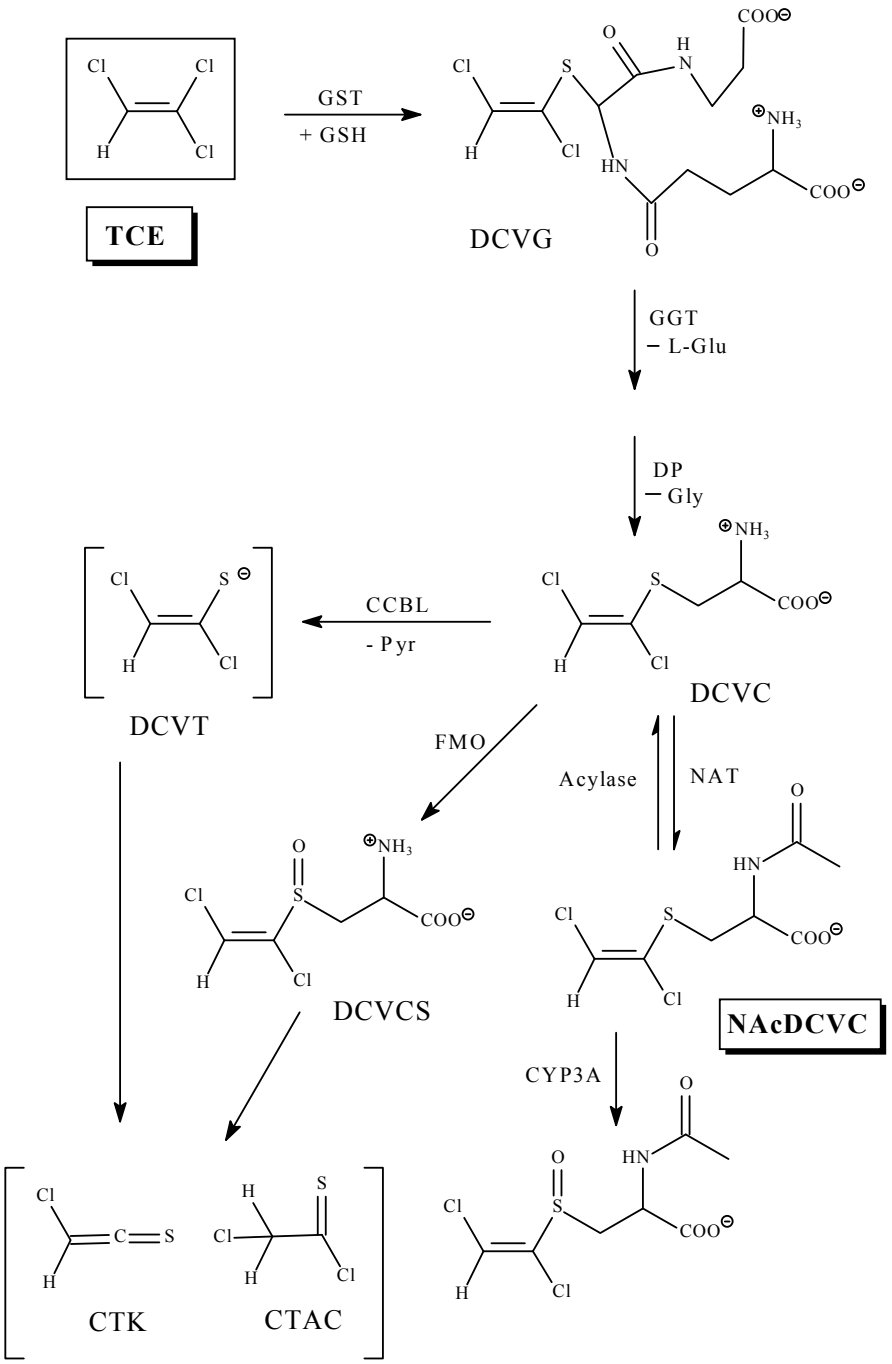
Compound or metabolite	Portal of entry or tissues where formed (A/H)	Systemic availability (A/H)
Trichloroethylene	Lung Gastrointestinal tract Skin	Yes (A,H)
Oxidative metabolites (CYP pathway)		
Trichloroethylene epoxide Dichloroacetyl chloride	Liver (A, H) Lung (A, H) Testes (A, H)	No
Chloral hydrate/chloral	Liver (A, H) Lung (A, H) Testes (A, H)	Yes
Trichloroethanol	Liver (A, H) Lung (A) GI (A, H) Testes (A,H)	Yes
Trichloroacetate	Liver (A, H) Lung (A,H) Testes (H)	Yes
Trichloroethanol glucuronide	Liver (A,H)	Yes
Dichloroacetate	Liver (A) Lung (A) Testes (H)	Yes (low amount)
GSH-conjugation metabolites		
DCVG	Liver (A, H) Kidney (A, H)	Yes
DCVC	Liver (A, H) Kidney (A, H)	Yes
DCVT DCVCS CTK/CTAC	Kidney (A, H) Haematopoietic (A)	No
NAcDCVC NAcDCVCS	Liver (A, H) Kidney (A, H)	Yes

A, animal; CTAC, chlorothionoacetyl chloride; CTK, chlorothioketene; DCA, dichloroacetate; DCAC, dichloroacetyl chloride; DCVC, *S*-(1,2-dichlorovinyl)-*L*-cysteine; DCVCS, DCVC sulfoxide; DCVG, *S*-(1,2-dichlorovinyl)glutathione; DCVT, *S*-(1,2-dichlorovinyl)-thiol; GSH, glutathione; GST, glutathione-*S*-transferase; H, human; NAcDCVC, *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine; NAcDCVCS, NAcDCVC sulfoxide

sulfoxide (DCVCS). Because of the reactive nature of the various intermediates from this pathway, only NAcDCVC has been recovered in urine of experimental animals (Dekant *et al.*, 1986b; Bernauer *et al.*, 1996) and humans (Birner *et al.*, 1993; Bernauer *et al.*, 1996) exposed to trichloroethylene or DCVC.

The mercapturate NAcDCVC can have three potential fates. Apart from being excreted into the urine, NAcDCVC can be deacetylated within the renal proximal tubular cell by aminoacylase III to reform DCVC (Uttamsingh & Anders, 1999; Uttamsingh *et al.*, 2000; Newman *et al.*, 2007). Additionally, mercapturates of several nephrotoxic haloalkenes, including NAcDCVC, are substrates for CYP3A enzymes, yielding sulfoxides (Werner *et al.*, 1995a, b, 1996). Thus, although NAcDCVC is considered a stable end product of the metabolism of trichloroethylene and is recovered in urine, it can undergo additional metabolic transformations that serve to reactivate it. [The Working Group noted that

Fig. 4.2 Scheme for glutathione-dependent metabolism of trichloroethylene



Trichloroethylene (trichloroethylene) undergoes conjugation with glutathione (GSH) to yield the GSH S-conjugate DCVG. After processing to yield the cysteine S-conjugate DCVC, three potential fates are detoxication to yield the mercapturate NAcDCVC or bioactivation by either the cysteine conjugate β-lyase to yield dichlorovinylthiol, which rearranges to yield thioacylating species, or the flavin-containing monooxygenase to yield DCVC sulfoxide. The mercapturate can also be deacetylated to regenerate DCVC or it can undergo CYP3A-dependent sulfoxidation. Names of metabolites than are recovered in urine are shown in boxes and those that are chemical unstable or reactive are shown in brackets. Abbreviations: CCBL, cysteine conjugate β-lyase; CYP3A, cytochrome P-450 3A; CTAC, chlorothionoacetyl chloride; CTK, chlorothioketene; DCVC, S-(1,2-dichlorovinyl)-L-cysteine; DCVG, *S*-(1,2-dichlorovinyl)glutathione; DCVCS, DCVC sulfoxide; DCVT, 1,2-dichlorovinylthiol; DP, dipeptidase; FMO, flavin-containing monooxygenase; GGT, γ-glutamyltransferase; GLy, glycine; GSH, glutathione; GST, GSH S-transferase; L-Glu, L-glutamic acid; NAcDCVC, *N*-acetyl-*S*-(1,2-dichlorovinyl)-*L*-cysteine; NAcDCVCS, NAcDCVC sulfoxide; NAT, *N*-acetyltransferase.

these additional fates of the putative end product of the GSH-conjugation pathway highlight both the complexity of the metabolism of trichloroethylene by this pathway and the potential difficulties in equating overall flux through the GSH-conjugation pathway when using urinary NAcDCVC as a surrogate measurement.]

A summary of the site of formation and systemic availability of the major metabolites from the GSH-conjugation pathway is presented in Table 4.1.

Of the two potential bioactivation pathways for DCVC, the CCBL and FMO reactions, the former has received the most study and is thought to account for most of the bioactivation activity for DCVC (Lash *et al.*, 2000a). CCBL activity is actually a catalytic function of a diverse array of enzymes (Cooper & Pinto, 2006; Lash, 2007). While CCBL activity has also been detected in liver and other tissues, only renal CCBL activity is toxicologically important for renal toxicity, because of the tissue localization of plasma-membrane transporters and several of the enzymes of the GSH-conjugation pathway that determine the distribution of trichloroethylene metabolites.

Studies in the mid-1960s first identified a thiol metabolite of a sulfonamide that was formed by a C–S lyase activity (Colucci & Buyske, 1965). Schultze and coworkers (Anderson & Schultze, 1965; Bhattacharya & Schultze, 1967) subsequently identified hepatic and renal enzymes that catalysed this reaction, and Tateishi *et al.* (1978) were the first to use the term "cysteine conjugate β-lyase" to describe this activity in rat liver. All the known CCBL enzymes contain pyridoxal 5′-phosphate as coenzyme. Although the overall β-lyase reaction mechanism is cleavage of a C–S bond to yield a reactive, thioacylating species, subsequent studies (Stevens *et al.*, 1986; Elfarra *et al.*, 1987) showed that the reaction mechanism can occur by either a direct β-elimination reaction or a transamination reaction with a suitable α-keto acid cosubstrate to yield either the thiolate or a propionic acid derivative, respectively; the latter is chemically unstable and rearranges to release the thiolate.

According to Cooper & Pinto (2006), there are 11 distinct mammalian enzymes capable of catalysing the CCBL reaction (Table 4.2). Some of the CCBL enzymes catalyse both β-elimination and transamination reactions, while others can only catalyse the former. The relative importance of each of these activities in the bioactivation of DCVC, however, is not presently known.

The FMOs, like the CYPs, represent a multigene family of enzymes. Both enzyme systems also share several other characteristics, including localization in the endoplasmic reticulum, requirement for NADPH as a reductant, and overall catalysis of a mixed-function oxidation reaction. Differences do exist, however, that make some of the functions of the FMOs rather distinctive. For example, although there are more than 50 individual functional CYP enzymes from more than 40 gene families in humans, there are only five FMO genes in mammals. FMOs catalyse the oxidation of chemicals containing sulfur, selenium, and nitrogen (Ziegler, 1993; Cashman & Zhang, 2006). Although FMOs and some CYPs share substrates and catalyse the same overall reactions, FMOs have some distinctive substrates, including cysteine *S*-conjugates of various haloalkenes and haloalkanes.

4.1.4 Excretion

(a) Humans

The main routes for the excretion of trichloroethylene are exhalation of the parent compound at high doses, and urinary excretion of metabolites. After exposure by inhalation, pulmonary excretion during and up to 5 days after exposure has been estimated to account for 19–35% of intake, with trichloroethanol and trichloroacetic acid in urine accounting for 24–39% of intake, and the balance still retained in the body at the end of the reporting period (Monster *et al.*, 1976; Opdam, 1989; Chiu *et al.*, 2007). The terminal

half-life of trichloroethylene in alveolar air has been estimated to be about 6–44 hours (Sato *et al.*, 1977; Opdam, 1989; Chiu *et al.*, 2007), reflecting release from storage in fat. The half-lives of trichloroethanol and trichloroacetate in urine have been estimated to be in the range of 15–50 and 36–73 hours, respectively (Bartonicek, 1962; Stewart *et al.*, 1970; Ikeda *et al.*, 1971; Nomiyama & Nomiyama, 1971; Ogata *et al.*, 1971; Ikeda & Imanura, 1973).

Limited information was available on faecal elimination of trichloroethylene and its metabolites. On the third day after exposure by inhalation, Bartonicek (1962) reported detecting trichloroacetate and trichloroethanol in the faeces at concentrations similar to those in urine. However, by the seventh day after exposure, neither substance was detected. Since daily faecal production is 10 times less than daily urine production, this implies that excretion in the urine is about 10-fold that in faeces.

(b) Experimental systems

Exhalation of trichloroethylene has been measured in rodents given trichloroethylene via inhalation, oral, and dermal exposure (Dekant *et al.*, 1984; Green & Prout, 1985; Prout *et al.*, 1985; Dekant *et al.*, 1986a; Dallas *et al.*, 1991; Poet *et al.*, 2000). Radiolabelling studies have also reported exhaled carbon dioxide as an excretion product in addition to unchanged parent (Dekant *et al.*, 1984, 1986a; Green & Prout, 1985; Prout *et al.*, 1985). In rats and mice, there is a general trend towards increasing exhalation of unchanged trichloroethylene with increasing dose from 2 to 2000 mg/kg bw, suggesting saturation of a metabolic pathway. In mice, the percentage recovered in air increased from 1–6% to 10–18% in this dose range, while in rats, the increase was from 1–3% to 43–78%. This was consistent with the greater overall metabolic capacity in mice than in rats. At exposures below saturation, most of the trichloroethylene administered is excreted in urine as metabolites. In addition, urinary excretion is relatively rapid in rodents, with reported half-lives of 14–17 hours (Ikeda & Imanura, 1973), and complete elimination within 1 or 2 days after exposure (Dekant *et al.*, 1984; Green & Prout, 1985; Prout *et al.*, 1985). [The Working Group noted that excretion rates in rodents are faster than those inferred from studies in humans.]

With respect to faecal elimination, Dekant *et al.* (1984) and Kim & Ghanayem (2006) reported that total radioactivity recovered in mouse and rat faeces after oral exposures accounted for about 1–5% of total radiolabel administered. However, Green & Prout (1985) and Prout *et al.* (1985) reported higher amounts of faecal excretion, about 8–24%, in various strains of mice.

4.2 Genotoxicity and related effects

4.2.1 Humans

(a) Chromosomal aberration, sister-chromatid exchange and related genotoxic effects

The limited number of studies on the genotoxicity of trichloroethylene have focused on the association between exposure to trichloroethylene and the development of chromosomal aberrations in exposed individuals. Chromosomal aberrations are disruptions in the normal content of the chromosomes and can include both numerical and structural changes, such as translocations and deletions (Natarajan *et al.*, 1996). There have been no additional studies of genotoxicity with trichloroethylene since the previous IARC evaluation in 1995 (IARC, 1995; Table 4.3; reviewed in Tabrez & Ahmad, 2009). The evidence for genotoxic effects of trichloroethylene in humans was limited and based on a small number of studies (IARC, 1995; Table 4.3). Exposure to trichloroethylene did not result in a significantly increased frequency of sister-chromatid exchange (SCE) in lymphocytes in one occupational study comparing trichloroethylene-exposed workers and controls, while in

Table 4.2 Mammalian enzymes capable of catalysing cysteine-conjugate β-lyase activity

Enzyme	Tissue (A/H)	Competing transamination	Subunit molecular weight (kDa)	Reference(s)
Cytoplasmic enzymes				
GTK/KAT (EC 2.6.1.64/EC 2.6.1.7)	Kidney (A/H)	Yes	45 (homodimer)	Stevens *et al.* (1986), Lash *et al.* (1990), Perry *et al.* (1993, 1995), Yamauchi *et al.* (1993)
GTK/KAT	Choroid plexus (A)	Yes	45 (homodimer)	Cooper *et al.* (1993), Alberati-Giani *et al.* (1995)
Kynureninase (EC 3.7.1.3)	Liver (A/H)	ND	55 (homodimer)	Tateishi *et al.* (1978), Stevens & Jakoby (1983), Stevens (1985), Tomisawa *et al.* (1986)
cytASAT (EC 2.6.1.1)	Heart (H)	No	45 (monomer)	Gaskin *et al.* (1995)
ALAT (EC 2.6.1.2)	Heart (H)	No		Gaskin *et al.* (1995)
$BCAT_c$ (EC 2.6.1.42)	Brain (H)	No	44 (homodimer)	Cooper *et al.* (2003)
High-molecular-weight CC β-lyase	Liver, kidney (A)	Yes	330	Abraham *et al.* (1995a)
Mitochondrial enzymes				
mitASAT (EC 2.6.1.1)	Liver (A)	Yes	45 (monomer)	Cooper *et al.* (2002)
$BCAT_m$ (EC 2.6.1.42)	Brain (H)	No	44 (homodimer)	Cooper *et al.* (2003)
GTK/KAT (EC 2.6.1.64/EC 2.6.1.7)	Brain (A)	Yes	45 (homodimer)	Malherbe *et al.* (1995)
High-molecular-weight CC β-lyase	Liver, kidney (A)	Yes	330	Abraham *et al.* (1995b), Cooper *et al.* (2001)

A, studies in experimental animals; ALAT, alanine aminotransferase; $BCAT_{c/m}$, branched-chain aminotransferase (cytoplasm/mitochondria); CC β-lyase, cysteine-conjugate β-lyase; cyt/mitASAT, cytosolic and mitochondrial aspartate aminotransferase; GTK, glutamine transaminase K; H, studies in human tissue; KAT, kynurenine aminotransferase; ND, not determined
Modified from Lash (2007).

another study it was reported that the frequency of structural aberrations and hyperdiploid cells in cultured lymphocytes was significantly increased in metal workers exposed to trichloroethylene compared with unexposed people (Tabrez & Ahmad, 2009). In another study, while quantitative estimates of exposure to trichloroethylene were not reported for degreasing workers, only workers with the highest levels of exposure (i.e. exposures > 20 hours per week) were included in the analysis (Rasmussen *et al.*, 1988). The reference group (not exposed) for this analysis was drawn from a population-based study and parents of offspring with stable chromosome abnormalities, and therefore may be less comparable to the exposed workers than in other studies. There is some evidence that the frequency of SCE may be significantly elevated in lymphocytes in male smokers exposed to trichloroethylene in an occupational setting, compared with male smokers in the control group, but these findings were based on only 15 subjects and similar associations were not observed for women or for nonsmokers (Seiji *et al.*, 1990). In this study, non-exposed workers were matched to exposed workers by age, sex, smoking habits, and working location in the factory. One study in India, including 97 trichloroethylene-exposed individuals and 220 individuals without exposure, evaluated genotoxicity in lymphocyte cultures *in vitro* in relation to polymorphisms in detoxifying genes (*CYP1A1, GSTM1, GSTT1, GSTP1;* Kumar *et al.*, 2009). Peripheral lymphocytes were treated *in vitro* with trichloroethylene at concentrations of 2, 4, and 6 mM and were examined for chromosomal aberration and micronucleus formation. [The Working Group noted that these high, non-physiological concentrations

of solvent typically cause non-specific effects.] Genotype frequencies for the detoxifying genes examined were similar in the solvent-exposed and control groups, and increased frequencies of chromosomal aberrations and micronucleus induction were generally not observed, except in rare cases at the higher levels of exposure to trichloroethylene, although no differences based on genotype were reported. Therefore while the available data from studies in humans provided inconclusive evidence of a genotoxic effect of trichloroethylene, the findings of an elevated frequency of structural chromosomal aberration and SCE in a subset of exposed individuals, combined with the weak mutagenic activity of trichloroethylene observed in some experimental studies, suggested that such effects cannot be ruled out (Tabrez & Ahmad, 2009).

(b) Mutations in the VHL gene

Several studies have evaluated the association between exposure to trichloroethylene and mutation of the von Hippel-Lindau (*VHL*) tumour suppressor gene, which has been reported in a relatively large percentage of cases of renal cell carcinoma (Table 4.3). *VHL* is thought to be a target for environmental carcinogens. The results regarding the association between renal cell carcinoma and mutations in the *VHL* gene were inconclusive.

The first study was of 23 patients in Germany with histologically confirmed renal cell carcinoma who had previously been exposed to trichloroethylene for an average of about 22 years, and who were estimated to have exposure levels exceeding 50 ppm based on reported symptoms (Brüning *et al.*, 1997a). Tumour tissue and normal tissue from each patient was isolated and individual *VHL* exons were amplified and subsequently analysed for single-strand conformation polymorphism (SSCP). All 23 patients had abnormal SSCP patterns in one of the exons considered when compared with wild-type *VHL* sequences, with most differences being in exon 2 (44% of patients), followed by exon 1 (30% of patients), and exon 3 (26% of patients). Further sequencing analysis of a subset of the aberrations confirmed these as *VHL* mutations. This frequency of mutation was higher than found in other studies reviewed below, and differed with that found in a later study in Germany in terms of the specific *VHL* exon that showed the highest frequency of mutation (Brauch *et al.*, 1999).

A case–control study in Germany enrolled 44 patients with renal cell carcinoma (a subset of whom were previously enrolled in the study by Brüning *et al.* 1997a) who had previously worked in metal-processing factories and had high cumulative exposures to trichloroethylene, and 107 control patients without reported exposure to trichloroethylene (Brauch *et al.*, 1999). [The Working Group noted that it did not see these two studies as independent.] The patients with exposure to trichloroethylene had a mean age of 24 years when they first began working with trichloroethylene, and were estimated to have exposure levels > 50 ppm based on reported clinical symptoms such as headache and nausea. Exposure intensity for the workers was scored on the basis of criteria that included the duration and frequency of working with trichloroethylene, associated clinical symptoms, and how the liquid trichloroethylene was regularly handled (Brauch *et al.*, 1999). *VHL* mutations were assessed by SSCP analysis, and microsatellite analysis was used to assess loss of heterozygosity (LOH). Of the 44 patients with renal cell carcinoma who had been exposed to trichloroethylene, *VHL* mutations were identified in 33 patients, and 14 of these had multiple, mainly missense (54%), mutations. The majority of mutations were found at exon 1 (52%), followed by exon 3 (28%), and exon 2 (20%) (Brauch *et al.*, 1999). Moreover, a mutational hot spot at nucleotide 454 of the *VHL* gene, which resulted in a C→T base change, was found in 39% of patients with a *VHL* mutation and 18 out of 18 renal cell carcinomas with intragenic somatic *VHL* mutation were reported to have LOH at the

Table 4.3 Genetic effects of trichloroethylene in humans

End-points evaluated	Total No. exposed	Total No. controls	Study design	Mean exposure to trichloroethylene	Notable effects	Comments	Reference, study location and period
Chromosomal aberrations in lymphocytes; sperm count and morphology, % two fluorescent bodies, structural aberrations, hyperdiploid cells	15	14 for semen examination and 669 for lymphocyte analysis	Population-based (all degreasing workers identified)	Trichloroethylene exposures > 20 hr per week in 15 workers included in genotoxic analyses; exposure level, NR	Exposed workers had significantly increased occurrence of structural aberrations and hyperdiploid cells compared with controls	No personal exposure monitoring conducted; exposure data collected via occupational medical interview; reference group for lymphocyte analysis was drawn from a population study and parents of offspring with stable chromosome abnormalities.	Rasmussen *et al.* (1988) Denmark
SCE in human lymphocytes *in vivo*	6	9	Workers exposed to trichloroethylene	Exposed level, NR	Exposed workers had significant increase in SCE	Personal exposures were assessed by the measure of trichloroethylene metabolites in the blood (trichloroethanol and trichloroacetate).	Gu *et al.* (1981)
SCE	22	22	Cross-sectional	Exposed: U-TTC, 183.6 mg/L	No differences between exposed and controls, including in smokers	Mean employment duration of workers was 9.7 years; U-TTC used as index for degree of exposure, no monitoring conducted; unexposed controls matched to exposed workers by sex, age, and smoking habits	Nagaya *et al.* (1989) Japan

Table 4.3 (continued)

End-points evaluated	Total No. exposed	Total No. controls	Study design	Mean exposure to trichloroethylene	Notable effects	Comments	Reference, study location and period
SCEs in peripheral blood lymphocytes.	38	51	Cross-sectional	Exposed: 7 ppm	No overall difference in SCEs in exposed workers and controls, but significant increase in male exposed smokers compared with male smoking controls.	Diffuse sampling techniques used to monitor workers in breathing zone as TWA for 8 h working day; concurrent controls matched to exposed workers by age, sex, smoking, location of factory	Seiji *et al.* (1990) Japan
VHL mutation	23	NA	Patients from two case–control studies	Semi-quantitative exposure estimates; average exposure time, 21.8 years	All 23 patients had aberrations of the *VHL* gene (exon 1, 30%; exon 2, 44%; exon 3, 26%)	Higher than expected mutation frequency; mutation prevalence highest in exon 2 whereas prevalence was highest in exon 1 in subsequent study (Brauch *et al.*, 1999)	Brüning *et al.* (1997a) Germany
VHL mutation	44	107	Case–control	Exposure determined by occupational hygenists and ranked as one of three levels. Scoring system took into account total exposure time, frequency, and duration of acute adverse effects.	75% mutation prevalence in trichloroethylene-exposed samples (multiple mutations, $n = 14$); 54% of mutations were missense; mutational hotspot at nucleotide 454 in 39% of patients and only in trichloroethylene-exposed.	Majority of mutations at exon 1 (52%); exon 1 is very difficult to sequence in FFPE tissue; mutations at nucleotide 454 were also observed in clear cell and papillary subtypes.	Brauch *et al.* (1999) Germany

Table 4.3 (continued)

End-points evaluated	Total No. exposed	Total No. controls	Study design	Mean exposure to trichloroethylene	Notable effects	Comments	Reference, study location and period
VHL mutation	17 (RCC cases exposed to trichloroethylene)	21 (RCC cases not exposed to trichloroethylene)	Case–control	Used same exposure scoring system as in previous study by Brauch *et al.* (1999).	Exposed cases had a higher frequency of mutations (82% versus 10% unexposed), multiple mutations (50% versus 0% unexposed), and 454 C→T mutation (38% versus 0% unexposed).	Reported methodological difficulties including insufficient DNA yield; 75% of *VHL* coding gene amplified; mutation rate only 10% in the unexposed cases.	Brauch *et al.* (2004) Germany
VHL mutation	69 cases of RCC	NA	Patients from a case–control study	Expert evaluated occupational questionnaires and used a task-exposure matrix; exposures assigned as low/ medium/high level.	No significant differences in frequency of mutations between RCC cases according to trichloroethylene exposure status; 4 mutations detected in 48 cases of RCC.	Potential trichloroethylene exposure misclassification, OR for trichloroethylene and RCC from case–control study was higher after restricting to higher confidence trichloroethylene exposures.	Charbotel *et al.* (2007) France
VHL mutation	470 sporadic clear cell RCC cases	NA	Case series	Exposure assessment based on expert review; estimated frequency, intensity, and cumulative exposure.	*VHL* inactivation reported in 86.6% of clear cell RCC cases, but differences in alteration prevalence according to trichloroethylene exposure status.	Only one unexposed case had a *VHL* mutation at nt454 and the *VHL* mutation prevalence was similar to trichloroethylene-exposed and unexposed cases.	Moore *et al.* (2011) Europe

FFPE, formalin-fixed, paraffin-embedded; NA, not applicable: normal tissue used as control or known control with additional band as positive control; nt, nucleotide; OR, odds ratio; RCC, renal cell carcinoma; SCE, sister-chromatid exchange; TWA, time-weighted average; U-TTC, urinary total trichloro compounds; ND, not determined.

3p allele. Notably, only patients with high and medium exposure to trichloroethylene, but not low exposure, had *VHL* mutations, and there was a significant correlation between severity of exposure and presence of multiple mutations.

A follow-up of the previous study compared the characteristics of *VHL* mutations in cases of renal cell carcinoma in people exposed to trichloroethylene (n = 17) and cases in people not exposed to trichloroethylene (n = 21) (Brauch *et al.*, 2004). Samples of tissues from tumour and non-tumour areas of the kidney were collected from the 38 cases, microdissected, and amplification and sequencing of the individual *VHL* exons was conducted using polymerase chain reaction (PCR). Cases of renal cell carcinoma associated with occupational exposure to trichloroethylene were reported to be diagnosed at a younger age (median, 57.5 years) compared with cases with no exposure to trichloroethylene (median, 67 years). In addition, mutation characteristics of the *VHL* gene differed according to trichloroethylene-exposure status, as exposed cases had a higher frequency of somatic mutations (82% in exposed versus 10% in unexposed), multiple mutations (50% in exposed versus 0% in unexposed), and frequency of the nucleotide 454 C→T hot spot mutation previously identified (38% in exposed versus 0% in unexposed). Methodological difficulties were reported relating to insufficient DNA yield, which may have explained the lower than expected frequency of *VHL* mutation in the unexposed cases (10%), compared with the 44% expected from published case series (Brauch *et al.*, 2004). Specifically, failure of the PCR amplification for the GC-rich *VHL* exon 1 occurred in 25% of cases, while the failure rate in cases was 76% for exon 3. This, combined with mutation-detection issues resulting from template disequilibrium, could have resulted in the observed frequency (Brauch *et al.*, 2004).

A study in France analysed somatic mutations in the *VHL* gene in 69 cases of renal cell carcinoma from a case–control study, of which 48 were clear cell renal cell carcinomas (Charbotel *et al.*, 2007). Formalin and Bouin's fixed paraffin-embedded tissue samples (n = 64) and frozen samples (n = 5) were obtained from patients, and screening for *VHL* mutation was conducted using PCR. The *VHL* gene was amplified and sequenced for all of the frozen samples, and for 71% and 38% of the formalin-fixed and Bouin's fixed samples, respectively, resulting in a complete sequence, with 100% of the coding region analysed, for 26 tumours (54%). There was no statistically significant difference between the trichloroethylene-exposed and not-exposed cases (P = 0.40) with respect to the frequency of mutations in codon 81, or the frequency of mutation overall. For the cases of clear cell renal cell carcinoma for which the *VHL* gene was fully sequenced (trichloroethylene-exposed, 15; not exposed, 11) and those for which the *VHL* gene was not entirely sequenced (trichloroethylene-exposed, 25; not exposed, 23), there were two mutations in each group. The low prevalence of alterations in *VHL* could be attributed to the method of DNA extraction, as formalin-fixing methods generally yield DNA of lower quality than that that obtained after freezing (Moore *et al.*, 2011). Another limitation was potential exposure misclassification, since sensitivity analyses from the case–control study that evaluated the association between exposure to trichloroethylene and renal cell carcinoma reported that the odds ratio increased when the analysis was restricted to cases for which exposure information was judged to be of good quality (Charbotel *et al.*, 2006).

A large case-series of clear cell renal cell carcinomas (n = 470) enrolled from a hospital-based case–control study in Europe evaluated *VHL* inactivation in relation to risk of sporadic clear cell renal cell carcinoma (Moore *et al.*, 2011). All cases were newly diagnosed and histologically confirmed. Exposure to chlorinated solvents was assessed using job-specific questionnaires, and expert review (blinded to disease status) was

conducted to estimate cumulative exposure, and frequency and intensity of exposure. In 86.6% of cases of sporadic clear cell renal cell carcinoma, *VHL* was inactivated through genetic (sequence alterations) or epigenetic (promoter methylation) mechanisms. The most common alterations observed were deletions (37.8%) and missense mutations (27.9%), and most alterations were found in exon 1 (36.7%). Consideration of exposure to trichloroethylene and the prevalence of *VHL* mutation did not reveal a difference between exposed and non-exposed cases with respect to overall frequency of alteration, or frequency of multiple mutation, and no specific hotspot mutation at nucleotide 454 (codon 81) was identified among cases exposed to trichloroethylene (Moore *et al.*, 2011).

The methodological issues relating to the interpretation of the results from these studies have been recently reviewed (EPA, 2011a). These issues include the method of tissue preparation (i.e. frozen, formalin-fixed), potential contamination of the tumour tissue with neighbouring healthy tissue resulting in a lower mutation frequency, and biases involving subject selection and misclassification. Furthermore, discrepancies between studies may be attributed to differences in levels of exposure to trichloroethylene (Moore *et al.*, 2011).

In summary, epidemiological studies of somatic mutations in the *VHL* gene have been inconclusive, as three studies conducted in Germany have reported alterations in the *VHL* gene associated with exposure to trichloroethylene, but have differed with respect to which exon was most commonly affected. These findings were not replicated in a study in France, or in a large case-series of clear cell renal cell carcinomas in Europe. Reported methodological difficulties and concern for bias in some studies prevented firm conclusions from being drawn.

4.2.2 Experimental systems

The genetic effects of trichloroethylene in experimental systems, as measured in DNA-binding studies; in bacterial systems; in fungal and yeast systems; and in mammalian systems have been studied. For information on the genotoxicity of some oxidative metabolites of trichloroethylene, see the *Monographs* on Chloral and Chloral Hydrate, Dichloroacetic Acid, and Trichloroacetic Acid in this Volume. The genetic toxicology of trichloroethylene has been reviewed (Baden & Simmon, 1980; Fabricant & Chalmers, 1980; Vainio *et al.*, 1985; Crebelli & Carere, 1989; Candura & Faustman, 1991; Jackson *et al.*, 1993; ECETOC, 1994; EPA, 2011a). The possible mechanisms for genotoxicity of trichloroethylene were discussed by Henschler (1987).

In considering the genotoxicity of trichloroethylene, it should be recognized that stabilizers, such as epichlorohydrin and 1,2-epoxybutane, are often used in commercial preparations of trichloroethylene. These stabilizers are mutagenic, rendering problematic the interpretation of positive results in assays for mutagenicity with trichloroethylene (McGregor *et al.*, 1989). Humans are exposed mostly, if not exclusively, to preparations of trichloroethylene containing stabilizers. Table 4.4 lists the known tests for genotoxicity that have been conducted on samples of pure trichloroethylene (without stabilizers), and Table 4.5 lists results of tests for genotoxicity with samples for which the content of stabilizers was unclear.

(a) Binding to DNA and protein

Evidence suggests that exposure to trichloroethylene can lead to binding to DNA and proteins, probably due to the conversion of trichloroethylene to reactive metabolites. In studies *in vitro*, ^{14}C-labelled trichloroethylene was found to bind to salmon sperm DNA and calf thymus DNA under conditions that favoured the presence of CYP enzymes and inhibited the activity

of epoxide hydrolase (Banerjee & Van Duuren, 1978; DiRenzo *et al.*, 1982). The work of Miller & Guengerich (1983) and Cai & Guengerich (2001a) indicated that liver microsomes or whole hepatocytes from phenobarbital-induced rats enhanced binding of trichloroethylene metabolites to DNA and to protein. The levels of DNA adducts (binding to calf thymus DNA) and protein adducts formed in the presence of microsomal preparations of human liver were approximately the same as those observed in liver microsomes from untreated rats. Trichloroethylene epoxide was found to be responsible for the binding to protein, and also to DNA to a lesser extent.

In studies in rodents *in vivo*, radiolabelling of protein was greater than of DNA (Stott *et al.*, 1982; Mazzullo *et al.*, 1992).

Covalent binding of radiolabel to hepatic and renal proteins in male F344 rats and $B6C3F_1$ mice after exposure to [^{14}C]trichloroethylene was studied by Eyre *et al.* (1995a). Association of radiolabel from [^{14}C]trichloroethylene was observed in liver (peak, 2–4 hours) and kidney (peak, 8 hours) proteins in rats and mice. The formation of acid-labile protein adducts from [^{14}C]trichloroethylene was approximately two times greater in mice than in rats. In the same study, there was no evidence for formation of these adducts in rats and mice treated with [^{14}C]-labelled trichloroacetic acid or [^{14}C]-labelled dichloroacetic acid, while treatment with [^{14}C]-labelled DCVC resulted in the formation of renal acid-labile protein adducts at a level that was greater by approximately 12-fold in male $B6C3F_1$ mice than in male F344 rats.

A low level of covalent interaction was reported with DNA from rat and mouse liver, kidney, lung, and stomach (estimated at 0.15 adducts per million nucleotides (Mazzullo *et al.*, 1992); and from mouse liver (maximum, 0.62 alkylations per million nucleotides, Stott *et al.*, 1982). Binding of [^{14}C]trichloroethylene to DNA *in vitro* was enhanced by the addition of GSH and was reduced by the addition of SKF-525-A, a CYP inhibitor (Mazzullo *et al.*, 1992). High-performance liquid chromatography indicated the possible presence of a DNA adduct, which was not identified. In other studies, DNA binding could not be demonstrated *in vivo* in several tissues of mice (Bergman, 1983), or in the liver of rats (Parchman & Magee, 1982); however, Bergman (1983) noted incorporation of radiolabel into nucleosides.

(b) *Mutation*

(i) *Bacterial systems*

See Table 4.4 and Table 4.5

The Ames assay for gene mutation has been conducted with trichloroethylene (pure, or of unspecified purity) in various strains of *Salmonella typhimurium*, with and without metabolic activation. Both positive and negative results have been reported, but several studies showed positive results with metabolic activation in strain TA100. The pure samples of trichloroethylene (non-technical grade) did not induce mutation in other strains.

Trichloroethylene (pure, or of unspecified purity) gave negative results in the SOS chromotest in *Escherichia coli* with or without metabolic activation, and in the Mutatox assay in the absence of metabolic activation. In the presence of metabolic activation, analytical-grade trichloroethylene induced *arg*$^+$reverse mutations, but not forward mutations or *gal*$^+$or *nad*$^+$ reversions, in *E. coli*.

(ii) *Fungi and yeast systems*

See Table 4.4 and Table 4.5

The ability of trichloroethylene to induce gene mutation, conversion, and recombination has been studied in fungi and yeast systems. Trichloroethylene (pure, or of unspecified purity) induced gene conversion in *Saccharomyces cerevisiae* in two out of three studies, and induced reverse mutation in the presence of metabolic activation in all four studies available. In a single study, pure trichloroethylene or trichloroethylene

Table 4.4 Genetic and related effects of trichloroethylene without mutagenic stabilizers in experimental systems

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SOS chromotest, *Escherichia coli* PQ37	–	–	7325[c]	Mersch-Sundermann *et al.* (1989)
Salmonella typhimurium BAL13, forward mutation (ara test)	–	–	190	Roldán-Arjona *et al.* (1991)
Salmonella typhimurium TA100, reverse mutation	–	+	160 vapour[c]	Simmon *et al.* (1977)
Salmonella typhimurium TA100, reverse mutation	–	+	160 vapour[c]	Baden *et al.* (1979)
Salmonella typhimurium TA100, reverse mutation	–	–	420 (8% vapour) 16 h	Bartsch *et al.* (1979)
Salmonella typhimurium TA100, reverse mutation	–	+	18 vapour	Crebelli *et al.* (1982)
Salmonella typhimurium TA100, reverse mutation	–	–	260 vapour[d]	Shimada *et al.* (1985)
Salmonella typhimurium TA100, reverse mutation	–	–	167[c]	Mortelmans *et al.* (1986)
Salmonella typhimurium TA100, reverse mutation	0	–	1050 vapour	McGregor *et al.* (1989)
Salmonella typhimurium TA1535, reverse mutation	–	–	2800 vapour[c]	Simmon *et al.* (1977)
Salmonella typhimurium TA1535, reverse mutation	–	–	526 vapour[c]	Baden *et al.* (1979)
Salmonella typhimurium TA1535, reverse mutation	(+)	0	50[c]	Kringstad *et al.* (1981)
Salmonella typhimurium TA1535, reverse mutation	(+)	–	50 vapour[d]	Shimada *et al.* (1985)
Salmonella typhimurium TA1535, reverse mutation	–	–	167[c]	Mortelmans *et al.* (1986)
Salmonella typhimurium TA1537, reverse mutation	–	–	167[c]	Mortelmans *et al.* (1986)
Salmonella typhimurium TA98, reverse mutation	–	–	167[c]	Mortelmans *et al.* (1986)
Salmonella typhimurium TA98, reverse mutation	0	–	1050 vapour	McGregor *et al.* (1989)
Salmonella typhimurium YG7108 pin 3ERb5, reverse mutation	0	–	3000 µg/plate	Emmert *et al.* (2006)
Saccharomyces cerevisiae D7, gene conversion	–	+	2600	Bronzetti *et al.* (1978)
Saccharomyces cerevisiae D7, reverse mutation	–	+	1300	Bronzetti *et al.* (1978)
Aspergillus nidulans, diploid yA2/+ strain 35×17, quiescent conidia, mitotic crossing-over	–	0	3660	Crebelli *et al.* (1985)
Aspergillus nidulans, diploid yA2/+ strain 35×17, growth-mediated assay, mitotic crossing-over	–	0	90 vapour	Crebelli *et al.* (1985)
Schizosaccharomyces pombe P1, stationary phase, forward mutation	–	–	3280	Rossi *et al.* (1983)
Schizosaccharomyces pombe P1, growing cells, forward mutation	–	–	13 140	Rossi *et al.* (1983)

Table 4.4 (continued)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Aspergillus nidulans, haploid strain 35, quiescent conidia, forward mutation (methionine suppressor)	–	0	100 vapour	Crebelli *et al.* (1985)
Aspergillus nidulans, haploid strain 35, 'growth-mediated assay', forward mutation (methionine suppressor)	+	0	13 vapour	Crebelli *et al.* (1985)
Aspergillus nidulans, diploid yA2/+ strain 35 × 17, quiescent conidia, nondisjunctional diploids	–	0	3660	Crebelli *et al.* (1985)
Aspergillus nidulans, diploid yA2/+ strain 35 × 17, quiescent conidia, haploids	–	0	3660	Crebelli *et al.* (1985)
Aspergillus nidulans, diploid yA2/+ strain 35 × 17, 'growth-mediated assay', nondisjunctional diploids	+	0	40 vapour	Crebelli *et al.* (1985)
Aspergillus nidulans, diploid yA2/+ strain 35 × 17, 'growth-mediated assay', haploids	+	0	90 vapour	Crebelli *et al.* (1985)
Drosophila melanogaster, sex-linked recessive lethal mutation	–		2500[c] injection	Foureman *et al.* (1994)
Drosophila melanogaster, sex-linked recessive lethal mutation	?		5000 feeding[c]	Foureman *et al.* (1994)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	0	130 vapour[d]	Shimada *et al.* (1985)
Unscheduled DNA synthesis, human lymphocytes *in vitro*	(+)	0	[7], 2.5–10µg/mL	Perocco & Prodi (1981)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	+	146[c]	Caspary *et al.* (1988)
Sister chromatid exchange, Chinese hamster ovary (CHO) cells *in vitro*	(+)	(+)	401[c]	Galloway *et al.* (1987)
Micronucleus induction, Chinese hamster ovary (CHO-K_1) cells *in vitro*	+	0	150 [0.8–1.4 ppm]	Wang *et al.* (2001)
Chromosomal aberrations, Chinese hamster ovary (CHO) cells *in vitro*	–[e]	–[e]	14 900[c]	Galloway *et al.* (1987)
Cell transformation, RLV/Fischer rat F1706 embryo cells *in vitro*	+	0	144	Price *et al.* (1978)
Micronucleus induction, human hepatoma Hep G2 cells	+	0	0.5 mM [65.7 µg/mL]	Hu *et al.* (2008)
Gene mutation, human lymphoblastoid TK6 cells *in vitro*	–	–	600	Caspary *et al.* (1988)
Inhibition of intercellular communication, B6C3F_1 mouse hepatocytes *in vitro*	+	0	1.3	Klaunig *et al.* (1989)

Table 4.4 (continued)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Inhibition of intercellular communication, F344 rat hepatocytes *in vitro*	–	0	13	Klaunig *et al.* (1989)
Host-mediated assay, gene conversion in *Saccharomyces cerevisiae* D4 recovered from CD-1 mouse liver, lungs and kidneys	+		400 po × 1[f]	Bronzetti *et al.* (1978)
Host-mediated assay, gene conversion in *Saccharomyces cerevisiae* D7 recovered from CD-1 mouse liver and kidneys	+		400 po × 1	Bronzetti *et al.* (1978)
Host-mediated assay, gene conversion in *Saccharomyces cerevisiae* D7 recovered from CD-1 mouse lungs	–		400 po × 1	Bronzetti *et al.* (1978)
HMM, Host-mediated assay, reverse mutation in *Saccharomyces cerevisiae* D7 from CD-1 mouse liver, lungs and kidneys	+		400 po × 1	Bronzetti *et al.* (1978)
Host-mediated assay, forward mutation in *Schizosaccharomyces pombe* P1, CD-1 × C57Bl hybrid mouse	–		2000 iv or ip × 1	Rossi *et al.* (1983)
Gene mutation, *Lac Z* transgenic mice, base change or small-deletion mutation in kidney, spleen, liver, lung, testis *in vivo*	–		3144 mg/kg bw, inhalation,[c] 6 h/day × 6 days	Douglas *et al.* (1999)
DNA single-strand breaks, mouse liver *in vivo*	–		2000 ip × 1	Parchman & Magee (1982)
DNA single-strand breaks (alkaline unwinding) in liver and kidney of male NMRI mice *in vivo*	+[g]		790 ip × 1	Walles (1986)
DNA single-strand breaks (alkaline unwinding), mouse liver *in vivo*	+		1500 po × 1[c]	Nelson & Bull (1988)
DNA single-strand breaks (alkaline unwinding), rat liver *in vivo*	+		3000 po × 1[c]	Nelson & Bull (1988)
Mouse spot test (DNA-alterations) *in vivo*	–		350 ip × 1	Fahrig (1977)
Unscheduled DNA synthesis, CD-1 mouse primary hepatocytes *in vivo*	–		1000 po × 1	Doolittle *et al.* (1987)
Micronucleus induction, mouse bone-marrow erythrocytes *in vivo*	+		750 po × 2	Duprat & Gradiski (1980)
Micronucleus induction, B6C3F$_1$ mouse bone-marrow erythrocytes *in vivo*	–		2500 ip × 3[c]	Shelby *et al.* (1993)
Micronucleus induction, mouse spermatocytes *in vivo* (spermatids examined) *in vivo*	–		565 inhalation 6 h/d × 5	Allen *et al.* (1994)
Micronucleus induction, mouse splenocytes *in vivo*	–		9800 inhalation 6 h	Kligerman *et al.* (1994)
Micronucleus induction, rat bone-marrow erythrocytes *in vivo*	+		5 inhalation 6 h	Kligerman *et al.* (1994)

Table 4.4 (continued)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus induction, rat bone-marrow erythrocytes *in vivo*	–		960 inhalation 6 h × 4	Kligerman *et al.* (1994)
Micronucleus induction, rat peripheral lymphocytes *in vivo*	–		8800 inhalation 6 h	Kligerman *et al.* (1994)
Micronucleus induction, rat peripheral lymphocytes *in vivo*	–		960 inhalation 6 h × 4	Kligerman *et al.* (1994)
Micronucleus induction, rat kidney cells *in vivo*	+		3591 po × 1	Robbiano *et al.* (2004)
Sister chromatid exchange, rat peripheral lymphocytes *in vivo*	–		8800 inhalation 6 h	Kligerman *et al.* (1994)
Sister chromatid exchange, rat peripheral lymphocytes *in vivo*	–		960 inhalation 6 h × 4	Kligerman *et al.* (1994)
Sister chromatid exchange, mouse splenocytes *in vivo*	–		9800 inhalation 6 h	Kligerman *et al.* (1994)
Chromosomal aberrations, rat peripheral lymphcytes *in vivo*	– [e]		8800 inhalation 6 h	Kligerman *et al.* (1994)
Chromosomal aberrations, rat peripheral lymphcytes *in vivo*	– [e]		960 inhalation 6 h × 4	Kligerman *et al.* (1994)
Chromosomal aberrations, mouse splenocytes *in vivo*	– [e]		9800 inhalation 6 h	Kligerman *et al.* (1994)
Dominant lethal mutation, male NMRI-Han/BGA mice *in vivo*	–		3400 inhalation 24 h[c]	Slacik-Erben *et al.* (1980)
Binding (covalent) to salmon sperm DNA *in vitro*	–	+	270	Banerjee & Van Duuren (1978)
Binding (covalent) to calf thymus DNA *in vitro*	–	+	340[c]	Bergman (1983)
Binding (covalent) to calf thymus DNA *in vitro*	0	+	13	Miller & Guengerich (1983)
Binding (covalent) to DNA of isolated rat hepatocytes *in vitro*	+	0	13	Miller & Guengerich (1983)
Binding (covalent) to DNA of isolated mouse hepatocytes *in vitro*	+	0	13	Miller & Guengerich (1983)
Binding (covalent) to calf thymus DNA *in vitro*	0	+	131	DiRenzo *et al.* (1982)
Binding (covalent) to RNA of NMRI mouse spleen, lung, liver, kidney, pancreas, testis and brain *in vivo*	–[h]		67 ip × 5[c]	Bergman (1983)

Table 4.4 (continued)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Binding (covalent) to DNA of NMRI mouse spleen, pancreas, lung, testis, kidney and brain *in vivo*	–[h]		67 ip × 5	Bergman (1983)
Binding (covalent) to DNA of NMRI mouse liver *in vivo*	?		67 ip × 5	Bergman (1983)
Binding (covalent) to DNA of B6C3F_1 mouse liver *in vivo*	?		1200 po × 1	Stott *et al.* (1982)
Binding (covalent) to DNA of B6C3F_1 mouse liver *in vivo*	?		250 ip × 1	Parchman & Magee (1982)
Binding (covalent) to DNA of Sprague-Dawley rat liver *in vivo*	?		1000 ip × 1	Parchman & Magee (1982)
Dichloroacetyl chloride				
λ Prophage induction, *Escherichia coli* WP2	–	–	10 000	DeMarini *et al.* (1994)
Salmonella typhimurium TA100, reverse mutation	+	–	3	DeMarini *et al.* (1994)
Trichloroethanol				
Salmonella typhimurium TA98, TA100, reverse mutation	–	–	7500	Waskell (1978)
Salmonella typhimurium TA100, TA1535, reverse mutation	–	–	8 µg/plate	Bignami *et al.* (1980)
Salmonella typhimurium TA100	–	–	0.5 µg/cm^3 vapour	DeMarini *et al.* (1994)
Salmonella typhimurium TA104, reverse mutation	–	+	2500 µg/plate	Beland (1999)
Sister-chromatid exchanges, human lymphocytes *in vitro*	+	0	178	Gu *et al.* (1981)

[a] +, considered to be positive; (+), considered to be weakly positive in an inadequate study; –, considered to be negative; ?, considered to be inconclusive (variable responses in several experiments within an inadequate study); 0, not tested.

