FINAL

Report on Carcinogens Background Document for

Trichloroethylene

December 13 - 14, 2000

Meeting of the
NTP Board of Scientific Counselors
Report on Carcinogens Subcommittee

Prepared for the:
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Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

U.S. Department of Health and Human Services
National Toxicology Program

Known to be Human Carcinogens:

There is sufficient evidence of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated to be Human Carcinogens:

There is limited evidence of carcinogenicity from studies in humans which indicates that causal interpretation is credible but that alternative explanations such as chance, bias or confounding factors could not adequately be excluded; or

There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species, or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a *known to be human carcinogen, or reasonably anticipated to be human carcinogen* or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

Summary of NIEHS Report on Carcinogens Review Group (RG1) and NTP Executive Committee Interagency Working Group for the Report on Carcinogens (RG2) actions for the nomination to upgrade trichloroethylene (TCE) to a *known to be human carcinogen* in the 10th RoC.

The RG1 reviewed the available carcinogenicity data for the nomination to upgrade TCE to a *known to be human carcinogen* in the 10th RoC. After applying the criteria for listing substances in the RoC, the RG1 passed a motion, by a vote of 7 in favor to 0 opposed, to recommend that TCE be upgraded in the 10th RoC to a *known to be a human carcinogen* based on the evidence of carcinogenicity from studies in humans. These findings are supported by evidence of malignant tumor formation in experimental animals, and convincing relevant information that TCE acts through mechanisms indicating that it would likely cause cancer in humans. The Summary Statement contained in the TCE draft background document for the December 13–15, 2000 NTP Board RoC Subcommittee meeting summarizes all the relevant information used by the RG1 to support their recommendation to upgrade TCE in the 10th RoC.

The RG2 reviewed the available carcinogenicity data for the nomination to upgrade TCE to a *known to be human carcinogen* in the 10th RoC. After applying the criteria for listing substances in the RoC, a motion recommending TCE be listed in the 10th RoC as a *known to be human carcinogen* was defeated by a vote of 3 in favor to 4 opposed. Reasons given by the RG2 members voting against the motion included the perception that the human studies do not provide sufficient evidence to list as a known human carcinogen mainly because of the lack of adequate exposure assessment information. Therefore, the recommendation going forward from the RG2 is that TCE should remain listed in the RoC as *reasonably anticipated to be a human carcinogen*. The current profile from the 9th RoC, where TCE is listed as *reasonably anticipated to be a human carcinogen*, is attached as appendix C to this background document.

Summary Statement

Trichloroethylene

CASRN 79-01-6

Carcinogenicity

Trichloroethylene (TCE) is *known to be a human carcinogen* based on evidence of carcinogenicity from studies in humans. These findings are supported by evidence of malignant tumor formation in experimental animals, and convincing relevant information that TCE acts through mechanisms indicating that it would likely cause cancer in humans.

A large and generally consistent body of epidemiologic findings provides support for the carcinogenicity of TCE in humans. In cohort studies, where the TCE exposures were best characterized, occupational exposure to TCE was associated with elevated incidence and mortality rates for cancer at several anatomical sites. A meta-analysis found elevated relative risks (RRs) for liver cancer (RR = 1.9, 95% CI 1.0 to 3.4) and kidney cancer (RR = 1.7, 95% CI 1.1 to 2.7), and somewhat less compelling results for non-Hodgkin's lymphoma (RR = 1.5, 95% CI 0.9 to 2.3), prostate cancer (RR = 1.3, 95% CI 1.0 to 1.6), and multiple myeloma (RR = 1.5, 95% CI 0.7 to 3.3) (Wartenberg *et al.* 2000). Although exposure was characterized less accurately in case-control studies, they also showed elevated odds ratios for kidney cancer (Dosemeci *et al*. 1999, Sinks *et al*. 1992, Vamvakas *et al*. 1998) and non-Hodgkin's lymphoma (Hardell *et al*. 1981, Hardell *et al*. 1984, Persson *et al*. 1989), supporting the findings of the cohort studies.

The findings in humans are supported by evidence of carcinogenicity in experimental animals at several of the same tissue sites as found in humans. In mice, TCE increased the incidences of benign and malignant tumors of the liver (NCI 1976; Maltoni *et al*. 1988; NTP 1990), lung (Maltoni *et al*. 1988), and blood (lymphoma) (Henschler *et al*. 1980). In rats, TCE-induced cancers of the kidney (Maltoni *et al*. 1988, NTP 1988, 1990), interstitial-cells of the testis (Maltoni *et al.* 1988, NTP 1988), and possibly leukemias (Maltoni *et al*.1988).

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

TCE is rapidly absorbed from the gastrointestinal tract and lung. Following absorption, TCE is distributed throughout the body and concentrates in lipophilic tissues (e.g., liver, brain, fat). Oxidation by cytochrome P-450 and conjugation with glutathione are the primary metabolic pathways. TCE metabolism in mice, rats, and humans is qualitatively similar producing the same primary metabolites. Chloral hydrate, dichloroacetic acid, and trichloroacetic acid are the primary toxic metabolites produced by the P-450 pathway and have been associated with liver and lung toxicity in rats and mice. Dichlorovinylcysteine is also a metabolite of the glutathione pathway and has been associated with kidney toxicity.

Renal-cell carcinomas from workers occupationally exposed to high levels of TCE exhibited somatic mutations of the von Hippel-Landau (VHL) tumor suppressor gene, a gene that has been associated with renal-cell carcinomas (Brauch *et al.* 1999). Of renal-cell carcinoma tissues from 44 TCE-exposed persons, 75% had mutations in the VHL gene. Sequencing analysis demonstrated that 39% of these tumors had a C to T transition at nucleotide (nt) 454, resulting in a Pro to Ser amino acid change at codon 81. The nt 454 mutation was found in the adjacent non-neoplastic kidney parenchyma in four patients. Moreover this mutation was both specific to TCE exposure, because it was not found in renal-cell carcinomas from non-exposed patients, and related to disease, because it was not found in germline DNA from either diseased or non-diseased individuals. It is biologically plausible that TCE exposure was related to the kidney tumors observed because (1) the site and histopathological characteristics of the tumors observed in humans and in experimental animals were identical (Vamvakas *et al.* 1993), (2) the molecular mechanism of this type of nephrocarcinogenicity has been elucidated (Dekant *et al.* 1986, cited in Bernauer *et al.* 1996), (3) the metabolites derived from the likely ultimate electrophilic intermediates of the bioactivation of TCE were identical in humans and experimental animals (Birner *et al*. 1993, cited in Clewell *et al*. 1995), and (4) taking the key urinary metabolites (mercapturic acids) as an indicator of the bioactivation of TCE (Birner *et al.* 1993, cited in Clewell *et al.* 1995), humans seem to be more sensitive than rats in developing the primary biochemical lesion leading to the induction of renal cancer.

In general, TCE and most of its major metabolites (chloral hydrate, dichloroacetic acid and trichloroacetic acid) were not potent genotoxins in a broad range of bacterial, lower eukaryotic, and *in vitro* and *in vivo* mammalian cell assays. In mammalian *in vitro* studies, TCE did not induce chromosomal aberrations in Chinese hamster ovary cells, unscheduled DNA synthesis in rat hepatocytes, or gene mutations in human lymphoblastoid cells but it did induce sister chromatid exchange in Chinese hamster ovary cells, gene mutations in mouse lymphoma cells and morphological transformation in rat embryo cells. In rodent *in vivo* studies, TCE did not induce unscheduled DNA synthesis, sister chromatid exchange, dominant lethal mutations or chromosome aberrations. TCE gave mixed results for DNA single-strand breaks or alkali-labile sites in mouse liver and positive results for micronucleus formation in mice. Studies of chromosomal aberrations, aneuploidy, and sister chromatid exchange in peripheral lymphocytes of workers exposed to TCE were considered inconclusive. In contrast to TCE, the dichlorovinylcysteine metabolite appears to be a more potent mutagen. Dichlorovinylcysteine was found to be mutagenic based on *Salmonella* assays and may induce primary DNA damage in mammalian cells *in vitro* and *in vivo*.

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1 Introduction

Trichloroethylene (TCE) is an industrial solvent used for vapor degreasing and cold cleaning of fabricated metal parts. Although no longer used with food, drugs, or cosmetics, TCE was used in the past as a carrier solvent for the active ingredients of insecticides and fungicides; as a solvent for waxes, fats, resins, and oils; as an anesthetic for medical and dental use; and for extraction of spice oleoresins and caffeine from coffee. TCE was listed in the Ninth Report on Carcinogens (RoC) as *reasonably anticipated to be a human carcinogen* based on limited evidence of carcinogenicity from studies in humans, sufficient evidence of malignant tumor formation in experimental animals, and convincing relevant information that TCE acts through mechanisms indicating that it would likely cause cancer in humans (NTP 2000a). TCE was nominated for upgrading to a *known human carcinogen* in the Tenth RoC by the National Institute of Environmental Health Sciences (NIEHS)/National Toxicology Program (NTP) RoC Review Group (RG1) based on recent publications of human studies consistently showing that occupational exposure to TCE results in elevated incidence and mortality rates for liver and kidney cancer in workers (Wartenberg *et al.* 2000).