[b] LED, lowest effective dose; HID, highest effective dose. In-vitro tests, µg/mL; in-vivo tests, mg/kg bw; ip, intraperitoneally; po, orally.

[c] Purity, 99% or greater.

[d] Stabilizers, 0.001%

[e] It should be noted that results of most assays for chromosomal aberration report the combined incidence of multiple effects, including chromatid breaks, isochromatid or chromosome breaks, chromatid exchanges, dicentric chromosomes, ring chromosomes, and other aberrations.

[f] Also positive by gavage at 150 mg/kg bw for 5 days per week, 22 times, with 400 mg/kg bw on the last day.

[g] No DNA strand breaks in lungs of mice treated at 1300 mg/kg bw ip × 1

[h] Metabolic incorporation of ^{14}C into nucleotides was observed.

containing stabilizers did not induce forward mutation in *Schizosaccharomyces pombe* (Rossi *et al.*, 1983). Pure trichloroethylene induced forward mutation in one study of growing cultures of *Aspergillus nidulans*, which are capable of some metabolic-activation reactions, but this effect was not seen in quiescent conidia. Trichloroethylene (of unspecified purity) induced aneuploidy in *S. cerevisiae* in the presence of growth-mediated metabolic activation, and the pure compound induced aneuploidy in *A. nidulans*. In a single study, trichloroethylene (of unspecified purity) induced gene mutation in *Tradescantia* (Schairer & Sautkulis, 1982). Pure trichloroethylene did not cause recessive lethal mutations in *Drosophila melanogaster* after injection, and equivocal results were obtained after feeding (Foureman *et al.*, 1994).

As with bacterial systems, the parent compound trichloroethylene was not itself genotoxic, but the metabolites of trichloroethylene appeared to be responsible for positive effects in the various assays. Yeast strains with a high CYP content showed an increase in frequencies of mitotic gene conversion and recombination after exposure to trichloroethylene, while a strain with a much lower CYP content did not (Callen *et al.*, 1980). However, Rossi *et al.* (1983) saw no change in mutation frequency in yeast exposed to trichloroethylene in the presence or absence of supernatant S9. Likewise, Koch *et al.* (1988) did not observe any change in mitotic gene conversion, or reverse mutation with or without S9 in yeast exposed to trichloroethylene.

(iii) Mammalian systems

See Table 4.4 and Table 4.5

There were several studies of mutation induced by trichloroethylene in mammalian systems. In a host-mediated assay, gene conversion and reverse mutation were induced in *S. cerevisiae* recovered from the liver, lungs and kidneys of mice treated orally with pure trichloroethylene. Forward mutation was weakly induced by trichloroethylene of unspecified purity in *Schizosaccharomyces pombe* cells injected into the peritoneum of mice in one of two studies; no effect was seen in the only study available in rats. *S. pombe* cells recovered from mice after intravenous injection showed no forward mutation in one study; a positive result was seen in another study in mouse liver, but not in kidneys or lungs, after treatment with trichloroethylene of unspecified purity. In a more direct test for mutagenicity in mammals (Douglas *et al.*, 1999), male and female transgenic *lac*Z mice were exposed by inhalation to trichloroethylene at varying levels and mutation frequencies in multiple tissues were determined after 60 days. No positive findings were observed. In a single study, pure trichloroethylene did not induce dominant-lethal mutation in mice (Slacik-Erben *et al.*, 1980).

(c) Cytogenetic effects

There were some reports of studies on trichloroethylene-induced chromosomal aberrations. No chromosomal aberrations were observed by Galloway *et al.* (1987) in trichloroethylene-exposed Chinese hamster ovary cells, with or without S9. Kligerman *et al.* (1994) exposed rats and mice to trichloroethylene at varying levels by inhalation and examined peripheral blood lymphocytes for chromosomal aberrations, SCE, and micronucleus formation. No chromosomal aberrations were observed.

Studies both *in vitro* and *in vivo* indicated that exposure to trichloroethylene can induce micronucleus formation. Wang *et al.* (2001) found a dose-dependent increase in micronucleus formation in trichloroethylene-exposed Chinese hamster ovary cells, while studies by Robbiano *et al.* (2004) observed increases in micronucleus formation in exposed, cultured rat kidney cells. Hu *et al.* (2008), using exposure concentrations similar to those used by Robbiano *et al.* (2004), found a trichloroethylene-induced increase in micronucleus frequency in cultured human hepatoma cells. In the inhalation studies

Table 4.5 Genetic and related effects of trichloroethylene containing mutagenic stabilizers or of unknown purity in experimental systems

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Escherichia coli PQ37, SOS chromotest	–	–	13 140	von der Hude *et al.* (1988)
Photobacterium phosphorium, mutatox assay, derepression of luminescence operon,	–	0	NR	Elmore & Fitzgerald (1990)
Salmonella typhimurium TA100, reverse mutation	–	–	14 650 plate incorporation assay	Henschler *et al.* (1977)
Salmonella typhimurium TA100 and TA100 reverse mutation	–	–	525 vapour	Waskell (1978)
Salmonella typhimurium TA100, reverse mutation	+	+	260 vapour[c]	Shimada *et al.* (1985)
Salmonella typhimurium TA100, reverse mutation	+	+	NR	Milman *et al.* (1988)
Salmonella typhimurium TA100, reverse mutation	(+)	(+)	130 vapour	McGregor *et al.* (1989)
Salmonella typhimurium TA1535, reverse mutation	+	+	50 vapour[c]	Shimada *et al.* (1985)
Salmonella typhimurium TA1535, reverse mutation	+	+	NR	Milman *et al.* (1988)
Salmonella typhimurium TA1535, reverse mutation	+	+	33 vapour	McGregor *et al.* (1989)
Salmonella typhimurium TA1537, reverse mutation	–	–	NR	Milman *et al.* (1988)
Salmonella typhimurium TA98, reverse mutation	–	–	525 vapour	Waskell (1978)
Salmonella typhimurium TA98, reverse mutation	–	–	0.00	Milman *et al.* (1988)
Salmonella typhimurium TA98, reverse mutation	–	–	65 vapour	McGregor *et al.* (1989)
Escherichia coli K12, forward mutation	–	–	434	Greim *et al.* (1975)
Escherichia coli K12, reverse mutation (*arg*$^+$)	–	+	434	Greim *et al.* (1975)
Escherichia coli K12, reverse mutation (*gal*$^+$) or (*nad*$^+$)	–	–	434	Greim *et al.* (1975)
Saccharomyces cerevisiae D7, log-phase cultures, gene conversion	0	+	1970[d]	Callen *et al.* (1980)
Saccharomyces cerevisiae D4, log-phase cultures, gene conversion	0	–	2900[e]	Callen *et al.* (1980)
Saccharomyces cerevisiae D7, log-phase and stationary cultures, gene conversion	–	–	2900[e]	Koch *et al.* (1988)
Saccharomyces cerevisiae XV185–^{14}C, reverse mutation (*lys*1–1, *his*1–7, *hom*3–10)	0	+	1460	Shahin & Von Borstel (1977)
Saccharomyces cerevisiae D7, log-phase cultures, reverse mutation	0	+	1970[d]	Callen *et al.* (1980)
Saccharomyces cerevisiae D7, log-phase cultures, mitotic recombinants or otherwise genetically altered colonies (*ade*2)	0	+	1970[d]	Callen *et al.* (1980)
Saccharomyces cerevisiae D7, log-phase and stationary cultures, reverse mutation	–	(+)	2900[e]	Koch *et al.* (1988)
Schizosaccharomyces pombe P1, stationary phase, forward mutation	–	–	3285	Rossi *et al.* (1983)
Schizosaccharomyces pombe P1, growing cells, forward mutation	–	–	13 140	Rossi *et al.* (1983)

Table 4.5 (continued)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Saccharomyces cerevisiae D61.M, growing cells, aneuploidy	+	+	725	Koch *et al.* (1988)
Tradescantia species, mutation	+	0	0.0003	Schairer & Sautkulis (1982)
DNA strand break, comet assay, rat kidney cells *in vitro*	+	0	130	Robbiano *et al.* (2004)
DNA strand break, comet assay, human kidney cells *in vitro*	+	0	130	Robbiano *et al.* (2004)
Unscheduled DNA synthesis, phenobarbital-induced rat hepatocytes *in vitro*	+	0	368	Costa & Ivanetich (1984)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	0	130 vapour	Shimada *et al.* (1985)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	0	NR	Milman *et al.* (1988)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	0	1445	Williams *et al.* (1989)
Unscheduled DNA synthesis, $B6C3F_1$ mouse primary hepatocytes *in vitro*	+	0	NR	Milman *et al.* (1988)
Sister chromatid exchange, Chinese hamster ovary (CHO) cells *in vitro*	0	–	9	White *et al.* (1979)
Chromosomal aberrations, Chinese hamster lung (CHL) cells *in vitro*	–	–	1000	Sofuni *et al.* (1985)
Micronucleus induction, rat kidney cells *in vitro*	+	0	16.5	Robbiano *et al.* (2004)
Cell transformation BALB/c–3T3 mouse cells, *in vitro*	(+)	0	250	Tu *et al.* (1985)
Morphological transformation Syrian hamster embryo cells, *in vitro*	(+)	0	25	Amacher & Zelljadt (1983)
Micronucleus induction, human kidney cells *in vitro*	+	0	16.5	Robbiano *et al.* (2004)
Host-mediated assay, forward mutation in *Schizosaccharomyces pombe* P1 recovered from CD-1 mouse kidneys and lungs	–	0	2000 po × 1	Loprieno & Abbondandolo (1980)
Host-mediated assay, forward mutation in *Schizosaccharomyces pombe* P1 recovered from CD-1 mouse liver	(+)	0	2000 po × 1	Loprieno & Abbondandolo (1980)
Host-mediated assay, *Schizosaccharomyces pombe* P1, forward mutation, in CD-1 mouse peritoneum	(+)	0	1000 po × 1	Loprieno & Abbondandolo (1980)
Host-mediated assay, *Schizosaccharomyces pombe* P1, forward mutation, in Spraque-Dawley rat peritoneum	–	0	1000 po × 1	Loprieno & Abbondandolo (1980)
Host-mediated assay, forward mutation in *Schizosaccharomyces pombe* P1CD-1 × 7BL hybrid mouse	–	0	2000 iv or ip × 1	Rossi *et al.* (1983)
DNA strand break, comet assay, Sprague-Dawley rat kidney *in vivo*	–		2000 ppm	Clay (2008)
Unscheduled DNA synthesis, Fischer 344 male rat hepatocytes *in vivo*	–		1000 po × 1	Mirsalis *et al.* (1989)

Table 4.5 (continued)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Unscheduled DNA synthesis, male and female B6C3F$_1$ mouse hepatocytes *in vivo*	–		1000 po × 1	Mirsalis *et al.* (1989)
Chromosomal aberrations, CD-1 mouse bone-marrow cells *in vivo*	–		1000 po × 1	Loprieno & Abbondandolo (1980)
Chromosomal aberrations, mouse bone-marrow cells *in vivo*	–		1200 po × 1	Sbrana *et al.* (1985) (abstract)
Chromosomal aberrations, mouse bone-marrow cells *in vivo*	–		795 inhalation 7 h × 5 d/wk, 10 wk	Sbrana *et al.* (1985) (abstract)
Micronucleus induction, mouse bone-marrow erythrocytes *in vivo*	+		1200 po × 1	Sbrana *et al.* (1985) (abstract)
Micronucleus induction, mouse bone-marrow erythrocytes *in vivo*	+		460 ip × 1[f]	Hrelia *et al.* (1994)
Binding (covalent) to calf thymus DNA *in vitro*	0	+	3.2	Mazzullo *et al.* (1992)
Binding (covalent to DNA of BALB/c mouse liver, kidney, lung and stomach *in vivo*	(+)		0.76 ip × 1	Mazzullo *et al.* (1992)
Binding (covalent) to DNA of Wistar rat liver, kidney, lung and stomach *in vivo*	(+)		0.76 ip × 1	Mazzullo *et al.* (1992)
Enzyme-altered foci in male Osborne-Mendel rat liver *in vivo*, promotion protocol, with and without NDEA as an initiator	–		1300 mg/kg bw, 5 d/wk, 7 wk	Milman *et al.* (1988)
Enzyme-altered foci in male Osborne-Mendel rat *liver in vivo*, initiation protocol, phenobarbital as promoter	–		1300 mg/kg bw	Milman *et al.* (1988)
S-phase induction, male and female B6C3F$_1$ mouse hepatocytes *in vivo*	+		200 mg/kg	Mirsalis *et al.* (1989)

[a] +, considered to be positive; (+), considered to be weakly positive in an inadequate study; –, considered to be negative; ?, considered to be inconclusive (variable responses in several experiments within an inadequate study); 0, not tested

[b] LED, lowest effective dose; HID, highest in effective dose. In-vitro tests, μg/mL; in-vivo tests, mg/kg bw; NR, dose not reported; ip, intraperitoneally; po, orally

[c] Purity, ≥ 99%

[d] CYP content fivefold greater in D7 strain

[e] High toxicity at 22 mM [2900 μg/mL]

[f] Correlate with trichloroethanol in urine

NDEA; *N*-nitrosodiethylamine

of Kligerman *et al.* (1994) in rats and mice, micronucleus formation was increased in the bone-marrow cells of rats, but not mice. A high oral dose of trichloroethylene in rats (Robbiano *et al.*, 2004) resulted in micronucleus formation in kidney cells. A high intraperitoneal dose (457 mg/kg bw) in mice resulted in an increase in micronucleus formation in bone-marrow cells, and the micronucleus frequency correlated with measures of urinary oxidative metabolites of trichloroethylene (Hrelia *et al.*, 1994).

Studies both *in vivo* and *in vitro* have reported that trichloroethylene induces SCE. In studies *in vitro* using Chinese hamster ovary cells, White *et al.* (1979) found no induction of SCE in response to trichloroethylene, while Galloway *et al.* (1987) saw a response in the same cell line both with and without metabolic activation. Gu *et al.* (1981) observed a positive response in cultured human lymphocytes. In studies conducted *in vivo*, no increase in the frequency of SCE was observed in peripheral lymphocytes of rats, or in splenocytes of mice exposed to trichloroethylene by inhalation (Kligerman *et al.* (1994). Analysis of peripheral blood lymphocytes in humans occupationally exposed to trichloroethylene showed no increase in the frequency of SCE (Nagaya *et al.*, 1989).

(d) Other types of DNA damage and related effects

Unscheduled DNA synthesis (UDS), DNA strand breaks and cell transformation have all been studied in relation to exposure to trichloroethylene. Primary cultures of hepatocytes from rats did not show any induction of UDS, even at relatively high exposures (Shimada *et al.*, 1985). In hepatocytes from rats treated with phenobarbital, exposure to trichloroethylene was found to induce UDS (Costa & Ivanetich, 1984). Mirsalis *et al.* (1989) found no induction of UDS in rats and mice exposed *in vivo*. UDS was studied in human lymphocytes cultured *in vitro* with and without metabolic activation from S9; an increase in UDS was observed only in the presence of metabolic activation (Perocco & Prodi, 1981).

In the studies by Robbiano *et al.* (2004), DNA strand breaks were observed in cultured rat and human kidney cells exposed to trichloroethylene, and in kidney cells of a rat exposed to trichloroethylene at a high dose. In contrast, a study by Clay (2008) in rats exposed by inhalation to trichloroethylene found no DNA strand breaks with the comet assay in rat kidney cells.

Assays for cell transformation with trichloroethylene showed weakly positive and negative responses. In three different assays, trichloroethylene (of unspecified purity, except in one study) weakly induced cell transformation in mouse, Syrian hamster and rat cells (pure trichloroethylene) *in vitro*, without exogenous metabolic activation. Pure trichloroethylene inhibited intercellular communication in mouse hepatocytes, but not in rat hepatocytes *in vitro*.

(e) Genotoxicity of metabolites of trichloroethylene

Several experimental studies of the genotoxicity of trichloroethylene metabolites were performed, and are reviewed here. For discussion of the genotoxicity of the trichloroethylene metabolites dichloroacetic acid, trichloroacetic acid, and chloral and chloral hydrate, see the corresponding *Monographs* in this Volume.

(i) Trichloroethanol

A limited number of studies on the genotoxicity of trichloroethanol were available (see Table 4.4). Trichloroethanol gave negative results in *S. typhimurium* strain TA100 (Waskell, 1978; Bignami *et al.*, 1980; DeMarini *et al.*, 1994). A study by Beland (1999) using *S. typhimurium* strain TA104 did not induce reverse mutations without exogenous metabolic activation; however, it did increase mutant frequency after preincubation of trichloroethanol at a dose of ≥ 2500 μg/plate with an exogenous metabolic activation system for 30 minutes before addition

of the tester strains. Trichloroethanol was not evaluated in the other recommended screening assays.

(ii) DCVG

See Table 4.6

Vamvakas *et al.* (1988a) investigated the mutagenicity of DCVG in *S. typhimurium* strain TA2638, using kidney subcellular fractions for metabolic activation and AOAA (a pyridoxal phosphate-dependent β-lyase inhibitor) to inhibit genotoxicity. DCVG exhibited direct-acting mutagenicity, with kidney mitochondria, cytosol, or microsomes enhancing the effects and AOAA diminishing, but not abolishing, the effects. Importantly, addition of liver subcellular fractions did not enhance the mutagenicity of DCVG, consistent with metabolism in situ (via GGT and dipeptidase) playing a significant role in the genotoxicity of the resulting cysteine conjugates in the kidney (Vamvakas *et al.*, 1988a).

(iii) DCVC

See Table 4.6

Dekant *et al.* (1986c) demonstrated mutagenicity of DCVC in S. *typhimurium* strains (TA100, TA2638, and TA98) using the Ames assay in the absence of S9. The effects were decreased with the addition of AOAA, a β-lyase inhibitor, suggesting that bioactivation by this enzyme plays a role in genotoxicity. Furthermore, Vamvakas *et al.* (1988a), in another experiment, investigated the mutagenicity of DCVC in *S. typhimurium* strain TA2638, using kidney subcellular fractions for metabolic activation and AOAA to inhibit genotoxicity. DCVC exhibited direct-acting mutagenicity, with kidney mitochondria or cytosol enhancing the effects and AOAA diminishing, but not abolishing, the effects.

DCVC has shown predominantly positive results in other available assays for genotoxicity *in vitro* and *in vivo*. Jaffe *et al.* (1985) reported DNA strand breaks after administration of DCVC *in vivo*, in isolated perfused kidneys (*ex vivo*), and in isolated proximal tubules of albino male rabbits (*ex vivo*). Vamvakas *et al.* (1989) reported dose-dependent increases in UDS in porcine kidney tubular epithelial LLC-PK1 cells at noncytotoxic concentrations. In addition, Vamvakas *et al.* (1996) reported that exposure of LLC-PK1 cells to DCVC at noncytotoxic concentrations for 7 weeks induced morphological and biochemical dedifferentiation of single cells (clones), which persisted for at least 30 passages after removal of the compound. This study also reported increased expression of the proto-oncogene c-*Fos* in the DCVC-derived clones. In Syrian hamster embryo fibroblasts, DCVC induced UDS, but not micronucleus formation (Vamvakas *et al.*, 1988b).

Two more recent studies are discussed in more detail. Mally *et al.* (2006) isolated primary rat kidney epithelial cells from *Tsc-2*$^{Ek/+}$ (Eker) rats, and reported increased transformation when the cells were exposed to DCVC at a concentration of 10 µM [2 µg/mL], like the genotoxic renal carcinogen *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (Horesovsky *et al.*, 1994). The frequency of transformation was variable, but consistently higher than background. No LOH of the tuberous sclerosis complex 2 tumour suppressor *Tsc-2* gene was reported either in these DCVC transformants or in renal tumours (which were not increased in incidence) in Eker rats treated with trichloroethylene. Mally *et al.* (2006) suggested that these data support a nongenotoxic mechanism, because a substantial fraction of spontaneous renal tumours in Eker rats showed LOH at this locus (Kubo *et al.*, 1994; Yeung *et al.*, 1995), and because LOH was demonstrated both *in vitro* and *in vivo* after treatment with 2,3,4-*tris*-(glutathione-*S*-yl)-hydroquinone in Eker rats (Yoon *et al.*, 2001). However, 2,3,4-*tris*-(glutathione-*S*-yl)-hydroquinone is not genotoxic in standard assays for mutagenicity (Yoon *et al.*, 2001), and Kubo *et al.* (1994) also reported that none of the renal tumours induced by the genotoxic carcinogen,

Table 4.6 Genetic and related effect of glutathione-derived metabolites of trichloroethylene in experimental systems

Test system/end-point	Result		Dose (LED or HID)	Comments	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation			
S-(1,2-dichlorovinyl)glutathione (DCVG)					
S. typhimurium, TA2638 gene mutations	+	+	50–300 nmol 20–120 µg/plate	Kidney subcellular fractions cytosol, mitochondria or microsomes used for activation, enhancing mutagenicity. AOAA diminished, but did not abolish, the effects. Liver subcellular fractions did not enhance mutagenicity.	Vamvakas *et al.* (1988a)
S-(1,2-dichlorovinyl)-*L*-cysteine (DCVC					
S. typhimurium, TA100, TA2638, TA98, gene mutations	+	ND	0.1–0.5 nmol 0.02–0.11 µg/plate	DCVC was mutagenic in all three strains of *S. typhimurium* without the addition of mammalian subcellular fractions. Decreased response when AOAA (β-lyase inhibitor) added.	Dekant *et al.*, (1986c)
S. typhimurium, TA2638, gene mutations	+	+	50–300 nmol 11–65 µg/plate	Kidney subcellular fractions mitochondria or cytosol used for activation, enhancing mutagenicity. AOAA diminished, but did not abolish, the effects.	Vamvakas *et al.* (1988a)
Gene mutation, LOH in *Tsc* gene, rat kidney epithelial cells *in vitro*	–	NA	10 µM [2 µg/mL]	Only 1/9 transformed cell lines showed LOH of the *Tsc-2* gene.	Mally *et al.* (2006)
Gene mutation, *Vhl* gene (exons 1–3), rat kidney epithelial cells *in vitro*	–	NA	10 µM [2 µg/mL]	No mutations in *Vhl* gene. Note: *Vhl* is not a target gene in rodent models of chemical-induced or spontaneous renal carcinogenesis.	Mally *et al.* (2006)
Unscheduled DNA synthesis, porcine kidney tubular epithelial cell line (LLC-PK1) *in vitro*	+	NA	2.5 µM [0.55 µg/mL], 5, 10, 15, 24 h; 2.5–100 µM [0.55–21.5 µg/mL]	Dose-dependent in UDS up to 24 h tested at 2.5 µM. Also, there was a dose-dependent increase at lower concentrations. Higher concentrations were cytotoxic as determined by cellular release of lactate dehydrogenase release.	Vamvakas *et al.* (1989)
Unscheduled DNA synthesis, Syrian hamster embryo fibroblasts *in vitro*	+	NA	2.5–10 µM [0.5–2 µg/mL]	Increase in UDS in treatment groups.	Vamvakas *et al.* (1988b)
DNA single-strand breaks, male rabbit renal tissue (perfused kidneys and proximal tubules)	+ +	ND	10–20 mg/kg, iv; 50–100 mg/kg bw, ip	Dose-dependent increase in DNA single-strand breaks after iv or ip injections (iv injections only at 10 and 20 mg/kg bw)	Jaffe *et al.* (1985)

Table 4.6 (continued)

Test system/end-point	Result		Dose (LED or HID)	Comments	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation			
DNA single-strand breaks, isolated perfused kidneys *in vitro*	ND	+	10–1000 µM	Perfusion of rabbit kidney (45-min exposure) and proximal tubules (30-min exposure) resulted in a dose-dependent difference in the amount of DNA single-strand breaks.	Jaffe *et al.* (1985)
DNA strand breaks, proximal tubules *in vitro*	ND	+	10–1000 µM [2–200 µg/mL]		
DNA strand breaks, comet assay, male Sprague-Dawley rats *in vivo*	NA	+/–	1 or 10 mg/kg bw, single oral dose, evaluated at 1, 2, and 16 h	Positive at 10 mg/kg bw, 2 h after treatment. No significant increase at other doses/time-points.	Clay (2008)
Micronucleus formation, Syrian hamster embryo fibroblasts *in vitro*	–	NA	10 µM [2 µg/mL]	No micronucleus formation	Vamvakas *et al.* (1988b)
Cell transformation, kidney tubular epithelial cell line (LLC-PK1)	+	NA	1 or 5 µM; 7 wk [0.2 or 1 µg/mL]	Induced morphological cell transformation at both concentrations tested. Furthermore, in clone cells biochemical and morphological alterations remained stable for 30 passages.	Vamvakas *et al.* (1996)
Cell transformation, rat kidney epithelial cells *in vitro*	+	NA	10 µM [2 µg/mL]; 24 h exposure, 7 wk post-incubation	Cell transformation was higher than in controls; however, cell survival ranged from 39% to 64%, indicating cytotoxicity.	Mally *et al.* (2006)
Gene expression, kidney tubular epithelial cell line (LLC-PK1)	+	NA	1 or 5 µM	Increased c-*Fos* expression in 1 and 5 µM DCVC-derived clones after 30, 60 or 90 min incubation without the compound.	Vamvakas *et al.* (1996)
N-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine (NAcDCVC)					
S. typhimurium, TA2638, gene mutation	+	+	5–100 nmol [1.3–26 µg/plate]; LED 25 nmol [6.5 µg/plate]	Kidney cytosolic fractions used for activation, enhancing mutagenicity. AOAA diminished, but did not abolish, the effects.	Vamvakas *et al.* (1987)

AOAA, aminooxyacetic acid; DCVC, *S*-(1,2-dichlorovinyl)-L-cysteine; DCVG, *S*-(1,2-dichlorovinyl)glutathione; HID, highest ineffective dose; LED, lowest effective dose; LOH, loss of heterozygosity; *N*-AcDCVC, *N*-acetylDCVC; NA, not applicable; ND, not determined.

N-ethyl-*N*-nitrosourea, showed LOH. Therefore, the lack of LOH at the *Tsc-2* locus after treatment with DCVC *in vitro*, or trichloroethylene *in vivo*, reported by Mally *et al.* (2006), was actually more similar to the response to the genotoxic carcinogen *N*-ethyl-*N*-nitrosourea than to the nongenotoxic carcinogen 2,3,4-*tris*-(glutathione-*S*-yl)-hydroquinone. Therefore, these data did not substantially contradict the body of evidence on the genotoxicity of DCVC.

Finally, Clay (2008) evaluated the genotoxicity of DCVC *in vivo* using the comet assay to assess DNA stand breakage in the proximal tubules of rat kidneys. Rats were given a single oral dose of DCVC (1 or 10 mg/kg bw) and killed 2 or 16 hours after dosing. There was no significant damage to DNA from rat kidney proximal tubules after treatment with DCVC at either dose after 16 hours, or with DCVC at a dose of 1 mg/kg bw after 2 hours. While Clay (2008) concluded that these data were insufficient to indicate a positive response in this assay, the study did report a statistically significant increase in percentage tail DNA 2 hours after treatment with DCVC at 10 mg/kg bw, despite the small number of rats at each dose (n = 5) and sampling time. Therefore, these data did not substantially contradict the body of evidence on the genotoxicity of DCVC.

Overall, DCVC has shown genotoxicity based on consistent results in several available studies. While some recent studies (Mally *et al.*, 2006; Clay, 2008) have reported a lack of positive responses in some measures of genotoxicity *in vivo*, these studies did not, due to the limitations discussed above, substantially contradict the body of evidence on the genotoxicity of DCVC. These metabolites are formed *in vivo* after exposure to trichloroethylene, specifically in the kidney, so they have the potential to contribute to the genotoxicity of trichloroethylene, especially in that tissue. Moreover, genotoxic responses associated with exposure to DCVC were enhanced when metabolic activation with kidney subcellular fractions was used (Vamvakas *et al.*, 1988a). Finally, the lack of similar responses in assays for genotoxicity with trichloroethylene *in vitro*, even with metabolic activation, was likely to be the result of the small yield (if any) of DCVC under conditions *in vitro*, since GGT and other bioactivating enzymes that form DCVC are present *in vivo* in higher concentrations in the kidney than in the liver, from which S9 fractions are typically derived.

(iv) NAcDCVC

See Table 4.6

Vamvakas *et al.* (1987) investigated the mutagenicity of NAcDCVC in *S. typhimurium* strain TA2638, using kidney subcellular fractions for metabolic activation and AOAA to inhibit genotoxicity. NAcDCVC exhibited direct-acting mutagenicity in the absence of exogenous metabolic activation, with kidney cytosol enhancing the effects and AOAA diminishing, but not abolishing, the effects.

4.3 Non-genotoxic mechanisms of carcinogenesis

The sections below describe the available data on non-genotoxic mechanisms of carcinogenesis for cancers of the kidney, liver, lung, immune system, and testes induced by trichloroethylene (see also Section 3).

4.3.1 Kidney

The available studies in humans and experimental animals have addressed multiple hypotheses for non-genotoxic mechanisms of carcinogenesis in the kidney associated with exposure to trichloroethylene. These include accumulation of α2u-globulin, activation of peroxisome proliferator-activated receptor α (PPARα), and sustained chronic nephropathy and regeneration (independent of α2u-globulin). The sections that follow address these three mechanisms in detail.

(a) Accumulation of α2u-globulin

Accumulation of α2u-globulin is a histopathological phenomenon elicited by long-term chemical exposure in the male rat kidney. The hypothesized sequence of key events comprises:

- Excessive accumulation of hyaline droplets containing α2u-globulin in renal proximal tubules;
- Subsequent cytotoxicity and single-cell necrosis of the tubule epithelium;
- Sustained regenerative tubule-cell proliferation
- Development of intralumenal granular casts from sloughed cellular debris associated with tubule dilatation and linear papillary mineralization;
- Foci of tubule hyperplasia in the convoluted proximal tubules;
- Tumours of the renal tubule.

Seven criteria have been specified for demonstrating that α2u-globulin is the sole mechanism for carcinogenesis in the male rat kidney (Swenberg & Lehman-McKeeman, 1999):

- Characteristic histopathology;
- Toxicity in the kidney is specific for male rats;
- Accumulation of α2u-globulin;
- Reversible binding to α2u-globulin;
- Increased, sustained cell proliferation;
- Similarities in dose–response relationships for histopathology and tumour outcome;
- Lack of genotoxicity.

This mechanism is posited to occur only in rodents; accordingly, only studies in experimental animals were identified, and are discussed below.

Experimental animals

Few studies of trichloroethylene in experimental animals were available, and the evidence did not meet the criteria for proof of this mechanism. Induction of α2u-globulin nephropathy has been investigated by Goldsworthy *et al.* (1988), who reported that trichloroethylene (1000 mg/kg bw for 10 days by gavage) did not induce increases in this urinary protein, nor did it stimulate tubular cellular proliferation in rats. Thus, direct evidence for several of the required criteria, including that trichloroethylene induces the characteristic histopathology, accumulation of α2u-globulin or reversible binding to α2u-globulin in a male rat-specific manner, was lacking. However, indirect evidence supporting an increase in α2u-globulin and associated histopathological changes was provided by a separate study in which groups of 60 male Fischer rats were exposed to the trichloroethylene metabolite trichloroethanol in drinking-water at concentrations of 50 and 1000 mg/L for 52 weeks (Green *et al.*, 2003). A dose-related increase in the incidence and severity of hyaline-droplet accumulation was observed. Increases in α2u-globulin were observed and were not considered sufficient to account for the renal pathology.

Concerning the remaining criteria, no studies were identified that would establish similarities in the dose–response relationships for histopathology and tumour outcome. Additionally, the evidence presented in Section 4.2 does not clearly rule out the potential role of genotoxicity, particularly in the kidney.

(b) Cytotoxicity/sustained chronic nephrotoxicity in the absence of α2u-globulin nephropathy

This hypothetical mechanism involves renal cytotoxicity and subsequent cellular proliferation in the absence of α2u-globulin accumulation. Evidence supporting this mechanism from studies in humans and experimental animals is summarized in the following sections.

(i) Humans

Epidemiological studies have demonstrated increased excretion of markers of nephrotoxicity (*N*-acetylglucosaminidase, NAG; protein,

albumin) at occupational (Green *et al.*, 2004) and higher (Brüning *et al.*, 1999a, b; Bolt *et al.*, 2004) levels of exposure to trichloroethylene. A more recent study that used the sensitive marker of kidney toxicity, kidney injury molecule-1 (Kim-1), demonstrated toxicity at low concentrations of trichloroethylene (22.2 ± 35.9 ppm; mean ± standard deviation), with exposures < 12 ppm (the median concentration of trichloroethylene) exhibiting a statistically significant elevation in urinary concentrations of Kim-1 (Vermeulen *et al.*, 2012).

Other evidence for renal toxicity associated with exposure to trichloroethylene came from studies of acute toxicity with GSH-derived metabolites in human cells *in vitro* (Chen *et al.*, 1990; Cummings & Lash, 2000; Cummings *et al.*, 2000b; Lash *et al.*, 2001a, 2003; Krause *et al.*, 2003; McGoldrick *et al.*, 2003). Additionally, changes in gene expression and mitochondrial disfunction induced by DCVC have been observed in primary human proximal tubular cells (Lash *et al.*, 2005; Lock *et al.*, 2006; Xu *et al.*, 2008).

(ii) Experimental animals

There was substantial evidence that trichloroethylene is nephrotoxic in experimental animals. Long-term bioassays have reported very high (nearly 100%) incidences of "nephrotoxicity" of the proximal tubule in rats (NTP, 1988, 1990) and mice (NCI, 1976; NTP, 1990) at the highest doses tested. Overt signs of subchronic nephropathy have been reported in multiple studies (see Section 3 for details) (Green *et al.*, 1997b, 1998). Maltoni *et al.* (1986) also reported cytomegaly and karyomegaly in rats exposed to trichloroethylene by inhalation. Studies i*n vivo* examining the effect of exposure to trichloroethylene on nephrotoxicity showed increased proximal tubule damage after intraperitoneal injection and inhalation of trichloroethylene in rats (Chakrabarti & Tuchweber, 1988) and intraperitoneal injection in mice (Cojocel *et al.*, 1989).

The available evidence also supported the nephrotoxicity of the trichloroethylene metabolite DCVC in rats (Terracini & Parker, 1965; Elfarra *et al.*, 1986) and mice (Jaffe *et al.*, 1984; Darnerud *et al.*, 1989). Studies *in vitro* showed that DCVC was more a more potent cytotoxic agent in the kidney than trichloroethylene (Stevens, 1985; Lash *et al.*, 1986; Stevens *et al.*, 1986). These studies also confirmed the greater susceptibility of male rats and mice to cytotoxicity induced by DCVC. Additionally, histological changes caused by DCVC in the kidney were similar to those induced by trichloroethylene, including cytokaryomegaly. Increased formation and urinary excretion of formic acid mediated by the oxidative metabolites trichloroacetic acid or trichloroethanol (Green *et al.*, 1998; Dow & Green, 2000; Green *et al.*, 2003) have also been posited to contribute to the observed nephrotoxicity of trichloroethylene. However, the contribution of these oxidative metabolites does not appear to be sufficient to explain the range of renal effects observed after exposure to trichloroethylene, particularly cytomegaly, karyomegaly, and flattening and dilation of the tubular epithelium. Multiple mechanisms of cytotoxicity, including alteration of calcium-ion homeostasis and mitochondrial dysfunction have been identified in kidney cells *in vitro* (Lash & Anders, 1986, 1987; Vamvakas *et al.*, 1990, 1992; van de Water *et al.*, 1993, 1994; Yu *et al.*, 1994) (see Section 4.5.1a).

The primary limitation to the evidence supporting this mechanism was that nephrotoxicity is observed in both mice and rats, in some cases with nearly 100% incidence in all dose groups, but tumours of the kidney are only observed at low incidence in rats at the highest doses tested (NCI, 1976; NTP, 1990). In rats carrying the Eker mutation (*Tsc-2*$^{EK/+}$), Mally *et al.* (2006) reported increased DNA synthesis as measured by bromodeoxyuridine (BrdU) incorporation in rats receiving the highest dose of trichloroethylene (1000 mg/kg bw per day) for 13 weeks, but there was no evidence of

clonal expansion or tumorigenesis in the form of increased preneoplastic or neoplastic lesions when compared with controls.

Therefore in studies of trichloroethylene exposure in rodents and humans, data demonstrating a causal link between compensatory proliferation and the induction of tumours in the kidney were lacking.

(c) Activation of PPARα

(i) Humans

No studies were identified addressing activation of PPARα in the human kidney. However, studies of transactivation *in vitro* have shown that human PPARα is activated by trichloroacetic acid and dichloroacetic acid, while trichloroethylene itself is relatively inactive (Zhou & Waxman, 1998; Maloney & Waxman, 1999). A limited number of studies have examined PPARα activation by the trichloroethylene metabolites dichloroacetic acid and trichloroacetic acid in cultured human liver cells (Walgren *et al.*, 2000). However, direct evidence of these effects from studies of human kidney *in vivo* or *in vitro* was lacking.

(ii) Experimental animals

No evidence was available concerning activation of PPARα in kidney. Peroxisome proliferation in the kidney has been evaluated in a single study of trichloroethylene (Goldsworthy & Popp, 1987), using increases in cyanide-insensitive palmitoyl-coenzyme A (palmitoyl-coA) oxidation activity as a marker in male rats and mice. Increases in renal palmitoyl-coA oxidation activity were observed in rats (3.0-fold) and mice (3.6-fold) treated with trichloroethylene by gavage at a dose of 1000 mg/kg bw per day for 10 days, with smaller increases in both species after treatment with trichloroacetic acid at 500 mg/kg bw per day for 10 days. No significant increases in kidney weight:body weight ratios were observed in either species.

(d) Conclusion

The kidney is a target organ in mammalian species for trichloroethylene and other related chlorinated ethanes and ethylenes, and trichloroethylene causes cancer of the kidney in male rats. There was inadequate evidence to support any of the three non-genotoxic mechanisms, that is, α2u-globulin nephropathy, cytotoxicity not associated with α2u-globulin accumulation, and PPARα activation. Very little evidence was available to support the hypothesized α2u-globulin nephropathy mechanism, and the identified evidence is insufficient to satisfy the criteria specified for establishing that this mechanism is operative. Concerning a mechanism dependent on sustained chronic nephrotoxicity, chronic exposure to trichloroethylene is indeed associated with damage to the kidney in humans as well as rats and mice. Nonetheless, no studies that have experimentally tested the hypothesis of ensuing cellular proliferation, or of mechanisms that would otherwise provide support for toxicity as a key step in carcinogenesis, were identified. One *in vivo* experimental study provides support for the view that short-term trichloroethylene exposure induces peroxisome proliferation in the kidney of exposed rodents. However, the effects occurred in both rats of mice whereas tumours occur only in male rats, suggesting a lack of species specificity of the mechanism. Additionally, direct evidence in the kidney is lacking from experimental animals (or humans) for activation of the PPAR receptor. Moreover, no studies have experimentally tested the link between peroxisome proliferation to kidney tumorigenesis for trichloroethylene or other compounds.

In conclusion, the overall evidence base supporting the three hypothesized mechanisms is inadequate in each case.

4.3.2 Liver

The available evidence for the non-genotoxic mechanisms for the induction of rodent (mouse) liver tumours by trichloroethylene comprised the following: (1) epigenetic effects (especially DNA hypomethylation); (2) cytotoxicity and oxidative stress; (3) alteration of proliferation and apoptosis, and clonal expansion; (4) PPARα activation; and (5) disruption of gap-junctional communication.

(a) Epigenetic effects

Experimental evidence for hypomethylation of DNA was limited to studies of trichloroacetic acid and dichloroacetic acid in mice (Tao *et al.*, 1998; 2004; Ge *et al.*, 2001). Since changes in methylation represent common early molecular events in most tumours (Zingg & Jones, 1997; Baylin *et al.*, 1998), these data supported the hypothesis that dysregulation of gene methylation plays a role in trichloroethylene-induced tumorigenesis. However, no data from studies in humans or experimental animals were available that specifically tested this hypothesis for trichloroethylene.

(b) Cytotoxicity and secondary oxidative stress

(i) Humans

Few studies on liver toxicity in humans and exposure to trichloroethylene were identified. Of these, three studies reported significant changes in serum liver-function tests (widely used in clinical settings partly to identify patients with liver disease) in metal degreasers whose exposure to trichloroethylene was assessed using urinary trichloro-compounds as biomarkers (Nagaya *et al.*, 1993; Rasmussen *et al.*, 1993a; Xu *et al.*, 2009). Two additional studies reported changes in plasma or serum bile acids (Driscoll *et al.*, 1992; Neghab *et al.*, 1997). The results of one study including subjects from a subregistry of the Agency for Toxic Substances and Disease Registry (ATSDR) were suggestive of liver disorders associated with exposure to trichloroethylene, but it was not possible to determine whether trichloroethylene caused these conditions given the limitations of this study (Davis *et al.*, 2005). Furthermore, several case reports existed of liver toxicity, including hepatitis accompanying immune-related generalized skin diseases described as a variation of erythema multiforme, Stevens–Johnson syndrome, toxic epidermal necrolysis, and hypersensitivity syndrome (Kamijima *et al.*, 2007), in addition to jaundice, hepatomegaly, hepatosplenomegaly, and liver failure in trichloroethylene-exposed workers (Thiele *et al.*, 1982). Cohort studies have examined mortality attributable to cirrhosis and exposure to either trichloroethylene (Garabrant *et al.*, 1988; Blair *et al.*, 1989; 1998; Morgan *et al.*, 1998; Boice *et al.*, 1999, 2006; Ritz, 1999; ATSDR, 2004; Radican *et al.*, 2008) or solvents (Leigh & Jiang, 1993), but were greatly limited by their use of death certificates for which there is a high degree (up to 50%) of under-reporting (Blake *et al.*, 1988), so these null findings did not rule out an effect of trichloroethylene on cirrhosis.

Overall, while some evidence exists for liver toxicity as assessed by tests for liver function, the data were inadequate for making conclusions regarding causality. Additionally, no data on secondary oxidative stress were identified.

(ii) Experimental animals

Numerous studies in experimental animals have demonstrated that trichloroethylene is hepatotoxic. In experimental animals, exposure to trichloroethylene is associated with a wide array of hepatotoxicity end-points. Like humans, experimental animals exposed to trichloroethylene have been observed to have increased levels of serum bile acids (Wang & Stacey, 1990; Bai & Stacey, 1993; Hamdan & Stacey, 1993), although the toxicological importance of this effect is unclear (see Section 4.5.2b). Increases in liver weight proportional to trichloroethylene dose have been consistently reported

in numerous studies and appear to be accompanied by periportal hepatocellular hypertrophy (Kjellstrand *et al.*, 1981, 1983a, b; Tucker *et al.*, 1982; Buben & O'Flaherty, 1985; Elcombe *et al.*, 1985; Goldsworthy & Popp, 1987; Melnick *et al.*, 1987; Merrick *et al.*, 1989; Goel *et al.*, 1992; Dees & Travis, 1993; Berman *et al.*, 1995; Nakajima *et al.*, 2000; Tao *et al.*, 2000; Nunes *et al.*, 2001; Laughter *et al.*, 2004).

Several studies have attempted to study oxidative stress and DNA damage resulting from exposure to trichloroethylene. Toraason *et al.* (1999) measured 8-hydrodeoxyguanosine adducts (8-OHdG) and a marker of oxidative damage to cell membranes, 8-epi-prostaglandin $F_{2\alpha}$ (8-epiPGF), excretion in the urine and thiobarbituric acid reactive substances (TBARS) (as an assessment of malondialdehyde and marker of lipid peroxidation) in the liver and kidney of male Fischer rats given single intraperitoneal injections of trichloroethylene. TBARs and the 8-OHdG/dG ratio were significantly elevated in the liver after treatment with trichloroethylene at 500 mg/kg bw, but there was significant toxicity and it was suggested that the animals would not have survived another 24 hours.

With regard to dichloroacetate and trichloroacetate, Larson & Bull (1992b) exposed male $B6C3F_1$ mice or F344 rats to single doses of trichloroacetate or dichloroacetate in distilled water by gavage (n = 4). In the first experiment, TBARS was measured from liver homogenates and assumed to be malondialdehyde equivalents. A preliminary experiment had shown that the maximum concentration of TBARS was increased 6 hours after dosing with dichloroacetate and 9 hours after dosing with trichloroacetate in mice, and that by 24 hours, TBARS concentrations had declined to control levels. Time-course information in rats was not presented. At a dose of 100 mg/kg bw, dichloroacetate (rats or mice) and trichloroacetate (mice) did not elevate concentrations of TBARS over those in liver of controls. However, trichloroacetate was not examined at this dose in rats. For trichloroacetate, there was a slight dose-related increase in concentration of TBARS over control values starting at 300 mg/kg bw in mice, with the increase in concentration of TBARS increasing at a rate that was lower than the magnitude of increase in dose. The induction of TBARS in mice was transient and subsided within 24 hours of a single dose of dichloroacetate or trichloroacetate. The response in mice appeared slightly greater with dichloroacetate than trichloroacetate at similar doses; for dichloroacetate, there was similar TBARS induction between rats and mice at similar dose levels.

Austin *et al.* (1996) is a follow-up publication of the preliminary experiment cited in Larson & Bull (1992b). Male $B6C3F_1$ mice were treated with single doses (300 mg/kg bw) of dichloroacetate or trichloroacetate via gavage and nuclear DNA from liver was examined for 8-OHdG as an indicator of oxidative stress. To reduce the number of animals used, controls were not employed at each time-point. A statistically significant increase in 8-OHdG for dichloroacetate in mice was seen at 4 and 6 hours (~1.4- and 1.5-fold levels for controls, respectively), but not 8 hours. For trichloroacetate, there was a statistically significant increase in 8-OHdG at 8 and 10 hours (~1.4- and 1.3-fold levels for controls, respectively).

Consistent results as to low, transient increases in markers of oxidative stress were also reported by Parrish *et al.* (1996), who in addition to examining oxidative stress alone, attempted to examine its possible relationship to palmitoyl-coA oxidation and liver weight in male $B6C3F_1$ mice exposed to trichloroacetate or dichloroacetate for 3 or 10 weeks (n = 6). While there was a dose-related increase in palmitoyl-coA oxidation activity at 21 days with trichloroacetate, there was only a statistically significant increase in palmitoyl-coA oxidation activity (~1.8-fold levels for controls) at 21 days with dichloroacetate at a concentration of 2.0 g/L. After 71 days of treatment, trichloroacetate induced dose-related increases in palmitoyl-coA oxidation activities

that were approximately twice those reported at 21 days. Treatment with dichloroacetate at 0.1 or 0.5 g/L produced statistically significant increases in palmitoyl-coA oxidation activity of ~1.5- and 2.5-fold levels for controls, respectively. The positive control, drinking-water containing clofibric acid at 1.25 g/L, produced increases of six- to sevenfold in palmitoyl-coA oxidation activity relative to controls after 21 and 71 days of exposure. Parrish *et al.* (1996) reported that the activity of laurate hydroxylase was elevated significantly only by trichloroacetate at 21 days, and to approximately the same extent (~1.4–1.6-fold levels for controls) at all doses tested. At 71 days, there was a statistically significant increase in the activity of laurate hydroxylase (i.e. 1.6- and 2.5-fold levels for controls, respectively) with trichloroacetate at concentrations of 0.5 and 2.0 g/L. No change in the activity of laurate hydroxylase was reported after exposure to dichloroacetate (activity was within the ranage for control values, varying 1.7-fold between days 21 and 71). Levels of 8-OHdG in isolated liver nuclei were not altered after exposure to trichloroacetate or dichloroacetate at concentrations of 0.1, 0.5, or 2.0 g/L for 21 days, and results remained negative even when treatment was extended to 71 days. The level of 8-OHdG increased in control mice with age (i.e. levels increased by about twofold between day 21 and day 71 in control mice).

Thus the increases in palmitoyl-coA oxidation activity reported after exposure to dichloroacetate or trichloroacetate were not associated with 8-OHdG levels (which were unchanged), nor with the observed changes in laurate hydrolase activity.

(c) Cell proliferation, apoptosis, and clonal expansion

(i) Humans

No studies were identified providing evidence of alteration of cell proliferation and apoptosis, or clonal expansion of initiated cells, in humans after exposure to trichloroethylene.

(ii) Experimental animals

Mirsalis *et al.* (1989) studied S-phase DNA synthesis in primary hepatocytes from male F344 rats and male and female B6C3F_1 mice given single doses (up to 1000 mg/kg bw) of trichloroethylene by gavage in corn oil. They reported that the only positive result was considered to be for a dose of 1000 mg/kg bw in male mice at 48 hours (~2.2% of hepatocytes), but no statistical analysis was performed. Dees & Travis (1993) reported elevated incorporation of tritiated thymidine in DNA from mouse liver after exposure to trichloroethylene at a dose of 250–1000 mg/kg bw. This study also reported that mitotic figures were more frequently observed after exposure to trichloroethylene. Channel *et al.* (1998) assessed liver-cell proliferation in B6C3F_1 mice given trichloroethylene orally at a dose of 0, 400, 800, or 1200 mg/kg bw per day in corn oil, 5 days per week, for 8 weeks. The number of proliferating cell nuclear antigen (PCNA)-positive cells, a measure of the number of cells that have undergone DNA synthesis, was elevated only on day 10 and only in the group at 1200 mg/kg per day. Laughter *et al.* (2004) found an increase in the level of DNA synthesis (BrdU labelling index) in mice given trichloroethylene by gavage at a dose of 500 and 1000 mg/kg bw for 3 weeks. Sano *et al.* (2009) examined cell proliferation in the liver using the K_i-67 antigen in male Sprague-Dawley rats and B6C3F_1 mice exposed by gavage to trichloroethylene at a dose of 1500 mg/kg bw per day for 14 days. A small number of K_i-67-positive hepatocytes and mitotic figures were

found in trichloroethylene-treated mice, but not in rats, but no quantitative analysis was reported.

With regard to changes in apoptosis, the study by Dees & Travis (1993) was the only one to report a qualitative increase in liver apoptotic bodies in $B6C3F_1$ mice exposed to trichloroethylene at a dose of 1000 mg/kg bw per day for 10 days. Channel *et al.* (1998), and Sano *et al.* (2009) observed no differences in liver apoptosis between control or trichloroethylene-treated animals.

Several studies have examined cell proliferation in *Pparα*-null mice. BrdU incorporation, a measure of DNA synthesis that may reflect cell division, polyploidization, or DNA repair, was diminished in null mice compared with wild-type mice given trichloroethylene at a dose of 500 or 1000 mg/kg bw per day (Laughter *et al.*, 2004). However, BrdU incorporation in *Pparα*-null mice was still higher by about threefold in than controls, although it was not statistically significantly different due to the small number of mice, high variability, and the baseline levels of BrdU incorporation in control *Pparα*-null mice that were two- to threefold those in control wild-type mice.

Other measures of proliferation, including liver-weight changes, have been examined. Trichloroethylene-induced increases in liver weight have been reported in male and female mice lacking a functional *Pparα* receptor (Nakajima *et al.*, 2000). On the other hand, Laughter *et al.* (2004) found no significant difference in liver weight in *Pparα*-null mice before and after exposure to trichloroethylene.

(d) Activation of PPARα

The sections below review the evidence that trichloroethylene or its metabolites induces PPARα activation or its markers.

(i) Humans

No studies were identified addressing peroxisome proliferation or the key events in the PPARα-activation mechanism in human liver. However, studies of transactivation *in vitro* have shown that human (as well as murine) versions of PPARα are activated by trichloroacetate and dichloroacetate, while trichloroethylene itself is relatively inactive (Zhou & Waxman, 1998; Maloney & Waxman, 1999). Limited studies have examined PPARα activation by the trichloroethylene metabolites dichloroacetate and trichloroacetate in cultured human cells of the liver (e.g. Walgren *et al.*, 2000).

(ii) Experimental animals

Numerous studies have reported that the administration by gavage of trichloroethylene to mice and rats leads to proliferation of peroxisomes in hepatocytes. Some studies have used changes in the volume and number of peroxisomes as measures of proliferation, while others have measured peroxisomal-enzyme activity, such as catalase and cyanide-insensitive palmitoyl-coA oxidation.

Elcombe *et al.* (1985) reported increases in the percentage of cytoplasm occupied by peroxisomes in $B6C3F_1$ and Alderley Park mice treated with trichloroethylene at 500–1500 mg/kg bw per day for 10 days. Although the increase compared with controls appeared greater in the $B6C3F_1$ strain, this was largely due to lower levels for controls in that strain. Nakajima *et al.* (2000) treated male wild type Sv/129 mice with trichloroethylene by gavage at 750 mg/kg bw per day for 14 days, and found even higher baseline values for the percentage peroxisomal volume, but with an absolute level after treatment similar to that reported by Channel *et al.* (1998) in $B6C3F_1$ mice treated with trichloroethylene at 1200 mg/kg bw per day for 14 days. Nakajima *et al.* (2000) also noted that the treatment-related increases were smaller for female wild-type mice, and that there were no increases in peroxisomal volume in male

or female *PPARα*-null mice, although levels for vehicle controls were slightly elevated (not statistically significant). Only Elcombe *et al.* (1985) examined peroxisomal volume in rats, and reported smaller treatment-related increases in two strains Osborne-Mendel and Alderley-Park, but higher and variable baseline levels.

In various strains of mice ($B6C3F_1$, Alderley-Park, Swiss albino, Sv/129 wild type) exposed to trichloroethylene at doses of 500–2000 mg/kg bw per day for 10–28 days, increases in catalase activity tended to be more modest (1.3–1.6-fold levels for controls) than increases in palmitoyl-coA oxidation activity (1.4–7.9-fold levels for controls) (Elcombe *et al.*, 1985; Goldsworthy & Popp, 1987; Goel *et al.*, 1992; Nakajima *et al.*, 2000; Watanabe & Fukui, 2000; Laughter *et al.*, 2004). In rats, Elcombe *et al.* (1985) reported no increases in catalase activity or palmitoyl-coA oxidation in Alderley-Park rats given trichloroethylene at 1000 mg/kg bw per day for 10 days. In F344 rats treated with trichloroethylene at 600–4800 mg/kg bw per day for 10–14 days, Goldsworthy & Popp (1987) and Melnick *et al.* (1987) reported increases of up to twofold in catalase activity, and 4.1-fold in palmitoyl-coA oxidation activity, relative to controls. The changes in catalase activity in rats were similar to those in mice at similar doses, with increases of 1.1–1.5-fold at doses of 1000–1300 mg/kg bw per day compared with controls (Elcombe *et al.*, 1985; Melnick *et al.*, 1987). However, the changes in palmitoyl-coA oxidation activity at these doses compared with controls were smaller, with increases of 1.1–1.8-fold in rats compared with 6.3–7.9-fold in mice (Goldsworthy & Popp, 1987; Melnick *et al.*, 1987).

Nakajima *et al.* (2000) and Laughter *et al.* (2004) investigated the dependence of these changes on PPARα using *Pparα*-null Sv/129 mice. Nakajima *et al.* (2000) reported that neither male nor female wild-type or null mice showed statistically significant increases in catalase activity after 14 days of treatment with trichloroethylene at 750 mg/kg bw per day. However, given the small number of mice (four per group) and the relatively small changes in catalase activity observed in other (wild-type) mouse strains, this study had limited power to detect such changes. Several other markers of peroxisome proliferation, including acyl-CoA oxidase and CYP4A1, were induced by trichloroethylene in male wild-type mice, but not in male *Pparα*-null mice or female mice of either type. Unfortunately, none of these markers have been investigated in female mice of any other strain, so it is unclear whether the lack of response to trichloroethylene is characteristic of female mice in general, or just in this strain. Interestingly, as noted above, increases in liver weight:body weight ratio were observed in male and female *Pparα*-null mice in this study. Laughter *et al.* (2004) only quantified activity of the peroxisome proliferation marker, palmitoyl-coA oxidation, in their study, and found a slight decrease (0.8 times lower than controls) in *Pparα*-null mice given trichloroethylene at 500 mg/kg bw per day, and an increase (1.5-fold levels for controls) at 1500 mg/kg bw per day after 3 weeks of treatment, with neither result being statistically significant (four to five mice per group). However, baseline activity of palmitoyl-coA oxidation was almost twofold higher in the null mice, and the treated wild-type and null mice differed in palmitoyl-coA oxidation activity by only about 1.5-fold.

In summary, oral administration of trichloroethylene for up to 28 days causes proliferation of peroxisomes in hepatocytes and associated increases in peroxisomal-enzyme activity in mice and rats. Male mice tend to be more sensitive to such effects: at similar doses, rats and female mice tend to exhibit weaker responses. For example, changes in peroxisomal volume and palmitoyl-coA oxidation were three to six times lower in rats than in mice, but changes in catalase activity were in F344 rats and mice. No longer-term studies or studies of inhalation were located, and only one study examined these

changes at more than one time-point. Therefore, little is known about the route-dependence, time course, and persistence of these changes. Finally, two studies (Nakajima *et al.*, 2000; Laughter *et al.*, 2004) found diminished responses in terms of increase in peroxisomal volume and peroxisomal enzyme activity in *Pparα*-null mice compared with wild-type mice, although there was some confounding due to baseline differences between null and wild-type mice in several measures.

The hypothesis that trichloroethylene-induced PPARα activation, along with its sequelae, is a key or causative event in trichloroethylene-induced hepatocarcinogenesis has not been directly tested (e.g. in bioassays with knockout mice or involving the blocking of hypothesized key events). Support for this hypothesis is based mainly on the observations that trichloroacetate induces tumours through PPARα activation, and that trichloroacetate is formed after exposure to trichloroethylene *in vivo*. To summarize, trichloroacetate activates PPARα, and induces proliferation of peroxisomes and hepatocytes. However, several inconsistencies and gaps in data reduce confidence in the conclusion that trichloroacetate induces hepatocarcinogenesis solely through a mechanism involving PPARα activation. First, while trichloroacetate induces peroxisome proliferation (a marker for PPARα agonism) in rats and in mice, it has been shown to be tumorigenic in B6C3F_1 mice, but not in a limited study in F344 rats (DeAngelo *et al.*, 1997) (the only strains tested for carcinogenicity). Second, the pattern of H-*ras* mutation in murine liver tumours induced by trichloroacetate differs from that in tumours induced by dichloroacetate and other peroxisome proliferators (Fox *et al.*, 1990; Hegi *et al.*, 1993; Stanley *et al.*, 1994; Bull *et al.*, 2002). Other effects of trichloroacetate, including increased expression of *c-myc* and hypomethylation of DNA (Tao *et al.*, 2000), are not specific to the PPARα-activation mechanism, and other data also contribute uncertainty as to whether PPARα-independent mechanisms may be involved in the induction of tumours by trichloroacetate in mice.

To conclude, trichloroethylene clearly activates PPARα. However, based on data from trichloroethylene and its metabolites alone, there is only limited evidence that activation of PPARα and its sequelae are key events in trichloroethylene-induced hepatocarcinogenesis. PPARα agonism may play a significant role in the induction of tumours in mouse liver by some compounds, such as Wy-14 643. However, recent studies suggest that di(2-ethylhexyl) phthalate (DEHP) can induce tumours in a PPARα-independent manner without any loss of potency (Ito *et al.*, 2007), and that PPARα agonism alone is not a sufficient cause for tumorigenesis in hepatocytes (Yang *et al.*, 2007). For trichloroethylene and most PPARα agonists, chemical-specific data to define the range of effects that may contribute to human carcinogenesis are insufficient.

4.3.3 Immune system

Tumours most strongly associated with exposure to trichloroethylene in humans include non-Hodgkin lymphoma. In experimental animals, an increased incidence of lymphoma was observed in female B6C3F_1 mice given trichloroethylene in corn oil by gavage (NTP, 1990), and the incidence of leukaemia was increased in male Sprague-Dawley rats and female August rats (Maltoni *et al.*, 1986, 1988; NTP, 1988). Evidence supporting hypothesized non-genotoxic mechanisms for these tumour types was limited to studies of immunological and haematological toxicity, as described in the sections that follow.

(a) Humans

Studies in humans provide evidence of associations between exposure to trichloroethylene and several immunotoxicological end-points. The relationship between occupational exposure to trichloroethylene and systemic autoimmune diseases, such as scleroderma, has been reported in several recent studies. A meta-analysis of

studies of scleroderma (Nietert *et al.*, 1998; Diot *et al.*, 2002; Garabrant *et al.*, 2003) conducted by the United States EPA resulted in a statistically significant combined odds ratio for any exposure in men (OR, 2.5; 95% CI, 1.1–5.4), with a lower odds ratio in women (OR, 1.2, 95% CI, 0.58–2.6) (Cooper *et al.*, 2009). The incidence of systemic sclerosis among men is very low (approximately 1 per 100 000 person-years), and is approximately 10 times lower than that in women (Cooper & Stroehla, 2003). Thus the available data for humans did not allow determination of whether the difference in effect estimates between men and women reflected the relatively low background risk of scleroderma in men, sex-related differences in exposure prevalence, or the reliability of exposure assessment (Messing *et al.*, 2003), a sex-related difference in susceptibility to the effects of trichloroethylene, or chance. Changes in levels of inflammatory cytokines were reported in a study of degreasers exposed occupationally to trichloroethylene (Iavicoli *et al.*, 2005), and in a study of infants exposed to trichloroethylene via indoor air (Lehmann *et al.*, 2001, 2002).

There were a large number of case reports of a severe hypersensitivity skin disorder, distinct from contact dermatitis and often accompanied by hepatitis, associated with occupational exposure to trichloroethylene, with prevalences as high as 13% among workers in the same location (Kamijima *et al.*, 2007, 2008; see Section 4.5.3b).

Several molecular epidemiology studies in humans have evaluated the effect of exposure to trichloroethylene on immune markers and potential for immunosuppressive effects. Most studies have been of workers exposed occupationally to trichloroethylene and unexposed control workers, and were conducted using a cross-sectional design with an exposure-monitoring period preceding collection of blood or other specimens (Table 4.7; Lan *et al.*, 2010; Hosgood *et al.*, 2011; Bassig *et al.*, 2013; Zhang *et al.*, 2013; see Section 4.5.3a).

(b) Experimental animals

Experimental studies provide additional support for the immunotoxicity of trichloroethylene. Numerous studies have demonstrated accelerated autoimmune responses in mice that are prone to autoimmune disease (Griffin *et al.*, 2000a, b; Blossom *et al.*, 2004, 2007, 2008; Cai *et al.*, 2008). With shorter exposure periods, effects include changes in cytokine concentrations similar to those reported in studies in humans. More severe effects, including autoimmune hepatitis, inflammatory skin lesions, and alopecia, were manifest at longer exposure periods, and these effects differed somewhat from "normal" expression in these mice. Immunotoxic effects (including increases in anti-double-stranded DNA antibodies in adult animals), decreased thymus weights, and decreased plaque-forming cell (PFC) response with prenatal and neonatal exposure, have been also reported in B6C3F_1 mice which are not known to be particularly susceptible to autoimmune disease (Peden-Adams *et al.*, 2006; Keil *et al.*, 2009). Recent mechanistic studies focused on the roles of various measures of oxidative stress in the induction of these effects by trichloroethylene (Wang *et al.*, 2007a, 2008).

Evidence of a treatment-related increase in delayed type hypersensitivity response accompanied by hepatic damage has been observed in guinea-pigs treated by intradermal injection (Tang *et al.*, 2002, 2008). Increase in delayed type and hypersensitivity response was also seen in mice offspring exposed to trichloroethylene in drinking-water between day 0 of gestation until age 8 weeks (Peden-Adams *et al.*, 2006).

Evidence for localized immunosuppression, as measured by pulmonary response to bacterial challenge (streptococcus aerosol infection), was seen in two studies of acute exposure in CD-1 mice (Aranyi *et al.*, 1986; Selgrade & Gilmour, 2010).

Table 4.7 Studies of the effects of exposure to trichloroethylene on the immune system, and autoimmune disease

Reference, study location	Total exposed	Total controls (unexposed)	Study design	Mean exposure level (mg/m³ or ppm)	End-points	Notable effects	Comments
Lan *et al.* (2010) China	80	96	Cross-sectional	Exposed: 22.19 ppm; < 12 ppm: mean, 5.19 ppm; ≥ 12 ppm: mean, 38.6 ppm	WBC, granulocytes, monocytes, lymphocytes, T-cells: $CD4^+$, $CD8^+$, B-cells, NK-cells, sCD27, sCD30	Exposed workers had reduced WBC (≥ 12 ppm only), lymphocytes, $CD4^+$ T cells, $CD8^+$ T cells, B cells, NK cells, T cells, sCD27, and sCD30 compared to controls.	Exposed workers had two or three full-shift personal air exposure measurements; subset analysed for other organic hydrocarbons; Controlled for potential confounders (age, sex, smoking, infection, alcohol, BMI) and characteristics were similar in exposed and non-exposed workers.
Iavicoli *et al.* (2005) Italy	35	70	Cross-sectional	Exposed: 5mg/m³;13.3mg/g creatinine urinary trichloroacetic acid	IL-4, IL-2, IFN-γ	Exposed workers had significant increase in IL-2 and IFN-γ; significant decrease in IL-4 levels compared to both control groups.	Urinary trichloroacetic acid levels measured in exposed and internal-control workers; Personal air sampling for 40% of exposed workers during three consecutive working shifts were collected using sorbent tubes; Analyses used both internal and external control group, but personal monitoring conducted on a subset of subjects and limited control for confounding.
Hosgood *et al.* (2011) China	80	96	Cross-sectional	Exposed: 22.19 ppm; < 12 ppm: 5.19 ppm; ≥ 12 ppm: 38.6 ppm	$CD4^+$ naïve, $CD4^+$ central and effector memory T cells, regulatory T cells subsets, $CD8^+$ naïve, $CD8^+$ central and effector memory cells	Exposed workers had reduced $CD4^+$ naïve (≥ 12 ppm) and $CD8^+$ naïve T cells, and $CD4^+$ effector memory T cells, compared with controls.	Exposed workers had two or three full-shift personal air exposure measurements; subset analysed for other organic hydrocarbons; Controlled for potential confounders (age, sex, smoking, infection, alcohol, BMI) and characteristics were similar in exposed and non-exposed workers.
Bassig *et al.* (2013) China	71	78	Cross-sectional	Exposed: 23.4 ppm; < 12 ppm: 5.1 ppm; ≥ 12 ppm: 41.2 ppm	IL-10, IL-6, TNF-α	Exposed workers had significantly reduced levels of serum IL-10, but not IL-6 or TNF-α, compared with controls	Exposed workers had two or three full-shift personal air exposure measurements; subset analysed for other organic hydrocarbons; Controlled for potential confounders (age, sex, smoking, infection, alcohol, BMI, lymphocyte counts) and characteristics were similar in exposed and non-exposed workers.

Table 4.7 (continued)

Reference, study location	Total exposed	Total controls (unexposed)	Study design	Mean exposure level (mg/m³ or ppm)	End-points	Notable effects	Comments
Zhang *et al.* (2013) China	80	45	Cross-sectional	Exposed: 22.2 ppm < 12 ppm: 5.2 ppm ≥ 12 ppm: 38.4 ppm	IgM, IgG, IgE	Exposed workers had reduced levels of serum IgM and IgG, but not IgE	Exposed workers had two or three full-shift personal air exposure measurements; subset analysed for other organic hydrocarbons; Controlled for potential confounders (age, sex, smoking, infection, alcohol, BMI) and characteristics were similar in exposed and non-exposed workers.
Lehmann *et al.* (2001) Germany	121	NA	Cohort	Median, 0.42 µg/m³	IgE antibodies, IL-4 + $CD3^+$ T cells, IFN-γ + $CD8^+$ T cells	Trichloroethylene not significantly associated with any end-point evaluated	Passive monitoring was conducted for VOCs for 4 weeks using 3M badges in the child's bedroom (children aged 36 months); Information on VOC exposure and IgE available for only 60.5% of children; VOCs highly correlated; population studied was children at risk for atopy, so external generalizability may be an issue.
Lehmann *et al.* (2002) Germany	85	NA	Cohort	Median, 0.6 µg/m³	IL-4, IL-2, TNF-α, and IFN-γ in cytokine-producing T cells.	Trichloroethylene associated with elevated IFN-γ OR, 3.6 (95% CI, 0.9–14.9) and reduced IL-4 OR, 4.4 (95% CI, 1.1–17.8) in multivariate analyses; reduced IL-2 in univariate analysis.	Passive monitoring was conducted for VOCs for 4 weeks using 3M badges in the child's bedroom (newborns).
Arif & Shah (2007) USA	550	NA	Cross-sectional	Geometric mean, 0.03 µg/m³	Physician diagnosed asthma/wheezing in previous 12 months	Physician-diagnosed asthma: OR, 0.94 (95% CI, 0.77–1.14); 1–2 wheezing attacks: OR, 1.29 (95% CI, 0.98–1.68); ≥ 3 wheezing attacks: OR, 0.21 (95% CI, 0.04–1.05).	Trichloroethylene and other VOCs measured in subjects using 3M personal monitoring for 48–72 h; limited ability to evaluate temporal relationship given cross-sectional design; no objective assessment of asthma available.

Table 4.7 (continued)

Reference, study location	Total exposed	Total controls (unexposed)	Study design	Mean exposure level (mg/m³ or ppm)	End-points	Notable effects	Comments
Nietert *et al.* (1998) USA	178	200	Case–control	NR	Scleroderma	Max. intensity, men: OR, 3.3 (95% CI, 1.0–10.3) Cumul. intensity, men: OR, 2.0 (95% CI, 0.7–5.3) Max. probability, men: OR, 5.1 (CI not calculated) Max. intensity, women: OR, 0.9 (95% CI, 0.3–2.3) Cumul. intensity, women: OR, 1.2 (95% CI, 0.5–2.6) Max. probability, women: OR, 0.7 (95% CI, 0.2–2.2)	Exposure assessment based on personal interviews and JEM, which was used to create semi-quantitative exposure scores based on intensity, probability of exposure, and cumulative intensity exposure score; used clinic-based control group but geographical location disregarded (> 97% controls lived in South Carolina versus 65% of cases).
Diot *et al.* (2002) France	80	160	Case–control	NR	Systemic sclerosis	Ever exposure: OR, 2.39 (95% CI, 1.04–5.22); high cumulative exposure: OR, 7.58 (95% CI, 1.54–37.36); ever exposure, women: OR, 2.10 (95% CI, 0.65–6.75); ever exposure, men: OR, 4.67 (95% CI, 0.99–21.89)	Expert review-assessed exposures and developed semi-quantitative exposure scores based on probability, frequency, intensity, and duration of exposure; the controls were matched to cases by age, sex, and smoking habits, and were hospitalized during the same time and in the same department as the cases.
Garabrant *et al.* (2003) USA	660	2227	Case–control	NR	Scleroderma	Exposed, self-report: OR, 2.0 (95% CI, 0.8–4.8) Exposed, expert review: OR, 1.9 (95% CI, 0.6–6.6)	Exposure assessment based on personal interviews and expert review which either confirmed or did not confirm reported exposures; controls frequency matched to cases by race, age, and residence and recruited using random-digit dialling.
Beaudreuil *et al.* (2005) France	60	120	Case–control	NR	ANCA (small vessel vasculitis)	No association: OR, 1.1 (95% CI, 0.5–2.4)	Exposure assessment based on self-reported exposures from personal interview and expert review developed exposure score (product of probability × intensity ×frequency × duration); In patient controls enrolled from same hospital as cases and matched by age and sex.

Table 4.7 (continued)

Reference, study location	Total exposed	Total controls (unexposed)	Study design	Mean exposure level (mg/m^3 or ppm)	End-points	Notable effects	Comments
Lacey *et al.* (1999) USA	205	2095	Case–control	NR	Undifferentiated connective tissue disease	Self-reported exposure: OR, 0.88 (95% CI, 0.11–6.95) Expert-review exposure: OR, 1.67 (95% CI, 0.19–14.90) Dry-cleaning: OR, 1.38 (95% CI, 0.68–2.78).	OR for self-report and expert review based on only 1 exposed case; exposure assessment based on personal interviews and expert review which either confirmed or did not confirm reported exposures; controls enrolled via random-digit dialling and matched to cases by age, race/ethnic group, and geographical region.

ANCA, antineutrophil cytoplasmic antibodies; BMI, body-mass index; CD, cluster of differentiation; CI, confidence interval; cumul., cumulative; IL, interleukin; IFN-γ, interferon-gamma; JEM, job-exposure matrix; max., maximum; NA, not available; NK, natural killer cells; NR, not reported; OR, odds ratio; sCD, soluble CD receptor, TNF-α, tumour necrosis factor-alpha; VOCs, volatile organic compounds, WBC, white blood cells

4.3.4 Lung

The hypothesized non-genotoxic mechanisms for induction of tumours of the lung by trichloroethylene include cytotoxicity. Evidence for this mechanism is limited to the demonstration of acute cytotoxicity in bronchiolar Clara cells and transient cell proliferation in mice exposed to trichloroethylene (Forkert *et al.*, 1985; Yost *et al.*, 1989; Forkert & Forkert, 1994; Henschler *et al.*, 1980; Fukuda *et al.*, 1983; Maltoni *et al.*, 1988). Because the cell type (or types) of origin for the observed tumours of the lung in mice has not been determined, the contribution to carcinogenicity of toxicity in Clara cells and subsequent regenerative cell division is largely unknown. Similarly, induction of dichloroacetyl-lysine protein adducts has only been studied over short duration and after intraperitoneal exposure (Forkert *et al.*, 2006), and the contribution of these adducts to tumorigenesis has not been investigated. Chloral hydrate, a genotoxic metabolite of trichloroethylene, is probably formed in the mouse lung and may therefore contribute to carcinogenicity (see the *Monograph* on Chloral Hydrate in this Volume). However, data were insufficient to determine whether chloral hydrate is formed in the human lung. Overall, the evidence for lung as a target tissue for trichloroethylene is moderate and there is only weak mechanistic support for trichloroethylene-induced carcinogenesis in the lung.

4.3.5 Testes

The data for trichloroethylene do not include an extensive characterization of the mechanisms for testicular tumorigenesis in the rat. Relevant evidence concerned a potential hormonal-disruption mechanism, for which key events leading to trichloroethylene-induced formation of testicular tumours were not specified. Two studies suggested that trichloroethylene causes hormonal disruption in male rats (Kumar *et al.*, 2000, 2001). Two studies in humans examined endocrine function in men (Chia *et al.*, 1997; Goh *et al.*, 1998), both studies followed up the cohort reported in Chia *et al.* (1996); increases in levels of the sulfated analogue of dehydroepiandrosterone, and decreases in levels of follicle-stimulating hormone, sex-hormone-binding globulin, and testosterone were seen with increased years of exposure to trichloroethylene. Other observed adverse effects on the male reproductive system included altered sperm morphology, hyperzoospermia, decreased sexual drive and function, and altered fertility (Bardodej & Vyskocil, 1956; El Ghawabi *et al.*, 1973; Saihan *et al.*, 1978).

4.4 Susceptibility data

4.4.1 Inter-individual variability

(a) Humans

The oxidative metabolism of trichloroethylene is largely dependent on CYP2E1, and clinically relevant genetic polymorphisms are known to exist in the gene encoding this enzyme (Catanzaro *et al.*, 2012; Daly, 2012). Neafsey *et al.* (2009) reviewed the literature and found that while a variety of *CYP2E1* variant alleles have been found, the functional significance of these variants is still unclear. Some, but not all, studies suggested that several upstream 5′ flanking mutations affect gene expression and response to inducers such as ethanol or obesity. None of the coding-region variants consistently affects enzyme function. The only indirect evidence for the potential role of *CYP2E1* polymorphisms in toxicity associated eith trichloroethylene has been reported in a study by Povey *et al.* (2001), who compared *CYP2E1* alleles in 7 patients who had developed scleroderma after exposure to solvents versus 71 patients with scleroderma without solvent exposure (“sporadic” disease) and 106 population controls. The *2E1*3* allele was found in two of the seven patients who had been exposed to organic solvents, with a greater

frequency than in either the disease controls or the population controls (OR, 9.1; [95% CI, 1.5–59.1]; and OR, 10.2 [95% CI, 1.8–62.2], respectively). [The Working Group commented that while this study provided some information on trichloroethylene exposure, *CYP2E1* polymorphisms and an adverse health effect (i.e. scleroderma), it did not evaluate the potential relationship to any cancer outcomes.]

GSH conjugation is a trichloroethylene-metabolism pathway that results in formation of several toxicologically relevant metabolites. Several transporter proteins in the kidney (e.g. organic anion transporters 1 and 3, OAT1 and OAT3) that are likely to be responsible for the uptake and cellular accumulation of the GSH metabolites DCVG and DCVC into the renal proximal tubular cells, are known to be polymorphic in humans (Erdman *et al.*, 2006; Lash *et al.*, 2006b; Urban *et al.*, 2006). Thus different subpopulations of humans may have a markedly different capacity to accumulate DCVG or DCVC, which may affect their susceptibility to nephrotoxicity.

While it has not been firmly established which GST enzymes are responsible for the metabolism of trichloroethylene, allelic polymorphisms of these enzymes in humans have been associated with variations in enzyme activity (Katoh *et al.*, 2008); such polymorphisms may thus affect the concentration of trichloroethylene metabolites in the body (Caldwell & Keshava, 2006).

Brüning *et al.* (1997b) investigated polymorphisms of *GSTM1* and *GSTT1* and risk of renal cell cancer in workers with long-term high occupational exposure to trichloroethylene [identified as "trichloroethene"]. The study comprised 45 cases with histologically verified renal cell cancer and a history of long-term occupational exposure to high concentrations of trichloroethylene. A reference group consisted of 48 cancer-free workers from the same geographical region with similar histories of occupational exposure to trichloroethylene. Among patients with renal cell carcinoma, 27 carried at least one functional *GSTM1* gene (*GSTM1*+) and 18 carried at least one functional *GSTT1* gene (*GSTT1*+). Among the 48 reference workers, 17 were *GSTM1*+ and 31 were *GSTT1*+. The odds ratios for renal cell cancer 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 study were re-evaluated by Wiesenhütter *et al.* (2007) who used data from additional control subjects to increase the size of the study population. No genetic influence on the development of renal cell cancer due to trichloroethylene (e.g. deletion polymorphisms of *GSTT1* and *GSTM1*, or *NAT2* rapid/slow acetylator) was found. There was a somewhat higher proportion of the homozygous *GSTP1* 313A wild type (*GSTP1*A*) among cases of renal cell cancer, although this was not statistically significant.

Moore *et al.* (2010) conducted a case–control study in central Europe (1097 cases and 1476 controls) to assess risk of renal carcinoma associated with occupational exposure to trichloroethylene (assessed from the work history). Increased risk was observed among subjects ever exposed to trichloroethylene. A significant association was found among trichloroethylene-exposed subjects with at least one intact *GSTT1* allele (active genotype), but not among subjects with two deleted alleles (null genotype). Similar associations for all exposure metrics including average intensity were observed among *GSTT1*-active subjects, but not among *GSTT1* nulls.

Bronley-DeLancey *et al.* (2006) used cryogenically preserved human hepatocytes to simultaneously evaluate the kinetics of chloral hydrate metabolism and aldehyde dehydrogenase (*ALDH*) or alcohol dehydrogenase (*ADH*) genotype. Thirteen samples of human hepatocytes were examined and large inter-individual variation in the V_{max} values for formation of trichloroethanol and trichloroacetate were reported; no correlation with *ADH*/*ALDH* genotype was apparent, although the sample size was limited.

Furthermore, despite the large variation in V_{max} values among individuals, disposition of chloral hydrate into downstream metabolites was found to be relatively constant. Therefore, cellular factors other than genotype may contribute to the observed variability in metabolism of chloral hydrate in human liver.

Among many inter-individual differences in lifestyle and nutrition, alcohol intake is the most prominent factor affecting susceptibility to trichloroethylene. Trichloroethylene is metabolized to chloral hydrate, and then to trichloroacetate by aldehyde dehydrogenase, and to trichloroethanol by alcohol dehydrogenase. The effects of trichloroethylene (probably through chloral hydrate) on alcohol and acetaldehyde metabolism have been suggested as a mechanism for the dramatic effects of coexposure to chlorinated solvents and alcohol. Such coexposures lead to more than additive sedative effects in humans (Sellers *et al.*, 1972). Adverse health effects indicative of elevated blood levels of acetaldehyde have been described as "degreaser's flush" (Stewart *et al.*, 1974). Additionally, aldehyde and alcohol dehydrogenases are polymorphic in humans, and these polymorphisms have a major impact on cancer susceptibility in humans who consume alcoholic beverages, especially in Asian countries (IARC, 2010, 2012). It has been suggested that polymorphisms in these metabolic pathways may yield subpopulations with greater than expected formation of trichloroacetate and enhanced risk of adverse health effects after exposure to chloral hydrate or other chlorinated solvents.

(b) Experimental animals

Bradford *et al.* (2011) studied the metabolic and genetic basis for differences in trichloroethylene toxicity using a panel of genetically diverse inbred mouse strains. Trichloroethylene (2100 mg/kg bw) or corn oil vehicle was given by gavage to male mice of 15 strains (age, 6–8 weeks). Serum and liver were collected 2, 8, and 24 hours after dosing and were analysed for trichloroethylene metabolites, hepatocellular injury, and hepatic gene expression. Metabolism of trichloroethylene through oxidative and conjugative pathways varied considerably between strains. Trichloroethylene-specific effects on hepatic gene expression were strongly dependent on genetic background. Conversely, effects on cell death, liver necrosis, and immune-mediated response pathways, which are altered in the liver by treatment with trichloroethylene, were largely independent of genetic background.

Chloral hydrate has been shown to be an inhibitor of aldehyde dehydrogenase (Wang *et al.*, 1999), thus suggesting that production of trichloroacetic acid from chloral hydrate may not increase in linear fashion with dose. An inhibitory effect of chloral hydrate on the activity of alcohol dehydrogenase in liver was also reported in studies in mice (Sharkawi *et al.*, 1983). In a short-term study in rats, Poon *et al.* (2002) showed that exposure to drinking-water containing chloral hydrate led to a significant reduction in the activity of liver aldehyde dehydrogenase, while the activity of liver aniline hydroxylase (associated with CYP2E1) was significantly elevated in males and females receiving chloral hydrate at a concentration of 200 ppm. This study confirmed previous findings (Wang *et al.*, 1999) that chloral hydrate was a potent inhibitor of liver aldehyde dehydrogenase *in vitro*, with a 50% inhibition concentration (IC_{50}) of 8 μM, while trichloroacetic acid was weakly inhibitory and trichloroethanol was without effect.

Coexposure to chlorinated solvents and alcohol lead to more than additive sedative effects in rodents (Sharkawi *et al.*, 1983), as also noted above in humans (Sellers *et al.*, 1972). Because alcohol consumption leads to increased activity of CYP2E1 in the liver (Bradford *et al.*, 2005), the metabolism of trichloroethylene via the oxidative pathway may be increased. Indeed, Nakajima *et al.* (1992a) observed an increase in metabolism of trichloroethylene in rat liver microsomes from rats pre-treated with alcohol.

4.4.2 Life-stage susceptibility

(a) Early life stages

Many studies have considered differences in exposure as an important element of early life-stage susceptibility to adverse health outcomes associated with trichloroethylene. It has been shown that trichloroethylene can be transferred to the fetus by the placenta in all mammalian species studied, including humans. Infants that are breastfed by mothers exposed to trichloroethylene by various routes (including inhalation) have been shown to receive appreciable amounts of trichloroethylene (Fisher *et al.*, 1990, 1997; Abbas & Fisher, 1997). Differences in diet between infants and adults may also be a factor in differences in exposure, since dairy products have been found to contain trichloroethylene and children eat a generally higher proportion of these foodstuffs than adults (Wu & Schaum, 2000). Exposure dermally and by inhalation was also estimated to be higher in children than in adults when bathing in trichloroethylene-containing water (Fan, 1988).

Since children have a higher ventilation rate than adults, and a relatively higher alveolar surface area for the first 2 years of life, absorption of trichloroethylene is expected to be greater in early life stages, although no data were available to prove this. As trichloroethylene is a lipophilic compound, the percentage of adipose tissue in the body will have an impact on distribution and retention of the absorbed dose; children, who have a lower percentage of body fat per unit body weight, may thus have higher concentrations of trichloroethylene in the body fat, although no data to corroborate this were available. While trichloroethylene has been found in the blood and tissues of fetuses born to exposed mothers, there is disagreement on whether such concentrations are higher or lower than those found in maternal tissues (Laham, 1970; Withey & Karpinski, 1985). More recent studies have demonstrated that the blood: air partition coefficient of trichloroethylene varies with age. Rodriguez *et al.* (2007) have demonstrated that in rats exposed to trichloroethylene via inhalation, blood concentrations of trichloroethylene were higher on postnatal day 10 than in adults, which may be attributed to the lower metabolic capacity of the pre-weaning rats. In addition, Mahle *et al.* (2007) reported that the tissue: air partition coefficient for trichloroethylene increases with age in rats, but decreases in humans.

It is well established that hepatic expression of most CYP and also GST enzymes is dramatically different in the fetus than in adults (Ring *et al.*, 1999). Within months after birth, the metabolic capacity of the human liver changes and becomes more similar to that in adults. Expression of CYP2E1 and GSTs in the developing fetus is detectable, albeit not in all samples, and is dependent on the stage of pregnancy (Carpenter *et al.*, 1996; McCarver & Hines, 2002; Johnsrud *et al.*, 2003). Thus differences in the metabolism of trichloroethylene between early life stages and adults represent a potential susceptibility factor.

Several early life stage-specific adverse health outcomes attributable to exposure to trichloroethylene during pregnancy or neonatal development have been reported in humans and other species. These include cardiac birth defects, neural tube defects, oral clefts, and choanal atresia (Bove *et al.*, 2002). It should be noted that a large number of epidemiological and experimental studies observed no association between trichloroethylene and these developmental abnormalities (Watson *et al.*, 2006). Other non-cancer adverse health effects in children or pre-weaning animals have been evaluated. Overall, the evidence base of available information was largely inconsistent with regard to whether early life stage is a susceptibility factor for adverse health outcomes in the nervous and immune systems, kidney, liver or lung.

Several studies have evaluated the potential for susceptibility to cancer outcomes in early life stages. Total incidence of childhood cancer, and

incidence of childhood leukaemia and tumours of the central nervous system have been considered. Most studies have found no evidence that children may be more susceptible than adults; however, these studies included small numbers of cases and poor characterization of exposure (see Section 2.2.2b, Section 2.2.3c; McKinney *et al.*, 1991; Shu *et al.*, 1999; De Roos *et al.*, 2001).

(b) Advanced life stages

Limited evidence existed to suggest that exposure to trichloroethylene in adults of advanced age (> 65 years for humans) may lead to greater adverse health effects than in younger people. Some studies have suggested that toxicokinetic parameters in later life stages are different from those in young adults (Benedetti *et al.*, 2007); however, there was little evidence to suggest that expression of CYP2E1 or GSTs differs with age in adults. While several studies have documented significant age-related declines in the amount, specific activity and rate of induction of liver microsomal mono-oxygenases in inbred male rats, on the basis of a variety of clinical tests, most liver functions in humans appear to be well preserved with age [reviewed in Schmucker (2001)]. There was some evidence suggesting a reduction in CYP2E1 activity in the elderly (O'Shea *et al.*, 1994; George *et al.*, 1995); however, studies *in vitro* that used non-human primate or human liver tissues, or isolated cells, did not detect age-related deficiencies in the activity of CYP-dependent microsomal mono-oxygenases [reviewed in Schmucker (2001)].

Mahle *et al.* (2007) observed that blood:air partition coefficients for trichloroethylene in the rat increased with age (age 60 days versus age 2 years). Another study used a modelling approach to predict age-appropriate pharmacokinetics of trichloroethylene in the rat (Rodriguez *et al.*, 2007). These authors predicted that the steady-state concentration of trichloroethylene in the blood would be reached more slowly and be higher at age 2 years than in young adults. In addition, they predicted that concentrations of trichloroethylene in the brain would increase with age. No experimental confirmation of this pharmacokinetic model was available.

4.4.3 Sex-specific differences

Several studies examined sex-specific differences in the toxicokinetic parameters of trichloroethylene using physiologically-based pharmacokinetic modelling. Sato *et al.* (1991) evaluated the influence of body size, body-fat content, and sex on the pharmacokinetic behaviour of trichloroethylene. Absorption, distribution, metabolism, and excretion of trichloroethylene were found to vary according to the different anatomical features of men and women. Body build (body weight and body-fat content) also affected the pharmacokinetic behaviour of trichloroethylene. In a follow-up study, Sato (1993) concluded that there was a sex difference in the pharmacokinetic profiles of trichloroethylene, and although retention of trichloroethylene in the body was greater in men than in women, the blood concentration of trichloroethylene in women was higher than in men 16 hours after exposure. Fisher *et al.* (1998) evaluated sex-specific differences in the uptake and metabolism of trichloroethylene using data on human exposure, and concluded that only minor differences existed in the toxicokinetics of trichloroethylene.

Lash *et al.* (2006a) evaluated the metabolism and tissue distribution of trichloroethylene in male and female Fischer 344 rats given doses of 2, 5, or 15 mmol/kg bw in corn oil by gavage, and monitored for trichloroethylene and its metabolites in the liver, kidneys, blood, and urine for up to 48 hours. Higher concentrations of trichloroethylene were generally observed in tissues of males at lower doses. Higher concentrations of oxidative metabolites were observed in the livers of males than in females, with the opposite pattern being observed in the kidneys.

DCVG was recovered in the liver and kidneys of female rats only, and in blood of males and females, with amounts being generally higher in females. DCVC, the nephrotoxic metabolite, was recovered in liver of males and females, kidneys of females, blood of males, and in the urine of males and females.

While most of the experimental systems used to study the mechanisms of DCVC-related nephrotoxicity have been derived from the male rat, studies have also been conducted in tissue from female rats and in mice. Darnerud *et al.* (1989) characterized the relationship between developmental stage, activities of CCBL and OAT, and nephrotoxicity. Female mice were found to be the most susceptible to DCVC-induced nephrotoxicity at low doses due to higher CCBL activity, while male mice were more susceptible at higher doses.

Nakajima *et al.* (1992b) used liver microsomes from male and female rats aged 3 and 18 weeks to study the metabolism of trichloroethylene (by measuring formation of chloral hydrate) *in vitro*. No differences between males and females were seen with age or concentration of trichloroethylene administered.

Expression and function of OAT1 and OAT3 and other organic anion transporters (such as OAT polypeptide 1) have been shown to exhibit sex-specific differences in humans and experimental animals (Gotoh *et al.*, 2001; Kato *et al.*, 2002; Kobayashi *et al.*, 2002; Buist *et al.*, 2003; Buist & Klaassen, 2004; Ljubojevic *et al.*, 2004), suggesting that transport differences may be another contributing factor to sex-specific differences in susceptibility to trichloroethylene metabolites.

No sex-specific susceptibility to toxicity in the liver or respiratory tract associated with exposure to trichloroethylene has been reported. Two studies evaluating kidney effects in humans have concluded that females may be more susceptible to kidney disease and diabetes associated with exposure to trichloroethylene (Davis *et al.*, 2005). However, males were reported to be more sensitive to renal toxicity in studies in rats (Lash *et al.*, 1998; Lash *et al.*, 2001b). With regard to data on immunotoxicity in humans, a meta-analysis of three case–control studies of scleroderma with a measure of occupational trichloroethylene exposure found that the combined odds ratio was 2.5 (95% CI, 1.1–5.4) in men and 1.2 (95% CI, 0.58–2.6) in women (Cooper *et al.*, 2009).