1.1 Chemical identification

Trichloroethylene (C_2HCl_3, CASRN 79-01-6, mol. wt. = 131.39) also is known by the following names:

ethene, trichloro- (9CI)	Flock Flip	Trichloran
ethylene, trichloro- (8CI)	Fluate	Trichloren
acetylene trichloride	Gemalgene	trichlorethylene
Algylen	Germalgen	trichloroethene
Anamenth	Germalgene	1,1,2-trichloroethene
Benzinol	Lanadin	1,1,2-trichloroethylene
Blacosolv	Lethurin	1,2,2-trichloroethylene
Blancosolv	Narcogen	trichloroethylene (CAN)
Cecolene	Narkogen	Tri-Clene
Chlorilen	Narkosoid	Trielene
1-chloro-2,2-dichloroethylene	Nialk	Trielin
Chlorylea	Perm-A-Chlor	Trieline
Chlorylen	Perm-a-Clor	Triklone

Chorylen	Petzinol	Trilen
Circosolv	Philex	Trilene
Crawhaspol	TCE	Triline
Densinfluat	Threthylen	Trimar
1,1-dichloro-2-chloroethylene	Threthylene	Triol
Dow-Tri	Trethylen	Tri-plus
Dukeron	Trethylene	Vestrol
ethinyl trichloride	Tri	Vitran
ethylene trichloride	Triad	Fleck-Flip
ethylene, 1,1,2-trichloro-	Trial	Westrosol

Trichloroethylene has a UN shipping number of UN1710 and RCRA waste number of U228. The chemical structure of TCE is illustrated in Figure 1-1, and its physical-chemical properties are listed in Table 1-1

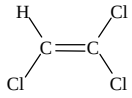

Figure 1-1. Structure of TCE

Table 1-1. Physical-Chemical chemical properties

Property	Information	Reference
Color	colorless, unless dyed blue	HSDB 1997
Physical state	liquid	HSDB 1997
Melting point (°C)	-84.8	Budavari 1996
Boiling point (°C)	86.9	Budavari 1996
Critical pressure (atm)	49.7	HSDB 1997
Critical temperature (°C)	271	HSDB 1997
Specific gravity (liquid) at	1.4642	Budavari 1996

Property	Information	Reference
20 °C or 4 °C		
Odor	ethereal odor, sweet chloroform-like odor	HSDB 1997
Odor threshold:		
Water	10 mg/L	Verschueren 1983, cited in in HSDB 1997
Air	ppm (115 mg/m^3)	Fazzalari 1978; cited in HSDB 1997
Solubility:		
in water at 25 °C	0.11 g/100 g	PPG Industries, Inc. 1997
in organic solvents	Soluble in chloroform, acetone, alcohol, and diethyl ether	HSDB 1997
Vapor density at 87°C and 760 mmHg (g/L)	4.45	PPG Industries, Inc. 1997
Vapor pressure (mm Hg)		
at 0°C	19.9	HSDB 1997
at 20°C	57.8	HSDB 1997

Upon combustion TCE produces irritants and toxic gases, which may include hydrogen chloride. In the presence of moisture and light, TCE decomposes by forming hydrochloric acid (HSDB 1997).

1.2 Packaging and shipping

TCE is generally shipped in 55-gal drums, tank trucks, or single compartment tank cars, with a capacity of 10,000 or 20,000 gal. PPG Industries, Inc., ships from its Louisiana plant and terminals located in New Jersey, Illinois, and California (PPG Industries, Inc. 1997).

2 Human Exposure

2.1 Use

TCE is used mainly as a degreaser for metal parts (CMR 1983, cited in Gist and Burg 1995). Five main industrial groups use TCE in vapor or cold degreasing operations: furniture and fixtures, fabricated metal products, electrical and electronic equipment, transport equipment, and miscellaneous manufacturing industries (IARC 1995e). TCE can be used as an extraction solvent and a chemical intermediate and as a component in adhesives, lubricants, paints, varnishes, paint strippers, pesticides, and cold metal cleaners (ATSDR 1995).

2.2 Production

The International Agency for Research on Cancer (IARC) (1995e) reported that in 1992, two companies in the United States produced a combined annual capacity of 160,000 tons of TCE (145,000 metric tons). The SRI *Directory of Chemical Producers, United States* listed only one producer (SRI 1996).