Sex-specific differences in susceptibility to cancer associated with exposure to trichloroethylene are well established. In rats, exposure to trichloroethylene by inhalation or gavage caused cancer of the kidney (tubular adenocarcinoma) only in males. Leukaemia was also observed in a single study in male rats, although low survival was noted as a challenge in interpreting this study (Maltoni *et al.*, 1988). Testicular tumours have also been observed in studies in rats. In studies in mice, no sex-specific differences in the incidence of cancer of the liver or lung were observed (Maltoni *et al.*, 1988). Lymphomas were reported in female mice (Henschler *et al.*, 1980).

Raaschou-Nielsen *et al.* (2003) evaluated risk of cancer among workers at Danish companies using trichloroethylene in a cohort study (see Section 2). No significant sex-specific differences in the incidence of tissue-specific cancer were observed in this study. Most other epidemiological studies of cancer and exposure to trichloroethylene did not report sex-specific differences.

4.5 Other adverse effects

4.5.1 Kidney: chronic nephropathy

(a) Humans

Brüning *et al.* (1996) detected substantially more tubular damage in patients with renal cell carcinoma who were also exposed to trichloroethylene than in those who were not exposed. In a second study, Brüning *et al.* (1999b) performed a retrospective analysis of 39 workers exposed to high levels of trichloroethylene for 19 years.

While the standard biomarkers of tubular function that indicate significant renal damage (i.e. total urinary protein, blood urea nitrogen, and urinary and serum creatinine) were not elevated, elevated urinary excretion of α1-microglobulin and GSTA were significantly elevated.

Vermeulen *et al.* (2012) studied a small group of 80 factory workers exposed to trichloroethylene and 45 non-exposed workers. Precise exposure information was obtained (mean ± standard deviation, 22.2 ± 35.9 ppm). All workers exposed to trichloroethylene were stratified according to their exposure level, with 12 ppm as the threshold. Significant elevation of urinary Kim-1 (a very sensitive and selective indicator of renal damage in humans and rodents) was observed (Hoffmann *et al.*, 2010; Harpur *et al.*, 2011). Even workers with exposures of < 12 ppm exhibited a statistically significant elevation in urinary Kim-1.

Green *et al.* (2004) investigated the nephrotoxic potential of trichloroethylene in a cross-sectional study of 70 workers currently exposed to trichloroethylene. Data from age- and sex-matched control populations were also available. The mean exposure to trichloroethylene, estimated from urinary concentrations of trichloroacetate, was 32 ppm (range, 0.5–252 ppm) with an average duration of exposure of 4.1 years (range, 1–20 years). Significant differences between the exposed and control populations were found for nephrotoxicity markers NAG, albumin, and formic acid. Neither NAG nor albumin showed any significant correlation with either the magnitude or duration of exposure to trichloroethylene. However, there was a significant correlation between urinary concentrations of formic acid and trichloroacetate. Within the exposed population there were dose-dependent increases in urinary concentrations of methylmalonic acid and urinary GSTA activity. Although not outside the range for controls, these changes were clearly dose-dependent.

(b) Experimental systems

Exposure of Eker rats to trichloroethylene in corn oil by gavage for 13 weeks (0, 100, 25, 500, and 1000 mg/kg bw) led to an increased incidence of nephrotoxicity, but no significant increases in the incidence of preneoplastic or neoplastic lesions when compared with controls (Mally *et al.*, 2006). Chronic nephropathy was also observed in male and female Osborne-Mendel rats exposed to trichloroethylene (549 and 1097 mg/kg bw per day) by gavage for 78 weeks (NCI, 1976).

Overt signs of short-term nephrotoxicity, such as changes in blood or urinary biomarkers, are primarily seen at higher doses, although histopathological changes are evident at lower doses. In Fischer 344 rats given trichloroethylene by gavage in corn oil at a dose of 2000 mg/kg bw per day for 42 days, there were increases of about twofold in urinary markers of nephrotoxicity such as urine volume and protein (both 1.8 times), NAG (1.6 times), glucose (2.2 times) and alkaline phosphatase (2.0 times) compared with controls (Green *et al.*, 1997b). No morphological changes were observed in the kidney of treated rats or controls (Green *et al.*, 1997b). At lower doses, Green *et al.* (1998) reported that plasma and urinary markers of nephrotoxicity were unchanged. In particular, after 28 days of exposure to trichloroethylene at a concentration of 250 or 500 ppm for 6 hours per day, there were no statistically significant differences in plasma concentrations of blood urea nitrogen, or in urinary concentrations of creatinine, protein, alkaline phosphatase, NAG, or GGT. However, increased urinary excretion of formic acid, accompanied by changes in urinary pH and increased ammonia, was found at these exposures. Interestingly, at the same exposure level of 500 ppm (6 hours per day, 5 days per week, for 6 months) in Long-Evans rats, Mensing *et al.* (2002) reported elevated excretion of low-molecular-weight proteins and NAG, biomarkers

of nephrotoxicity, but after the longer exposure duration of 6 months.

Numerous studies have reported histological changes after short- and long-term exposure trichloroethylene (Maltoni *et al.*, 1986; NTP, 1988, 1990; Mensing *et al.*, 2002).

After 1–2 years of exposure to trichloroethylene by gavage (NCI, 1976; NTP, 1988, 1990) or inhalation (Maltoni *et al.*, 1986, 1988), mice and rats exhibited lesions in the tubular epithelial cells of the inner renal cortex that are characterized by cytomegaly, karyomegaly, and "toxic nephrosis" (see Section 3 for details). These long-term studies reported cytomegaly and karyomegaly of tubular cells. NTP (1990) specified the area of damage as the pars recta, located in the corticomedullary region. These effects are distinct from the chronic nephropathy and inflammation observed in control mice and rats (NCI, 1976; Maltoni *et al.*, 1986, 1988; Lash *et al.*, 2000b).

These effects of trichloroethylene on the kidney appear to be progressive. Maltoni *et al.* (1986, 1988) and NTP (1988, 1990) noted that the incidence and degree of renal toxicity increased with exposure duration and with time from the start of treatment.

Wallin *et al.* (1992) characterized the renal cellular response to DCVC in male Sprague-Dawley rats. Rats given ^{35}S-labelled DCVC at a dose of 30 mg/kg bw exhibited a doubling of blood urea nitrogen levels within 2 days. Formation of covalent adducts was seen within 3 hours and peaked at 6 hours, but was still detectable after 120 hours. BrdU staining indicative of cell proliferation increased within 24 hours, and the increase of vimentin-positive cells indicative of loss of differentiation was evident.

Eyre *et al.* (1995b) demonstrated that administration of DCVC (1, 5, or 25 mg/kg bw) or trichloroethylene (1000 mg/kg bw) to male F344 rats or male B6C3F$_1$ mice resulted in increased cell proliferation, as indicated by BrdU staining. Moreover, they observed greater formation in mice than in rats of acid-labile adducts from both DCVC and trichloroethylene that correlated with a higher proliferative response. These results were consistent with the documented greater rates of GSH-dependent metabolism and CCBL-dependent bioactivation in mice than in rats.

Cummings *et al.* (2000c) studied cytotoxicity of trichloroethylene and DCVC in primary cultures of rat proximal tubular cells and found that exposure of cells for 72 hours to trichloroethylene (10 mM) or DCVC (10 µM) resulted in the appearance of vimentin-positive cells, indicating loss of differentiation.

Mally *et al.* (2006) assessed renal cell proliferation and transformation *in vivo* and *in vitro* in Eker rats (a strain that carries the Eker mutation, *Tsc-2*$^{Ek/+}$, and is thus extremely susceptible to renal carcinogens). *In vivo*, exposure to trichloroethylene (0, 100, 250 500, and 1000 mg/kg bw) by gavage, 5 days per week, for 13 weeks significantly increased cell proliferation, but did not enhance formation of preneoplastic lesions or renal tumours. *In vitro,* exposure of primary cultures of kidney epithelial cells to DCVC (10 µM) reduced cell viability to ~50% after a 24-hour incubation, and caused transformation. These effects were not associated with known, carcinogen-specific mutations in either the *VHL* or *Tsc-2* tumour suppressor genes.

Mehendale and colleagues studied tissue repair and survival in male Swiss-Webster mice exposed to DCVC in a series of studies (Korrapati *et al.*, 2005, 2006, 2007; Dnyanmote *et al.*, 2006). These studies showed that intraperitoneal injection of a sublethal dose of DCVC could stimulate renal repair processes and protect mice from subsequent exposure to normally lethal doses of either DCVC or other nephrotoxicants, such as mercuric chloride. These studies also identified potential mechanisms for this stimulation of renal repair, including changes in the expression of certain cyclins and cyclin-dependent kinases.

Counts *et al.* (1995) demonstrated that incubation of rabbit proximal tubules with a relatively high concentration of DCVC inhibited the ability of the renal cells to undergo repair and regeneration.

Nowak and colleagues (Nowak *et al.*, 1999; Nowak, 2003; Shaik *et al.*, 2008) provided insight into how the compensatory repair and proliferation response might occur in rabbit proximal tubules, by showing that activation of certain regulatory enzymes, such as protein kinase C and protein kinase B (Akt), or epidermal growth factor promoted recovery of renal mitochondrial function and promoted repair after DCVC-induced injury.

4.5.2 Liver

(a) Cytotoxic injury and hepatitis

(i) Humans

In a cross-sectional epidemiological study of the early hepatic effects of long-term exposure to low levels of trichloroethylene, Nagaya *et al.* (1993) evaluated serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and activity of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and GGT (called GGtranspeptidase in this report) in 148 workers occupationally exposed to trichloroethylene by inhalation. Of the main cohort, 13 workers were followed for 2 years. Exposure to trichloroethylene was ascertained from the urinary concentrations of total trichloro-compounds, and subjects were divided into groups with low, moderate, and high exposure. Slight, but not statistically significant, increases in total and HDL cholesterol were observed with increasing exposure levels. No effect was found on the activities of the serum enzymes. In the follow-up study, concurrent fluctuations in urinary concentrations of total trichloro-compounds and subclinical (e.g. not exceeding the normal range in this study) changes in HDL cholesterol, AST, and GGT were observed. While there was some evidence for liver toxicity of trichloroethylene in occupationally exposed individuals, these effects were subclinical and reversible.

Rasmussen *et al.* (1993a) examined dose–response relationships between exposure to degreasing solvents (mainly trichloroethylene) and liver function. The study included 99 metal degreasers using trichloroethylene (range of full-time degreasing, between 1 month and 36 years); no control group was used. Based on the estimated total number of hours of exposure, subjects were divided into four groups. Present or recent exposure to trichloroethylene was quantified by blood and urine analyses of the metabolites trichloroacetate and trichloroethanol. Of several serum markers of liver injury, a dose–response relationship was found to be statistically significant only for GGT. However, when age and alcohol abuse were included as covariates in the multiple-regression analysis, this association was no longer significant.

Xu *et al.* (2009) evaluated liver-injury markers in 21 subjects with severe hypersensitivity dermatitis who were employed as metal degreasers. Exposure was evaluated from workplace air measurements and further documented by urinary concentrations of trichloroacetate. In most subjects, exposure was classified as exceeding recommended occupational levels. In 76–90% of subjects, an increase in serum activities of liver enzymes (ALT, AST) and total bilirubin was detected.

Kamijima *et al.* (2007) performed a comprehensive literature analysis to evaluate the possible relationship between idiosyncratic generalized skin disorders and accompanying hepatitis, and occupational exposure to trichloroethylene. They reported, based on primary evidence presented in Xia *et al.* (2004), that hepatitis was diagnosed in 46–94% of trichloroethylene-exposed subjects who also had various types of dermatitis ranging from hypersensitivity syndrome to erythema

multiforme, Stevens–Johnson syndrome, or toxic epidermal necrolysis.

(ii) Experimental systems

Acute exposure to trichloroethylene, even at high doses, is not known to cause significant hepatocellular necrosis. Okino *et al.* (1991) exposed male Wistar rats to trichloroethylene via inhalation for 2 hours (up to 8000 ppm) or 8 hours (up to 2000 ppm). Mild hepatocellular necrosis, evaluated by histopathology, and small increases in serum enzyme markers of liver injury were observed only at the highest concentrations. In a study of oral exposure (up to 5000 mg/kg bw by gavage), Berman *et al.* (1995) reported no elevation in serum enzyme activities, but histopathological evidence of mild hepatocellular necrosis in female F344 rats. Sano *et al.* (2009) observed no liver injury in either male Sprague-Dawley rats or $B6C3F_1$ mice given trichloroethylene at 1500 mg/kg bw by gavage.

Several, although not all, studies reported an increased incidence of liver necrosis after short-term exposure to trichloroethylene. Buben & O'Flaherty (1985) observed central lobular necrosis in male Swiss-Cox mice exposed to trichloroethylene at a dose of 1600 mg/kg bw per day for 6 weeks. Melnick *et al.* (1987) reported single-cell necrosis in male F344 rats fed or gavaged with trichloroethylene at doses of 2.2 g/kg bw per day or higher for 14 days. Merrick *et al.* (1989) found that the use of corn oil as vehicle for trichloroethylene (4 weeks at 600, 1200 and 2400 mg/kg bw per day for males and 450, 900 and 1800 mg/kg bw per day for females) promotes liver necrosis in male, but not female $B6C3F_1$ mice. When aqueous vehicle was used, no liver necrosis was found. In addition, mild increases in serum enzymes were found in male mice treated with trichloroethylene diluted in corn oil. Dees & Travis (1993) observed slight to mild liver necrosis in $B6C3F_1$ mice treated with trichloroethylene at doses of up to 1000 mg/kg bw per day for 10 days. Berman *et al.* (1995) reported histopathological evidence for hepatocellular necrosis in female F344 rats exposed to trichloroethylene at a dose of 1500 mg/kg bw per day or higher for 14 days. Ramdhan *et al.* (2008, 2010) found an increase in serum enzyme activities (ALT and AST) in Sv/129 mice exposed to trichloroethylene at concentrations exceeding 1000 ppm via inhalation for 7 days.

Other investigators reported no evidence of liver necrosis after short-term exposure to trichloroethylene. These included a study in $B6C3F_1$ mice given doses of up to 1200 mg/kg bw per day in corn oil for up to 8 weeks (Channel *et al.*, 1998); a study in rats given a dose of 2000 mg/kg bw per day in corn oil for 7 days (Nunes *et al.*, 2001); a study in Sv/129 mice given doses of up to 1500 mg/kg bw per day for 3 weeks (Laughter *et al.*, 2004); and a study in male Sprague-Dawley rats and $B6C3F_1$ mice given doses of up to 1500 mg/kg bw per day in corn oil for 14 days (Sano *et al.*, 2009). In a 2-year cancer bioassay (NTP, 1990), no evidence of increased focal necrosis in the liver was found in $B6C3F_1$ male and female mice, although the incidence of neoplasms of the liver was increased.

Two studies examined end-points for liver injury in MRL mice, which are prone to autoimmune disease. Kaneko *et al.* (2000) observed dose-dependent mild liver inflammation after exposure to trichloroethylene (up to 2000 ppm) via inhalation for up to 8 weeks (4 hours per day, 6 days per week). Gilbert *et al.* (2009) evaluated the effects of long-term (32-week) low-level exposure to trichloroethylene (0.5 mg/mL in drinking-water) in female MRL mice; trichloroethylene-induced autoimmune hepatitis could be detected in as little as 26 weeks. Exposure to trichloroethylene also generated a time-dependent increase in the number of antibodies specific for liver proteins, and altered the hepatic expression of selected genes associated with immunity and inflammation.

Several studies reported liver inflammation and/or evidence for inflammatory cell infiltrates.

Kjellstrand *et al.* (1983b) exposed male and female NRMI mice to trichloroethylene at 150 ppm, and found inflammatory cell infiltration and an increase in the number and size of Kupffer cells in mice exposed for 120 days. Elcombe *et al.* (1985) observed isolated inflammatory foci in livers of two strains of rats exposed to trichloroethylene at 1500 mg/kg bw per day for 10 days. Goel *et al.* (1992) found "proliferation of hepatic sinusoidal endothelial cells" in male Swiss mice exposed to trichloroethylene at 1000–2000 mg/kg bw per day for 28 days.

(b) Cholestatic injury

(i) Humans

Driscoll *et al.* (1992) evaluated concentrations of individual serum or plasma bile acids in subjects exposed occupationally to trichloroethylene. The study cohort of 22 volunteers (21 men) was divided into two groups: exposed (n = 16) and unexposed (n = 6) to trichloroethylene on the basis of their job tasks. Blood was collected at the beginning of a shift after an overnight fast. Highly significant increases in the exposed group, after controlling for age and alcohol intake, were seen for plasma concentrations of chenodeoxycholic acid, and its glycine and taurine conjugate, and for total glycine, taurine conjugates, and total cholate, and total chenodeoxycholate (free plus conjugates), and total bile acids.

Neghab *et al.* (1997) examined hepatobiliary function in subjects exposed occupationally to trichloroethylene. This study included 10 healthy workers (5 unexposed controls and 5 exposed) and evaluated end-points before (first day of the working week) and after exposure (2 days into the working week). Statistically significant elevations in concentrations of total serum bile acids, particularly deoxycholic acid and the subtotal of free bile acids, among "exposed" subjects were reported before exposure compared with after exposure. Furthermore, serum bile acid concentrations correlated with level of exposure to trichloroethylene (r = 0.94).

(ii) Experimental systems

Several studies of exposure to trichloroethylene by intraperitoneal injection from one laboratory reported increases in serum bile-acid concentrations in male rats exposed to trichloroethylene (Wang & Stacey, 1990; Bai & Stacey, 1993; Hamdan & Stacey, 1993). Serum increases in cholic, chenodeoxycholic, deoxycholic, taurocholic, and tauroursodeoxycholic acids in serum were dose-related in male Sprague-Dawley rats treated with high doses of trichloroethylene via intraperitoneal injection (up to 1300 mg/kg bw per day for 3 days) (Wang & Stacey, 1990). Studies *in vitro* supported a dose-related suppression of initial rates of uptake of cholic and taurocholic acids, with no significant effect on enzyme leakage or intracellular potassium-ion content (Bai & Stacey, 1993). The inhibition of uptake of cholic and taurocholic acids was confirmed and shown to be reversible in accompanying studies *in vivo*.

(c) Cirrhosis

(i) Humans

There is mixed evidence with regard to a possible association between exposure to trichloroethylene and cirrhosis of the liver. A cohort study on deaths from cirrhosis in California between 1979 and 1981 (Leigh & Jiang, 1993), which examined various occupational risk factors from job titles, reported elevated risks of cirrhosis among white males with occupational titles of sheet metal workers and metalworkers. Ala *et al.* (2006) found that the prevalence ratio for primary biliary cirrhosis, an uncommon liver disease of unknown etiology that has been linked to environmental factors, was significantly higher in zip codes containing or adjacent to Superfund sites (a discarded site where hazardous waste is located). However, no specific link to trichloroethylene was made in this study.

Contrary to the findings listed above, a deficit in mortality from cirrhosis was observed in three epidemiological studies that evaluated potential occupational exposure to trichloroethylene and cirrhosis (Garabrant *et al.*, 1988; Morgan *et al.*, 1998; Boice *et al.*, 1999, 2006), while several other studies found either null or non-significantly elevated associations (Blair *et al.*, 1989, 1998; Ritz, 1999).

(ii) Experimental systems

No data were available to the Working Group.

(d) Hepatomegaly

(i) Humans

No data were available to the Working Group.

(ii) Experimental systems

Increases in liver weight after single, short-term and long-term exposure to trichloroethylene have been observed in numerous studies in mice, rats and other species. However, single-exposure studies with trichloroethylene, even at doses exceeding 1500 mg/kg bw, in male Sprague-Dawley rats and $B6C3F_1$ mice (Sano *et al.*, 2009), or in a panel of inbred mouse strains (Bradford *et al.*, 2011) found no elevation in relative liver weight. Nonetheless, trichloroethylene-induced increases in liver weight have been reported to occur as early as after 2 days of exposure by inhalation in NMRI mice (Kjellstrand *et al.*, 1981). Laughter *et al.* (2004) found increased liver weight in Sv/129 mice after 3 days of treatment by gavage, and Tao *et al.* (2000) reported an increase in liver weight in female $B6C3F_1$ mice after 5 days. Elcombe *et al.* (1985) and Dees & Travis (1993) reported that mice and rats given trichloroethylene by gavage for 10 days showed significant increases in relative liver weight. Tucker *et al.* (1982) observed that male CD-1 mice given trichloroethylene by gavage at 24 or 240 mg/kg bw for 14 days showed a dose-dependent increase in liver weight. Sano *et al.* (2009) found an increase in relative liver weight in male Sprague-Dawley rats and $B6C3F_1$ mice given trichloroethylene at 1500 mg/kg bw per day by gavage for 14 days, but they reported greater increases in mice than in rats.

Studies have provided little evidence for a major role of PPARα in hepatomegaly associated with treatment with trichloroethylene. Nakajima *et al.* (2000) treated groups of Sv/129 wild-type and *Pparα*-null male and female mice (n = 6) with trichloroethylene at a dose of 750 mg/kg bw by gavage for 2 weeks. Relative liver weight increased by 1.5-fold in wild-type males, and by 1.3-fold in *Pparα*-null males. In female mice, the increase was about 1.25-fold in both strains. Laughter *et al.* (2004) treated Sv/129 wild-type and *Pparα*-null male mice with three daily doses of trichloroethylene at in 0.1% methyl cellulose for either 3 days (trichloroethylene, 1500 mg/kg bw) or 3 weeks (trichloroethylene, 0, 10, 50, 125, 500, 1000, or 1500 mg/kg bw, 5 days per week). After 3 days, the percentage liver weight:body weight ratio was 1.4-fold control levels in the wild-type mice and 1.07-fold control levels in the null mice. After 3 weeks of exposure to trichloroethylene at varying concentrations, wild-type mice were reported to have percentage liver weight:body weight ratios that were close to control values, with the exception of the groups at 1000 and 1500 mg/kg bw (increased by 1.18- and 1.30-fold, respectively, compared with controls). For the *Pparα*-null mice, the variability in percentage liver weight:body weight ratios was reported to be greater than that of the wild-type mice in most of the groups receiving trichloroethylene, and the baseline levels of percentage liver weight:body weight ratio for control mice were 1.16-fold that of wild-type mice. Of note was the higher toxicity of trichloroethylene in *Pparα*-null mice; some mice at 1500 mg/kg bw per day died during the study. Ramdhan *et al.* (2010) exposed male wild-type, *Pparα*-null, and humanized *PPARα* mice on an Sv/129 genetic background to trichloroethylene at 0, 1000, and 2000 ppm by inhalation, 8 hours per day, for 7 days. Hepatomegaly was induced

in all strains to a similar extent after exposure to trichloroethylene.

Several studies showed that hepatomegaly in mice is reversible after cessation of treatment with trichloroethylene for up to 30 days (Kjellstrand *et al.*, 1981, 1983b; Elcombe *et al.*, 1985).

4.5.3 Immune system

(a) Immune markers and immunosuppression

(i) Humans

As mentioned in Section 4.3.3a, several molecular epidemiology studies have evaluated the effect of exposure to trichloroethylene on concentrations of immune markers in humans and the potential for immunosuppressive effects. Most studies have been of workers exposed occupationally to trichloroethylene, with the controls being unexposed workers, and have been of a cross-sectional design with an exposure-monitoring period preceding collection of blood or other specimens (Table 4.7; Lan *et al.*, 2010; Selgrade & Gilmour, 2010; Hosgood *et al.*, 2011; Bassig *et al.*, 2013; Zhang *et al.*, 2013).

A cross-sectional study in China evaluated peripheral blood-cell counts, total lymphocyte counts and subsets, and specific markers of B-cell activation in relation to exposure to trichloroethylene among 80 healthy exposed factory workers and 96 unexposed controls in separate factories in the same geographical area in Guangdong (Lan *et al.*, 2010). Two to three full-shift air-exposure measurements for all exposed subjects and a subset of controls were collected over 3 weeks using 3M organic-vapour monitoring badges, and additional monitoring for other organic solvents was completed to rule out potential confounding exposures. The mean exposure level in the exposed workers was 22.19 ppm (± 35.94). Specific markers evaluated included peripheral blood cell counts, lymphocyte subsets, and soluble levels of CD27 (sCD27) and CD30 (sCD30), which regulate immune-cell activation (Table 4.7). Compared with unexposed factory workers, total lymphocytes (15% reduction), specific lymphocyte subsets including $CD4^+$ T-cells (8%), $CD8^+$ T-cells (14%), B-cells (24%), natural killer (NK) cells (30%), and plasma levels of sCD27 (62%) and sCD30 (34%) were significantly decreased in a dose-dependent manner after adjustment for age, sex, and other demographic variables, including smoking.

Subsequent analyses using data from this cross-sectional study examined levels of $CD4^+$ and $CD8^+$ T-cell subsets (Hosgood *et al.*, 2011), levels of key cytokines with a role in immune regulation and suspected to be relevant to lymphomagenesis (Bassig *et al.*, 2013), and serum levels of immunoglobulins (Zhang *et al.*, 2013) in the exposed workers and controls. Significant decreases in $CD4^+$ naïve, $CD8^+$ naïve, and in $CD4^+$ effector memory T-cell counts were reported in exposed workers compared with controls, and significantly decreased trends were demonstrated for each of these end-points. Conversely, levels of $CD8^+$ memory T-cells, $CD4^+$ central memory T-cells, or T-cell regulation subsets were not significantly different in exposed and unexposed workers. This study provided evidence that exposure to trichloroethylene may result in immunosuppressive effects, and potentially a reduced capacity to respond to antigen-related inflammation (Hosgood *et al.*, 2011) and immune dysregulation.

Serum concentrations of the cytokines IL-6, IL-10, and TNF-α were examined in a subset of exposed and unexposed workers in the cross-sectional study, including a total of 71 exposed and 78 unexposed workers (Bassig *et al.*, 2013). Compared with the unexposed workers, the concentration of IL-10 in exposed workers was significantly reduced by about 70%, but there were no significant differences in IL-6 or TNF-α. The decrease in IL-10 concentration was similar in workers exposed to trichloroethylene at < 12 ppm or ≥ 12 ppm compared with unexposed workers, suggesting that these effects may occur

at relatively low exposures. IL-10 is an anti-inflammatory cytokine that is involved in the regulation of T-cell mediated immune inflammation, and previous studies have indicated that altered levels of this marker are associated with risk of lymphoma. A subsequent analysis of the same study population (Zhang *et al.*, 2013) found that workers exposed to trichloroethylene had significant declines in serum concentrations of IgG (18% reduction) and IgM (38% reduction), but not in levels of IgE compared with unexposed workers, after adjustment for demographic variables. These reductions were apparent in workers exposed to trichloroethylene at < 12 ppm or ≥ 12 ppm. For each of these analyses in the cross-sectional study, potential confounding by age, sex, smoking, alcohol consumption, recent infections, and body-mass index was evaluated, and personal exposure monitoring was available for each of the enrolled subjects. Therefore, confounding with respect to these variables was unlikely.

A cross-sectional study in Italy (Iavicoli *et al.*, 2005) evaluated serum concentrations of the cytokines IL-2, IL-4, and INF-γ in 35 healthy male workers employed in the printing sector who were exposed to trichloroethylene in the degreasing process (exposed group) and in two groups of control workers. The first group included 30 healthy male workers in the same factory, but not involved in degreasing or any work involving exposure to trichloroethylene (internal controls), while the second group included 40 healthy male office workers in the same factory (external controls). Personal air monitoring of trichloroethylene was conducted for four exposed workers and four workers in the internal control group for three consecutive working shifts, with a total of 24 samples. Urinary concentrations of trichloroacetate were higher in exposed workers (13.3 ± 5.9 mg/g creatinine) than in the internal control group (0.02 ± 0.02 mg/g creatinine). Compared with workers in the internal or external control groups, exposed workers had significantly increased levels of type-1 cytokines IL-2 (11.5% increase compared with the internal control group, 8.5% increase compared with the external control group) and INF-γ (38% increase compared with internal or external control groups), and a significantly decreased concentration of the type-2 cytokine IL-4 (52% decrease compared with internal or external control groups). There were no significant differences in cytokine concentrations between the two control groups.

Two studies of children in Germany evaluated levels of IgE antibodies to food and allergens and of cytokines in relation to exposure to trichloroethylene and other volatile organic compounds (Lehmann *et al.*, 2001, 2002). In the first study, blood samples were taken during a medical examination; serum concentrations of IgE were measured in 121 children, and cytokine measurements (IFN-γ, IL-4) were available for 28 children (Lehmann *et al.*, 2001). The subjects were drawn from those enrolled in the birth cohort study at the age of 36 months, and subjects were at high risk of atopy based on a family history of atopy and cord-blood levels of IgE of > 0.9 kU/L. Trichloroethylene and other exposures were passively monitored using 3M badges for 4 weeks in each child's bedroom, and the median level of exposure to trichloroethylene was reported to be 0.42 μg/m^3. Indoor exposure to trichloroethylene in these children was not significantly associated with allergic sensitization to indoor or outdoor allergens, or with cytokine-producing T-cells (Lehmann *et al.*, 2001).

In a subsequent study, exposure to volatile organic compounds and levels of cytokines in cord blood were evaluated for 85 neonates randomly selected from 976 children enrolled in a prospective cohort study in Germany (Lehmann *et al.*, 2002). Intracellular-cytokine staining was used to assess cord blood T-cell function, and levels of trichloroethylene and other volatile organic compounds were monitored passively using 3M badges for 4 weeks in each child's bedroom.

Median concentrations of trichloroethylene were reported to be 0.6 μg/m³. Exposure to trichloroethylene was associated with increased levels of IFN-γ (OR for > 75th percentile, 3.6; 95% CI, 0.9–14.9) and reduced levels of IL-4 (OR for < 25th percentile, 4.4; 95% CI, 1.1–17.8) after the multivariate adjustment. Moreover, a reduction in levels of IL-2 was reported for higher degrees ofexposure to trichloroethylene in the univariate analysis, but there was no significant association after adjustment for family history of atopy, sex, and maternal smoking during pregnancy (Lehmann *et al.*, 2002). However, the findings of this study of children known to be at high risk of atopy may not be generalizable to children not at high risk (Lehmann *et al.*, 2001). There was no association between exposure to trichloroethylene and levels of cytokines in these children at high risk for atopy.

Apart from the studies evaluated above, there were limited data available concerning the immunosuppressive effects of trichloroethylene in humans. Some studies of residents of Woburn, MA, USA, which was the site of several industrial facilities, examined immunosuppressive effects associated with ingestion of drinking-water contaminated with chlorinated solvents (Lagakos *et al.*, 1986; Byers *et al.*, 1988). Environmental testing of municipal wells in the town in 1979 suggested levels of exposure to trichloroethylene of 267 ppb, although tetrachloroethylene and other chemicals were also detected (Byers *et al.*, 1988). These studies provided some evidence for a positive association between higher levels of cumulative exposure and a history of infections involving the kidney, urinary tract, and respiratory system, including asthma, chronic bronchitis, and pneumonia (Lagakos *et al.*, 1986). A study of 23 family members of patients with leukaemia in Woburn, and 70 control subjects living in Boston, also suggested that levels of T-cells, CD4, and CD8 T-cells were elevated and the CD4:CD8 ratio was reduced in the family members compared with the controls (Byers *et al.*, 1988). However, cell counts in the family members declined and were no longer statistically different from those in control subjects on reassessment 18 months later. These findings suggested that there may be alterations in lymphocyte subpopulations and altered susceptibility to infection in subjects exposed to chlorinated solvents.

In summary, these findings provided evidence that exposure to trichloroethylene at concentrations of < 12 ppm can result in an altered immune response, as indicated by a decrease in cell count for lymphocytes and specifically $CD4^+$ T-cells, and immune dysregulation via altered levels of cytokines that mediate the Th1/Th2 immune response in otherwise healthy individuals. There is a well established connection between immune status and carcinogenesis, particularly for lymphoma, as an increased risk of cancer has been associated with a history of use of immunosuppressive medication, with certain chronic infections such as HIV, and with certain autoimmune diseases and lifestyle factors that result in immune alterations and abnormalities (Whiteside, 2006). Furthermore, more subtle changes in immune function, including imbalances in Th1/Th2 responses resulting from cytokine alterations, have been implicated in the oncogenic process via regulation of transcriptional factors and of tumour growth, angiogenesis, and cell differentiation and survival (Tan & Coussens, 2007).

(ii) Experimental systems

Several studies investigated the effect of exposure to trichloroethylene by inhalation on pulmonary defences as measured by the degree of susceptibility to respiratory bacterial infections. Aranyi *et al.* (1986) exposed female CD-1 mice to trichloroethylene at various concentrations (up to 50 ppm) as a single dose (3 hours) or as repeated doses (5 days, 3 hours per day) by inhalation. Susceptibility to *Streptococcus zooepidemicus* aerosol infection and pulmonary

bactericidal activity to inhaled *Klebsiella pneumoniae* were evaluated. Single 3-hour exposures to trichloroethylene at 10 ppm or more resulted in significant increases in mortality after infection. Pulmonary bactericidal activity was significantly decreased after a single exposure at 10 ppm. A similar experimental design was used by Selgrade & Gilmour (2010). Female CD-1 mice were exposed by inhalation to filtered air (control) or trichloroethylene (5 to 200 ppm) for 3 hours. Immediately after exposure to trichloroethylene, mice were challenged with an aerosol of *Streptococcus zooepidemicus* and monitored for clearance of bacteria from the lung and for mortality. In separate experiments, exposed mice were injected intratracheally with viable bacteria and phagocytic function was evaluated in macrophages obtained from lung washes 30 minutes later. Mortality due to infection was significantly increased with exposure to trichloroethylene at concentrations of 50 ppm and higher. Significant differences in clearance of bacteria from the lung were noted in mice exposed to trichloroethylene at concentrations greater than 50 ppm, and differences in alveolar macrophage phagocytic index were noted at concentrations greater than 100 ppm.

Humoral immunity end-points were assessed in studies in animals exposed to trichloroethylene in drinking-water. Groups of female and male mice were exposed to trichloroethylene at a concentration of 0.1, 1.0, 2,5, and 5 mg/mL for up to 6 months. The immunological parameters assessed were humoral immunity, cell-mediated immunity, lymphocyte responsiveness, bone-marrow function, and macrophage function. Females were more affected than males by exposure to trichloroethylene, particularly after 4 months. In females, cell-mediated immunity and bone-marrow stem-cell colonization were inhibited by trichloroethylene at all concentrations, while humoral immunity was inhibited only at the highest concentrations. The males were relatively unaffected after exposure for 4 or 6 months.

In a study by Peden-Adams *et al.* (2006), $B6C3F_1$ mice were given drinking-water containing trichloroethylene (0, 1400, 14 000 ppb) from day 0 of gestation until age 8 weeks. Decreased sheep-erythrocyte-specific production of IgM (plaque-forming cell response) was noted in male offspring at age 3 or 8 weeks and at both concentrations. Plaque-forming cell responses in female offspring were suppressed by treatment with trichloroethylene at 14 000 ppb at both ages assessed and at 1400 ppb at 8 weeks. Splenic numbers of B220 cells were only decreased in pups aged 3 weeks exposed to trichloroethylene at 14 000 ppb. The most pronounced alteration in T-cell subpopulations was increases in all thymic T-cell types ($CD4^+$, $CD8^+$, $CD4^+/CD8^+$, and $CD4^-/CD8^-$) in mice aged 8 weeks. Delayed-type hypersensitivity (DTH) was increased in females at both concentrations of trichloroethylene and in males at the higher dose only.

Blossom & Doss (2007) studied central and peripheral immune function in autoimmune disease-prone $MRL^{+/+}$ mice given occupationally relevant doses of trichloroethylene (0, 0.5, or 2.5 mg/mL in drinking-water) throughout development until adulthood (i.e. age 7–8 weeks). Decreased spleen cellularity and reduced numbers of $CD4^+$, $CD8^+$, and $B220^+$ lymphocyte subpopulations were observed in the post-weaning offspring. Thymocyte development was altered by exposure to trichloroethylene, as shown by significant alterations in the proportions of double-negative ($CD4^-/CD8^-$) subpopulations and inhibition of apoptosis in immature thymocytes *in vitro*. Trichloroethylene was also shown to induce a dose-dependent increase in IFN-γ production by $CD4^+$ and $CD8^+$ T-lymphocytes in peripheral blood by age 4–5 weeks, although these effects were no longer observed at age 7–8 weeks. Serum anti-histone autoantibodies and total IgG_{2a} were significantly increased in

trichloroethylene-exposed offspring; however, no histopathological signs of autoimmunity were observed in the liver and kidneys.

In a follow-up study, Blossom *et al.* (2008) exposed MRL$^{+/+}$ mice to trichloroethylene (0.1 mg/mL in drinking-water) from mating until postnatal day 42. Offspring were evaluated at various time-points. Evaluation of the thymus identified a significant treatment-related increase in cellularity, accompanied by alterations in thymocyte subset distribution, at postnatal day 20 (sexes combined). Treatment with trichloroethylene also appeared to promote T-cell differentiation and maturation at postnatal day 42, and evaluation of cultured thymocytes *ex vivo* indicated increased generation of reactive oxygen species at postnatal day 20. Evaluation of peripheral blood indicated that splenic CD4$^+$ T-cells from trichloroethylene-exposed mice at postnatal day 42 produced significantly greater levels of IFN-γ and IL-2 in males and TNF-α in both sexes.

Peden-Adams *et al.* (2008) investigated lifetime exposure to trichloroethylene (up to 14 000 ppb in drinking-water) in MRL$^{+/+}$ mice from day 0 of gestation until age 12 months. Splenic CD4$^-$/CD8$^-$ cells were altered in female mice (but not males) at 1400 ppb only. Splenic T-cell populations, numbers of B220$^+$ cells, and lymphocyte proliferation were not affected by treatment. Populations of thymic T-cell subpopulations (CD8$^+$, CD4$^-$/CD8$^-$, and CD4$^+$) were significantly decreased in male but not female mice after exposure to trichloroethylene at 14 000 ppb, and CD4$^+$/CD8$^+$ cells were significantly reduced in males by treatment with trichloroethylene. Autoantibody levels (anti-dsDNA and anti-glomerular antigen) were not increased in the offspring over the course of the study, indicating that trichloroethylene did not contribute to the development of autoimmune disease markers after developmental exposures that continued into adult life.

(b) Autoimmune disease and allergy

(i) Humans

Several epidemiological studies have evaluated the association between exposure to trichloroethylene and autoimmune disease (Nietert *et al.*, 1998; Lacey *et al.*, 1999; Diot *et al.*, 2002; Garabrant *et al.*, 2003; Beaudreuil *et al.*, 2005; Table 4.7). Exposure to trichloroethylene has been associated with generalized skin disorders with accompanying hepatitis that resemble drug hypersensitivities, as previously reviewed in Section 4.3.3 (Kamijima *et al.*, 2007). The clinical manifestations of this disorder generally involve the presence of rash on the extremities, neck, trunk, or face and potentially fever that occurs within 2 weeks to 2 months after first occupational exposure (Kamijima *et al.*, 2007). The disorders have been classified into four groups, including exfoliative dermatitis, erythema multiforme, Stevens-Johnson syndrome, and toxic epidermal necrolysis, depending on the specific skin manifestation. While the incidence of these disorders has been estimated to be between 1–13% in trichloroethylene-exposed workers, being most common in Asia, between 9–13% of cases have been reported to be fatal in a review of case series (Kamijima *et al.*, 2007). A cross-sectional study in China included 19 hospitalized patients with these disorders who worked with trichloroethylene in factories, three to six healthy workers from each factory who performed job duties similar to those of the hospitalized patients, and two control workers from each factory not exposed to trichloroethylene (Kamijima *et al.*, 2008). Levels of trichloroethylene metabolites were measured in the patients, and exposure assessments at the factories included both personal and area monitoring for trichloroethylene and other volatile organic compounds (VOCs). This study provided evidence that the skin hypersensitivity disorders were attributable to trichloroethylene, given the absence of common impurities in the factories, and suggested based on the exposure assessments

that urinary trichloroacetate concentrations of less than 50 mg/L may be needed to reduce the risk of these disorders (Kamijima *et al.*, 2008).

A case–control study conducted in France evaluated the risk of systemic sclerosis in 80 case patients and 160 controls matched by age, sex, and smoking habits (Diot *et al.*, 2002). The study assessed exposure to organic solvents and other occupational exposures, and an expert review panel was used to semiquantitatively assess exposures in cases and controls. This assessment involved the use of an exposure score that considered the probability, intensity, frequency, and duration of exposure. A significantly elevated risk of systemic sclerosis was observed for ever exposure to trichloroethylene as well as for high cumulative exposure, and stratified analyses by sex suggested a higher risk in males than females (Diot *et al.*, 2002; Table 4.7).

A higher risk of scleroderma in men was similarly reported in a case–control study of 178 case patients and 200 controls conducted in the USA, while there was no elevated risk in women (Nietert *et al.*, 1998). The exposure assessment for trichloroethylene and other organic solvents was conducted using a job exposure matrix and semiquantitative exposure scores were assigned based on the probability and intensity of exposure. The exposure intensity and probability were each defined as none, low, medium, or high. Risk of systemic sclerosis associated with exposure to trichloroethylene was evaluated according to the cumulative and maximum exposure intensities and the maximum probability of exposure, which was the highest probability score of exposure for all of a given subject's jobs (Nietert *et al.*, 1998). An increased risk of systemic sclerosis was reported for each of these trichloroethylene exposure metrics (Table 4.7; Nietert *et al.*, 1998). Sensitivity analyses taking into account geographic residence suggested similar results (Nietert *et al.*, 1998).

A case–control study in the USA evaluated the risk of systemic sclerosis in relation to trichloroethylene exposure only in women (Garabrant *et al.*, 2003). The study included 660 cases of scleroderma (8 self-reported exposed trichloroethylene cases) and 2227 frequency matched controls. Self-reported exposures that were not considered plausible or were judged to be "trivial" with respect to frequency, intensity, or duration were not classified as exposures by the expert review (Garabrant *et al.*, 2003). Risk estimates for scleroderma were similar for self-reported and expert confirmed trichloroethylene exposure, and were non-significantly elevated for both exposure metrics (Table 4.7).

An evaluation of community exposure to chlorinated solvents was conducted among residents of Tucson, Arizona, where ground water contamination of the Santa Cruz River aquifer resulted from percolation of metal-cleaning products (Kilburn & Warshaw, 1992). This study included 345 residents of Tucson who agreed to provide blood samples and a regional comparison group consisting of 158 residents of Phoenix. Levels of antinuclear antibodies measured by fluorescence (FANA) and the prevalence of symptoms related to systemic lupus erythematosus were compared between the Tucson residents and reference subjects in Phoenix. Residents of Tucson reported higher frequencies of all examined symptoms, with significant differences reported for arthralgia, Raynards's phenomenon, malar rash, skin lesions, seizure or convulsion (Kilburn & Warshaw, 1992). Furthermore, exposed subjects had increased frequencies of having three and four or more symptoms and an increased prevalence of FANA titre levels consistent with an autoimmune response. However, the Tucson residents were exposed to multiple chemicals and therefore the effects of trichloroethylene specifically could not be evaluated.

There were few data concerning the risk of other autoimmune conditions associated with exposure to trichloroethylene, other than a small case–control study in France, which found

no association between exposure to trichloroethylene and antineutrophil cytoplasmic autoantibodies (ANCA) (Beaudreuil *et al.*, 2005). Cases included hospital in-patients admitted between 1990 and 2000 who were ANCA-positive, and in-patient controls admitted between 2000 and 2001 were matched to cases by age (± 5 years) and sex. The exposure assessment included both qualitative and semiquantitative methods and exposures were assessed by a panel of experts blinded to the disease status of the patients. The associated odds ratio for ANCA-positivity was based on 11 cases exposed to trichloroethylene and was null (Table 4.7). One additional study has evaluated the risk of undifferentiated connective tissue disease (UCTD) in women in relation to self-reported solvent exposure, including trichloroethylene (Lacey *et al.*, 1999). All self-reported exposures were subsequently examined and confirmed by expert review. While exposure to trichloroethylene was not significantly associated with UCTD for either self-reported or expert confirmed trichloroethylene exposures (Table 4.7), these results were based on only one exposed case. Similar null findings were observed for dry-cleaning workers, including nine cases exposed to trichloroethylene.

One study using data from the National Health and Nutrition Examination Survey (NHANES) has evaluated the association between exposure to trichloroethylene and physician-diagnosed asthma and wheezing episodes during the previous 12 months (Arif & Shah, 2007). A subsample of the subjects participating in the NHANES survey were selected to undergo personal-exposure monitoring and subjects with non-missing data for VOCs were included in the study. The sample included a total of 550 subjects each of whom were monitored for 48–72 hours using 3M organic-vapour monitors for 10 VOCs, including trichloroethylene and tetrachloroethylene. The geometric mean exposure to trichloroethylene in the population was 0.03 μg/m^3 (95% CI, 0.02–0.04). Exposure to trichloroethylene was not significantly associated with physician-diagnosed asthma or with episodes of wheezing in the previous 12 months (Table 4.7). The cross-sectional design made it difficult to evaluate the temporal relationship between asthma and exposure to VOCs in this study, and furthermore the self-reported diagnosis of asthma could be subject to some misclassification in the absence of objective assessments of lung function (Arif & Shah, 2007).

Overall, while case–control studies of autoimmune disease have included a larger number of subjects compared with the considered cross-sectional studies of immune parameters, a relatively small number of trichloroethylene-exposed cases has been included and therefore the available evidence was based on small samples. Furthermore, the implications of the stronger trichloroethylene-related effect for autoimmune disease in men, particularly for scleroderma, are unclear, but may be attributed to the lower background risk of this condition in men, differences in prevalence of exposure to trichloroethylene, or genuine differences in susceptibility for trichloroethylene-induced toxicity (Cooper *et al.*, 2009) (see Section 4.3.3 and Section 4.4.3 in this *Monograph*). Moreover, differences in the magnitude of risk between men and women may partially be attributed to exposure misclassification, particularly in studies without quantitative exposure assessments and personal monitoring. While personal-exposure monitoring was generally not available in the studies of autoimmune disease, the exposure assessment was improved through the use of expert review. Nevertheless, these studies have provided some evidence for an elevated risk of autoimmunity associated with exposure to trichloroethylene, particularly for scleroderma.

(ii) Experimental systems

Skin hypersensitivity and immune-mediated hepatitis attributable to exposure to trichloroethylene were studied in guinea-pigs. Tang

et al. (2002) evaluated the contact allergenicity potential of trichloroethylene in FMMU strain. Oedema and erythema indicative of skin sensitization (and confirmed by histopathology) were observed. Sensitization rates were reported to be 71.4% for trichloroethylene, when compared with a reference positive-control response rate (i.e. 100% for 2,4-dinitrochlorobenzene). Trichloroethylene was judged to be a strong allergen.

In a second study, female FMMU guinea-pigs were given trichloroethylene as an intradermal injection at 0, 167, 500, 1500, or 4500 mg/kg bw, or as a dermal patch at 0 or 900 mg/kg bw, and killed 48 hours after treatment Tang *et al.* (2008). With regard to dermal hypersensitivity, treatment with trichloroethylene resulted in dermal erythema and oedema, and the sensitization rate was 66% (i.e. classified as a strong sensitizer). In addition, liver end-points associated with hypersensitive skin reaction were evaluated. At the intradermal dose of 1500 mg/kg bw, a significant increase ($P < 0.05$) in serum AST levels was observed. At 4500 mg/kg bw, significantly ($P < 0.01$) increased ALT and AST levels were reported, and total protein and globulin decreased significantly ($P < 0.05$). Histopathological examination of the liver revealed fatty degeneration, hepatic sinusoid dilation, and inflammatory cell infiltration. No changes were observed at the intradermal doses of ≤ 500 mg/kg bw, or the dermal patch dose of 900 mg/kg bw.

Peden-Adams *et al.* (2006) exposed $B6C3F_1$ mice to drinking-water containing trichloroethylene (0, 1400, 14 000 ppb) from day 0 of gestation to up to age 8 weeks and reported an increased delayed hypersensitivity response in female offspring aged 8 weeks at both concentrations and in males at the higher concentration of 14 000 ppb.

Seo *et al.* (2008) investigated trichloroethylene exposure-associated antigen-induced histamine release and inflammatory mediator production *in vivo* and *in vitro*. Male Wistar rats were injected with trichloroethylene (0.1, 1 and 10 mg/kg bw) intraperitoneally, together with 0.1 mL of anti-dinitrophenol (DNP) monoclonal IgE antibody (anti-DNP IgE) diluted to 1:1000 or 1:4000, injected subcutaneously into both abdominal sides. After 48 hours, 1 mL of antigen (DNP-conjugated bovine serum albumin B5A)-Evans blue solution was injected into the tail vein. The rats were killed after 30 minutes, and passive cutaneous anaphylaxis was evaluated. A significant dose-dependent increase in cutaneous anaphylaxis was seen after exposure to trichloroethylene. In addition, a part of this study *in vitro* used non-purified rat peritoneal mast cells (NPMC) and rat basophilic leukaemia (RBL-2H3) cells that were sensitized with anti-DNP IgE antibody and then stimulated with DNP-BSA plus trichloroethylene. Trichloroethylene enhanced antigen-induced histamine release from NPMC and RBL-2H3 cells in a dose-related manner, and increased IL-4 and TNF-α production from the RBL-2H3 cells.

A series of studies evaluated the ability of trichloroethylene to promote autoimmune reactions in mouse strains that are prone to autoimmune disease. $MRL^{+/+}$ mice treated with trichloroethylene either by intraperitoneal injection (Khan *et al.*, 1995; Wang *et al.*, 2007b, 2008), or via drinking-water (Gilbert *et al.*, 1999; Griffin *et al.*, 2000a, b; Cai *et al.*, 2008). Both short-term and long-term exposure scenarios were evaluated and a range of trichloroethylene doses were used. All the studies have observed that exposure to trichloroethylene was associated with increases in antinuclear-, anti-ssDNA-, and anti-dsDNA antibodies. Increased activation of splenic $CD4^+$ cells, increased weights of spleen and increases in IgG levels were also commonly reported. Interestingly, Keil *et al.* (2009) showed trichloroethylene autoimmune-mediating effects in $B6C3F_1$ mice, which are not prone to spontaneous autoimmune disorders. Mice were exposed to trichloroethylene (0, 1, 1400 or 14 000 ppb) via drinking-water for 30 weeks.

During the exposure period, serum levels of total IgG, and autoantibodies (anti-ssDNA, -dsDNA, and -glomerular antigen) were monitored. Trichloroethylene did not alter NK cell activity, or T- and B-cell proliferation. Numbers of activated T-cells ($CD4^+/CD44^+$) were increased in the $B6C3F_1$ mice. Serum levels of autoantibodies to dsDNA and ssDNA were also increased.

5. Summary of Data Reported

5.1 Exposure data

Trichloroethylene, a chlorinated solvent, has been produced commercially since the 1920s by chlorination of ethylene or acetylene. In the 1930s, it was introduced in the dry-cleaning industry, but was replaced by tetrachloroethylene in the 1950s. Trichloroethylene has had numerous other uses, including as an anaesthetic and in veterinary medicine. Its use as a degreasing agent began in the 1930s, reached its peak in 1990s, and declined thereafter. Currently, trichloroethylene is still used as a spot remover in dry cleaning, but the main use of trichloroethylene is as feedstock to produce chlorinated chemicals. Trichloroethylene has been found in both outdoor and indoor air, water, soil, food, and animal tissues; exposure from environmental sources (including hazardous waste sites and contaminated water) is common throughout the United States and elsewhere in the world. Exposure in humans occurs mainly by inhalation. The most heavily occupationally exposed people are those involved in degreasing of metals and other materials. In Europe and North America, exposure levels have declined by at least one order of magnitude since the 1940s, and the number of exposed workers has also declined.

5.2 Human carcinogenicity data

In its evaluation of the epidemiological data, the Working Group recognized positive associations between trichloroethylene and cancer of the kidney, non-Hodgkin lymphoma, and cancer of the liver. The Working Group concluded that the evidence for cancer of the kidney was stronger than that for non-Hodgkin lymphoma or cancer of the liver.

5.2.1 Cancer of the kidney

The largest body of evidence regarding the carcinogenicity of trichloroethylene comes from studies of cancer of the kidney. In the previous evaluation by IARC (Volume 63), information regarding carcinogenicity in the kidney came from a small cohort of workers in a cardboard-manufacturing factory in Germany and two Nordic cohorts monitored for exposure to trichloroethylene using biological measurements in urine, which gave conflicting results. Since that time, studies of several cohorts of aircraft and aerospace workers in the USA and seven case–control studies in several countries have reported data relevant to the association of cancer of the kidney and trichloroethylene. In general, the case–control studies adjusted for smoking and other potential confounders, but such adjustments seldom had a notable impact on the risk estimates. Although these adjustments were typically not possible in cohort studies, confounding by smoking was unlikely as relative risks for cancer of the lung were not elevated in most cohorts or in a meta-analysis.

A significant amount of new evidence had become available from cohort studies of aircraft and aerospace workers in the USA and a study of workers employed in industries using trichloroethylene in Denmark. Some of these cohort studies reported modestly elevated relative risks for cancer of the kidney, with an indication of an exposure–response relationship in one study.

The other important group of cohort studies comprised biologically monitored workers from three Nordic countries. These studies showed little evidence of increased risk of cancer of the kidney. However, the Working Group noted that the cohorts included workers recruited at different points in time from diverse industries with a broad range of exposures and only a small number of measurements per worker, on average. The studies were also relatively small and may have had limited ability to detect a modest increase in risk. A small elevation in the risk of cancer of the lung was also noted in the Danish study.

Case–control studies provide stronger and more consistent evidence than cohort studies of a positive association between kidney cancer and exposure to trichloroethylene. The most detailed exposure assessments were carried out for two studies in France and eastern Europe. Both studies evaluated confounding for several risk factors for cancer of the kidney, and both provided evidence for an exposure–response relationship. The study in France was conducted in an area with a high prevalence of occupational exposure to trichloroethylene and assessed the potentially confounding effects of exposure to cutting oils. These analyses found a non-statistically significant odds ratio of 1.62 (95% CI, 0.76–3.44) for the association with trichloroethylene in workers not exposed to cutting oils, and a statistically significant odds ratio of 2.70 (95% CI, 1.02–7.17) for workers in the highest category of exposure to trichloroethylene who were also exposed to cutting oils. The study in eastern Europe was larger than the study in France, but the prevalence of exposure to trichloroethylene was lower. The odds ratio for any exposure to trichloroethylene was 1.63 (95% CI, 1.04–2.54), and in the highest category of exposure intensity it was 2.34 (95% CI, 1.05–2.51).

Two recent, independently conducted meta-analyses based on a largely similar set of case–control and cohort studies of cancer of the kidney reported statistically significant meta-relative risks (meta-RR) for cancer of the kidney and exposure to trichloroethylene of 1.3 and 1.4. One meta-analysis reported a higher meta-RR of 1.6 (95% CI, 1.3–2.0) for groups with a higher exposure. Neither analysis found significant heterogeneity between studies.

5.2.2 Non-Hodgkin lymphoma

Information on non-Hodgkin lymphoma was available from eight independent cohort studies and eight case–control studies. Cohort studies of aircraft and aerospace workers in the USA reported modestly elevated relative risks for non-Hodgkin lymphoma. The cohort studies of biologically monitored workers in the Nordic countries show evidence of modestly increased risk for non-Hodgkin lymphoma, in contrast to the findings for cancer of the kidney.

Interpretation of the results from case–control studies on non-Hodgkin lymphoma was complicated by the variety of systems used to classify the lymphomas. Modest positive associations of non-Hodgkin lymphoma with exposure to trichloroethylene were observed in several case–control studies, but the results were inconsistent overall. There were some indications of an exposure–response relationship, but these were also inconsistent and generally not statistically significant.

A meta-analysis of cohort and case–control studies of non-Hodgkin lymphoma reported statistically significant meta-RRs of 1.2 (95% CI, 1.1–1.4) for non-Hodgkin lymphoma and any exposure to trichloroethylene and 1.4 (95% CI, 1.1–1.8) for higher exposure. The meta-RR was 1.33 (95% CI, 1.13–1.58) for cohort studies and 1.11 (95% CI, 0.89–1.38) for case–control studies and heterogeneity between studies was found. There was also some indication of publication bias. [The Working Group emphasized the results of the meta-analysis in its evaluation and noted

that the evidence from case–control studies was weaker than that from cohort studies.]

5.2.3 Cancer of the liver

Information about the association of cancer of the liver with exposure to trichloroethylene was available from one case–control study and nine cohort studies. Although some positive associations were observed in cohort studies, the results were somewhat inconsistent. The cohort studies were unable to adjust for other risk factors, such as consumption of alcoholic beverages. The only case–control study available had only one exposed case and was considered uninformative. A meta-analysis reported similar meta-RRs for overall exposure (meta-RR, 1.29; 95% CI, 1.07–1.56) and for higher exposure (meta-RR, 1.28; 95% CI, 0.93–1.77).

5.2.4 Other sites

Statistically significant excess risks of cancers of the lung, cervix and oesophagus were also observed in isolated studies, but these observations were considered insufficient to make an evaluation.

5.3 Animal carcinogenicity data

In four studies in $B6C3F_1$ mice, treatment with trichloroethylene by gavage increased the incidence of hepatocellular adenoma and hepatocellular carcinoma in males and/or females. In one of these studies, there was also an increase in the incidence of Harderian gland adenoma in males. In one study in Swiss mice treated by inhalation, there was an increase in the incidence of malignant hepatoma in males.

In one study in female mice treated by inhalation, there was an increase in the incidence of lung adenocarcinoma. In two other studies of exposure by inhalation, there was an increase in the incidence of pulmonary tumours (mainly adenomas) in male Swiss mice and female $B6C3F_1$ mice. In one study in $B6C3F_1$ mice treated by gavage, there was also an increase in the incidence of bronchioloalveolar adenoma in females. Increased incidences of malignant lymphoma were reported in two strains of mice in one study of exposure by gavage and one study of exposure by inhalation.

In three gavage studies and one inhalation study in rats, increases in the incidence of benign interstitial cell tumours of the testis were reported in trichloroethylene-treated rats of four different strains, along with the occurrence of a few rare malignant interstitial cell tumours in two of these studies.

In female rats, an increasing trend in the incidence of leukaemia (of the monocytic type or not otherwise specified) has been reported in one study in rats treated by gavage, and an increased incidence of lymphoma was observed in one study in rats treated by inhalation. The incidence of subcutaneous tissue sarcoma was also increased in male rats treated with trichloroethylene by gavage.

In three studies in three different strains of rats, treatment with trichloroethylene by gavage increased the incidences of adenoma or carcinoma (combined) of the kidney in males or females, while incidence of kidney carcinoma also increased in one of these studies. Overall, nine studies of exposure by gavage or inhalation in several different strains reported rare kidney adenoma or carcinoma in at least one or more rats treated with trichloroethylene per study.

After adjustment for survival, an increase in the incidence of malignant peritoneal mesothelioma was observed in male rats in one gavage study, and an increase in the incidence of adrenal cortex adenoma was observed in females in another gavage study. Although not statistically significant, rare liver tumours (i.e. haemangiosarcoma, cholangioma, cholangiocarcinoma, and hepatocellular carcinoma) were reported in several trichloroethylene-treated groups of three

different strains of rats, and some rare bronchioloalveolar tumours were also reported in five groups of treated rats of three different strains.

5.4 Mechanistic and other relevant data

A comprehensive body of literature exists to characterize the absorption, distribution, metabolism and excretion of trichloroethylene in humans and in experimental animals; it is clear that qualitative similarities are evident between humans and rodents. Two major metabolic pathways of trichloroethylene have been characterized in humans and laboratory animals. The major pathway is cytochrome P450 (CYP)-mediated oxidation, resulting in formation of a variety of short- and long-lived metabolites. Subsequent processing of oxidative metabolites involves alcohol and aldehyde dehydrogenases and glucuronidation. In all species, trichloroacetic acid and trichloroethanol/trichloroethanol glucuronide are measured in vastly larger amounts than other oxidative metabolites. Evidence exists supporting quantitative differences between species in the extent of oxidative metabolism of trichloroethylene at higher exposures, but at lower exposures oxidation is limited by hepatic blood flow. Glutathione (GSH) conjugation is another important metabolic pathway resulting in formation of short-lived and reactive metabolites. The initial conjugation reactions primarily occur in the liver to form *S*-(1,2-dichlorovinyl) glutathione (DCVG). Subsequent processing of DCVG occurs primarily in the kidney. In humans and rodents, urinary excretion of stable end products is orders of magnitude greater for the oxidative pathway than the GSH-conjugation pathway. However, this is not an accurate indication of the overall flux through each pathway, because it does not account for the formation of reactive and chemically unstable metabolites by the GSH-conjugation pathway.

Data from studies in humans have suggested possible mutagenicity of trichloroethylene leading to *VHL* gene mutations in renal cell carcinoma, but only a limited number of studies have reported an association. Carefully controlled studies evaluating trichloroethylene alone found it to be incapable of inducing gene mutations in most standard assays for bacterial mutagenesis. Therefore, it appears unlikely that trichloroethylene is a direct-acting mutagen, although trichloroethylene has shown the potential to affect DNA and chromosomal structure. There is strong evidence that the GSH-conjugation metabolites of trichloroethylene, *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC), and to a lesser degree, DCVG and *N*-acetyl-*S*-(1,2-dichloroWvinyl)-L-cysteine (NAcDCVC), are genotoxic on the basis of consistent results in several studies. For oxidative metabolites of trichloroethylene, the Working Group concluded that weak to moderate evidence was available to suggest that dichloroacetic acid may cause genotoxicity (see *Monograph* on Dichloroacetic Acid in this Volume for details); that evidence suggested that trichloroacetic acid is not genotoxic (see *Monograph* on Trichloroacetic Acid in this Volume for details); and that strong evidence was available to suggest that chloral hydrate may cause genotoxicity (see *Monograph* on Chloral and Chloral Hydrate in this Volume for details). The data on genotoxicity of trichloroethanol were limited. Overall, there was strong evidence to conclude that, after metabolism, trichloroethylene can be genotoxic, particularly in the kidney where metabolism in situ occurs.

Trichloroethylene has been shown to be associated with adverse health outcomes in the kidney, liver, and lung, and in the immune, central nervous, and reproductive systems. The evidence for kidney as a target tissue for trichloroethylene, from cancer and toxicity findings in humans and experimental animals was strong. The data supporting the non-genotoxic mechanisms of kidney carcinogenesis were limited.

However, strong evidence of genotoxicity of DCVC, the metabolite of trichloroethylene in the kidney, supported an overall conclusion that the evidence of mechanisms of carcinogenesis in kidney is strong. There was strong evidence for liver as a target tissue for trichloroethylene from cancer and toxicity findings in experimental animals. The evidence for non-genotoxic and/or genotoxic mechanisms of liver carcinogenesis was moderate. The available data suggested that multiple non-genotoxic mechanisms of carcinogenesis exist, and that there is the potential for genotoxic mechanisms from trichloroethylene metabolites dichloroacetic acid and chloral hydrate. The evidence for the immune system as a target tissue for trichloroethylene from findings of a generalized hypersensitivity syndrome and of alterations of immune response in humans and experimental animals was strong. Evidence from studies in humans and experimental animals identifying active metabolites or the mechanisms for cancers of the immune system was weak, being limited to studies of immunological and haematological toxicity in humans and experimental animals. The evidence for the lung as a target tissue for trichloroethylene, from cancer and toxicity findings in experimental animals, was moderate. The data supporting the mechanisms of carcinogenesis in the lung were weak. The evidence for the nervous system as a target tissue for trichloroethylene on the basis of a variety of neurobehavioural effects in studies in humans and experimental animals was strong. The relevance of these effects to the potential cancer hazard of trichloroethylene in the nervous system is unknown. The data regarding the mechanism of carcinogenesis of trichloroethylene in the central nervous system were inconclusive. Trichloroethylene has been shown to adversely affect the male and female reproductive systems. The evidence for the male reproductive system as a target tissue for trichloroethylene was strong, on the basis of studies of toxicity in humans and experimental animals and studies of cancer in rats. The overall data supporting the mechanisms of carcinogenesis of trichloroethylene in the testes were weak, with limited data from humans and experimental animals available to support a mechanism involving hormonal disruption for trichloroethylene-induced testicular tumours. The overall support for an association between exposure to trichloroethylene and reproductive toxicity in females was weak.

The carcinogenicity and toxicity of trichloroethylene, particularly in the liver and kidney, are associated with its metabolism. Inter-individual differences in the formation of trichloroethylene metabolites that are thought to be responsible for toxic and carcinogenic effects of trichloroethylene in the kidney and liver exist in humans and rodents. Susceptibility to the adverse health effects of trichloroethylene may be influenced by genetics, sex, life stage and other conditions that influence the extent and nature of its metabolism. Polymorphisms in genes involved in oxidative metabolism (e.g. *CYP2E1*, *ADH*, *ALDH*) and glutathione conjugation (e.g. GSTs) have been studied in connection with susceptibility to toxicity and carcinogenicity caused by trichloroethylene. Polymorphisms in genes for plasma-membrane transporters (e.g. *OAT1* and *OAT3*) may also influence rates or extent of cellular accumulation of key metabolites. With respect to life-stage susceptibility, data were available to support conclusions concerning differences in exposure (e.g. transplacental transfer or exposure through breast milk in early life stages) or life-stage-specific differences in toxicokinetics. Lifestyle factors (e.g. consumption of alcoholic beverages) may also affect the metabolism of trichloroethylene, while nutrition or obesity may affect internal concentrations of trichloroethylene and its metabolites.

6. Evaluation

6.1 Cancer in humans

There is *sufficient evidence* in humans for the carcinogenicity of trichloroethylene. Trichloroethylene causes cancer of the kidney. A positive association has been observed between exposure to trichloroethylene and non-Hodgkin lymphoma and liver cancer.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

Trichloroethylene is *carcinogenic to humans (Group 1).*

6.4 Rationale

The Working Group was unanimous in its conclusion that trichloroethylene is a Group 1 carcinogen.

The majority of the Working Group concluded that the epidemiological data were sufficient; however, a minority had concerns because most of the positive evidence came from case–control studies, while the data from cohort studies were weaker.

In reaching unanimous agreement, the Working Group took into consideration the following supporting evidence:

- The absorption, distribution, metabolism and excretion of trichloroethylene are well characterized in experimental animals and humans.
- In experimental animals and humans, oxidative metabolism of trichloroethylene is catalysed by cytochrome P450 enzymes and GSH conjugation of trichloroethylene is catalysed by GST enzymes.
- The formation of reactive metabolites of trichloroethylene in the kidney from processing of GSH-conjugation metabolites in situ has been observed in experimental animals and in human kidney cells.
- The reactive GSH-conjugation metabolites of trichloroethylene are genotoxic on the basis of consistent results in several available test systems.

Consistent with the importance of the GSH-conjugation metabolic pathway for kidney carcinogenesis, one study demonstrated a statistically significant association among trichloroethylene-exposed people with at least one intact *GSTT1* allele, but not among those with two deleted alleles.

References

Abbas R & Fisher JW (1997). A physiologically based pharmacokinetic model for trichloroethylene and its metabolites, chloral hydrate, trichloroacetate, dichloroacetate, trichloroethanol, and trichloroethanol glucuronide in B6C3F1 mice. *Toxicol Appl Pharmacol*, 147: 15–30. doi:10.1006/taap.1997.8190 PMID:9356303

Abraham DG, Patel PP, Cooper AJL (1995a). Isolation from rat kidney of a cytosolic high molecular weight cysteine-S-conjugate β-lyase with activity toward leukotriene E4. *J Biol Chem*, 270: 180–188. doi:10.1074/jbc.270.1.180 PMID:7814371

Abraham DG, Thomas RJ, Cooper AJL (1995b). Glutamine transaminase K is not a major cysteine S-conjugate β-lyase of rat kidney mitochondria: evidence that a high-molecular weight enzyme fulfills this role. *Mol Pharmacol*, 48: 855–860. PMID:7476916

Abrahamsson K, Ekdahl A, Collén J, Pedersén M (1995). Marine algae- a source of trichloroethylene and perchloroethylene. *Limnol Oceanogr*, 40: 1321–1326. doi:10.4319/lo.1995.40.7.1321

Adgate JL, Eberly LE, Stroebel C *et al.* (2004). Personal, indoor, and outdoor VOC exposures in a probability sample of children. *J Expo Anal Environ Epidemiol*, 14: Suppl 1: S4–S13. doi:10.1038/sj.jea.7500353 PMID:15118740

Ahlmark A, Gerhardsson G, Holm A (1963). *Trichloroethylene exposure in Swedish engineering workshops*. In: *Proceedings of the 14th International Congress on Occupational Health*. London, United Kingdom: Permanent Commission and International Association on Occupational Health, pp. 448–450

Ala A, Stanca CM, Bu-Ghanim M *et al.* (2006). Increased prevalence of primary biliary cirrhosis near Superfund toxic waste sites. *Hepatology*, 43: 525–531. doi:\\ PMID:16496326

Alberati-Giani D, Malherbe P, Köhler C *et al.* (1995). Cloning and characterization of a soluble kynurenine aminotransferase from rat brain: identity with kidney cysteine conjugate β-lyase. *J Neurochem*, 64: 1448–1455. doi:10.1046/j.1471-4159.1995.64041448.x PMID:7891071

Alexander DD, Kelsh MA, Mink PJ *et al.* (2007). A meta-analysis of occupational trichloroethylene exposure and liver cancer. *Int Arch Occup Environ Health*, 81: 127–143. doi:10.1007/s00420-007-0201-4 PMID:17492303

Alexander DD, Mink PJ, Mandel JH *et al.* (2006). A meta-analysis of occupational trichloroethylene exposure and multiple myeloma or leukaemis. *Occup Med*, 56: 485–493. doi:10.1093/occmed/kql083

Allen JW, Collins BW, Evansky PA (1994). Spermatid micronucleus analyses of trichloroethylene and chloral hydrate effects in mice. *Mutat Res*, 323: 81–88. doi:10.1016/0165-7992(94)90049-3 PMID:7508572

Almaguer O, Kramkowski RS, Orris P (1984). *Johnson Controls Inc., Watertown, WL (Health Hazard Evaluation Report No. 83-296-1491)*. Cincinnati, OH: United States National Institute for Occupational Safety and Health

Amacher DE & Zelljadt I (1983). The morphological transformation of Syrian hamster embryo cells by chemicals reportedly nonmutagenic to Salmonella typhimurium. *Carcinogenesis*, 4: 291–295. doi:10.1093/carcin/4.3.291 PMID:6339095

Andelman JB (1985). Inhalation exposure in the home to volatile organic contaminants of drinking water. *Sci Total Environ*, 47: 443–460. doi:10.1016/0048-9697(85)90349-3 PMID:4089611

Anderson PM & Schultze MO (1965). Cleavage of S-(1,2-dichlorovinyl)-L-cysteine by an enzyme of bovine origin. *Arch Biochem Biophys*, 111: 593–602. doi:10.1016/0003-9861(65)90240-7 PMID:5862209

Anna CH, Maronpot RR, Pereira MA *et al.* (1994). *ras* proto-oncogene activation in dichloroacetic acid-, trichloroethylene- and tetrachloroethylene-induced liver tumors in B6C3F1 mice. *Carcinogenesis*, 15: 2255–2261. doi:10.1093/carcin/15.10.2255 PMID:7955063

Anttila A, Pukkala E, Sallmén M *et al.* (1995a). Cancer incidence among Finnish workers exposed to halogenated hydrocarbons. *J Occup Environ Med*, 37: 797–806. doi:10.1097/00043764-199507000-00008 PMID:7552463

Anttila A, Riala R, Pukkala E et al. (1995b). *Occupational exposure to solvents and risk of cancer*. Final report, Project No. 92293. Helsinki, Finland: The Finnish working environment fund, pp. 1–74.

Aranyi C, O'Shea WJ, Graham JA, Miller FJ (1986). The effects of inhalation of organic chemical air contaminants on murine lung host defenses. *Fundam Appl Toxicol*, 6: 713–720. doi:10.1016/0272-0590(86)90184-3 PMID:3519345

Arif AA & Shah SM (2007). Association between personal exposure to volatile organic compounds and asthma among US adult population. *Int Arch Occup Environ Health*, 80: 711–719. doi:10.1007/s00420-007-0183-2 PMID:17357796

Arizona Department of Health Services (1995). *Update of the Incidence of Childhood Cancers and Testicular Cancer in Southwest Tuscon: 1987–1991*. Phoenix, AZ.

Ashley DL, Bonin MA, Cardinali FL *et al.* (1992). Determining volatile organic compounds in human blood from a large sample population by using purge and trap gas chromatography/mass spectrometry. *Anal Chem*, 64: 1021–1029. doi:10.1021/ac00033a011 PMID:1590585

Astrand I & Ovrum P (1976). Exposure to trichloroethylene I. Uptake and distribution in man. *Scand J Work Environ Health*, 2: 199–211. doi:10.5271/sjweh.2803 PMID:1019594

ATSDR; Agency for Toxic Substances and Disease Registry (1997). *Toxicological Profile for Tetrachloroethylene*. Atlanta, GA: Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services, Public Health Service, pp. 1–318. Available at: http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=265&tid=48. Accessed 19 November 2013.

ATSDR; Agency for Toxic Substances and Disease Registry (2004). *Feasibility investigation of worker exposure to trichloroethylene at the View-Master factory in Beaverton, Oregon*. Atlanta, GA, USA: Department of Health and Human Services.

ATSDR; Agency for Toxic Substances and Disease Registry (2006). *Health statistics review: Cancer and birth outcome analysis: Endicott area investigation: Endicott area, Town of Union, Broome County, New York*. Atlanta, GA: US Department of Health and Humans Services, pp. 7–6. Available at: http://www.atsdr.cdc.gov/HAC/pha/EndicottAreaInvestigation/EndicottHealthStatsReviewHC052606.pdf

ATSDR; Agency for Toxic Substances and Disease Registry (2008). *Health consultation: Health statistics review follow-up: Cancer and birth outcome analysis: Endicott area investigation, Endicott area, Town of Union, Broome County, New York*. Atlanta, GA: US Department of Health and Human Services. Available at: http://www.atsdr.cdc.gov/

hac/pha//EndicottAreaInvestigationFollowUp/EndicottAreaHC051508.pdf

Austin EW, Parrish JM, Kinder DH, Bull RJ (1996). Lipid peroxidation and formation of 8-hydroxydeoxyguanosine from acute doses of halogenated acetic acids. *Fundam Appl Toxicol*, 31: 77–82. doi:10.1006/faat.1996.0078 PMID:8998956

Axelson O, Andersson K, Hogstedt C *et al.* (1978). A cohort study on trichloroethylene exposure and cancer mortality. *J Occup Med*, 20: 194–196. doi:10.1097/00043764-197803000-00008 PMID:627938

Axelson O, Seldén A, Andersson K, Hogstedt C (1994). Updated and expanded Swedish cohort study on trichloroethylene and cancer risk. *J Occup Med*, 36: 556–562. PMID:8027881

Baden JM, Kelley M, Mazze RI, Simmon VF (1979). Mutagenicity of inhalation anaesthetics: trichloroethylene, divinyl ether, nitrous oxide and cyclopropane. *Br J Anaesth*, 51: 417–421. doi:10.1093/bja/51.5.417 PMID:375954

Baden JM & Simmon VF (1980). Mutagenic effects of inhalational anesthetics. *Mutat Res*, 75: 169–189. doi:10.1016/0165-1110(80)90013-5 PMID:6102737

Bahr DE, Aldrich TE, Seidu D *et al.*; Paducah Gaseous Diffusion Plant Project Team; ; Contractors (2011). Occupational exposure to trichloroethylene and cancer risk for workers at the Paducah Gaseous Diffusion Plant. *Int J Occup Med Environ Health*, 24: 67–77. doi:10.2478/s13382-011-0007-1 PMID:21468904

Bai CL & Stacey NH (1993). Mechanism of trichloroethylene-induced elevation of individual serum bile acids. II. In vitro and in vivo interference by trichloroethylene with bile acid transport in isolated rat hepatocytes. *Toxicol Appl Pharmacol*, 121: 296–302. doi:10.1006/taap.1993.1157 PMID:8346546

Banerjee S & Van Duuren BL (1978). Covalent binding of the carcinogen trichloroethylene to hepatic microsomal proteins and to exogenous DNA *in vitro*. *Cancer Res*, 38: 776–780. PMID:626981

Bardodej Z & Vyskocil J (1956). The problem of trichloroethylene in occupational medicine; trichloroethylene metabolism and its effect on the nervous system evaluated as a means of hygienic control. *AMA Arch Ind Health*, 13: 581–592. PMID:13312688

Barton HA, Creech JR, Godin CS *et al.* (1995). Chloroethylene mixtures: pharmacokinetic modeling and *in vitro* metabolism of vinyl chloride, trichloroethylene, and *trans*-1,2-dichloroethylene in rat. *Toxicol Appl Pharmacol*, 130: 237–247. doi:10.1006/taap.1995.1029 PMID:7871537

Bartonicek V (1962). Metabolism and excretion of trichloroethylene after inhalation by human subjects. *Br J Ind Med*, 19: 134–141. PMID:13865497

Bartsch H, Malaveille C, Barbin A, Planche G (1979). Mutagenic and alkylating metabolites of halo-ethylenes, chlorobutadienes and dichlorobutenes produced by rodent or human liver tissues. Evidence for oxirane formation by P450-linked microsomal mono-oxygenases. *Arch Toxicol*, 41: 249–277. doi:10.1007/BF00296896 PMID:373707

Bassig BA, Zhang L, Tang X *et al.* (2013). Occupational exposure to trichloroethylene and serum concentrations of IL-6, IL-10, and TNF-alpha. *Environ Mol Mutagen*, 54: 450–454. doi:10.1002/em.21789 PMID:23798002

Baylin SB, Herman JG, Graff JR *et al.* (1998). Alterations in DNA methylation: a fundamental aspect of neoplasia. *Adv Cancer Res*, 72: 141–196. doi:10.1016/S0065-230X(08)60702-2 PMID:9338076

Beaudreuil S, Lasfargues G, Lauériere L *et al.* (2005). Occupational exposure in ANCA-positive patients: a case-control study. *Kidney Int*, 67: 1961–1966. doi:10.1111/j.1523-1755.2005.00295.x PMID:15840044

Beland FA (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302–17–0). Administered by gavage to F344/N rats and B6C3F1 mice. *Toxic Rep Ser*, 59: 1–66, A1–E7. PMID:11803702

Belanger PL, Coye MJ (1984). *Basic Tool and Supply, Oakland, CA (Health Hazard Evaluation Report No. 84-196-1527)*. Cincinnati, OH: United States National Institute for Occupational Safety and Health

Benedetti MS, Whomsley R, Canning M (2007). Drug metabolism in the paediatric population and in the elderly. *Drug Discov Today*, 12: 599–610. doi:10.1016/j.drudis.2007.06.011 PMID:17706541

Bergman K (1983). Interactions of trichloroethylene with DNA in vitro and with RNA and DNA of various mouse tissues in vivo. *Arch Toxicol*, 54: 181–193. doi:10.1007/BF01239202 PMID:6197950

Berman E, Schlicht M, Moser VC, MacPhail RC (1995). A multidisciplinary approach to toxicological screening: I. Systemic toxicity. *J Toxicol Environ Health*, 45: 127–143. doi:\\ PMID:7783250

Bernauer U, Birner G, Dekant W, Henschler D (1996). Biotransformation of trichloroethene: dose-dependent excretion of 2,2,2-trichloro-metabolites and mercapturic acids in rats and humans after inhalation. *Arch Toxicol*, 70: 338–346. doi:10.1007/s002040050283 PMID:8975632

Bhattacharya RK & Schultze MO (1967). Enzymes from bovine and turkey kidneys which cleave S-(1,2-dichlorovinyl)-L-cysteine. *Comp Biochem Physiol*, 22: 723–735. doi:10.1016/0010-406X(67)90765-7 PMID:6053640

Bi E, Liu Y, He J *et al.* (2012). Screening of emerging volatile organic contaminants in shallow groundwater in east China. *Ground Water Monit Remediat*, 32: 53–58. doi:10.1111/j.1745-6592.2011.01362.x

Bignami M, Conti G, Conti L *et al.* (1980). Mutagenicity of halogenated aliphatic hydrocarbons in *Salmonella typhimurium*, *Streptomyces coelicolor* and *Aspergillus*

nidulans. *Chem Biol Interact*, 30: 9–23. doi:10.1016/0009-2797(80)90110-6 PMID:6991142

Billionnet C, Gay E, Kirchner S *et al.* (2011). Quantitative assessments of indoor air pollution and respiratory health in a population-based sample of French dwellings. *Environ Res*, 111: 425–434. doi:10.1016/j.envres.2011.02.008 PMID:21397225

Birkenfeld F, Gastl D, Heblich D et al. (2005). *Product ban versus risk management by setting emission and technology requirements: The effect of different regulatory schemes taking the use of trichloroethylene in Sweden and Germany as an example*. Diskussionsbeitrag Nr. V-37-0; Universitat Passau.

Birner G, Vamvakas S, Dekant W, Henschler D (1993). Nephrotoxic and genotoxic *N*-acetyl-*S*-dichlorovinyl-*L*-cysteine is a urinary metabolite after occupational 1,1,2-trichloroethene exposure in humans: implications for the risk of trichloroethene exposure. *Environ Health Perspect*, 99: 281–284. doi:10.1289/ehp.9399281 PMID:8319644

Blair A, Haas T, Prosser R *et al.* (1989). Mortality among United States Coast Guard marine inspectors. *Arch Environ Health*, 44: 150–156. doi:\\ PMID:2751350

Blair A, Hartge P, Stewart PA *et al.* (1998). Mortality and cancer incidence of aircraft maintenance workers exposed to trichloroethylene and other organic solvents and chemicals: extended follow up. *Occup Environ Med*, 55: 161–171. doi:\\ PMID:9624267

Blake JE, Compton KV, Schmidt W, Orrego H (1988). Accuracy of death certificates in the diagnosis of alcoholic liver cirrhosis. *Alcohol Clin Exp Res*, 12: 168–172. doi:10.1111/j.1530-0277.1988.tb00154.x PMID:3279851

Bloom TF, Kramkowski RS, Cromer JW (1974). *Essex Wire Corp., Kenton, OH (Health Hazard Evaluation Determination Report No. 73-151-141)*. Cincinnati, OH: United States National Institute for Occupational Safety and Health

Blossom SJ & Doss JC (2007). Trichloroethylene alters central and peripheral immune function in autoimmune-prone MRL(+/+) mice following continuous developmental and early life exposure. *J Immunotoxicol*, 4: 129–141. doi:10.1080/15476910701337035 PMID:18958721

Blossom SJ, Doss JC, Gilbert KM (2007). Chronic exposure to a trichloroethylene metabolite in autoimmune-prone MRL+/+ mice promotes immune modulation and alopecia. *Toxicol Sci*, 95: 401–411. doi:10.1093/toxsci/kfl149 PMID:17077186

Blossom SJ, Doss JC, Hennings LJ *et al.* (2008). Developmental exposure to trichloroethylene promotes $CD4^+$ T cell differentiation and hyperactivity in association with oxidative stress and neurobehavioral deficits in MRL+/+ mice. *Toxicol Appl Pharmacol*, 231: 344–353. doi:10.1016/j.taap.2008.05.009 PMID:18579175

Blossom SJ, Pumford NR, Gilbert KM (2004). Activation and attenuation of apoptosis of CD4+ T cells following in vivo exposure to two common environmental toxicants, trichloroacetaldehyde hydrate and trichloroacetic acid. *J Autoimmun*, 23: 211–220. doi:10.1016/j.jaut.2004.06.007 PMID:15501392

Boice JD Jr, Marano DE, Cohen SS *et al.* (2006). Mortality among Rocketdyne workers who tested rocket engines, 1948–1999. *J Occup Environ Med*, 48: 1070–1092. doi:\\ PMID:17033507

Boice JD Jr, Marano DE, Fryzek JP *et al.* (1999). Mortality among aircraft manufacturing workers. *Occup Environ Med*, 56: 581–597. doi:10.1136/oem.56.9.581 PMID:10615290

Bolt HM, Lammert M, Selinski S, Brüning T (2004). Urinary alpha1-microglobulin excretion as biomarker of renal toxicity in trichloroethylene-exposed persons. *Int Arch Occup Environ Health*, 77: 186–190. doi:10.1007/s00420-003-0500-3 PMID:14986001

Bomhard E & Mohr U (1989). Spontaneous tumours in NMRI mice from carcinogenicity studies. *Exp Pathol*, 36: 129–145. doi:10.1016/S0232-1513(89)80090-8 PMID:2759194

Boutonnet JC, De Rooij C, Garny V *et al.* (1998). Euro Chlor risk assessment for the marine environment OSPARCOM region: North sea-trichloroethylene. *Environ Monit Assess*, 53: 467–487. doi:10.1023/A:1006066600350

Bove F, Shim Y, Zeitz P (2002). Drinking water contaminants and adverse pregnancy outcomes: a review. *Environ Health Perspect*, 110: Suppl 1: 61–74. doi:10.1289/ehp.02110s1161 PMID:11834464

Bradford BU, Kono H, Isayama F *et al.* (2005). Cytochrome P450 CYP2E1, but not nicotinamide adenine dinucleotide phosphate oxidase, is required for ethanol-induced oxidative DNA damage in rodent liver. *Hepatology*, 41: 336–344. doi:10.1002/hep.20532 PMID:15660387

Bradford BU, Lock EF, Kosyk O *et al.* (2011). Interstrain differences in the liver effects of trichloroethylene in a multistrain panel of inbred mice. *Toxicol Sci*, 120: 206–217. doi:\\ PMID:21135412

Brauch H, Weirich G, Hornauer MA *et al.* (1999). Trichloroethylene exposure and specific somatic mutations in patients with renal cell carcinoma. *J Natl Cancer Inst*, 91: 854–861. doi:10.1093/jnci/91.10.854 PMID:10340905

Brauch H, Weirich G, Klein B *et al.* (2004). *VHL* mutations in renal cell cancer: does occupational exposure to trichloroethylene make a difference? *Toxicol Lett*, 151: 301–310. doi:10.1016/j.toxlet.2003.12.074 PMID:15177666

Brix AE, Nyska A, Haseman JK *et al.* (2005). Incidences of selected lesions in control female Harlan Sprague-Dawley rats from two-year studies performed by the National Toxicology Program. *Toxicol Pathol*, 33: 477–483. doi:10.1080/01926230590961836 PMID:16036865

Bronley-DeLancey A, McMillan DC, McMillan JM *et al.* (2006). Application of cryopreserved human hepatocytes in trichloroethylene risk assessment: relative disposition of chloral hydrate to trichloroacetate and trichloroethanol. *Environ Health Perspect*, 114: 1237–1242. doi:10.1289/ehp.9047 PMID:16882532

Bronzetti G, Zeiger E, Frezza D (1978). Genetic activity of trichloroethylene in yeast. *J Environ Pathol Toxicol*, 1: 411–418. PMID:363965

Brüning T, Golka K, Makropoulos V, Bolt HM (1996). Preexistence of chronic tubular damage in cases of renal cell cancer after long and high exposure to trichloroethylene. *Arch Toxicol*, 70: 259–260. doi:10.1007/s002040050271 PMID:8825688

Brüning T, Lammert M, Kempkes M *et al.* (1997b). 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: 596–599. doi:10.1007/s002040050432 PMID:9285043

Brüning T, Mann H, Melzer H *et al.* (1999a). Pathological excretion patterns of urinary proteins in renal cell cancer patients exposed to trichloroethylene. *Occup Med (Lond)*, 49: 299–305. doi:10.1093/occmed/49.5.299 PMID:10628055

Brüning T, Pesch B, Wiesenhütter B *et al.* (2003). Renal cell cancer risk and occupational exposure to trichloroethylene: results of a consecutive case-control study in Arnsberg, Germany. *Am J Ind Med*, 43: 274–285. doi:10.1002/ajim.10185 PMID:12594774

Brüning T, Sundberg AGM, Birner G *et al.* (1999b). Glutathione transferase alpha as a marker for tubular damage after trichloroethylene exposure. *Arch Toxicol*, 73: 246–254. doi:10.1007/s002040050613 PMID:10463390

Brüning T, Vamvakas S, Makropoulos V, Birner G (1998). Acute intoxication with trichloroethene: clinical symptoms, toxicokinetics, metabolism, and development of biochemical parameters for renal damage. *Toxicol Sci*, 41: 157–165. doi:10.1093/toxsci/41.2.157 PMID:9520351

Brüning T, Weirich G, Hornauer MA *et al.* (1997a). Renal cell carcinomas in trichloroethene (TRI) exposed persons are associated with somatic mutations in the von Hippel-Lindau (VHL) tumour suppressor gene. *Arch Toxicol*, 71: 332–335. doi:10.1007/s002040050394 PMID:9137812

Buben JA & O'Flaherty EJ (1985). Delineation of the role of metabolism in the hepatotoxicity of trichloroethylene and perchloroethylene: a dose-effect study. *Toxicol Appl Pharmacol*, 78: 105–122. doi:10.1016/0041-008X(85)90310-2 PMID:2994252

Buist SCN, Cherrington NJ, Klaassen CD (2003). Endocrine regulation of rat organic anion transporters. *Drug Metab Dispos*, 31: 559–564. doi:10.1124/dmd.31.5.559 PMID: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: 620–625. doi:10.1124/dmd.32.6.620 PMID:15155553

Bull RJ, Orner GA, Cheng RS *et al.* (2002). Contribution of dichloroacetate and trichloroacetate to liver tumor induction in mice by trichloroethylene. *Toxicol Appl Pharmacol*, 182: 55–65. doi:10.1006/taap.2002.9427 PMID:12127263

Burgess WA (1981). *Recognition of Health Hazards in Industry. A Review of Materials and Processes*. New York: John Wiley & Sons, pp. 20–32

Burroughs GE (1980). *Protective Coatings Corporation, Fort Wayne, IN (Health Hazard Evaluation Report No. 79-96-729)*. Cincinnati, OH: United States National Institute for Occupational Safety and Health

Burroughs GE, Moody PL (1982). *Industrial Plastics, Valley City, OH (Health Hazard Evaluation Report No. 81-029-1088)*. Cincinnati, OH: United States National Institute for Occupational Safety and Health

Burton DT, Dilorenzo JL, Shedd TR, Wrobel JG (2002). Aquatic hazard assessment of a contaminated surficial aquifer discharge into the Bush river, Maryland (U.S.A.). *Water Air Soil Pollut*, 139: 159–182. doi:10.1023/A:1015855924228

Byers VS, Levin AS, Ozonoff DM, Baldwin RW (1988). Association between clinical symptoms and lymphocyte abnormalities in a population with chronic domestic exposure to industrial solvent-contaminated domestic water supply and a high incidence of leukaemia. *Cancer Immunol Immunother*, 27: 77–81. doi:10.1007/BF00205762 PMID:3260823

Cai H & Guengerich FP (2001a). Reaction of trichloroethylene oxide with proteins and DNA: instability of adducts and modulation of functions. *Chem Res Toxicol*, 14: 54–61. doi:10.1021/tx000185n PMID:11170508

Cai P, König R, Boor PJ *et al.* (2008). Chronic exposure to trichloroethene causes early onset of SLE-like disease in female MRL +/+ mice. *Toxicol Appl Pharmacol*, 228: 68–75. doi:\\ PMID:18234256

Caldwell JC & Keshava N (2006). Key issues in the modes of action and effects of trichloroethylene metabolites for liver and kidney tumorigenesis. *Environ Health Perspect*, 114: 1457–1463. doi:10.1289/ehp.8692 PMID:16966105

Callen DF, Wolf CR, Philpot RM (1980). Cytochrome P-450 mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in *Saccharomyces cerevisiae*. *Mutat Res*, 77: 55–63. doi:10.1016/0165-1218(80)90120-2 PMID:6767185

Candura SM & Faustman EM (1991). Trichloroethylene: toxicology and health hazards. *G Ital Med Lav*, 13: 17–25. PMID:1845454

CAREX Canada (2012). *Trichloroethylene*. Available at: http://www.carexcanada.ca/en/trichloroethylene/occupational_estimate/. Accessed 19 November 2013.

Carpenter SP, Lasker JM, Raucy JL (1996). Expression, induction, and catalytic activity of the ethanol-inducible cytochrome P450 (CYP2E1) in human fetal liver and hepatocytes. *Mol Pharmacol*, 49: 260–268. PMID:8632758

Cashman JR & Zhang J (2006). Human flavin-containing monooxygenases. *Annu Rev Pharmacol Toxicol*, 46: 65–100. doi:10.1146/annurev.pharmtox.46.120604.141043 PMID:16402899

Caspary WJ, Langenbach R, Penman BW *et al.* (1988). The mutagenic activity of selected compounds at the TK locus: rodent vs. human cells. *Mutat Res*, 196: 61–81. doi:10.1016/0165-1110(88)90028-0 PMID:3292900

Catanzaro I, Naselli F, Saverini M *et al.* (2012). Cytochrome P450 2E1 variable number tandem repeat polymorphisms and health risks: a genotype-phenotype study in cancers associated with drinking and/or smoking. *Mol Med Report*, 6: 416–420. PMID:22614694

Chakrabarti SK & Tuchweber B (1988). Studies of acute nephrotoxic potential of trichloroethylene in Fischer 344 rats. *J Toxicol Environ Health*, 23: 147–158. doi:10.1080/15287398809531102 PMID:3343692

Chang YM, Tai CF, Yang SC *et al.* (2003). A cohort mortality study of workers exposed to chlorinated organic solvents in Taiwan. *Ann Epidemiol*, 13: 652–660. doi:10.1016/S1047-2797(03)00038-3 PMID:14732305

Chang YM, Tai CF, Yang SC *et al.* (2005). Cancer incidence among workers potentially exposed to chlorinated solvents in an electronics factory. *J Occup Health*, 47: 171–180. doi:10.1539/joh.47.171 PMID:15824483

Channel SR, Latendresse JR, Kidney JK *et al.* (1998). A subchronic exposure to trichloroethylene causes lipid peroxidation and hepatocellular proliferation in male B6C3F1 mouse liver. *Toxicol Sci*, 43: 145–154. doi:\\ PMID:9710956

Charbotel B, Fevotte J, Hours M *et al.* (2006). Case-control study on renal cell cancer and occupational exposure to trichloroethylene. Part II: Epidemiological aspects. *Ann Occup Hyg*, 50: 777–787. doi:10.1093/annhyg/mel039 PMID:16840435

Charbotel B, Fevotte J, Martin JL, Bergeret A (2009). [Renal cell carcinoma and exposure to trichloroethylene: are the French limits of occupational exposure relevant?] *Rev Epidemiol Sante Publique*, 57: 41–47. doi:10.1016/j.respe.2008.09.008 PMID:19155150

Charbotel B, Gad S, Caïola D *et al.* (2007). Trichloroethylene exposure and somatic mutations of the *VHL* gene in patients with Renal Cell Carcinoma. *J Occup Med Toxicol*, 2: 13 doi:10.1186/1745-6673-2-13 PMID:17997830

Charbotel B, Massardier-Pilonchery A, Fort E *et al.* (2013). Occupational trichloroethylene exposure and cervical pathology: a case-control study. *Ann Occup Hyg*, 57: 407–416. doi:10.1093/annhyg/mes075 PMID:23091111

Chen JC, Stevens JL, Trifillis AL, Jones TW (1990). Renal cysteine conjugate β-lyase-mediated toxicity studied with primary cultures of human proximal tubular cells. *Toxicol Appl Pharmacol*, 103: 463–473. doi:10.1016/0041-008X(90)90319-P PMID:2339419

Chia SE, Goh VH, Ong CN (1997). Endocrine profiles of male workers with exposure to trichloroethylene. *Am J Ind Med*, 32: 217–222. doi:10.1002/(SICI)1097-0274(199709)32:3<217::AID-AJIM6>3.0.CO;2-U PMID:9219650

Chia S-E, Ong C-N, Tsakok MF, Ho A (1996). Semen parameters in workers exposed to trichloroethylene. *Reprod Toxicol*, 10: 295–299. doi:10.1016/0890-6238(96)00058-5 PMID:8829252

Chiu WA, Micallef S, Monster AC, Bois FY (2007). Toxicokinetics of inhaled trichloroethylene and tetrachloroethylene in humans at 1 ppm: empirical results and comparisons with previous studies. *Toxicol Sci*, 95: 23–36. doi:10.1093/toxsci/kfl129 PMID:17032701

Chiu WA, Okino MS, Lipscomb JC, Evans MV (2006). Issues in the pharmacokinetics of trichloroethylene and its metabolites. *Environ Health Perspect*, 114: 1450–1456. doi:10.1289/ehp.8691 PMID:16966104

Christensen KY, Vizcaya D, Richardson H *et al.* (2013). Risk of selected cancers due to occupational exposure to chlorinated solvents in a case-control study in Montreal. *J Occup Environ Med*, 55: 198–208. doi:10.1097/JOM.0b013e3182728eab PMID:23147555

Class Th & Ballschmiter K (1986). Chemistry of organic traces in air VI: Distribution of chlorinated C1 - C4 hydrocarbons in air over the northern and southern Atlantic Ocean. *Chemosphere*, 15: 413–427. doi:10.1016/0045-6535(86)90535-7

Clay P (2008). Assessment of the genotoxicity of trichloroethylene and its metabolite, S-(1,2-dichlorovinyl)-L-cysteine (DCVC), in the comet assay in rat kidney. *Mutagenesis*, 23: 27–33. doi:10.1093/mutage/gem034 PMID:18003627

Cocco P, t'Mannetje A, Fadda D *et al.* (2010). Occupational exposure to solvents and risk of lymphoma subtypes: results from the Epilymph case-control study. *Occup Environ Med*, 67: 341–347. doi:10.1136/oem.2009.046839 PMID:20447988

Cohn P, Klotz J, Bove F *et al.* (1994). Drinking Water Contamination and the Incidence of Leukemia and Non-Hodgkin's Lymphoma. *Environ Health Perspect*, 102: 556–561. doi:10.1289/ehp.94102556 PMID:9679115

Cojocel C, Beuter W, Müller W, Mayer D (1989). Lipid peroxidation: a possible mechanism of trichloroethylene-induced nephrotoxicity. *Toxicology*, 55: 131–141. doi:10.1016/0300-483X(89)90180-7 PMID:2711399

Colucci DF & Buyske DA (1965). The biotransformation of a sulfonamide to a mercaptan and to mercapturic acid and glucuronide conjugates. *Biochem Pharmacol*,

14: 457–466. doi:10.1016/0006-2952(65)90218-2 PMID:14322970

Commission for Health and Safety at Work (2012). [*Trichloroéthylène, CAS No 79-01-06.*] Montreal, QC, Canada: pp. 1–19. Available at: http://www.reptox.csst.qc.ca/Produit.asp?no_produit=1065. Accessed 19 November 2013.

Cooper AJL, Abraham DG, Gelbard AS *et al.* (1993). High activities of glutamine transaminase K (dichlorovinylcysteine β-lyase) and ω-amidase in the choroid plexus of rat brain. *J Neurochem*, 61: 1731–1741. doi:10.1111/j.1471-4159.1993.tb09810.x PMID:8228989

Cooper AJL, Bruschi SA, Conway M, Hutson SM (2003). Human mitochondrial and cytosolic branched-chain aminotransferases are cysteine S-conjugate β-lyases, but turnover leads to inactivation. *Biochem Pharmacol*, 65: 181–192. doi:10.1016/S0006-2952(02)01513-7 PMID:12504794

Cooper AJL, Bruschi SA, Iriarte A, Martinez-Carrion M (2002). Mitochondrial aspartate aminotransferase catalyses cysteine *S*-conjugate *β*-lyase reactions. *Biochem J*, 368: 253–261. doi:10.1042/BJ20020531 PMID:12137566

Cooper AJL & Pinto JT (2006). Cysteine S-conjugate β-lyases. *Amino Acids*, 30: 1–15. doi:10.1007/s00726-005-0243-4 PMID: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: 1345–1353. doi:10.1016/S0006-2952(01)00802-4 PMID:11709194

Cooper GS, Makris SL, Nietert PJ, Jinot J (2009). Evidence of autoimmune-related effects of trichloroethylene exposure from studies in mice and humans. *Environ Health Perspect*, 117: 696–702. PMID:19479009

Cooper GS & Stroehla BC (2003). The epidemiology of autoimmune diseases. *Autoimmun Rev*, 2: 119–125. doi:10.1016/S1568-9972(03)00006-5 PMID:12848952

Coopman VAE, Cordonnier JA, De Letter EA, Piette MHA (2003). Tissue distribution of trichloroethylene in a case of accidental acute intoxication by inhalation. *Forensic Sci Int*, 134: 115–119. doi:10.1016/S0379-0738(03)00131-2 PMID:12850404

Costa AK & Ivanetich KM (1984). Chlorinated ethylenes: their metabolism and effect on DNA repair in rat hepatocytes. *Carcinogenesis*, 5: 1629–1636. doi:10.1093/carcin/5.12.1629 PMID:6499114

Costas K, Knorr RS, Condon SK (2002). A case-control study of childhood leukemia in Woburn, Massachusetts: the relationship between leukemia incidence and exposure to public drinking water. *Sci Total Environ*, 300: 23–35. doi:10.1016/S0048-9697(02)00169-9 PMID:12685468

Counts RS, Nowak G, Wyatt RD, Schnellmann RG (1995). Nephrotoxicant inhibition of renal proximal tubule cell regeneration. *Am J Physiol*, 269: F274–F281. PMID:7653601

Coyle YM, Hynan LS, Euhus DM, Minhajuddin AT (2005). An ecological study of the association of environmental chemicals on breast cancer incidence in Texas. *Breast Cancer Res Treat*, 92: 107–114. doi:10.1007/s10549-004-8268-z PMID:15986119

Crebelli R, Bignami M, Conti L, Carere A (1982). Mutagenicity of trichloroethylene in Salmonella typhimurium TA100. *Ann Ist Super Sanita*, 18: 117–121. PMID:6763484

Crebelli R & Carere A (1989). Genetic toxicology of 1,1,2-trichloroethylene. *Mutat Res*, 221: 11–37. doi:10.1016/0165-1110(89)90043-2 PMID:2664493

Crebelli R, Conti G, Conti L, Carere A (1985). Mutagenicity of trichloroethylene, trichloroethanol and chloral hydrate in Aspergillus nidulans. *Mutat Res*, 155: 105–111. doi:10.1016/0165-1218(85)90126-0 PMID:3883153

Cummings BS & Lash LH (2000). Metabolism and toxicity of trichloroethylene and *S*-(1,2-dichlorovinyl)-*L*-cysteine in freshly isolated human proximal tubular cells. *Toxicol Sci*, 53: 458–466. doi:10.1093/toxsci/53.2.458 PMID:10696794

Cummings BS, Lasker JM, Lash LH (2000b). Expression of glutathione-dependent enzymes and cytochrome P450s in freshly isolated and primary cultures of proximal tubular cells from human kidney. *J Pharmacol Exp Ther*, 293: 677–685. PMID:10773044

Cummings BS, Parker JC, Lash LH (2000a). Role of cytochrome P450 and glutathione *S*-transferase alpha in the metabolism and cytotoxicity of trichloroethylene in rat kidney. *Biochem Pharmacol*, 59: 531–543. doi:10.1016/S0006-2952(99)00374-3 PMID:10660119

Cummings BS, Parker JC, Lash LH (2001). Cytochrome P450-dependent metabolism of trichloroethylene in rat kidney. *Toxicol Sci*, 60: 11–19. doi:10.1093/toxsci/60.1.11 PMID:11222868

Cummings BS, Zangar RC, Novak RF, Lash LH (2000c). Cytotoxicity of trichloroethylene and S-(1, 2-dichlorovinyl)-L-cysteine in primary cultures of rat renal proximal tubular and distal tubular cells. *Toxicology*, 150: 83–98. doi:10.1016/S0300-483X(00)00252-3 PMID:10996665

D'Souza RW, Bruckner JV, Feldman S (1985). Oral and intravenous trichloroethylene pharmacokinetics in the rat. *J Toxicol Environ Health*, 15: 587–601. doi:10.1080/15287398509530688 PMID:4046066

Dallas CE, Gallo JM, Ramanathan R *et al.* (1991). Physiological pharmacokinetic modeling of inhaled trichloroethylene in rats. *Toxicol Appl Pharmacol*, 110: 303–314. doi:10.1016/S0041-008X(05)80013-4 PMID:1891776

Daly AK (2012). Genetic polymorphisms affecting drug metabolism: recent advances and clinical aspects.

Adv Pharmacol, 63: 137–167. doi:10.1016/B978-0-12-398339-8.00004-5 PMID:22776641

Darnerud PO, Brandt I, Feil VJ, Bakke JE (1989). Dichlorovinyl cysteine (DCVC) in the mouse kidney: tissue-binding and toxicity after glutathione depletion and probenecid treatment. *Arch Toxicol*, 63: 345–350. doi:10.1007/BF00303121 PMID:2818197

Davis SI, Laszlo Pallos L, Wu JQ *et al.* (2005). ATSDR's trichloroethylene subregistry methods and results: 1989–2000. *Arch Environ Occup Health*, 60: 130–139. doi:10.3200/AEOH.60.3.130-139 PMID:17153085

De Baere S, Meyer E, Dirinck I *et al.* (1997). Tissue distribution of trichloroethylene and its metabolites in a forensic case. *J Anal Toxicol*, 21: 223–227. PMID:9171208

De Roos AJ, Olshan AF, Teschke K *et al.* (2001). Parental occupational exposures to chemicals and incidence of neuroblastoma in offspring. *Am J Epidemiol*, 154: 106–114. doi:10.1093/aje/154.2.106 PMID:11447042

DeAngelo AB, Daniel FB, Most BM, Olson GR (1997). Failure of monochloroacetic acid and trichloroacetic acid administered in the drinking water to produce liver cancer in male F344/N rats. *J Toxicol Environ Health*, 52: 425–445. PMID:9388534

Dees C & Travis C (1993). The mitogenic potential of trichloroethylene in B6C3F1 mice. *Toxicol Lett*, 69: 129–137. doi:10.1016/0378-4274(93)90098-I PMID:8212055

Dehon B, Humbert L, Devisme L *et al.* (2000). Tetrachloroethylene and trichloroethylene fatality: case report and simple headspace SPME-capillary gas chromatographic determination in tissues. *J Anal Toxicol*, 24: 22–26. PMID:10654565

Dekant W, Berthold K, Vamvakas S *et al.* (1988). Thioacylating intermediates as metabolites of *S*-(1,2-dichlorovinyl)-*L*-cysteine and *S*-(1,2,2-trichlorovinyl)-*L*-cysteine formed by cysteine conjugate β-lyase. *Chem Res Toxicol*, 1: 175–178. doi:10.1021/tx00003a008 PMID:2979728

Dekant W, Metzler M, Henschler D (1984). Novel metabolites of trichloroethylene through dechlorination reactions in rats, mice and humans. *Biochem Pharmacol*, 33: 2021–2027. doi:10.1016/0006-2952(84)90568-9 PMID:6743352

Dekant W, Metzler M, Henschler D (1986b). Identification of S-1,2-dichlorovinyl-N-acetyl-cysteine as a urinary metabolite of trichloroethylene: a possible explanation for its nephrocarcinogenicity in male rats. *Biochem Pharmacol*, 35: 2455–2458. doi:10.1016/0006-2952(86)90039-0 PMID:3741453

Dekant W, Schulz A, Metzler M, Henschler D (1986a). Absorption, elimination and metabolism of trichloroethylene: a quantitative comparison between rats and mice. *Xenobiotica*, 16: 143–152. doi:10.3109/00498258609043517 PMID:3962335

Dekant W, Vamvakas S, Berthold K *et al.* (1986c). Bacterial beta-lyase mediated cleavage and mutagenicity of cysteine conjugates derived from the nephrocarcinogenic alkenes trichloroethylene, tetrachloroethylene and hexachlorobutadiene. *Chem Biol Interact*, 60: 31–45. doi:10.1016/0009-2797(86)90015-3 PMID:3536138

Delinsky AD, Bruckner JV, Bartlett MG (2005). A review of analytical methods for the determination of trichloroethylene and its major metabolites chloral hydrate, trichloroacetic acid and dichloroacetic acid. *Biomed Chromatogr*, 19: 617–639. doi:10.1002/bmc.488 PMID:15828053

DeMarini DM, Perry E, Shelton ML (1994). Dichloroacetic acid and related compounds: induction of prophage in E. coli and mutagenicity and mutation spectra in Salmonella TA100. *Mutagenesis*, 9: 429–437. doi:10.1093/mutage/9.5.429 PMID:7837977

Demeestere K, Dewulf J, De Witte B, Van Langenhove H (2007). Sample preparation for the analysis of volatile organic compounds in air and water matrices. *J Chromatogr A*, 1153: 130–144. doi:10.1016/j.chroma.2007.01.012 PMID:17258752

Deng Q, Zheng T, Lan Q *et al.* (2012). Occupational solvent exposure, genetic variation in immune genes, and the risk for non-Hodgkin lymphoma. *Eur J Cancer Prev*, 2012: 18 PMID:22609637

Dinse GE, Peddada SD, Harris SF, Elmore SA (2010). Comparison of NTP historical control tumour incidence rates in female Harlan Sprague Dawley and Fischer 344/N Rats. *Toxicol Pathol*, 38: 765–775. doi:10.1177/0192623310373777 PMID:20622195

Diot E, Lesire V, Guilmot JL *et al.* (2002). Systemic sclerosis and occupational risk factors: a case-control study. *Occup Environ Med*, 59: 545–549. doi:10.1136/oem.59.8.545 PMID:12151611

DiRenzo AB, Gandolfi AJ, Sipes IG (1982). Microsomal bioactivation and covalent binding of aliphatic halides to DNA. *Toxicol Lett*, 11: 243–252. doi:10.1016/0378-4274(82)90157-6 PMID:7101318

Dnyanmote AV, Sawant SP, Lock EA *et al.* (2006). Diabetic mice are protected from normally lethal nephrotoxicity of S-1,2-dichlorovinyl-L-cysteine (DCVC): role of nephrogenic tissue repair. *Toxicol Appl Pharmacol*, 211: 133–147. doi:10.1016/j.taap.2005.07.015 PMID:16125744

Doherty RE (2000). A history of the production and use of carbon tetrachloride, tetrachloroethylene, trichloroethylene and 1,1,1-trichloroethane in the United States, Part 2 Trichloroethylene and 1,1,1-trichloroethane *J Environmental Forensics*, 1: 82–93.

Doolittle DJ, Muller G, Scribner HE (1987). The *in vivo-in vitro* hepatocyte assay for assessing DNA repair and DNA replication: studies in the CD-1 mouse. *Food Chem Toxicol*, 25: 399–405. doi:10.1016/0278-6915(87)90176-1 PMID:3609980

Dosemeci M, Cocco P, Chow WH (1999). Gender differences in risk of renal cell carcinoma and occupational exposures to chlorinated aliphatic hydrocarbons. *Am J Ind Med*, 36: 54–59.

doi:10.1002/(SICI)1097-0274(199907)36:1<54::AID-AJIM8>3.0.CO;2-0 PMID:10361587

Douglas GR, Gingerich JD, Soper LM *et al.* (1999). Evidence for the lack of base-change and small-deletion mutation induction by trichloroethylene in *lacZ* transgenic mice. *Environ Mol Mutagen*, 34: 190–194. doi:10.1002/(SICI)1098-2280(1999)34:2/3<190::AID-EM18>3.0.CO;2-# PMID:10529743

Dow JL & Green T (2000). Trichloroethylene induced vitamin B_{12} and folate deficiency leads to increased formic acid excretion in the rat. *Toxicology*, 146: 123–136. doi:10.1016/S0300-483X(00)00156-6 PMID:10814845

DOW Chemical (2012). *DOW Customer Help: Trichloroethylene*. Midland, MI: DOW Chemical Company

Driscoll TR, Hamdan HH, Wang G *et al.* (1992). Concentrations of individual serum or plasma bile acids in workers exposed to chlorinated aliphatic hydrocarbons. *Br J Ind Med*, 49: 700–705. PMID:1419858

Duprat P & Gradiski D (1980). Cytogenetic effect of trichloroethylene in the mouse as evaluated by the micronucleus test. *IRCS Med Sci*, 8: 182

ECETOC; European Centre for Ecotoxicology and Toxicology of Chemicals (1994). *Trichloroethylene: Assessment of Human Carcinogenic Hazard*. Available at: http://www.ecetoc.org/index.php?mact=MCSoap,cntnt01,details,0&cntnt01by_category=5&cntnt01template=display_list_v2&cntnt01order_by=Reference%20Desc&cntnt01display_template=display_details_v2&cntnt01document_id=238&cntnt01returnid=89. Accessed 18.10.2013.

ECSA; European Chlorinated Solvent Association (2012). *Trichloroethylene Legislation, Markets and Uses*. Available at: http://www.eurochlor.org/media/8198/trichloroethylene-legislation-markets-uses.pdf. Accessed 19 November 2013.

El Ghawabi SM, Mansoor MB, El Gamel MS *et al.* (1973). Chronic trichloroethylene exposure. *J Egypt Med Assoc*, 56: 715–724. PMID:4618847

Elcombe CR, Rose MS, Pratt IS (1985). Biochemical, histological, and ultrastructural changes in rat and mouse liver following the administration of trichloroethylene: possible relevance to species differences in hepatocarcinogenicity. *Toxicol Appl Pharmacol*, 79: 365–376. doi:10.1016/0041-008X(85)90135-8 PMID:4035684

Elfarra AA, Jakobson I, Anders MW (1986). Mechanism of *S*-(1,2-dichlorovinyl)glutathione-induced nephrotoxicity. *Biochem Pharmacol*, 35: 283–288. doi:10.1016/0006-2952(86)90527-7 PMID:2867768

Elfarra AA, Lash LH, Anders MW (1987). α-ketoacids stimulate rat renal cysteine conjugate *β*-lyase activity and potentiate the cytotoxicity of *S*-(1,2-dichlorovinyl)-*L*-cysteine. *Mol Pharmacol*, 31: 208–212. PMID:3807895

Elmore E & Fitzgerald MP (1990). Evaluation of the bioluminescence assays as screens for genotoxic chemicals. *Prog Clin Biol Res*, 340D: 379–387. PMID:2371306

Emmert B, Bünger J, Keuch K *et al.* (2006). Mutagenicity of cytochrome P450 2E1 substrates in the Ames test with the metabolic competent *S. typhimurium* strain YG7108pin3ERb5. *Toxicology*, 228: 66–76. doi:10.1016/j.tox.2006.08.013 PMID:16978761

EPA (1985). *Health Assessment Document for Trichloroethylene (Final Report)*. Washington, DC: Research Triangle Park, NC, No. EPA-600/8–82006-F; US NTIS PB85–249696

EPA (1988) *Methods for the Determination of Organic Compounds in Drinking Water*. Cincinnati, OH: Environmental Monitoring Systems Laboratory, No. EPA-600/4–88–039; US NTIS PB89–220461, 5–87, 255–323

EPA (1989). *Locating and estimating air emissions from sources of perchloroethylene and trichloroethylene*. No. EPA-450/2–89–013

EPA (1995a). *Volatile Organic Compounds in Water by Purge and Trap Capillary Column Gas Chromatography with Photoionization and Electrolytic Conductivity Detectors in Series*. United States Environmental Protection Agency, Cincinnati, OH: Environmental Monitoring Systems Laboratory, No. (Method 502.2, Rev 2.1)

EPA (1995b). *Measurement of Purgeable Organic Compounds in Water by Capillary Column Gas Chromatography/Mass Spectrometry*. United States Environmental Protection Agency, Cincinnati, OH: Environmental Monitoring Systems Laboratory, No. (Method 524.2, Rev 4.1)

EPA (1995c). *Determination of Chlorination Disinfection Byproducts, Chlorinated Solvents, and Halogenated Pesticides/Herbicides in Drinking Water by Liquid-liquid Extraction and Gas Chromatography with Electron Capture Detection*. United States Environmental Protection Agency, Cincinnati, OH: Environmental Monitoring Systems Laboratory, No. (Method 551.1, Rev 1.0), 1–61

EPA (1996). *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*. United States Environmental Protection Agency, Cincinnati, OH: Environmental Monitoring Systems Laboratory, No. SW-846, Method 8021B

EPA (1999a). *Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air, Determination of Volatile Organic Compounds (VOCs) in Air Collected in Specially-Prepared Canisters and Analyzed by Gas Chromatography/Mass Spectrometry (GC/MS)*. United States Environmental Protection Agency, Cincinnati, OH: No. 2nd Ed. Method TO-15

EPA (1999b). *Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air. Determination of Volatile Organic Compounds in*

Ambient Air Using Active Sampling Onto Sorbent Tubes. United States Environmental Protection Agency, Cincinnati, OH: No. 2nd Ed. Method TO-17

EPA (2011a). *Toxicological Review of Trichloroethylene: In support of summary information on the integrated risk information system (IRIS)*. EPA/635/R-09/011F. Washington, DC: US Environmental Protection Agency. Available at: http://www.epa.gov/iris/toxreviews/0199tr/0199tr.pdf. Accessed 18 October 2013.

EPA (2011b). *Toxicological Review of Trichloroethylene (CAS No. 79–01–6), Appendices*. In Support of Summary Information on the Integrated Risk Information System (IRIS). EPA/635/R-09/011F. Available at: www.epa.gov/iris/supdocs/0199index.html. Accessed August 2013.

Erdman AR, Mangravite LM, Urban TJ *et al.* (2006). The human organic anion transporter 3 (OAT3; *SLC22A8*): genetic variation and functional genomics. *Am J Physiol Renal Physiol*, 290: F905–F912. PMID:16291576

Eyre RJ, Stevens DK, Parker JC, Bull RJ (1995a). Acid-labile adducts to protein can be used as indicators of the cysteine S-conjugate pathway of trichloroethene metabolism. *J Toxicol Environ Health*, 46: 443–464. doi:10.1080/15287399509532048 PMID:8523471

Eyre RJ, Stevens DK, Parker JC, Bull RJ (1995b). Renal activation of trichloroethene and *S*-(1,2-dichlorovinyl)-*L*-cysteine and cell proliferative responses in the kidneys of F344 rats and B6C3F1 mice. *J Toxicol Environ Health*, 46: 465–481. doi:10.1080/15287399509532049 PMID:8523472

Fabricant JD, Chalmers JH Jr (1980). *Evidence of the mutagenicity of ethylene dichloride and structurally related compounds*. In *Ethylene Dichloride: A Potential Health Risk (Banbury Report 5)*. Ames B, Infante P, Reitz R, editors. Cold Spring Harbor, NY, USA: CSH Press, pp. 309–329.

Fagliano J, Berry M, Bove F, Burke T (1990). Drinking water contamination and the incidence of leukemia: an ecologic study. *Am J Public Health*, 80: 1209–1212. doi:10.2105/AJPH.80.10.1209 PMID:2400032

Fahrig R (1977). The mammalian spot test (Fellfleckentest) with mice. *Arch Toxicol*, 38: 87–98. doi:10.1007/BF00293666 PMID:199140

Fan AM (1988). Trichloroethylene: water contamination and health risk assessment. *Rev Environ Contam Toxicol*, 101: 55–92. doi:10.1007/978-1-4612-3770-9_2 PMID:3275994

Fan C, Wang GS, Chen YC, Ko CH (2009). Risk assessment of exposure to volatile organic compounds in groundwater in Taiwan. *Sci Total Environ*, 407: 2165–2174. doi:10.1016/j.scitotenv.2008.12.015 PMID:19167026

FDA (1977). Trichloroethylene. Removal from food additive use. *Fed Regist*, 42: 49465–49471.

Fernández JG, Droz PO, Humbert BE, Caperos JR (1977). Trichloroethylene exposure. Simulation of uptake, excretion, and metabolism using a mathematical model. *Br J Ind Med*, 34: 43–55. PMID:843463

Fevotte J, Charbotel B, Muller-Beauté P *et al.* (2006). Case-control study on renal cell cancer and occupational exposure to trichloroethylene. Part I: Exposure assessment. *Ann Occup Hyg*, 50: 765–775. doi:10.1093/annhyg/mel040 PMID:16840434

Fiserova-Bergerova V, Tichy M, Di Carlo FJ (1984). Effects of biosolubility on pulmonary uptake and disposition of gases and vapors of lipophilic chemicals. *Drug Metab Rev*, 15: 1033–1070. doi:10.3109/03602538409033557 PMID:6396052

Fishbein L (1976). Industrial mutagens and potential mutagens I. Halogenated aliphatic derivatives. *Mutat Res*, 32: 267–307. doi:10.1016/0165-1110(76)90003-8 PMID:785243

Fisher J, Mahle D, Bankston L *et al.* (1997). Lactational transfer of volatile chemicals in breast milk. *Am Ind Hyg Assoc J*, 58: 425–431. doi:10.1080/15428119791012667 PMID:9183837

Fisher JW, Gargas ML, Allen BC, Andersen ME (1991). Physiologically based pharmacokinetic modeling with trichloroethylene and its metabolite, trichloroacetic acid, in the rat and mouse. *Toxicol Appl Pharmacol*, 109: 183–195. doi:10.1016/0041-008X(91)90167-D PMID:2068722

Fisher JW, Mahle D, Abbas R (1998). A human physiologically based pharmacokinetic model for trichloroethylene and its metabolites, trichloroacetic acid and free trichloroethanol. *Toxicol Appl Pharmacol*, 152: 339–359. doi:10.1006/taap.1998.8486 PMID:9853003

Fisher JW, Whittaker TA, Taylor DH *et al.* (1989). Physiologically based pharmacokinetic modeling of the pregnant rat: a multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. *Toxicol Appl Pharmacol*, 99: 395–414. doi:10.1016/0041-008X(89)90149-X PMID:2749729

Fisher JW, Whittaker TA, Taylor DH *et al.* (1990). Physiologically based pharmacokinetic modeling of the lactating rat and nursing pup: a multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. *Toxicol Appl Pharmacol*, 102: 497–513. doi:10.1016/0041-008X(90)90045-V PMID:2315918

Fleming-Jones ME & Smith RE (2003). Volatile organic compounds in foods: a five year study. *J Agric Food Chem*, 51: 8120–8127. doi:10.1021/jf0303159 PMID:14690406

Flood TJ, Aickin M, Lucier JL, Petersen NJ (1990). *Incidence Study of Childhood Cancer in Maricopa County 1965–1986* (Final Report). Phoenix, Arizona: Department of Health Services

Forand SP, Lewis-Michl EL, Gomez MI (2012). Adverse birth outcomes and maternal exposure to trichloroethylene and tetrachloroethylene through soil vapor intrusion in New York State. *Environ Health Perspect*, 120: 616–621. doi:10.1289/ehp.1103884 PMID:22142966

Ford ES, Rhodes S, McDiarmid M *et al.* (1995). Deaths from acute exposure to trichloroethylene. *J Occup*

Environ Med, 37: 749–754. doi:10.1097/00043764-199506000-00019 PMID:7670923

Forkert P-G, Baldwin RM, Millen B *et al.* (2005). Pulmonary bioactivation of trichloroethylene to chloral hydrate: relative contributions of CYP2E1, CYP2F, and CYP2B1. *Drug Metab Dispos*, 33: 1429–1437. doi:10.1124/dmd.105.005074 PMID:15987776

Forkert PG & Forkert L (1994). Trichloroethylene induces pulmonary fibrosis in mice. *Can J Physiol Pharmacol*, 72: 205–210. doi:10.1139/y94-032 PMID:8069766

Forkert PG, Lash L, Tardif R *et al.* (2003). Identification of trichloroethylene and its metabolites in human seminal fluid of workers exposed to trichloroethylene. *Drug Metab Dispos*, 31: 306–311. doi:10.1124/dmd.31.3.306 PMID:12584157

Forkert PG, Lash LH, Nadeau V *et al.* (2002). Metabolism and toxicity of trichloroethylene in epididymis and testis. *Toxicol Appl Pharmacol*, 182: 244–254. doi:10.1006/taap.2002.9421 PMID:12183104

Forkert PG, Millen B, Lash LH *et al.* (2006). Pulmonary bronchiolar cytotoxicity and formation of dichloroacetyl lysine protein adducts in mice treated with trichloroethylene. *J Pharmacol Exp Ther*, 316: 520–529. doi:10.1124/jpet.105.093062 PMID:16269531

Forkert PG, Sylvestre PL, Poland JS (1985). Lung injury induced by trichloroethylene. *Toxicology*, 35: 143–160. doi:10.1016/0300-483X(85)90028-9 PMID:4002240

Foureman P, Mason JM, Valencia R, Zimmering S (1994). Chemical mutagenesis testing in Drosophila. IX. Results of 50 coded compounds tested for the National Toxicology Program. *Environ Mol Mutagen*, 23: 51–63. doi:10.1002/em.2850230109 PMID:8125083

Fox TR, Schumann AM, Watanabe PG *et al.* (1990). Mutational analysis of the H-*ras* oncogene in spontaneous C57BL/6 x C3H/He mouse liver tumors and tumors induced with genotoxic and nongenotoxic hepatocarcinogens. *Cancer Res*, 50: 4014–4019. PMID:2191770

Fredriksson M, Bengtsson NO, Hardell L, Axelson O (1989). Colon cancer, physical activity, and occupational exposures. A case-control study. *Cancer*, 63: 1838–1842. doi:10.1002/1097-0142(19900501)63:9<1838::AID-CNCR2820630930>3.0.CO;2-4 PMID:2702592

Fukuda K, Takemoto K, Tsuruta H (1983). Inhalation carcinogenicity of trichloroethylene in mice and rats. *Ind Health*, 21: 243–254. doi:10.2486/indhealth.21.243 PMID:6654707

Galloway SM, Armstrong MJ, Reuben C *et al.* (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. *Environ Mol Mutagen*, 10: Suppl 10: 1–175. doi:10.1002/em.2850100502 PMID:3319609

Garabrant DH, Held J, Langholz B, Bernstein L (1988). Mortality of aircraft manufacturing workers in southern California. *Am J Ind Med*, 13: 683–693. doi:\\ PMID:3389363

Garabrant DH, Lacey JV Jr, Laing TJ *et al.* (2003). Scleroderma and solvent exposure among women. *Am J Epidemiol*, 157: 493–500. doi:10.1093/aje/kwf223 PMID:12631538

Gargas ML, Burgess RJ, Voisard DE *et al.* (1989). Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. *Toxicol Appl Pharmacol*, 98: 87–99. doi:10.1016/0041-008X(89)90137-3 PMID:2929023

Gaskin PJ, Adcock HJ, Buckberry LD *et al.* (1995). The C-S lysis of L-cysteine conjugates by aspartate and alanine aminotransferase enzymes. *Hum Exp Toxicol*, 14: 422–427. doi:10.1177/096032719501400506 PMID:7612304

Ge R, Yang S, Kramer PM *et al.* (2001). The effect of dichloroacetic acid and trichloroacetic acid on DNA methylation and cell proliferation in B6C3F1 mice. *J Biochem Mol Toxicol*, 15: 100–106. doi:10.1002/jbt.5 PMID:11284051

George J, Byth K, Farrell GC (1995). Age but not gender selectively affects expression of individual cytochrome P450 proteins in human liver. *Biochem Pharmacol*, 50: 727–730. doi:10.1016/0006-2952(95)00192-3 PMID:7669077

GESTIS (2012). *GESTIS International Limit Values – Databases hazardous substances*. Institute for Work Protection of the German Accident Insurance. Available at http://limitvalue.ifa.dguv.de/Webform_gw.aspx. Accessed 19 November 2013.

Gilbert KM, Griffin JM, Pumford NR (1999). Trichloroethylene activates $CD4^+$ T cells: potential role in an autoimmune response. *Drug Metab Rev*, 31: 901–916. doi:\\ PMID:10575554

Gilbert KM, Przybyla B, Pumford NR *et al.* (2009). Delineating liver events in trichloroethylene-induced autoimmune hepatitis. *Chem Res Toxicol*, 22: 626–632. doi:\\ PMID:19254012

Gilles D, Philbin E (1976). *TRW Inc., Philadelphia, PA (Health Hazard Evaluation Determination Report No. 76-61-337)*. Cincinnati, OH: United States National Institute for Occupational Safety and Health

Glauser J, Ishikawa Y (2008). *Chemical Economics Handbook Marketing Research Report: C_2 Chlorinated Solvents*. Menlo Park, CA: SRI Consulting. Available at: http://chemical.ihs.com/nl/Public/2008Aug.pdf. Accessed 15 November 2013.

Goel SK, Rao GS, Pandya KP, Shanker R (1992). Trichloroethylene toxicity in mice: a biochemical, hematological and pathological assessment. *Indian J Exp Biol*, 30: 402–406. PMID:1459618

Goh VH, Chia SE, Ong CN (1998). Effects of chronic exposure to low doses of trichloroethylene on steroid hormone and insulin levels in normal men. *Environ Health Perspect*, 106: 41–44. doi:10.1289/ehp.9810641 PMID:9417767

Gold LS, Stewart PA, Milliken K *et al.* (2011). The relationship between multiple myeloma and occupational exposure to six chlorinated solvents. *Occup Environ Med*, 68: 391–399. doi:10.1136/oem.2009.054809 PMID:20833760

Goldsworthy TL, Lyght O, Burnett VL, Popp JA (1988). Potential role of α-2 mu-globulin, protein droplet accumulation, and cell replication in the renal carcinogenicity of rats exposed to trichloroethylene, perchloroethylene, and pentachloroethane. *Toxicol Appl Pharmacol*, 96: 367–379. doi:10.1016/0041-008X(88)90095-6 PMID:2461605

Goldsworthy TL & Popp JA (1987). Chlorinated hydrocarbon-induced peroxisomal enzyme activity in relation to species and organ carcinogenicity. *Toxicol Appl Pharmacol*, 88: 225–233. doi:10.1016/0041-008X(87)90008-1 PMID:3564041

Gorman R, Rinsky R, Stein G, Anderson K (1984). *Pratt & Whitney Aircraft, West Palm Beach, FL (HETA Report No. 82-075-1545)*. Cincinnati, OH: United States National Institute for Occupational Safety and Health

Gotoh Y, Kato Y, Stieger B *et al.* (2001). Gender difference in the Oatp1-mediated tubular reabsorption of estradiol 17β-D-glucuronide in rats. *Am J Physiol*, 282: E1245–E1254.

Grandjean E, Munchinger R, Turrian V *et al.* (1955). Investigations into the effects of exposure to trichlorethylene in mechanical engineering. *Br J Ind Med*, 12: 131–142. PMID:14363593

Green T, Dow J, Ellis MK *et al.* (1997b). The role of glutathione conjugation in the development of kidney tumours in rats exposed to trichloroethylene. *Chem Biol Interact*, 105: 99–117. doi:10.1016/S0009-2797(97)00040-9 PMID:9251723

Green T, Dow J, Foster J (2003). Increased formic acid excretion and the development of kidney toxicity in rats following chronic dosing with trichloroethanol, a major metabolite of trichloroethylene. *Toxicology*, 191: 109–119. doi:10.1016/S0300-483X(03)00206-3 PMID:12965114

Green T, Dow J, Foster JR, Hext PM (1998). Formic acid excretion in rats exposed to trichloroethylene: a possible explanation for renal toxicity in long-term studies. *Toxicology*, 127: 39–47. doi:10.1016/S0300-483X(98)00020-1 PMID:9699792

Green T, Dow J, Ong CN *et al.* (2004). Biological monitoring of kidney function among workers occupationally exposed to trichloroethylene. *Occup Environ Med*, 61: 312–317. doi:10.1136/oem.2003.007153 PMID:15031388

Green T, Mainwaring GW, Foster JR (1997a). Trichloroethylene-induced mouse lung tumors: studies of the mode of action and comparisons between species. *Fundam Appl Toxicol*, 37: 125–130. doi:10.1006/faat.1997.2312 PMID:9242585

Green T & Prout MS (1985). Species differences in response to trichloroethylene. II. Biotransformation in rats and mice. *Toxicol Appl Pharmacol*, 79: 401–411. doi:10.1016/0041-008X(85)90138-3 PMID:3929429

Greenberg MS, Burton GA, Fisher JW (1999). Physiologically based pharmacokinetic modeling of inhaled trichloroethylene and its oxidative metabolites in B6C3F1 mice. *Toxicol Appl Pharmacol*, 154: 264–278. doi:10.1006/taap.1998.8594 PMID:9931286

Greenland S, Salvan A, Wegman DH *et al.* (1994). A case-control study of cancer mortality at a transformer-assembly facility. *Int Arch Occup Environ Health*, 66: 49–54. doi:10.1007/BF00386579 PMID:7927843

Greim H, Bonse G, Radwan Z *et al.* (1975). Mutagenicity in vitro and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxiran formation. *Biochem Pharmacol*, 24: 2013–2017. doi:10.1016/0006-2952(75)90396-2 PMID:1108881

Griffin JM, Blossom SJ, Jackson SK *et al.* (2000a). Trichloroethylene accelerates an autoimmune response by Th1 T cell activation in MRL +/+ mice. *Immunopharmacology*, 46: 123–137. doi:10.1016/S0162-3109(99)00164-2 PMID:10647871

Griffin JM, Gilbert KM, Lamps LW, Pumford NR (2000b). $CD4^+$ T-cell activation and induction of autoimmune hepatitis following trichloroethylene treatment in MRL+/+ mice. *Toxicol Sci*, 57: 345–352. doi:10.1093/toxsci/57.2.345 PMID:11006364

Gu ZW, Sele B, Jalbert P *et al.* (1981). [Induction of sister chromatide exchange by trichloroethylene and its metabolites (author's transl)] *Toxicol Eur Res*, 3: 63–67. PMID:7245187

Gunter BJ (1977). *FMC Corp., Bloomfield, CO* (*HETA Report No. 76-101-376*). Cincinnati, OH: United States National Institute for Occupational Safety and Health

Hajimiragha H, Ewers U, Jansen-Rosseck R, Brockhaus A (1986). Human exposure to volatile halogenated hydrocarbons from the general environment. *Int Arch Occup Environ Health*, 58: 141–150. doi:10.1007/BF00380765 PMID:3744568

Hamdan H & Stacey NH (1993). Mechanism of trichloroethylene-induced elevation of individual serum bile acids. I. Correlation of trichloroethylene concentrations to bile acids in rat serum. *Toxicol Appl Pharmacol*, 121: 291–295. doi:10.1006/taap.1993.1156 PMID:8346545

Hansch C, Leo A, Hoekman D (1995). *Exploring QSAR: Hydrophobic, electronic, and steric constants*. Washington, DC: American Chemical Society, p. 4.

Hansen J, Raaschou-Nielsen O, Christensen JM *et al.* (2001). Cancer incidence among Danish workers exposed to trichloroethylene. *J Occup Environ Med*, 43: 133–139. doi:10.1097/00043764-200102000-00012 PMID:11227631

Hardell L, Eriksson M, Degerman A (1994). Exposure to phenoxyacetic acids, chlorophenols, or organic solvents in relation to histopathology, stage, and anatomical localization of non-Hodgkin's lymphoma. *Cancer Res*, 54: 2386–2389. PMID:8162585

Hardie DWF (1964). *Chlorocarbons and chlorohydrocarbons. 1,1,2,2-Tetrachloroethane*. In: *Encyclopedia of Chemical Technology*. Kirk RE, Othmer DF, editors. New York: John Wiley & Sons, pp. 159–164.

Hargarten JJ, Hetrick GH, Fleming AJ (1961). Industrial safety experience with trichlorethylene. Its use as a vapor degreasing solvent 1948–1957. *Arch Environ Health*, 3: 461–467. PMID:13904693

Harlan (2011). *Database for Wistar Rats from 2-Year Bioassays (Planned Sacrifice Schedule:> 103 Weeks) Individual incidence data in 50 studies conducted between 1980 and 2004–2006*. Available at: https://webapps.harlan.com/wistarhannover/. Accessed 11 November 2013.

Harpur E, Ennulat D, Hoffman D *et al.*; HESI Committee on Biomarkers of Nephrotoxicity (2011). Biological qualification of biomarkers of chemical-induced renal toxicity in two strains of male rat. *Toxicol Sci*, 122: 235–252. doi:10.1093/toxsci/kfr112 PMID:21593213

Haseman JK, Hailey JR, Morris RW (1998). Spontaneous neoplasm incidences in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: a National Toxicology Program update. *Toxicol Pathol*, 26: 428–441. doi:10.1177/019262339802600318 PMID:9608650

Hayden PJ, Ichimura T, McCann DJ *et al.* (1991). Detection of cysteine conjugate metabolite adduct formation with specific mitochondrial proteins using antibodies raised against halothane metabolite adducts. *J Biol Chem*, 266: 18415–18418. PMID:1917965

Hayden PJ, Welsh CJ, Yang Y *et al.* (1992). Formation of mitochondrial phospholipid adducts by nephrotoxic cysteine conjugate metabolites. *Chem Res Toxicol*, 5: 231–237. doi:10.1021/tx00026a013 PMID:1643252

Haynes WM (2012). *CRC Handbook of Chemistry and Physics, (Internet Version 2012)*. Boca Raton, FL: CRC Press/Taylor and Francis, No. EPA/625/R-96/010b, 92nd Ed. Available at: http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/8260b.pdf

Hegi ME, Fox TR, Belinsky SA *et al.* (1993). Analysis of activated protooncogenes in B6C3F1 mouse liver tumors induced by ciprofibrate, a potent peroxisome proliferator. *Carcinogenesis*, 14: 145–149. doi:10.1093/carcin/14.1.145 PMID:8425263

Hein MJ, Waters MA, Ruder AM *et al.* (2010). Statistical modeling of occupational chlorinated solvent exposures for case-control studies using a literature-based database. *Ann Occup Hyg*, 54: 459–472. doi:10.1093/annhyg/meq027 PMID:20418277

Heineman EF, Cocco P, Gómez MR *et al.* (1994). Occupational exposure to chlorinated aliphatic hydrocarbons and risk of astrocytic brain cancer. *Am J Ind Med*, 26: 155–169. doi:10.1002/ajim.4700260203 PMID:7977393

Henschler D (1987). *Mechanisms of genotoxicity of chlorinated aliphatic hydrocarbons*. In: *Selectivity and Molecular Mechanisms of Toxicity*. De Matteis F, Lock EA, editors. New York, USA: MacMillan, pp. 153–181.

Henschler D, Eder E, Neudecker T, Metzler M (1977). Carcinogenicity of trichloroethylene: fact or artifact? *Arch Toxicol*, 37: 233–236. doi:10.1007/BF00355492 PMID:332116

Henschler D, Elsässer H, Romen W, Eder E (1984). Carcinogenicity study of trichloroethylene, with and without epoxide stabilizers, in mice. *J Cancer Res Clin Oncol*, 107: 149–156. doi:10.1007/BF01032599 PMID:6736101

Henschler D, Romen W, Elsässer HM *et al.* (1980). Carcinogenicity study of trichloroethylene by longterm inhalation in three animal species. *Arch Toxicol*, 43: 237–248. doi:10.1007/BF00366179 PMID:7387385

Henschler D, Vamvakas S, Lammert M *et al.* (1995). Increased incidence of renal cell tumors in a cohort of cardboard workers exposed to trichloroethene. *Arch Toxicol*, 69: 291–299. doi:10.1007/s002040050173 PMID:7654132

Herren-Freund SL, Pereira MA, Khoury MD, Olson G (1987). The carcinogenicity of trichloroethylene and its metabolites, trichloroacetic acid and dichloroacetic acid, in mouse liver. *Toxicol Appl Pharmacol*, 90: 183–189. doi:10.1016/0041-008X(87)90325-5 PMID:3629594

Hiatt MH & Pia JH (2004). Screening processed milk for volatile organic compounds using vacuum distillation/gas chromatography/mass spectrometry. *Arch Environ Contam Toxicol*, 46: 189–196. PMID:15106669

Hoffmann D, Adler M, Vaidya VS *et al.* (2010). Performance of novel kidney biomarkers in preclinical toxicity studies. *Toxicol Sci*, 116: 8–22. doi:\\ PMID:20118187

Horesovsky G, Ginsler J, Everitt J, Walker C (1994). Increased susceptibility to *in vitro* transformation of cells carrying the Eker tumor susceptibility mutation. *Carcinogenesis*, 15: 2183–2187. doi:10.1093/carcin/15.10.2183 PMID:7955051

Hosgood HD 3rd, Zhang L, Tang X *et al.* (2011). Decreased numbers of $CD4^+$ naive and effector memory T cells, and $CD8^+$ naïve T cells, are associated with trichloroethylene exposure. *Front Oncol*, 1: 53 doi:10.3389/fonc.2011.00053 PMID:22649769

Hrelia P, Maffei F, Vigagni F *et al.* (1994). Interactive effects between trichloroethylene and pesticides at metabolic and genetic level in mice. *Environ Health Perspect*, 102: Suppl 9: 31–34. doi:10.1289/ehp.94102s931 PMID:7698080

Hu C, Jiang L, Geng C *et al.* (2008). Possible involvement of oxidative stress in trichloroethylene-induced genotoxicity in human HepG2 cells. *Mutat Res*, 652: 88–94. doi:10.1016/j.mrgentox.2008.01.002 PMID:18289923

Huybrechts T, Dewulf J, Van Langenhove H (2005). Priority volatile organic compounds in surface waters of the southern North Sea. *Environ Pollut*, 133: 255–264. doi:10.1016/j.envpol.2004.05.039 PMID:15519456

IARC (1979). Some halogenated hydrocarbons. *IARC Monogr Eval Carcinog Risk Chem Hum*, 20: 1–609. PMID:296120

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. PMID:3482203

IARC (1995). Dry-cleaning, some chlorinated solvents and other industrial chemicals. *IARC Monogr Eval Carcinog Risks Hum*, 63: 1–551.PMID:9139128

IARC (2010). Alcohol consumption and ethyl carbamate. *IARC Monogr Eval Carcinog Risks Hum*, 96: 1–1428. PMID:21735939

IARC (2012). Personal habits and indoor combustions. *IARC Monogr Eval Carcinog Risks Hum*, 100E: 1–575. PMID:23193840

Iavicoli I, Marinaccio A, Carelli G (2005). Effects of occupational trichloroethylene exposure on cytokine levels in workers. *J Occup Environ Med*, 47: 453–457. doi:\\ PMID:15891523

Ikeda M & Imanura T (1973). Biological half-life of trichloroethylene and tetrachloroethylene in human subjects. *Int Arch Arbeitsmed*, 31: 209–224. doi:10.1007/BF00539241 PMID:4593976

Ikeda M, Otsuji H, Kawai H, Kuniyoshi M (1971). Excretion kinetics of urinary metabolites in a patient addicted to trichloroethylene. *Br J Ind Med*, 28: 203–206. PMID:5572691

Imbriani M, Niu Q, Negri S, Ghittori S (2001). Trichloroethylene in urine as biological exposure index. *Ind Health*, 39: 225–230. doi:10.2486/indhealth.39.225 PMID:11499997

Isacson P, Bean JA, Splinter R *et al.* (1985). Drinking water and cancer incidence in Iowa. III. Association of cancer with indices of contamination. *Am J Epidemiol*, 121: 856–869. PMID:4014178

Ito Y, Yamanoshita O, Asaeda N *et al.* (2007). Di(2-ethylhexyl)phthalate induces hepatic tumorigenesis through a peroxisome proliferator-activated receptor alpha-independent pathway. *J Occup Health*, 49: 172–182. doi:10.1539/joh.49.172 PMID:17575397

Jackson MA, Stack HF, Waters MD (1993). The genetic toxicology of putative nongenotoxic carcinogens. *Mutat Res*, 296: 241–277. doi:10.1016/0165-1110(93)90014-E PMID:7680106

Jaffe DR, Gandolfi AJ, Nagle RB (1984). Chronic toxicity of *S-(trans*-1,2-dichlorovinyl)-L-cysteine in mice. *J Appl Toxicol*, 4: 315–319. doi:10.1002/jat.2550040607 PMID:6520320

Jaffe DR, Hassall CD, Gandolfi AJ, Brendel K (1985). Production of DNA single strand breaks in rabbit renal tissue after exposure to 1,2-dichlorovinyl-cysteine. *Toxicology*, 35: 25–33. doi:10.1016/0300-483X(85)90129-5 PMID:4002236

Jakubowski M & Wieczorek H (1988). The effects of physical effort on pulmonary uptake of selected organic compound vapours. *Pol J Occup Med*, 1: 62–71. PMID:2980152

Jia C, Yu X, Masiak W (2012). Blood/air distribution of volatile organic compounds (VOCs) in a nationally representative sample. *Sci Total Environ*, 419: 225–232. doi:10.1016/j.scitotenv.2011.12.055 PMID:22285084

Johnson P (1980). *Miami Carey, Inc., Monroe, OH (Health Hazard Evaluation Report No. 80-48-689)*. Cincinnati, OH: United States National Institute for Occupational Safety and Health

Johnsrud EK, Koukouritaki SB, Divakaran K *et al.* (2003). Human hepatic CYP2E1 expression during development. *J Pharmacol Exp Ther*, 307: 402–407. doi:10.1124/jpet.102.053124 PMID:14500779

Kamijima M, Hisanaga N, Wang H, Nakajima T (2007). Occupational trichloroethylene exposure as a cause of idiosyncratic generalized skin disorders and accompanying hepatitis similar to drug hypersensitivities. *Int Arch Occup Environ Health*, 80: 357–370. doi:10.1007/s00420-006-0147-y PMID:17106739

Kamijima M, Wang H, Huang H *et al.* (2008). Trichloroethylene causes generalized hypersensitivity skin disorders complicated by hepatitis. *J Occup Health*, 50: 328–338. doi:\\ PMID:18540116

Kaneko T, Saegusa M, Tasaka K, Sato A (2000). Immunotoxicity of trichloroethylene: a study with MRL-*lpr/lpr* mice. *J Appl Toxicol*, 20: 471–475. doi:10.1002/1099-1263(200011/12)20:6<471::AID-JAT716>3.0.CO;2-E PMID:11180269

Karami S, Lan Q, Rothman N *et al.* (2012In press). Occupational trichloroethylene exposure and kidney cancer risk: a meta-analysis. *Occup Environ Med*,

Kato Y, Kuge K, Kusuhara H *et al.* (2002). Gender difference in the urinary excretion of organic anions in rats. *J Pharmacol Exp Ther*, 302: 483–489. doi:10.1124/jpet.102.033878 PMID:12130705

Katoh T, Yamano Y, Tsuji M, Watanabe M (2008). Genetic polymorphisms of human cytosol glutathione *S*-transferases and prostate cancer. *Pharmacogenomics*, 9: 93–104. doi:10.2217/14622416.9.1.93 PMID:18154451

Kauppinen T, Toikkanen J, Pedersen D *et al.* (2000). Occupational exposure to carcinogens in the European Union. *Occup Environ Med*, 57: 10–18. doi:10.1136/oem.57.1.10 PMID:10711264

Keenan KP, Soper KA, Smith PF *et al.* (1995). Diet, overfeeding, and moderate dietary restriction in control Sprague-Dawley rats: I. Effects on spontaneous neoplasms. *Toxicol Pathol*, 23: 269–286. doi:10.1177/019262339502300305 PMID:7659952

Keil DE, Peden-Adams MM, Wallace S *et al.* (2009). Assessment of trichloroethylene (trichloroethylene) exposure in murine strains genetically-prone and non-prone to develop autoimmune disease. *J Environ Sci Health A Tox Hazard Subst Environ Eng*, 44: 443–453. doi:\\ PMID:19241258

Kelsh MA, Alexander DD, Mink PJ, Mandel JH (2010). Occupational trichloroethylene exposure and kidney cancer: a meta-analysis. *Epidemiology*, 21: 95–102. doi:10.1097/EDE.0b013e3181c30e92 PMID:20010212

KEMI; Swedish Chemicals Agency (2012). *Chlorinated solvents*. Available at: http://www.kemi.se/en/Content/Statistics/Statistics-in-brief/Statistics-in-brief---Substances-and-substance-groups/Chlorinated-solvents/. Accessed 19 November 2013.

Kernan GJ, Ji BT, Dosemeci M *et al.* (1999). Occupational risk factors for pancreatic cancer: a case-control study based on death certificates from 24 U.S. states. *Am J Ind Med*, 36: 260–270. doi:10.1002/(SICI)1097-0274(199908)36:2<260::AID-AJIM5>3.0.CO;2-P PMID:10398934

Keys DA, Bruckner JV, Muralidhara S, Fisher JW (2003). Tissue dosimetry expansion and cross-validation of rat and mouse physiologically based pharmacokinetic models for trichloroethylene. *Toxicol Sci*, 76: 35–50. doi:10.1093/toxsci/kfg212 PMID:12915716

Kezic S, Monster AC, Krüse J, Verberk MM (2000). Skin absorption of some vaporous solvents in volunteers. *Int Arch Occup Environ Health*, 73: 415–422. doi:10.1007/s004200000161 PMID:11007346

Kezic S, Monster AC, van de Gevel IA *et al.* (2001). Dermal absorption of neat liquid solvents on brief exposures in volunteers. *AIHAJ*, 62: 12–18. doi:10.1080/15298660108984604 PMID:11258863

Khan MF, Kaphalia BS, Prabhakar BS *et al.* (1995). Trichloroethene-induced autoimmune response in female MRL +/+ mice. *Toxicol Appl Pharmacol*, 134: 155–160. doi:10.1006/taap.1995.1179 PMID:7676450

Kilburn KH & Warshaw RH (1992). Prevalence of symptoms of systemic lupus erythematosus (SLE) and of fluorescent antinuclear antibodies associated with chronic exposure to trichloroethylene and other chemicals in well water. *Environ Res*, 57: 1–9. doi:10.1016/S0013-9351(05)80014-3 PMID:1740091

Kim D & Ghanayem BI (2006). Comparative metabolism and disposition of trichloroethylene in Cyp2e1-/- and wild-type mice. *Drug Metab Dispos*, 34: 2020–2027. doi:10.1124/dmd.106.010538 PMID:16959879

Kioski C, Heubach S, Petersen NJ (1990). *The Incidence of Childhood Leukemia and Testicular Cancer in Pima County: 1970–1986, Tucson*. Arizona Department of Health Services.

Kjellstrand P, Holmquist B, Alm P *et al.* (1983b). Trichloroethylene: further studies of the effects on body and organ weights and plasma butyrylcholinesterase activity in mice. *Acta Pharmacol Toxicol (Copenh)*, 53: 375–384. doi:10.1111/j.1600-0773.1983.tb03438.x PMID:6659967

Kjellstrand P, Holmquist B, Mandahl N, Bjerkemo M (1983a). Effects of continuous trichloroethylene inhalation on different strains of mice. *Acta Pharmacol Toxicol (Copenh)*, 53: 369–374. doi:10.1111/j.1600-0773.1983.tb03437.x PMID:6659966

Kjellstrand P, Kanje M, Månsson L *et al.* (1981). Trichloroethylene: effects on body and organ weights in mice, rats and gerbils. *Toxicology*, 21: 105–115. doi:10.1016/0300-483X(81)90121-9 PMID:7281199

Klaunig JE, Ruch RJ, Lin EL (1989). Effects of trichloroethylene and its metabolites on rodent hepatocyte intercellular communication. *Toxicol Appl Pharmacol*, 99: 454–465. doi:10.1016/0041-008X(89)90153-1 PMID:2749732

Kligerman AD, Bryant MF, Doerr CL *et al.* (1994). Inhalation studies of the genotoxicity of trichloroethylene to rodents. *Mutat Res*, 322: 87–96. doi:10.1016/0165-1218(94)00013-1 PMID:7519326

Kobayashi Y, Hirokawa N, Ohshiro N *et al.* (2002). Differential gene expression of organic anion transporters in male and female rats. *Biochem Biophys Res Commun*, 290: 482–487. doi:10.1006/bbrc.2001.6180 PMID:11779196

Koch R, Schlegelmilch R, Wolf HU (1988). Genetic effects of chlorinated ethylenes in the yeast Saccharomyces cerevisiae. *Mutat Res*, 206: 209–216. doi:10.1016/0165-1218(88)90162-0 PMID:3050501

Koizumi A (1989). Potential of physiologically based pharmacokinetics to amalgamate kinetic data of trichloroethylene and tetrachloroethylene obtained in rats and man. *Br J Ind Med*, 46: 239–249. PMID:2713280

Kominsky JR (1976). *Dana Corp., Tipton, IN (Health Hazard Evaluation Report No. 76-24-350; US NTIS PB-273716)*. Cincinnati, OH: United States National Institute for Occupationa1 Safety and Health

Korrapati MC, Chilakapati J, Lock EA *et al.* (2006). Preplaced cell division: a critical mechanism of autoprotection against S-1,2-dichlorovinyl-L-cysteine-induced acute renal failure and death in mice. *Am J Physiol Renal Physiol*, 291: F439–F455. doi:10.1152/ajprenal.00384.2005 PMID:16495211

Korrapati MC, Chilakapati J, Witzmann FA *et al.* (2007). Rao, C., Lock, E.A., and Mehendale, H.M. Proteomics of *S*-(1,2-dichlorovinyl)-*L*-cysteine-induced acute renal failure and autoprotection in mice. *Am J Physiol*, 293: F994–F1006. doi:10.1152/ajprenal.00114.2007

Korrapati MC, Lock EA, Mehendale HM (2005). Molecular mechanisms of enhanced renal cell division in protection against S-1,2-dichlorovinyl-L-cysteine-induced acute renal failure and death. *Am J Physiol Renal Physiol*, 289: F175–F185. doi:10.1152/ajprenal.00418.2004 PMID:15741605

Kostopoulou MN, Golfinopoulos SK, Nikolaou AD *et al.* (2000). Volatile organic compounds in the surface waters of northern Greece. *Chemosphere*, 40: 527–532. doi:10.1016/S0045-6535(99)00293-3 PMID:10665390

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 PMID:12490590

Kringstad KP, Ljungquist PO, De Sousa F, Stroemberg LM (1981). Identification and mutagenic properties of some chlorinated aliphatic compounds in the spent liquor from kraft pulp chlorination. *Environ Sci Technol*, 15: 562–566. doi:10.1021/es00087a006 PMID:22283948

Kromhout H, Swuste P, Boleij JSM (1994). Empirical modelling of chemical exposure in the rubber-manufacturing industry. *Ann Occup Hyg*, 38: 3–22. doi:10.1093/annhyg/38.1.3 PMID:8161092

Kroneld R (1989). Volatile pollutants in the environment and human tissues. *Bull Environ Contam Toxicol*, 42: 873–877. doi:10.1007/BF01701629 PMID:2743020

Kronoveter KJ, Boiano JL (1984). *Charlies' Taxidermy and Gifts, Fleetwood, PA (Health Hazard Evaluation Report No. 83-276-1499)*. Cincinnati, OH: United States National Institute for Occupational Safety and Health

Kubo Y, Mitani H, Hino O (1994). Allelic loss at the predisposing gene locus in spontaneous and chemically induced renal cell carcinomas in the Eker rat. *Cancer Res*, 54: 2633–2635. PMID:8168090

Kumar M, Tewari S, Sharma P *et al.* (2009). Study of genetic polymorphism in solvent exposed population and its correlation to in vitro effect of trichloroethylene on lymphocytes. *J Environ Biol*, 30: 685–691. PMID:20136049

Kumar P, Prasad AK, Dutta KK (2000). Steroidogenic alterations in testes and sera of rats exposed to trichloroethylene (trichloroethylene) by inhalation. *Hum Exp Toxicol*, 19: 117–121. doi:10.1191/096032700678815657 PMID:10773841

Kumar P, Prasad AK, Mani U *et al.* (2001). Trichloroethylene induced testicular toxicity in rats exposed by inhalation. *Hum Exp Toxicol*, 20: 585–589. doi:10.1191/096032701718620882 PMID:11926613

Lacey JV Jr, Garabrant DH, Laing TJ *et al.* (1999). Petroleum distillate solvents as risk factors for undifferentiated connective tissue disease (UCTD). *Am J Epidemiol*, 149: 761–770. doi:10.1093/oxfordjournals.aje.a009885 PMID:10206626

Lagakos S, Wessen B, Zelen M (1986). An analysis of contaminated well water and health effects in Woburn, Massachusetts. *J Am Stat Assoc*, 81: 583–596. doi:10.1080/01621459.1986.10478307

Laham S (1970). Studies on placental transfer. Trichlorethylene. *IMS Ind Med Surg*, 39: 46–49. PMID:5263053

Lan Q, Zhang L, Tang X *et al.* (2010). Occupational exposure to trichloroethylene is associated with a decline in lymphocyte subsets and soluble CD27 and CD30 markers. *Carcinogenesis*, 31: 1592–1596. doi:10.1093/carcin/bgq121 PMID:20530238

Landrigan PJ, Kominsky JR, Stein GF *et al.* (1987). Common-source community and industrial exposure to trichloroethylene. *Arch Environ Health*, 42: 327–332. doi:10.1080/00039896.1987.9934354 PMID:3439809

Larson JL & Bull RJ (1992b). Metabolism and lipoperoxidative activity of trichloroacetate and dichloroacetate in rats and mice. *Toxicol Appl Pharmacol*, 115: 268–277. doi:10.1016/0041-008X(92)90332-M PMID:1641860

Lash LH (2007). Glutathione-dependent bioactivation *Curr Protoc Toxicol*, 34: 7901–7916.

Lash LH & Anders MW (1986). Cytotoxicity of *S*-(1,2-dichlorovinyl)glutathione and *S*-(1,2-dichlorovinyl)-*L*-cysteine in isolated rat kidney cells. *J Biol Chem*, 261: 13076–13081. PMID:2875994

Lash LH & Anders MW (1987). Mechanism of *S*-(1,2-dichlorovinyl)-*L*-cysteine- and *S*-(1,2-dichlorovinyl)-*L*-homocysteine-induced renal mitochondrial toxicity. *Mol Pharmacol*, 32: 549–556. PMID:3670284

Lash LH, Elfarra AA, Anders MW (1986). Renal cysteine conjugate β-lyase. Bioactivation of nephrotoxic cysteine S-conjugates in mitochondrial outer membrane. *J Biol Chem*, 261: 5930–5935. PMID:3700378

Lash LH, Fisher JW, Lipscomb JC, Parker JC (2000a). Metabolism of trichloroethylene. *Environ Health Perspect*, 108: Suppl 2: 177–200. doi:10.1289/ehp.00108s2177 PMID:10807551

Lash LH, Hueni SE, Putt DA (2001a). Apoptosis, necrosis, and cell proliferation induced by *S*-(1,2-dichlorovinyl)-*L*-cysteine in primary cultures of human proximal tubular cells. *Toxicol Appl Pharmacol*, 177: 1–16. doi:\\ PMID:11708895

Lash LH, Nelson RM, Van Dyke RA, Anders MW (1990). Purification and characterization of human kidney cytosolic cysteine conjugate β-lyase activity. *Drug Metab Dispos*, 18: 50–54. PMID:2139845

Lash LH, Parker JC, Scott CS (2000b). Modes of action of trichloroethylene for kidney tumorigenesis. *Environ Health Perspect*, 108: Suppl 2: 225–240. doi:10.1289/ehp.00108s2225 PMID:10807554

Lash LH, Putt DA, Cai H (2006b). Membrane transport function in primary cultures of human proximal tubular cells. *Toxicology*, 228: 200–218. doi:10.1016/j.tox.2006.08.035 PMID:16997449

Lash LH, Putt DA, Hueni SE *et al.* (2003). Roles of necrosis, Apoptosis, and mitochondrial dysfunction in *S*-(1,2-dichlorovinyl)-*L*-cysteine sulfoxide-induced cytotoxicity in primary cultures of human renal proximal tubular cells. *J Pharmacol Exp Ther*, 305: 1163–1172. doi:\\ PMID:12626654

Lash LH, Putt DA, Hueni SE, Horwitz BP (2005). Molecular markers of trichloroethylene-induced toxicity in human kidney cells. *Toxicol Appl Pharmacol*, 206: 157–168. doi:10.1016/j.taap.2004.09.023 PMID:15967204

Lash LH, Putt DA, Parker JC (2006a). Metabolism and tissue distribution of orally administered trichloroethylene in male and female rats: identification of glutathione- and cytochrome P-450-derived metabolites in liver, kidney, blood, and urine. *J Toxicol Environ Health*

A, 69: 1285–1309. doi:10.1080/15287390500360133 PMID:16754541

Lash LH, Qian W, Putt DA *et al.* (1998). Glutathione conjugation of trichloroethylene in rats and mice: sex-, species-, and tissue-dependent differences. *Drug Metab Dispos*, 26: 12–19. PMID:9443846

Lash LH, Qian W, Putt DA *et al.* (2001b). Renal and hepatic toxicity of trichloroethylene and its glutathione-derived metabolites in rats and mice: sex-, species-, and tissue-dependent differences. *J Pharmacol Exp Ther*, 297: 155–164. PMID:11259540

Laughter AR, Dunn CS, Swanson CL *et al.* (2004). Role of the peroxisome proliferator-activated receptor alpha (PPARalpha) in responses to trichloroethylene and metabolites, trichloroacetate and dichloroacetate in mouse liver. *Toxicology*, 203: 83–98. doi:10.1016/j.tox.2004.06.014 PMID:15363585

Lee LJ, Chan CC, Chung CW *et al.* (2002). Health risk assessment on residents exposed to chlorinated hydrocarbons contaminated in groundwater of a hazardous waste site. *J Toxicol Environ Health A*, 65: 219–235. doi:10.1080/15287390252800828 PMID:11911487

Lee S, Parkinson D (1982). *Rola-Esmark Co., Dubois, PA (Health Hazard Evaluation Report No. 80-168-1204).* Cincinnati, OH: United States National Institute for Occupational Safety and Health

Lehmann I, Rehwagen M, Diez U *et al.*; Leipzig Allergy Risk Children Study (2001). Enhanced in vivo IgE production and T cell polarization toward the type 2 phenotype in association with indoor exposure to VOC: results of the LARS study. *Int J Hyg Environ Health*, 204: 211–221. doi:10.1078/1438-4639-00100 PMID:11833293

Lehmann I, Thoelke A, Rehwagen M *et al.* (2002). The influence of maternal exposure to volatile organic compounds on the cytokine secretion profile of neonatal T cells. *Environ Toxicol*, 17: 203–210. doi:10.1002/tox.10055 PMID:12112628

Leigh JP & Jiang WY (1993). Liver cirrhosis deaths within occupations and industries in the California occupational mortality study. *Addiction*, 88: 767–779. doi:10.1111/j.1360-0443.1993.tb02091.x PMID:8329968

Linak E, Leder A, Yoshida Y (1992). *Chlorinated Solvents.* In: *Chemical Economies Handbook.* Menlo Park, CA: SRI International, 632.3000–632.3001.

Lindbohm ML, Sallmén M, Kyyrönen P *et al.* (2009). Risk of liver cancer and exposure to organic solvents and gasoline vapors among Finnish workers. *Int J Cancer*, 124: 2954–2959. doi:10.1002/ijc.24309 PMID:19319983

Lipworth L, Sonderman JS, Mumma MT *et al.* (2011). Cancer mortality among aircraft manufacturing workers: an extended follow-up. *J Occup Environ Med*, 53: 992–1007. doi:10.1097/JOM.0b013e31822e0940 PMID:21866047

Ljubojevic M, Herak-Kramberger CM, Hagos Y *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: F124–F138. doi:10.1152/ajprenal.00029.2004 PMID:15010355

Lock EA, Barth JL, Argraves SW, Schnellmann RG (2006). Changes in gene expression in human renal proximal tubule cells exposed to low concentrations of *S*-(1,2-dichlorovinyl)-*l*-cysteine, a metabolite of trichloroethylene. *Toxicol Appl Pharmacol*, 216: 319–330. doi:10.1016/j.taap.2006.06.002 PMID:16844155

Loprieno N, Abbondandolo A (1980). *Comparative mutagenic evaluation of some industrial compounds.* In: *Short-term Test Systems for Detecting Carcinogens.* Norpoth KH, Garner RC, editors. Berlin, Germany: Springer-Verlag, pp. 333–356.

Love JR, Kern M (1981). *Metro Bus Maintenance Shop, Washington, DC (Health Hazard Evaluation Report No. 81-065-938).* Cincinnati, OH: United States National Institute for Occupational Safety and Health

Lowengart RA, Peters JM, Cicioni C *et al.* (1987). Childhood leukemia and parents' occupational and home exposures. *J Natl Cancer Inst*, 79: 39–46. PMID:3474448

Ludwig HR (1981). *Occupational exposure to perchloroethylene in the dry-cleaning industry.* Cincinnati, Ohio: NIOSH, pp. 1–102

Mahle DA, Gearhart JM, Grigsby CC *et al.* (2007). Age-dependent partition coefficients for a mixture of volatile organic solvents in Sprague-Dawley rats and humans. *J Toxicol Environ Health A*, 70: 1745–1751. doi:10.1080/15287390701458991 PMID:17885931

Maita K, Hirano M, Harada T *et al.* (1988). Mortality, major cause of moribundity, and spontaneous tumours in CD-1 mice. *Toxicol Pathol*, 16: 340–349. doi:10.1177/019262338801600305 PMID:3194656

Malek B, Kromarova B, Rodova O (1979).[An epidemiological study of hepatic tumour incidence in subjects working with trichloroethylene. II. Negative result of retrospective investigations in dry-cleaners]*Prac Lek*, 31: 124–126.

Malherbe P, Alberati-Giani D, Köhler C, Cesura AM (1995). Identification of a mitochondrial form of kynurenine aminotransferase/glutamine transaminase K from rat brain. *FEBS Lett*, 367: 141–144. doi:10.1016/0014-5793(95)00546-L PMID:7796908

Mallin K (1990). Investigation of a bladder cancer cluster in northwestern Illinois. *Am J Epidemiol*, 132: Suppl: S96–S106. PMID:2356842

Mally A, Walker CL, Everitt JI *et al.* (2006). Analysis of renal cell transformation following exposure to trichloroethene in vivo and its metabolite *S*-(dichlorovinyl)-*L*-cysteine *in vitro*. *Toxicology*, 224: 108–118. doi:10.1016/j.tox.2006.04.036 PMID:16730402

Maloney EK & Waxman DJ (1999). *trans*-Activation of PPARalpha and PPARgamma by structurally diverse environmental chemicals. *Toxicol Appl Pharmacol*, 161: 209–218. doi:10.1006/taap.1999.8809 PMID:10581215

Maltoni C, Lefemine G, Cotti G (1986). *Experimental research on trichloroethylene carcinogenesis, Volume 5*. Princeton, NJ: Princeton Scientific Publishing, pp. 1–393.

Maltoni C, Lefemine G, Cotti G, Perino G (1988). Long-term carcinogenicity bioassays on trichloroethylene administered by inhalation to Sprague-Dawley rats and Swiss and B6C3F1 mice. *Ann N Y Acad Sci*, 53: 4:: 316–342. doi:10.1111/j.1749-6632.1988.tb30120.x PMID:3389663

Mandel JH, Kelsh MA, Mink PJ *et al.* (2006). Occupational trichloroethylene exposure and non-Hodgkin's lymphoma: a meta-analysis and review. *Occup Environ Med*, 63: 597–607. doi:10.1136/oem.2005.022418 PMID:16644896

Marano DE, Boice JD Jr, Fryzek JP *et al.* (2000). Exposure assessment for a large epidemiological study of aircraft manufacturing workers. *Appl Occup Environ Hyg*, 15: 644–656. doi:10.1080/10473220050075653 PMID:10957820

Martin SA, Simmons MB, Ortiz-Serrano M *et al.* (2005). Environmental exposure of a community to airborne trichloroethylene. *Arch Environ Occup Health*, 60: 314–316. doi:10.3200/AEOH.60.6.314-316 PMID:17447576

Mazzullo M, Bartoli S, Bonora B *et al.* (1992). In vivo and in vitro interaction of trichloroethylene with macromolecules from various organs of rat and mouse. *Res Commun Chem Pathol Pharmacol*, 76: 192–208. PMID:1376481

McCarthy MC, Hafner HR, Montzka SA (2006). Background concentrations of 18 air toxics for North America. *J Air Waste Manag Assoc*, 56: 3–11. doi:10.1080/10473289.2006.10464436 PMID:16499141

McCarver DG & Hines RN (2002). The ontogeny of human drug-metabolizing enzymes: phase II conjugation enzymes and regulatory mechanisms. *J Pharmacol Exp Ther*, 300: 361–366. doi:10.1124/jpet.300.2.361 PMID:11805192

McConnell G, Ferguson DM, Pearson CR (1975). Chlorinated hydrocarbons and the environment. *Endeavour*, 34: 13–18. doi:10.1016/0160-9327(75)90062-9 PMID:54249

McGoldrick TA, Lock EA, Rodilla V, Hawksworth GM (2003). Renal cysteine conjugate C-S lyase mediated toxicity of halogenated alkenes in primary cultures of human and rat proximal tubular cells. *Arch Toxicol*, 77: 365–370. doi:10.1007/s00204-003-0459-6 PMID:12700887

McGregor DB, Reynolds DM, Zeiger E (1989). Conditions affecting the mutagenicity of trichloroethylene in Salmonella. *Environ Mol Mutagen*, 13: 197–202. doi:10.1002/em.2850130302 PMID:2651116

McKinney PA, Alexander FE, Cartwright RA, Parker L (1991). Parental occupations of children with leukaemia in west Cumbria, north Humberside, and Gateshead. *BMJ*, 302: 681–687. doi:10.1136/bmj.302.6778.681 PMID:2021741

McMartin DN, Sahota PS, Gunson DE *et al.* (1992). Neoplasms and related proliferative lesions in control Sprague-Dawley rats from carcinogenicity studies. Historical data and diagnostic considerations. *Toxicol Pathol*, 20: 212–225. doi:10.1177/019262339202000208 PMID:1475582

Melnick RL, Jameson CW, Goehl TJ *et al.* (1987). Application of microencapsulation for toxicology studies. II. Toxicity of microencapsulated trichloroethylene in Fischer 344 rats. *Fundam Appl Toxicol*, 8: 432–442. doi:10.1016/0272-0590(87)90129-1 PMID:3111923

Mensing T, Welge P, Voss B *et al.* (2002). Renal toxicity after chronic inhalation exposure of rats to trichloroethylene. *Toxicol Lett*, 128: 243–247. doi:10.1016/S0378-4274(01)00545-8 PMID:11869834

Merrick BA, Robinson M, Condie LW (1989). Differing hepatotoxicity and lethality after subacute trichloroethylene exposure in aqueous or corn oil gavage vehicles in B6C3F1 mice. *J Appl Toxicol*, 9: 15–21. doi:10.1002/jat.2550090105 PMID:2926094

Mersch-Sundermann V, Müller G, Hofmeister A (1989). Examination of mutagenicity of organic microcontaminations of the environment. IV. Communication: the mutagenicity of halogenated aliphatic hydrocarbons with the SOS-chromotest. [in German]. *Zbl. Hyg*, 189: 266–271.

Mertens JA (1993). *Chlorocarbons and chlorohydrocarbons*. In: *Kirk-Othmer Encyclopedia of Chemical Technology, 4th Ed.* Kroschwitz JI, Howe-Grant M, editors. New York: John Wiley & Sons, pp. 40–50.

Messing K, Punnett L, Bond M *et al.* (2003). Be the fairest of them all: challenges and recommendations for the treatment of gender in occupational health research. *Am J Ind Med*, 43: 618–629. doi:10.1002/ajim.10225 PMID:12768612

Miligi L, Costantini AS, Benvenuti A *et al.* (2006). Occupational exposure to solvents and the risk of lymphomas. *Epidemiology*, 17: 552–561. doi:10.1097/01.ede.0000231279.30988.4d PMID:16878041

Miller RE & Guengerich FP (1983). Metabolism of trichloroethylene in isolated hepatocytes, microsomes, and reconstituted enzyme systems containing cytochrome P-450. *Cancer Res*, 43: 1145–1152. PMID:6825087

Milman HA, Story DL, Riccio ES *et al.* (1988). Rat liver foci and *in vitro* assays to detect initiating and promoting effects of chlorinated ethanes and ethylenes. *Ann N Y Acad Sci*, 534:: 521–530. doi:10.1111/j.1749-6632.1988.tb30143.x PMID:3389679

Mirabelli D & Kauppinen T (2005). Occupational exposures to carcinogens in Italy: an update of CAREX database. *Int J Occup Environ Health*, 11: 53–63. PMID:15859192

Mirsalis JC, Tyson CK, Steinmetz KL *et al.* (1989). Measurement of unscheduled DNA synthesis and S-phase synthesis in rodent hepatocytes following in vivo treatment: testing of 24 compounds. *Environ Mol Mutagen*, 14: 155–164. doi:10.1002/em.2850140305 PMID:2792091

Mirza T, Gérin M, Bégin D, Drolet D (2000). A study on the substitution of trichloroethylene as a spot remover in the textile industry. *AIHAJ*, 61: 431–438. doi:10.1080/15298660008984555 PMID:10885896

Monster AC, Boersma G, Duba WC (1976). Pharmacokinetics of trichloroethylene in volunteers, influence of workload and exposure concentration. *Int Arch Occup Environ Health*, 38: 87–102. doi:10.1007/BF00378619 PMID:1002309

Moon CS, Lee JT, Chun JH, Ikeda M (2001). Use of solvents in industries in Korea: experience in Sinpyeong-Jangrim industrial complex. *Int Arch Occup Environ Health*, 74: 148–152. doi:10.1007/s004200000197 PMID:11317709

Moore LE, Boffetta P, Karami S *et al.* (2010). Occupational trichloroethylene exposure and renal carcinoma risk: evidence of genetic susceptibility by reductive metabolism gene variants. *Cancer Res*, 70: 6527–6536. doi:10.1158/0008-5472.CAN-09-4167 PMID:20663906

Moore LE, Nickerson ML, Brennan P *et al.* (2011). Von Hippel-Lindau (*VHL*) Inactivation in Sporadic Clear Cell Renal Cancer: Associations with Germline *VHL* Polymorphisms and Etiologic Risk Factors. *PLoS Genet*, 7: e1002312 doi:10.1371/journal.pgen.1002312 PMID:21998597

Moore OR, Walker SL, Ansari R (1991). *Canadian Water Quality Guidelines for Trichloroethylene*. Ottawa, Canada: Water Quality Branch, Inland Waters Directorate, Scientific Series No. 183

Moran MJ, Zogorski JS, Squillace PJ (2007). Chlorinated solvents in groundwater of the United States. *Environ Sci Technol*, 41: 74–81. doi:10.1021/es061553y PMID:17265929

Morgan JW & Cassady RE (2002). Community cancer assessment in response to long-time exposure to perchlorate and trichloroethylene in drinking water. *J Occup Environ Med*, 44: 616–621. doi:10.1097/00043764-200207000-00005 PMID:12134524

Morgan RW, Kelsh MA, Zhao K, Heringer S (1998). Mortality of aerospace workers exposed to trichloroethylene. *Epidemiology*, 9: 424–431. doi:10.1097/00001648-199807000-00013 PMID:9647907

Morse KM & Goldberg L (1943). Chlorinated solvent exposures at degreasing operations. *Ind. Med.*, 12: 706–713.

Mortelmans K, Haworth S, Lawlor T *et al.* (1986). *Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ Mutagen*, 8: Suppl 7: 1–119. doi:10.1002/em.2860080802 PMID:3516675

Mroueh U-M (1993). *[Solvent use in Finland]*. Helsinki, Finland: National Board of Waters and the Environment.

Müller M, Birner G, Dekant W (1998a). Reactivity of haloketenes and halothioketenes with nucleobases: chemical characterization of reaction products. *Chem Res Toxicol*, 11: 454–463. doi:10.1021/tx9701438 PMID:9585476

Müller M, Birner G, Sander M, Dekant W (1998b). Reactivity of haloketenes and halothioketenes with nucleobases: reactions in vitro with DNA. *Chem Res Toxicol*, 11: 464–470. doi:10.1021/tx9701440 PMID:9585477

Nagasawa Y, Ukai H, Okamoto S *et al.* (2011a). Organic solvent use in enterprises in Japan. *Ind Health*, 49: 534–541. doi:10.2486/indhealth.MS1244 PMID:21697614

Nagasawa Y, Ukai H, Okamoto S *et al.* (2011b). Organic solvent use in research institutions in Japan. *Ind Health*, 49: 421–426. doi:10.2486/indhealth.MS1232 PMID:21697627

Nagaya T, Ishikawa N, Hata H (1989). Sister-chromatid exchanges in lymphocytes of workers exposed to trichloroethylene. *Mutat Res*, 222: 279–282. doi:10.1016/0165-1218(89)90144-4 PMID:2922011

Nagaya T, Ishikawa N, Hata H, Otobe T (1993). Subclinical and reversible hepatic effects of occupational exposure to trichloroethylene. *Int Arch Occup Environ Health*, 64: 561–563. doi:10.1007/BF00517701 PMID:8314614

Nakajima T, Kamijo Y, Usuda N *et al.* (2000). Sex-dependent regulation of hepatic peroxisome proliferation in mice by trichloroethylene via peroxisome proliferator-activated receptor alpha (PPARalpha). *Carcinogenesis*, 21: 677–682. doi:10.1093/carcin/21.4.677 PMID:10753203

Nakajima T, Wang RS, Elovaara E *et al.* (1992a). A comparative study on the contribution of cytochrome P450 isozymes to metabolism of benzene, toluene and trichloroethylene in rat liver. *Biochem Pharmacol*, 43: 251–257. doi:10.1016/0006-2952(92)90285-Q PMID:1739413

Nakajima T, Wang R-S, Katakura Y *et al.* (1992b). Sex-, age- and pregnancy-induced changes in the metabolism of toluene and trichloroethylene in rat liver in relation to the regulation of cytochrome P450IIE1 and P450IIC11 content. *J Pharmacol Exp Ther*, 261: 869–874. PMID:1602390

Natarajan AT, Boei JJ, Darroudi F *et al.* (1996). Current cytogenetic methods for detecting exposure and effects of mutagens and carcinogens. *Environ Health Perspect*, 104: Suppl 3: 445–448. PMID:8781361

National Research Council (2006). *Assessing the Human Health Risks of Trichloroethylene: Key Scientific Issues*. Washington, DC, USA: The National Academies Press

National Research Council (2009). *Contaminated Water Supplies at Camp Lejeune: Assessing Potential Health Effects*. Washington DC: National Academics Press. Available at: http://www.nap.edu/catalog.php?record_id=12618. Accessed 19 November 2013.

NCI; National Cancer Institute (1976). *Carcinogenesis bioassay of trichloroethylene*. Bethesda, MD: US Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Tech Rep Series 2, pp 1–215. Available at: http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr002.pdf

Neafsey P, Ginsberg G, Hattis D *et al.* (2009). Genetic polymorphism in CYP2E1: Population distribution of CYP2E1 activity. *J Toxicol Environ Health B Crit Rev*, 12: 362–388. doi:10.1080/10937400903158359 PMID:20183527

Neghab M, Qu S, Bai CL *et al.* (1997). Raised concentration of serum bile acids following occupational exposure to halogenated solvents, 1,1,2-trichloro-1,2,2-trifluoroethane and trichloroethylene. *Int Arch Occup Environ Health*, 70: 187–194. doi:10.1007/s004200050205 PMID:9298401

Nelson MA & Bull RJ (1988). Induction of strand breaks in DNA by trichloroethylene and metabolites in rat and mouse liver in vivo. *Toxicol Appl Pharmacol*, 94: 45–54. doi:10.1016/0041-008X(88)90335-3 PMID:3376113

Neta G, Stewart PA, Rajaraman P *et al.* (2012). Occupational exposure to chlorinated solvents and risks of glioma and meningioma in adults. [Epub ahead of print] *Occup Environ Med*, doi:10.1136/oemed-2012-100742 PMID:22864249

Newman D, Abuladze N, Scholz K *et al.* (2007). Specificity of aminoacylase III-mediated deacetylation of mercapturic acids. *Drug Metab Dispos*, 35: 43–50. doi:10.1124/dmd.106.012062 PMID:17012540

NICNAS; National Industrial Chemicals Notification and Assessment Scheme (2000). *Trichloroethylene: Priority Existing Chemical Assessment Report No. 8*. Sydney, Australia. Available at: http://www.nicnas.gov.au/__data/assets/pdf_file/0004/4369/PEC_8_Trichloroethylene_Full_Report_PDF.pdf. Accessed 19 November 2013.

Nietert PJ, Sutherland SE, Silver RM *et al.* (1998). Is occupational organic solvent exposure a risk factor for scleroderma? *Arthritis Rheum*, 41: 1111–1118. doi:10.1002/1529-0131(199806)41:6<1111::AID-ART19>3.0.CO;2-J PMID:9627022

NIOSH (1994a). *Pocket Guide to Chemical Hazards*. Cincinnati, OH: United States National Institute for Occupational Safety and Health, DHHS Publ. 94–116), 314–315

NIOSH (1994b). *National Occupational Exposure Survey (1981–1983)*. Cincinnati, OH.

Nomiyama K & Nomiyama H (1971). Metabolism of trichloroethylene in human. Sex difference in urinary excretion of trichloroacetic acid and trichloroethanol. *Int Arch Arbeitsmed*, 28: 37–48. doi:10.1007/BF00539728 PMID:5088555

Nordström M, Hardell L, Magnuson A *et al.* (1998). Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukaemia evaluated in a case-control study. *Br J Cancer*, 77: 2048–2052. doi:10.1038/bjc.1998.341 PMID:9667691

Nowak G (2003). Protein kinase C mediates repair of mitochondrial and transport functions after toxicant-induced injury in renal cells. *J Pharmacol Exp Ther*, 306: 157–165. doi:10.1124/jpet.103.050336 PMID:12665543

Nowak G, Keasler KB, McKeller DE, Schnellmann RG (1999). Differential effects of EGF on repair of cellular functions after dichlorovinyl-L-cysteine-induced injury. *Am J Physiol*, 276: F228–F236. PMID:9950953

NTP; National Toxicology Program (1988). NTP Toxicology and Carcinogenesis Studies of Trichloroethylene (CAS No. 79–01–6) in Four Strains of Rats (ACI, August, Marshall, Osborne-Mendel) (Gavage Studies). *Natl Toxicol Program Tech Rep Ser*, 273: 1–299. PMID:12748681

NTP; National Toxicology Program (1990). NTP Carcinogenesis Studies of Trichloroethylene (Without Epichlorohydrin) (CAS No. 79–01–6) in F344/N Rats and B6C3F1 Mice (Gavage Studies). *Natl Toxicol Program Tech Rep Ser*, 243: 1–174. PMID:12750750

NTP; National Toxicology Program (2000). *Final Report on Carcinogens Background Document for Trichloroethylene*. Research Triangle Park, NC, USA: National Toxicology Program. Available at: http://ntp.niehs.nih.gov/ntp/newhomeroc/roc10/trichloroethylene.pdf

NTP; National Toxicology Program (2011). NTP 12th Report on Carcinogens. *Rep Carcinog*, 12: iii-499 PMID:21822324

NTP; National Toxicology Program (2011a). Trichloroethylene. *Rep Carcinog*, 12: 420–424. PMID:21863114

NTP; National Toxicology Program (2011b). *Summary report of the National Toxicology Program and Environmental Protection Agency-Sponsored Review of Pathology Materials from Selected Ramazzini Institute Rodent Cancer Bioassays*. Research Triangle Park, NC: National Toxicology Program. Available at: http://ntp.niehs.nih.gov/NTP/About_NTP/Partnerships/International/SummaryPWG_Report_RI_Bioassays.pdf. Accessed 13 November 2013.

NTP; National Toxicology Program (2011c). *Amended Pathology Quality Assessment Review and PWG Coordinator's Report for Lifetime Carcinogenicity Study of Methyl-tertiary-butyl Ether (MTBE) in Sprague-Dawley Rats Conducted at the Cancer Research Center, European Ramazzini Foundation for Oncology and Environmental Sciences, Bologna, Italy*. Research Triangle Park, NC: National Toxicology Program. Available at: http://ntp.niehs.nih.gov/ntp/foia/pwgMTBE_508.pdf. Accessed 13 November 2013.

Nunes J, Ehrich M, Robertson J (2001). Toxicosis associated with dual oral exposure of rats to lead and trichloroethylene. *Toxicol Pathol*, 29: 451–457. doi:10.1080/01926230152499809 PMID:11560250

O'Neil MJ, Heckelman PE, Roman CB (2006). *The Merck Index*. 14th Ed. Whitehouse Station, NJ: Merck & Co. Monograph Number 09639.

O'Shea D, Davis SN, Kim RB, Wilkinson GR (1994). Effect of fasting and obesity in humans on the 6-hydroxylation of chlorzoxazone: a putative probe of CYP2E1 activity. *Clin Pharmacol Ther*, 56: 359–367. doi:10.1038/clpt.1994.150 PMID:7955797

Odum J, Foster JR, Green T (1992). A mechanism for the development of Clara cell lesions in the mouse lung after exposure to trichloroethylene. *Chem Biol Interact*, 83: 135–153. doi:10.1016/0009-2797(92)90042-J PMID:1505057

Ogata M, Takatsuka Y, Tomokuni K (1971). Excretion of organic chlorine compounds in the urine of persons exposed to vapours of trichloroethylene and tetrachloroethylene. *Br J Ind Med*, 28: 386–391. PMID:5124840

Ohura T, Amagai T, Senga Y, Fusaya M (2006). Organic air pollutants inside and outside residences in Shimizu, Japan: levels, sources and risks. *Sci Total Environ*, 366: 485–499. doi:10.1016/j.scitotenv.2005.10.005 PMID:16298419

Okada Y, Nakagoshi A, Tsurukawa M *et al.* (2012). Environmental risk assessment and concentration trend of atmospheric volatile organic compounds in Hyogo Prefecture, Japan. *Environ Sci Pollut Res Int*, 19: 201–213. doi:10.1007/s11356-011-0550-0 PMID:21717170

Okawa MT, Blejer HP, Solomon RM (1978). *Trans World Airlines Corp., Los Angeles, CA (Health Hazard Evaluation Determination Report No. 78-38-312)*. Cincinnati, OH: United States National Institute for Occupational Safety and Health

Okino T, Nakajima T, Nakano M (1991). Morphological and biochemical analyses of trichloroethylene hepatotoxicity: differences in ethanol- and phenobarbital-pretreated rats. *Toxicol Appl Pharmacol*, 108: 379–389. doi:10.1016/0041-008X(91)90084-R PMID:2020965

Ömür-Özbek P, Gallagher DL, Dietrich AM (2011). Determining human exposure and sensory detection of odorous compounds released during showering. *Environ Sci Technol*, 45: 468–473. doi:10.1021/es1030068 PMID:21141853

Opdam JJ (1989). Intra and interindividual variability in the kinetics of a poorly and highly metabolising solvent. *Br J Ind Med*, 46: 831–845. PMID:2611156

Pannett B, Coggon D, Acheson ED (1985). A job-exposure matrix for use in population based studies in England and Wales. *Br J Ind Med*, 42: 777–783. PMID:4063222

Papdullo RF, Shareef SA, Kincaid L et al. (1985). *Survey of Trichloroethylene Emission Sources*. (EPA-450/3-85-021; PB86-107943). Research Triangle Park, NX: Office of Air Quality Planning and Standards, Environmental Protection Agency.

Parchman LG & Magee PN (1982). Metabolism of [^{14}C] trichloroethylene to $^{14}CO_2$ and interaction of a metabolite with liver DNA in rats and mice. *J Toxicol Environ Health*, 9: 797–813. doi:10.1080/15287398209530204 PMID:6811765

Parrish JM, Austin EW, Stevens DK *et al.* (1996). Haloacetate-induced oxidative damage to DNA in the liver of male B6C3F1 mice. *Toxicology*, 110: 103–111. doi:10.1016/0300-483X(96)03342-2 PMID:8658551

Peden-Adams MM, Eudaly JG, Heesemann LM *et al.* (2006). Developmental immunotoxicity of trichloroethylene (trichloroethylene): studies in B6C3F1 mice. *J Environ Sci Health A Tox Hazard Subst Environ Eng*, 41: 249–271. doi:10.1080/10934520500455289 PMID:16484062

Peden-Adams MM, Eudaly JG, Lee AM *et al.* (2008). Lifetime exposure to trichloroethylene (trichloroethylene) does not accelerate autoimmune disease in MRL +/+ mice. *J Environ Sci Health A Tox Hazard Subst Environ Eng*, 43: 1402–1409. doi:10.1080/10934520802232063 PMID:18780217

Pellizzari ED, Hartwell TD, Harris BS 3rd *et al.* (1982). Purgeable organic compounds in mother's milk. *Bull Environ Contam Toxicol*, 28: 322–328. doi:10.1007/BF01608515 PMID:7082873

Perbellini L, Olivato D, Zedde A, Miglioranzi R (1991). Acute trichloroethylene poisoning by ingestion: clinical and pharmacokinetic aspects. *Intensive Care Med*, 17: 234–235. doi:10.1007/BF01709883 PMID:1744309

Perocco P & Prodi G (1981). DNA damage by haloalkanes in human lymphocytes cultured in vitro. *Cancer Lett*, 13: 213–218. doi:10.1016/0304-3835(81)90020-3 PMID:6457684

Perry SJ, Harries H, Scholfield C *et al.* (1995). Molecular cloning and expression of a cDNA for human kidney cysteine conjugate β-lyase. *FEBS Lett*, 360: 277–280. doi:10.1016/0014-5793(95)00123-Q PMID:7883047

Perry SJ, Schofield MA, MacFarlane M *et al.* (1993). Isolation and expression of a cDNA coding for rat kidney cytosolic cysteine conjugate β-lyase. *Mol Pharmacol*, 43: 660–665. PMID:8502223

Persson B, Dahlander AM, Fredriksson M *et al.* (1989). Malignant lymphomas and occupational exposures. *Br J Ind Med*, 46: 516–520. PMID:2775671

Persson B & Fredrikson M (1999). Some risk factors for non-Hodgkin's lymphoma. *Int J Occup Med Environ Health*, 12: 135–142. PMID:10465904

Persson B, Fredriksson M, Olsen K *et al.* (1993). Some occupational exposures as risk factors for malignant lymphomas. *Cancer*, 72: 1773–1778. doi:10.1002/1097-0142(19930901)72:5<1773::AID-CNCR2820720542>3.0.CO;2-6 PMID:8348507

Pesch B, Haerting J, Ranft U *et al.* (2000a). Occupational risk factors for renal cell carcinoma: agent-specific results from a case-control study in Germany. MURC Study Group. Multicenter urothelial and renal cancer study. *Int J Epidemiol*, 29: 1014–1024. doi:10.1093/ije/29.6.1014 PMID:11101542

Pesch B, Haerting J, Ranft U *et al.* (2000b). Occupational risk factors for urothelial carcinoma: agent-specific results from a case-control study in Germany. MURC Study Group. Multicenter Urothelial and Renal Cancer. *Int J Epidemiol*, 29: 238–247. doi:10.1093/ije/29.2.238 PMID:10817119

Pfaffenberger CD, Peoples A, Briggle TV (1984). *Blood plasma levels of volatile chlorinated solvents and metabolites in occupationally exposed workers*. In: *The Biosphere: Problems and Solutions, Amsterdam*. Veziroglu TN, editor. Elsevier Science Publishers, pp. 59–569.

Pfaffenberger CD, Peoples AJ, Enos HF (1980). Distribution of volatile halogenated organic compounds between rat blood serum and adipose tissue. *Int J Environ Anal Chem*, 8: 55–65. doi:10.1080/03067318008071879 PMID:7399799

Poet TS, Corley RA, Thrall KD *et al.* (2000). Assessment of the percutaneous absorption of trichloroethylene in rats and humans using MS/MS real-time breath analysis and physiologically based pharmacokinetic modeling. *Toxicol Sci*, 56: 61–72. doi:10.1093/toxsci/56.1.61 PMID:10869454

Poon R, Nakai J, Yagminas A *et al.* (2002). Subchronic toxicity of chloral hydrate on rats: a drinking water study. *J Appl Toxicol*, 22: 227–236. doi:10.1002/jat.843 PMID:12210539

Povey A, Guppy MJ, Wood M *et al.* (2001). Cytochrome P2 polymorphisms and susceptibility to scleroderma following exposure to organic solvents. *Arthritis Rheum*, 44: 662–665. doi:10.1002/1529-0131(200103)44:3<662::AID-ANR115>3.0.CO;2-P PMID:11263781

Pratt GC, Palmer K, Wu CY *et al.* (2000). An assessment of air toxics in Minnesota. *Environ Health Perspect*, 108: 815–825. doi:10.1289/ehp.00108815 PMID:11017885

Price PJ, Hassett CM, Mansfield JI (1978). Transforming activities of trichloroethylene and proposed industrial alternatives. *In Vitro*, 14: 290–293. doi:10.1007/BF02616038 PMID:669729

Prout MS, Provan WM, Green T (1985). Species differences in response to trichloroethylene. I. Pharmacokinetics in rats and mice. *Toxicol Appl Pharmacol*, 79: 389–400. doi:10.1016/0041-008X(85)90137-1 PMID:4035686

Pukkala E, Guo J, Kyyrönen P *et al.* (2005). National job-exposure matrix in analyses of census-based estimates of occupational cancer risk. *Scand J Work Environ Health*, 31: 97–107. doi:10.5271/sjweh.856 PMID:15864903

Purdue MP, Bakke B, Stewart P *et al.* (2011). A case-control study of occupational exposure to trichloroethylene and non-Hodgkin lymphoma. *Environ Health Perspect*, 119: 232–238. doi:10.1289/ehp.1002106 PMID:21370516

Raaschou-Nielsen O, Hansen J, Christensen JM *et al.* (2001). Urinary concentrations of trichloroacetic acid in Danish workers exposed to trichloroethylene, 1947–1985. *Am J Ind Med*, 39: 320–327. doi:10.1002/1097-0274(200103)39:3<320::AID-AJIM1020>3.0.CO;2-8 PMID:11241565

Raaschou-Nielsen O, Hansen J, McLaughlin JK *et al.* (2003). Cancer risk among workers at Danish companies using trichloroethylene: a cohort study. *Am J Epidemiol*, 158: 1182–1192. doi:10.1093/aje/kwg282 PMID:14652303

Raaschou-Nielsen O, Hansen J, Thomsen BL *et al.* (2002). Exposure of Danish workers to trichloroethylene, 1947–1989. *Appl Occup Environ Hyg*, 17: 693–703. doi:10.1080/10473220290096186 PMID:12363210

Radican L, Blair A, Stewart P, Wartenberg D (2008). Mortality of aircraft maintenance workers exposed to trichloroethylene and other hydrocarbons and chemicals: extended follow-up. *J Occup Environ Med*, 50: 1306–1319. doi:10.1097/JOM.0b013e3181845f7f PMID:19001957

Ramdhan DH, Kamijima M, Wang D *et al.* (2010). Differential response to trichloroethylene-induced hepatosteatosis in wild-type and PPARalpha-humanized mice. *Environ Health Perspect*, 118: 1557–1563. doi:10.1289/ehp.1001928 PMID:20709644

Ramdhan DH, Kamijima M, Yamada N *et al.* (2008). Molecular mechanism of trichloroethylene-induced hepatotoxicity mediated by CYP2E1. *Toxicol Appl Pharmacol*,231:300–307.doi:10.1016/j.taap.2008.04.020 PMID:18565563

Ramírez N, Cuadras A, Rovira E *et al.* (2012). Chronic risk assessment of exposure to volatile organic compounds in the atmosphere near the largest Mediterranean industrial site. *Environ Int*, 39: 200–209. doi:10.1016/j.envint.2011.11.002 PMID:22208760

Rantala, K., Riipinen, H. Anttila A (1992). [*Exposure at work: 22. Halogenated hydrocarbons*]. Helsinki, Finland: Finnish Institute of Occupational Health, Finnish Work Environment Fund

Rappaport SM & Kupper LL (2004). Variability of environmental exposures to volatile organic compounds. *J Expo Anal Environ Epidemiol*, 14: 92–107. doi:10.1038/sj.jea.7500309 PMID:14726948

Rasmussen K, Brogren CH, Sabroe S (1993a). Subclinical affection of liver and kidney function and solvent exposure. *Int Arch Occup Environ Health*, 64: 445–448. doi:10.1007/BF00517951 PMID:8458661

Rasmussen K, Sabroe S, Wohlert M *et al.* (1988). A genotoxic study of metal workers exposed to trichloroethylene. Sperm parameters and chromosome aberrations in lymphocytes. *Int Arch Occup Environ Health*, 60: 419–423. doi:10.1007/BF00381389 PMID:3410552

Rich AL (2011). *Air emissions from natural gas exploration and mining in the Barnett shale geologic reservoir.* Unpublished dissertation. Arlington, TX: University of Texas.

Ring JA, Ghabrial H, Ching MS *et al.* (1999). Fetal hepatic drug elimination. *Pharmacol Ther*, 84: 429–445. doi:10.1016/S0163-7258(99)00046-7 PMID:10665839

Ritz B (1999). Cancer mortality among workers exposed to chemicals during uranium processing. *J Occup Environ Med*, 41: 556–566. doi:10.1097/00043764-199907000-00004 PMID:10412097

Ritz B, Morgenstern H, Froines J, Moncau J (1999). Chemical exposures of rocket-engine test-stand personnel and cancer mortality in a cohort of aerospace workers. *J Occup Environ Med*, 41: 903–910. doi:10.1097/00043764-199910000-00011 PMID:10529946

Robbiano L, Baroni D, Carrozzino R *et al.* (2004). DNA damage and micronuclei induced in rat and human kidney cells by six chemicals carcinogenic to the rat kidney. *Toxicology*, 204: 187–195. doi:10.1016/j.tox.2004.06.057 PMID:15388244

Rodriguez CE, Mahle DA, Gearhart JM *et al.* (2007). Predicting age-appropriate pharmacokinetics of six volatile organic compounds in the rat utilizing physiologically based pharmacokinetic modeling. *Toxicol Sci*, 98: 43–56. doi:10.1093/toxsci/kfm082 PMID:17426107

Roldán-Arjona T, García-Pedrajas MD, Luque-Romero FL *et al.* (1991). An association between mutagenicity of the Ara test of Salmonella typhimurium and carcinogenicity in rodents for 16 halogenated aliphatic hydrocarbons. *Mutagenesis*, 6: 199–205. doi:10.1093/mutage/6.3.199 PMID:1881351

Rosa C (2003). Exposure to trichloroethylene in an insignia manufacturing facility. *Appl Occup Environ Hyg*, 18: 646–648. doi:10.1080/10473220301373 PMID:12909531

Rossi AM, Migliore L, Barale R, Loprieno N (1983). In vivo and in vitro mutagenicity studies of a possible carcinogen, trichloroethylene, and its two stabilizers, epichlorohydrin and 1,2-epoxybutane. *Teratog Carcinog Mutagen*, 3: 75–87. doi:10.1002/1520-6866(1990)3:1<75::AID-TCM1770030109>3.0.CO;2-5 PMID:6132459

Ruder AM, Yiin JH, Waters MA *et al.*; Brain Cancer Collaborative Study Group (2013). The Upper Midwest Health Study: gliomas and occupational exposure to chlorinated solvents. *Occup Environ Med*, 70: 73–80. doi:10.1136/oemed-2011-100588 PMID:23104734

Ruhe RL (1982). *Synthes Ltd., Monument, CO (Health Hazard Evaluation Report No. 82-040-1119)*. Cincinnati, OH: United States National Institute for Occupational Safety and Health

Ruhe RL, Donohue M (1980). *Duralectra Inc., Natick, MA (Health Hazard Evaluation Report No. 79-147-702)*. Cincinnati, OH: United States National Institute for Occupational Safety and Health

Ruhe RL, Watanabe A, Stein G (1981). *Superior Tube Company, Collegeville, PA (Health Hazard Evaluation Report No. 80-49-808)*. Cincinnati, OH, United States National lnstitute for Occupational Safety and Health

Sabel GV & Clark TP (1984). Volatile organic compounds as indicators of municipal solid waste leachate contamination. *Waste Manag Res*, 2: 119–130.

Sack TM, Steele DH, Hammerstrom K *et al.* (1992). A survey of household products for volatile organic compounds. *Atmos Environ*, 26A: 1063–1070.

Sadtler Research Laboratories (1980). *1980 Cumulative Index*. Philadelphia, PA

Saihan EM, Burton JL, Heaton KW (1978). A new syndrome with pigmentation, scleroderma, gynaecomastia, Raynaud's phenomenon and peripheral neuropathy. *Br J Dermatol*, 99: 437–440. doi:10.1111/j.1365-2133.1978.tb06184.x PMID:213095

Sano Y, Nakashima H, Yoshioka N *et al.* (2009). Trichloroethylene liver toxicity in mouse and rat: microarray analysis reveals species differences in gene expression. *Arch Toxicol*, 83: 835–849. doi:10.1007/s00204-009-0431-1 PMID:19448997

Sato A (1993). Confounding factors in biological monitoring of exposure to organic solvents. *Int Arch Occup Environ Health*, 65: Suppl: S61–S67. doi:10.1007/BF00381309 PMID:8406940

Sato A, Endoh K, Kaneko T, Johanson G (1991). A simulation study of physiological factors affecting pharmacokinetic behaviour of organic solvent vapours. *Br J Ind Med*, 48: 342–347. PMID:2039747

Sato A & Nakajima T (1978). Differences following skin or inhalation exposure in the absorption and excretion kinetics of trichloroethylene and toluene. *Br J Ind Med*, 35: 43–49. PMID:629888

Sato A & Nakajima T (1979). A vial-equilibration method to evaluate the drug-metabolizing enzyme activity for volatile hydrocarbons. *Toxicol Appl Pharmacol*, 47: 41–46. doi:10.1016/0041-008X(79)90069-3 PMID:34248

Sato A, Nakajima T, Fujiwara Y, Murayama N (1977). A pharmacokinetic model to study the excretion of trichloroethylene and its metabolites after an inhalation exposure. *Br J Ind Med*, 34: 56–63. PMID:843464

Savolainen H, Pfäffli P, Tengén M, Vainio H (1977). Trichloroethylene and 1,1,1-trichloroethane: effects on brain and liver after five days intermittent inhalation. *Arch Toxicol*, 38: 229–237. doi:10.1007/BF00293657 PMID:578726

Sbrana I, Lascialfari D, Loprieno N (1985). *Trichloroethylene induces micronuclei but not chromosomal aberrations in mouse bone marrow cells* (Abstract). Ramel CE, editor. *Fourth International Conference on Environmental Mutagens, Stockholm, June 24–28, 1985*. New York, USA: Alan R Liss, p. 163.

Schairer LA & Sautkulis RC (1982). Detection of ambient levels of mutagenic atmospheric pollutants with the higher plant. *Tradescantia*. *Environ. Mutag. Carcinog. Plant Biol*, 2: 154–194.

Schmucker DL (2001). Liver function and phase I drug metabolism in the elderly: a paradox. *Drugs Aging*, 18: 837–851. doi:10.2165/00002512-200118110-00005 PMID:11772124

SCOEL/SUM (2009). *Recommendation from the Scientific Committee on Occupational Exposure Limits for Trichloroethylene*. Report No. 142. Available at: http://www.ec.europa.eu/social/BlobServlet?docId=6405&langId=en. Accessed 20 November 2013.

Scott CS & Jinot J (2011). Trichloroethylene and cancer: systematic and quantitative review of epidemiologic evidence for identifying hazards. *Int J Environ Res Public Health*, 8: 4238–4272. doi:10.3390/ijerph8114238 PMID:22163205

Seidler A, Möhner M, Berger J *et al.* (2007). Solvent exposure and malignant lymphoma: a population-based case-control study in Germany. *J Occup Med Toxicol*, 2: 2 doi:10.1186/1745-6673-2-2 PMID:17407545

Seiji K, Jin C, Watanabe T *et al.* (1990). Sister chromatid exchanges in peripheral lymphocytes of workers exposed to benzene, trichloroethylene, or tetrachloroethylene, with reference to smoking habits. *Int Arch Occup Environ Health*, 62: 171–176. doi:10.1007/BF00383594 PMID:2323835

Selgrade MK & Gilmour MI (2010). Suppression of pulmonary host defenses and enhanced susceptibility to respiratory bacterial infection in mice following inhalation exposure to trichloroethylene and chloroform. *J Immunotoxicol*, 7: 350–356. doi:\\ PMID:20925451

Sellers EM, Lang M, Koch-Weser J *et al.* (1972). Interaction of chloral hydrate and ethanol in man. I. Metabolism. *Clin Pharmacol Ther*, 13: 37–49. PMID:4550322

Seo M, Ikeda K, Okamura T *et al.* (2008). Enhancing effect of chlorinated organic solvents on histamine release and inflammatory mediator production. *Toxicology*, 243: 75–83. doi:10.1016/j.tox.2007.09.024 PMID:18006135

Sexton K, Adgate JL, Church TR *et al.* (2005). Children's exposure to volatile organic compounds as determined by longitudinal measurements in blood. *Environ Health Perspect*, 113: 342–349. doi:10.1289/ehp.7412 PMID:15743726

Shahin MM & Von Borstel RC (1977). Mutagenic and lethal effects of alpha-benzene hexachloride, dibutyl phthalate and trichloroethylene in Saccharomyces cerevisiae. *Mutat Res*, 48: 173–180. doi:10.1016/0027-5107(77)90157-9 PMID:69268

Shaik ZP, Fifer EK, Nowak G (2008). Akt activation improves oxidative phosphorylation in renal proximal tubular cells following nephrotoxicant injury. *Am J Physiol*, 294: F423–F432.

Sharkawi M, De Saint Blanquat G, Elfassy B (1983). Inhibition of alcohol dehydrogenase by chloral hydrate and trichloroethanol: possible role in the chloral hydrate-ethanol interaction. *Toxicol Lett*, 17: 321–328. doi:10.1016/0378-4274(83)90245-X PMID:6353674

Shelby MD, Erexson GL, Hook GJ, Tice RR (1993). Evaluation of a three-exposure mouse bone marrow micronucleus protocol: results with 49 chemicals. *Environ Mol Mutagen*, 21: 160–179. doi:10.1002/em.2850210210 PMID:8444144

Shimada T, Swanson AF, Leber P, Williams GM (1985). Activities of chlorinated ethane and ethylene compounds in the Salmonella/rat microsome mutagenesis and rat hepatocyte/DNA repair assays under vapor phase exposure conditions. *Cell Biol Toxicol*, 1: 159–179. doi:10.1007/BF00120162 PMID:3917197

Shindell S & Ulrich S (1985). A cohort study of employees of a manufacturing plant using trichlorethylene. *J Occup Med*, 27: 577–579. doi:10.1097/00043764-198508000-00015 PMID:4032094

Shipman AJ & Whim BP (1980). Occupational exposure to trichloroethylene in metal cleaning processes and to tetrachloroethylene in the drycleaning industry in the U.K. *Ann Occup Hyg*, 23: 197–204. doi:10.1093/annhyg/23.2.197 PMID:7436231

Shu XO, Stewart P, Wen WQ *et al.* (1999). Parental occupational exposure to hydrocarbons and risk of acute lymphocytic leukemia in offspring. *Cancer Epidemiol Biomarkers Prev*, 8: 783–791. PMID:10498397

Siemiatycki J (1991). *Risk factors for cancer in the workplace*, Boca Raton, FL:CRC Press, pp 325.

Simmon V, Kauhanen K, Tardiff R (1977). *Mutagenic activity of chemicals identified in drinking water*. In: *Progress in Genetic Toxicology, vol. 2*. Scott D, BBFS, editor. New York; Amsterdam: Elsevier/North Holland Press, pp. 249–258.

Simmons JE, Boyes WK, Bushnell PJ *et al.* (2002). A physiologically based pharmacokinetic model for trichloroethylene in the male long-evans rat. *Toxicol Sci*, 69: 3–15. doi:10.1093/toxsci/69.1.3 PMID:12215655

Sittig M (1985). *Handbook of Toxic and Hazardous Chemicals and Carcinogens*. Park Ridge, New Jersey: Noyes Publications.

Slacik-Erben R, Roll R, Franke G, Uehleke H (1980). Trichloroethylene vapours do not produce dominant lethal mutations in male mice. *Arch Toxicol*, 45: 37–44. doi:10.1007/BF00303293 PMID:6893145

Sofuni T, Hayashi M, Matsuoka A *et al.* (1985). Mutagenicity tests on organic chemical contaminants in city water and related compounds. II.Chromosome aberration tests in cultured mammalian cells. *Bull.natl Inst. Hyg.Sci. (Tokyo)*, 103: 64–75.

Soh SC & Abdullah MP (2007). Determination of volatile organic compounds pollution sources in malaysian drinking water using multivariate analysis. *Environ Monit Assess*, 124: 39–50. doi:10.1007/s10661-006-9207-8 PMID:16967208

Solleveld HA & Boorman GA (1986). Spontaneous renal lesions in five rat strains. *Toxicol Pathol*, 14: 168–174. doi:10.1177/019262338601400204 PMID:3764314

Sonnenfeld N, Hertz-Picciotto I, Kaye WE (2001). Tetrachloroethylene in drinking water and birth outcomes at the US Marine Corps Base at Camp

Lejeune, North Carolina. *Am J Epidemiol*, 154: 902–908. doi:10.1093/aje/154.10.902 PMID:11700244

Soucek B & Vlachova D (1960). Excretion of trichloroethylene metabolites in human urine. *Br J Ind Med*, 17: 60–64. PMID:13832993

Spirtas R, Stewart PA, Lee JS *et al.* (1991). Retrospective cohort mortality study of workers at an aircraft maintenance facility. I. Epidemiological results. *Br J Ind Med*, 48: 515–530. PMID:1878308

Staats DA, Fisher JW, Connolly RB (1991). Gastrointestinal absorption of xenobiotics in physiologically based pharmacokinetic models. A two-compartment description. *Drug Metab Dispos*, 19: 144–148. PMID:1673388

Stanley LA, Blackburn DR, Devereaux S *et al.* (1994). *Ras* mutations in methylclofenapate-induced B6C3F1 and C57BL/10J mouse liver tumours. *Carcinogenesis*, 15: 1125–1131. doi:10.1093/carcin/15.6.1125 PMID:8020144

Stevens JL (1985). Isolation and characterization of a rat liver enzyme with both cysteine conjugate β-lyase and kynureninase activity. *J Biol Chem*, 260: 7945–7950. PMID:4008484

Stevens JL & Jakoby WB (1983). Cysteine conjugate β-lyase. *Mol Pharmacol*, 23: 761–765. PMID:6865918

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: 15529–15537. PMID:3782077

Stewart PA, Lee JS, Marano DE *et al.* (1991). Retrospective cohort mortality study of workers at an aircraft maintenance facility. II. Exposures and their assessment. *Br J Ind Med*, 48: 531–537. PMID:1878309

Stewart RD, Dodd HC, Gay HH, Erley DS (1970). Experimental human exposure to trichloroethylene. *Arch Environ Health*, 20: 64–71. PMID:5409662

Stewart RD, Hake CL, Peterson JE (1974). "Degreaser's flush". *Arch Environ Health*, 29: 1–5. PMID:4276444

Stott WT, Quast JF, Watanabe PG (1982). The pharmacokinetics and macromolecular interactions of trichloroethylene in mice and rats. *Toxicol Appl Pharmacol*, 62: 137–151. doi:10.1016/0041-008X(82)90110-7 PMID:7064149

Sung TI, Chen PC, Jyuhn-Hsiarn Lee L *et al.* (2007). Increased standardized incidence ratio of breast cancer in female electronics workers. *BMC Public Health*, 7: 102 doi:10.1186/1471-2458-7-102 PMID:17559641

Swenberg JA & Lehman-McKeeman LD (1999). alpha 2-Urinary globulin-associated nephropathy as a mechanism of renal tubule cell carcinogenesis in male rats. *IARC Sci Publ*, 147: 95–118. PMID:10457913

Tabrez S & Ahmad M (2009). Toxicity, biomarkers, genotoxicity, and carcinogenicity of trichloroethylene and its metabolites: a review. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev*, 27: 178–196. doi:10.1080/10590500903091340 PMID:19657920

Tan TT & Coussens LM (2007). Humoral immunity, inflammation and cancer. *Curr Opin Immunol*, 19: 209–216. doi:10.1016/j.coi.2007.01.001 PMID:17276050

Tang X, Que B, Song X *et al.* (2008). Characterization of liver injury associated with hypersensitive skin reactions induced by trichloroethylene in the guinea pig maximization test. *J Occup Health*, 50: 114–121. doi:\\ PMID:18403861

Tang XJ, Li LY, Huang JX, Deng YY (2002). Guinea pig maximization test for trichloroethylene and its metabolites. *Biomed Environ Sci*, 15: 113–118. PMID:12244752

Tao L, Kramer PM, Ge R, Pereira MA (1998). Effect of dichloroacetic acid and trichloroacetic acid on DNA methylation in liver and tumors of female B6C3F1 mice. *Toxicol Sci*, 43: 139–144. doi:10.1093/toxsci/43.2.139 PMID:9710955

Tao L, Li Y, Kramer PM *et al.* (2004). Hypomethylation of DNA and the insulin-like growth factor-II gene in dichloroacetic and trichloroacetic acid-promoted mouse liver tumors. *Toxicology*, 196: 127–136. doi:10.1016/j.tox.2003.11.011 PMID:15036762

Tao L, Yang S, Xie M *et al.* (2000). Effect of trichloroethylene and its metabolites, dichloroacetic acid and trichloroacetic acid, on the methylation and expression of c-*Jun* and c-*Myc* protooncogenes in mouse liver: prevention by methionine. *Toxicol Sci*, 54: 399–407. doi:\\ PMID:10774822

Tateishi M, Suzuki S, Shimizu H (1978). Cysteine conjugate β-lyase in rat liver. A novel enzyme catalyzing formation of thiol-containing metabolites of drugs. *J Biol Chem*, 253: 8854–8859. PMID:721818

Terracini B & Parker VH (1965). A pathological study on the toxicity of s-dichlorovinyl-l-cysteine. *Food Cosmet Toxicol*, 3: 67–74. doi:10.1016/S0015-6264(65)80010-4 PMID:14342797

Teschke K, Ahrens W, Andersen A *et al.* (1999). Occupational exposure to chemical and biological agents in the nonproduction departments of pulp, paper, and paper product mills: an international study. *Am Ind Hyg Assoc J*, 60: 73–83. doi:10.1080/00028899908984424 PMID:10028618

Thiele DL, Eigenbrodt EH, Ware AJ (1982). Cirrhosis after repeated trichloroethylene and 1,1,1-trichloroethane exposure. *Gastroenterology*, 83: 926–929. PMID:7106523

Tola S, Vilhunen R, Järvinen E, Korkala ML (1980). A cohort study on workers exposed to trichloroethylene. *J Occup Med*, 22: 737–740. PMID:7441393

Tomisawa H, Ichihara S, Fukazawa H *et al.* (1986). Purification and characterization of human hepatic cysteine-conjugate β-lyase. *Biochem J*, 235: 569–575. PMID:3741406

Toraason M, Clark J, Dankovic D *et al.* (1999). Oxidative stress and DNA damage in Fischer rats following acute exposure to trichloroethylene or perchloroethylene. *Toxicology*, 138: 43–53. doi:\\ PMID:10566590

Tu AS, Murray TA, Hatch KM *et al.* (1985). In vitro transformation of BALB/c-3T3 cells by chlorinated ethanes and ethylenes. *Cancer Lett*, 28: 85–92. doi:10.1016/0304-3835(85)90096-5 PMID:4027960

Tucker AN, Sanders VM, Barnes DW *et al.* (1982). Toxicology of trichloroethylene in the mouse. *Toxicol Appl Pharmacol*, 62: 351–357. doi:10.1016/0041-008X(82)90137-5 PMID:7071854

Ulander A, Seldén A, Ahlborg G Jr (1992). Assessment of intermittent trichloroethylene exposure in vapor degreasing. Am. ind. *Hyg. Assoc.*, 1: 53,742–743.

Urban TJ, Sebro R, Hurowitz EH *et al.* (2006). Functional genomics of membrane transporters in human populations. *Genome Res*, 16: 223–230. doi:10.1101/gr.4356206 PMID:16354753

Uttamsingh V & Anders MW (1999). Acylase-catalyzed deacetylation of haloalkene-derived mercapturates. *Chem Res Toxicol*, 12: 937–942. doi:10.1021/tx990090p PMID:10525269

Uttamsingh V, Baggs RB, Krenitsky DM, Anders MW (2000). Immunohistochemical localization of the acylases that catalyze the deacetylation of N-acetyl-L-cysteine and haloalkene-derived mercapturates. *Drug Metab Dispos*, 28: 625–632. PMID:10820133

Vainio H, Waters MD, Norppa H (1985). Mutagenicity of selected organic solvents. *Scand J Work Environ Health*, 11: Suppl 1: 75–82. PMID:3906872

Vamvakas S, Bittner D, Dekant W, Anders MW (1992). Events that precede and that follow *S*-(1,2-dichlorovinyl)-L-cysteine-induced release of mitochondrial Ca^{2+} and their association with cytotoxicity to renal cells. *Biochem Pharmacol*, 44: 1131–1138. doi:10.1016/0006-2952(92)90377-U PMID:1417936

Vamvakas S, Brüning T, Thomasson B *et al.* (1998). Renal cell cancer correlated with occupational exposure to trichloroethene. *J Cancer Res Clin Oncol*, 124: 374–382. doi:10.1007/s004320050186 PMID:9719500

Vamvakas S, Dekant W, Berthold K *et al.* (1987). Enzymatic transformation of mercapturic acids derived from halogenated alkenes to reactive and mutagenic intermediates. *Biochem Pharmacol*, 36: 2741–2748. doi:10.1016/0006-2952(87)90258-9 PMID:3307787

Vamvakas S, Dekant W, Henschler D (1989). Assessment of unscheduled DNA synthesis in a cultured line of renal epithelial cells exposed to cysteine *S*-conjugates of haloalkenes and haloalkanes. *Mutat Res*, 222: 329–335. doi:10.1016/0165-1218(89)90108-0 PMID:2704384

Vamvakas S, Dekant W, Schiffmann D, Henschler D (1988b). Induction of unscheduled DNA synthesis and micronucleus formation in Syrian hamster embryo fibroblasts treated with cysteine S-conjugates of chlorinated hydrocarbons. *Cell Biol Toxicol*, 4: 393–403. doi:10.1007/BF00117768 PMID:3228710

Vamvakas S, Elfarra AA, Dekant W *et al.* (1988a). Mutagenicity of amino acid and glutathione S-conjugates in the Ames test. *Mutat Res*, 206: 83–90. doi:10.1016/0165-1218(88)90144-9 PMID:2901035

Vamvakas S, Richter H, Bittner D (1996). Induction of dedifferentiated clones of LLC-PK1 cells upon long-term exposure to dichlorovinylcysteine. *Toxicology*, 106: 65–74. doi:10.1016/0300-483X(95)03166-D PMID:8571403

Vamvakas S, Sharma VK, Sheu S-S, Anders MW (1990). Perturbations of intracellular calcium distribution in kidney cells by nephrotoxic haloalkenyl cysteine *S*-conjugates. *Mol Pharmacol*, 38: 455–461. PMID:2233687

van de Water B, Zoeteweij JP, de Bont HJGM *et al.* (1994). Role of mitochondrial Ca^{2+} in the oxidative stress-induced dissipation of the mitochondrial membrane potential. Studies in isolated proximal tubular cells using the nephrotoxin 1,2-dichlorovinyl-L-cysteine. *J Biol Chem*, 269: 14546–14552. PMID:8182062

van de Water B, Zoetewey JP, de Bont HJ *et al.* (1993). The relationship between intracellular Ca^{2+} and the mitochondrial membrane potential in isolated proximal tubular cells from rat kidney exposed to the nephrotoxin 1,2-dichlorovinyl-cysteine. *Biochem Pharmacol*, 45: 2259–2267. doi:\\ PMID:8517866

Van Duuren BL, Goldschmidt BM, Loewengart G *et al.* (1979). Carcinogenicity of halogenated olefinic and aliphatic hydrocarbons in mice. *J Natl Cancer Inst*, 63: 1433–1439. PMID:292813

Van Duuren BL, Kline SA, Melchionne S, Seidman I (1983). Chemical structure and carcinogenicity relationships of some chloroalkene oxides and their parent olefins. *Cancer Res*, 43: 159–162. PMID:6847764

Vandervort R, Polakoff A (1973). *Dunham-Bush Inc., West Hartford, CN (Health Hazard Evaluation/Toxicity Determination Report No 72-84-31; US NTIS PB-229-627*). Cincinnati, OH: United States National Institute for Occupational Safety and Health

Vartiainen T, Pukkala E, Rienoia T *et al.* (1993). Population exposure to tri- and tetrachloroethene and cancer risk: two cases of drinking water pollution. *Chemosphere*, 27: 1171–1181. doi:10.1016/0045-6535(93)90165-2

Vedrina-Dragojević I & Dragojević D (1997). Trichloroethene and tetrachloroethene in ground waters of Zagreb, Croatia. *Sci Total Environ*, 203: 253–259. doi:10.1016/S0048-9697(97)00153-8 PMID:9260311

Vermeulen R, Zhang L, Spierenburg A *et al.* (2012). Elevated urinary levels of kidney injury molecule-1 among Chinese factory workers exposed to trichloroethylene. *Carcinogenesis*, 33: 1538–1541. doi:10.1093/carcin/bgs191 PMID:22665366

Vesterberg O & Astrand I (1976). Exposure to trichloroethylene monitored by analysis of metabolites in blood and urine. *J Occup Med*, 18: 224–226. PMID:1271124

Vesterberg O, Gorczak J, Krasts M (1976). Exposure to trichloroethylene II. Metabolites in blood and urine.

Scand J Work Environ Health, 2: 212–219. doi:10.5271/sjweh.2802 PMID:1019595

Vizcaya D, Christensen KY, Lavoué J, Siemiatycki J (2013). Risk of lung cancer associated with six types of chlorinated solvents: results from two case-control studies in Montreal, Canada. *Occup Environ Med*, 70: 81–85. doi:10.1136/oemed-2012-101155 PMID:23104733

Völkel W & Dekant W (1998). Chlorothioketene, the ultimate reactive intermediate formed by cysteine conjugate β-lyase-mediated cleavage of the trichloroethene metabolite S-(1,2-Dichlorovinyl)-L-cysteine, forms cytosine adducts in organic solvents, but not in aqueous solution. *Chem Res Toxicol*, 11: 1082–1088. doi:10.1021/tx980084d PMID:9760283

von der Hude W, Behm C, Gürtler R, Basler A (1988). Evaluation of the SOS chromotest. *Mutat Res*, 203: 81–94. doi:10.1016/0165-1161(88)90023-4 PMID:3280989

von Grote J, Hürlimann C, Scheringer M, Hungerbühler K (2003). Reduction of occupational exposure to perchloroethylene and trichloroethylene in metal degreasing over the last 30 years: influences of technology innovation and legislation. *J Expo Anal Environ Epidemiol*, 13: 325–340. doi:10.1038/sj.jea.7500288 PMID:12973361

Walgren JE, Kurtz DT, McMillan JM (2000). Expression of PPAR(alpha) in human hepatocytes and activation by trichloroacetate and dichloroacetate. *Res Commun Mol Pathol Pharmacol*, 108: 116–132. PMID:11758968

Walles SA (1986). Induction of single-strand breaks in DNA of mice by trichloroethylene and tetrachloroethylene. *Toxicol Lett*, 31: 31–35. doi:10.1016/0378-4274(86)90191-8 PMID:3715914

Wallin A, Zhang G, Jones TW *et al.* (1992). Mechanism of the nephrogenic repair response. Studies on proliferation and vimentin expression after ^{35}S-1,2-dichlorovinyl-L-cysteine nephrotoxicity in vivo and in cultured proximal tubule epithelial cells. *Lab Invest*, 66: 474–484. PMID:1374823

Wang G, Ansari GA, Khan MF (2007b). Involvement of lipid peroxidation-derived aldehyde-protein adducts in autoimmunity mediated by trichloroethene. *J Toxicol Environ Health A*, 70: 1977–1985. doi:10.1080/15287390701550888 PMID:17966069

Wang G, Cai P, Ansari GA, Khan MF (2007a). Oxidative and nitrosative stress in trichloroethene-mediated autoimmune response. *Toxicology*, 229: 186–193. doi:10.1016/j.tox.2006.10.014 PMID:17123686

Wang G, König R, Ansari GA, Khan MF (2008). Lipid peroxidation-derived aldehyde-protein adducts contribute to trichloroethene-mediated autoimmunity via activation of CD4+ T cells. *Free Radic Biol Med*, 44: 1475–1482. doi:10.1016/j.freeradbiomed.2008.01.012 PMID:18267128

Wang GF & Stacey NH (1990). Elevation of individual serum bile acids on exposure to trichloroethylene or alpha-naphthylisothiocyanate. *Toxicol Appl Pharmacol*, 105: 209–215. doi:\\ PMID:2219116

Wang J-L, Chen W-L, Tsai S-Y *et al.* (2001). An in vitro model for evaluation of vaporous toxicity of trichloroethylene and tetrachloroethylene to CHO-K1 cells. *Chem Biol Interact*, 137: 139–154. doi:10.1016/S0009-2797(01)00226-5 PMID:11551530

Wang R, Zhang Y, Lan Q *et al.* (2009). Occupational exposure to solvents and risk of non-Hodgkin lymphoma in Connecticut women. *Am J Epidemiol*, 169: 176–185. doi:10.1093/aje/kwn300 PMID:19056833

Wang RS, Nakajima T, Honma T (1999). Trichloroethylene inhibits aldehyde dehydrogenase only for aliphatic aldehydes of short chains in rats. *Toxicology*, 132: 9–18. doi:10.1016/S0300-483X(98)00132-2 PMID:10199577

Ward PM (2012). Brown and black grease suitability for incorporation into feeds and suitability for biofuels. *J Food Prot*, 75: 731–737. doi:10.4315/0362-028X.JFP-11-221 PMID:22488062

Ward TJ, Underberg H, Jones D *et al.* (2009). Indoor/ambient residential air toxics results in rural western Montana. *Environ Monit Assess*, 153: 119–126. doi:10.1007/s10661-008-0342-2 PMID:18548326

Wartenberg D, Reyner D, Scott CS (2000). Trichloroethylene and cancer: epidemiologic evidence. *Environ Health Perspect*, 108: Suppl 2: 161–176. doi:10.1289/ehp.00108s2161 PMID:10807550

Waskell L (1978). A study of the mutagenicity of anesthetics and their metabolites. *Mutat Res*, 57: 141–153. doi:10.1016/0027-5107(78)90261-0 PMID:351387

Watanabe S & Fukui T (2000). Suppressive effect of curcumin on trichloroethylene-induced oxidative stress. *J Nutr Sci Vitaminol (Tokyo)*, 46: 230–234. doi:10.3177/jnsv.46.230 PMID:11234915

Watson RE, Jacobson CF, Williams AL *et al.* (2006). Trichloroethylene-contaminated drinking water and congenital heart defects: a critical analysis of the literature. *Reprod Toxicol*, 21: 117–147. doi:10.1016/j.reprotox.2005.07.013 PMID:16181768

Weast RC, Astle MJ (1985). *CRC Handbook of Data on Organic Compounds*: *Trichloroethylene*. 57th Ed. Boca Raton, FL: CRC Press

Weisel CP, Alimokhtari S, Sanders PF (2008). Indoor air VOC concentrations in suburban and rural New Jersey. *Environ Sci Technol*, 42: 8231–8238. doi:10.1021/es8005223 PMID:19068799

Werner M, Birner G, Dekant W (1995a). The role of cytochrome P4503A1/2 in the sex-specific sulfoxidation of the hexachlorobutadiene metabolite, N-acetyl-S-(pentachlorobutadienyl)-L-cysteine in rats. *Drug Metab Dispos*, 23: 861–868. PMID:7493554

Werner M, Birner G, Dekant W (1996). Sulfoxidation of mercapturic acids derived from tri- and tetrachloroethene by cytochromes P450 3A: a bioactivation reaction in addition to deacetylation and cysteine conjugate β-lyase mediated cleavage. *Chem Res Toxicol*, 9: 41–49. doi:10.1021/tx950075u PMID:8924615

Werner M, Guo Z, Birner G *et al.* (1995b). The sulfoxidation of the hexachlorobutadiene metabolite N-acetyl-S-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine is catalyzed by human cytochrome P450 3A enzymes. *Chem Res Toxicol*, 8: 917–923. doi:10.1021/tx00049a004 PMID:8555406

White AE, Takehisa S, Eger EI 2nd *et al.* (1979). Sister chromatid exchanges induced by inhaled anesthetics. *Anesthesiology*, 50: 426–430. doi:10.1097/00000542-197905000-00010 PMID:453561

Whiteside TL (2006). Immune suppression in cancer: effects on immune cells, mechanisms and future therapeutic intervention. *Semin Cancer Biol*, 16: 3–15. doi:10.1016/j.semcancer.2005.07.008 PMID:16153857

WHO (1985). *Trichloroethylene*. Environmental Health Criteria No. 50. Geneva, Switzerland: World Health Organization

Wiesenhütter B, Selinski S, Golka K *et al.* (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: 247–251. doi:10.1007/s00420-007-0200-5 PMID:17479278

Williams GM, Mori H, McQueen CA (1989). Structure-activity relationships in the rat hepatocyte DNA-repair test for 300 chemicals. *Mutat Res*, 221: 263–286. doi:10.1016/0165-1110(89)90039-0 PMID:2682231

Withey JR, Collins BT, Collins PG (1983). Effect of vehicle on the pharmacokinetics and uptake of four halogenated hydrocarbons from the gastrointestinal tract of the rat. *J Appl Toxicol*, 3: 249–253. doi:10.1002/jat.2550030506 PMID:6662999

Withey JR & Karpinski K (1985). The fetal distribution of some aliphatic chlorinated hydrocarbons in the rat after vapor phase exposure. *Biol Res Pregnancy Perinatol*, 6: 79–88. PMID:4016168

Wolf K, Morris M (2007). *Spotting Chemicals: Alternatives to Perchloroethylene and Trichloroethylene in the Textile Cleaning Industry*. California Environmental Agency, Department of Toxic Substances Control and US Environmental Protection Agency

Wu C & Schaum J (2000). Exposure assessment of trichloroethylene. *Environ Health Perspect*, 108: Suppl 2: 359–363. doi:10.1289/ehp.00108s2359 PMID:10807565

Wu C, Schaum J (2001). *Sources, emissions and exposures for trichloroethylene and related chemicals*. Washington, DC: US Environmental Protection Agency

Wu CF, Liu LJ, Cullen A *et al.* (2011). Spatial-temporal and cancer risk assessment of selected hazardous air pollutants in Seattle. *Environ Int*, 37: 11–17. doi:10.1016/j.envint.2010.06.006 PMID:20619894

Xia LH, Huang HL, Kuang SR *et al.* (2004). A clinical analysis of 50 cases of medicament-like dermatitis due to trichloroethylene] *Chinese journal of industrial hygiene and occupational diseases*, 22: 207–210.

Xu F, Papanayotou I, Putt DA *et al.* (2008). Role of mitochondrial dysfunction in cellular responses to *S*-(1,2-dichlorovinyl)-*L*-cysteine in primary cultures of human proximal tubular cells. *Biochem Pharmacol*, 76: 552–567. doi:10.1016/j.bcp.2008.05.016 PMID:18602084

Xu X, Yang R, Wu N *et al.* (2009). Severe hypersensitivity dermatitis and liver dysfunction induced by occupational exposure to trichloroethylene. *Ind Health*, 47: 107–112. doi:10.2486/indhealth.47.107 PMID:19367038

Yamauchi A, Stijntjes GJ, Commandeur JNM, Vermeulen NPE (1993). Purification of glutamine transaminase K/cysteine conjugate β-lyase from rat renal cytosol based on hydrophobic interaction HPLC and gel permeation FPLC. *Protein Expr Purif*, 4: 552–562. doi:10.1006/prep.1993.1073 PMID:8286953

Yang Q, Ito S, Gonzalez FJ (2007). Hepatocyte-restricted constitutive activation of PPAR alpha induces hepatoproliferation but not hepatocarcinogenesis. *Carcinogenesis*, 28: 1171–1177. doi:10.1093/carcin/bgm046 PMID:17331954

Yeung RS, Xiao GH, Everitt JI *et al.* (1995). Allelic loss at the tuberous sclerosis 2 locus in spontaneous tumors in the Eker rat. *Mol Carcinog*, 14: 28–36. doi:10.1002/mc.2940140107 PMID:7546222

Yoon HS, Monks TJ, Walker CL, Lau SS (2001). Transformation of kidney epithelial cells by a quinol thioether via inactivation of the tuberous sclerosis-2 tumor suppressor gene. *Mol Carcinog*, 31: 37–45. doi:10.1002/mc.1037 PMID:11398196

Yoshida M, Fukabori S, Hara K *et al.* (1996). Concentrations of trichloroethylene and its metabolites in blood and urine after acute poisoning by ingestion. *Hum Exp Toxicol*, 15: 254–258. doi:10.1177/096032719601500312 PMID:8839215

Yost GS, Buckpitt AR, Roth RA, McLemore TL (1989). Mechanisms of lung injury by systemically administered chemicals. *Toxicol Appl Pharmacol*, 101: 179–195. doi:10.1016/0041-008X(89)90268-8 PMID:2510367

Yu K, Chen Q, Liu H *et al.* (1994). Signalling the molecular stress response to nephrotoxic and mutagenic cysteine conjugates: differential roles for protein synthesis and calcium in the induction of c-*fos* and c-*myc* mRNA in LLC-PK1 cells. *J Cell Physiol*, 161: 303–311. doi:10.1002/jcp.1041610215 PMID:7962114

Zahm SH (1992). *Computerized occupational referent population system (corps): study documentation*. Anonymous. Anonymous. Rockville, MD:National Cancer Institute & National Institute for Occupational Safety and Health.

Zhang L, Bassig BA, Mora JL *et al.* (2013). Alterations in serum immunoglobulin levels in workers occupationally exposed to trichloroethylene. *Carcinogenesis*, 34: 799–802. doi:10.1093/carcin/bgs403 PMID:23276795

Zhao Y, Krishnadasan A, Kennedy N *et al.* (2005). Estimated effects of solvents and mineral oils on cancer incidence and mortality in a cohort of aerospace

workers. *Am J Ind Med*, 48: 249–258. doi:10.1002/ajim.20216 PMID:16167347

Zhou YC & Waxman DJ (1998). Activation of peroxisome proliferator-activated receptors by chlorinated hydrocarbons and endogenous steroids. *Environ Health Perspect*, 106: Suppl 4: 983–988. PMID:9703482

Zhu J, Newhook R, Marro L, Chan CC (2005). Selected volatile organic compounds in residential air in the city of Ottawa, Canada. *Environ Sci Technol*, 39: 3964–3971. doi:10.1021/es050173u PMID:15984771

Ziegler DM (1993). Recent studies on the structure and function of multisubstrate flavin-containing monooxygenases. *Annu Rev Pharmacol Toxicol*, 33: 179–199. doi:10.1146/annurev.pa.33.040193.001143 PMID:8494339

Zingg JM & Jones PA (1997). Genetic and epigenetic aspects of DNA methylation on genome expression, evolution, mutation and carcinogenesis. *Carcinogenesis*, 18: 869–882. doi:10.1093/carcin/18.5.869 PMID:9163670

Zoccolillo L, Amendola L, Insogna S (2009). Comparison of atmosphere/aquatic environment concentration ratio of volatile chlorinated hydrocarbons between temperate regions and Antarctica. *Chemosphere*, 76: 1525–1532. doi:10.1016/j.chemosphere.2009.05.044 PMID:19541344