2.3 Analysis

NIOSH has two methods to determine TCE levels in relatively non-complex atmospheres. NIOSH Method #1022 is meant to measure TCE levels for STEL determinations. It has a working range of 27 to 875 ppm (150 to 4,700 mg/m^3) and is measured using a solid sorbent tube and a gas chromatograph, FID. NIOSH Method #3701 is meant to measure TCE using a tedlar air bag and a portable gas chromatograph with a photoionization detector. This method has a working range of 10 to 1000 ppm (54 to 5400 mg/m^3) (NIOSH 1994).

2.4 Environmental occurrence

Releases of TCE into the environment occur during its manufacture, use, and disposal. Although TCE can be formed by one red microalga and in temperate, subtropical, and tropical algae, nonanthropogenic releases of TCE are negligible (Wu and Schaum 2000).

2.4.1 Air

Most TCE emissions into the atmosphere are from vapor degreasing operations. Releases of TCE to air will also occur at sewage treatment and disposal facilities, water treatment facilities, and landfills. TCE has also been detected in stack emissions from municipal and hazardous waste incinerators (Wu and Schaum 2000).

The Toxic Chemical Release Inventory (TRI) for 1995 (TRI95 1997) contains reports on environmental releases of TCE from 717 U.S. facilities. Of these, 591 reported releases to the atmosphere of more than 2,000 lb (900 kg). Among the 591 facilities, 132 each released 2,000 to 10,000 lb (900 to 4,500 kg), 328 released 10,000 to 50,000 lb (4,500 to 22,700 kg), 114 released 50,000 to 200,000 lb (22,700 to 90,900 kg), and 17 released > 200,000 lb (> 90,900 kg). The total amount of TCE released in 1995 by the 717 facilities was 25,484,235 lb (11,600,000 kg), while the 17 greatest emitters together released 6,100,000 lb (2,800,000 kg). The greatest releases generally were from metalworking facilities, with three sites each reporting under Standard Industrial Classification (SIC) codes 3317 (steel pipe and tubes) and 3714 (motor

vehicle parts and accessories). Other facilities (one each) reported under SICs 3089 (plastics and plastic products, not elsewhere classified), 3671 (electron tubes), and 3721 (aircraft).

2.4.2 Water

Industrial discharges of wastewater streams are the primary releases of TCE into aquatic systems. TCE can also be released to groundwaters via leaching from landfills (Wu and Schaum 2000). TRI95 (1997) included data from 28 facilities that had each released more than 10 lb (4.5 kg) of TCE to water in 1995. Five facilities each released 250 to 280 lb (114 to 127 kg). The total release of TCE to water was 1,477 lb (670 kg). Four of the five facilities were metalworking plants, and one was a plant that produced TCE as a by-product and for onsite use and processing.

2.4.3 Soil

The total releases of TCE to land and underground injection wells in 1995 were 3,577 lb (1,626 kg) and 550 lb (250 kg), respectively (TRI95 1997).

2.5 Environmental fate

2.5.1 Air

TCE in the atmosphere is expected to be present primarily in the vapor phase rather than sorbed to particulates because of its high vapor pressure. Some removal by scavenging during wet precipitation is expected because of the moderate solubility of TCE in water (1.1 g/L). The major degradation process affecting vapor-phase TCE is photooxidation by hydroxyl radicals, with a half-life on the order of one to 11 days (Wu and Schaum 2000).

2.5.2 Soil

The dominant terrestrial fate of TCE is volatilization to air. Because of its moderate water solubility, TCE introduced into soil (e.g., via landfills) has the potential to migrate through the soil into groundwater. Biodegradation in soil is thought to be slow, with a half-life on the order of months to years (Wu and Schaum 2000).

2.5.3 Water

The dominant fate of TCE in water is volatilization with a predicted half-life of minutes to hours. Bioconcentration, biodegradation, and sorption to sediments and suspended solids are not through to be significant (Wu and Schaum 2000).

2.6 Environmental exposure

Because TCE is pervasive in the environment, most people are likely to be exposed to TCE by one or more of the following pathways: ingestion of drinking water, inhalation of ambient air, or ingestion of food. The Third National Health and Nutrition Examination Survey (NHANES III) suggests that about 10% of the population has detectable levels of TCE in their blood. The NHANES III examined TCE concentrations in 677 nonoccupationally exposed individuals from 1988 to 1994. These individuals were selected to represent a ranges of population characteristics such as age, race, gender, and region of residence. TCE levels in whole blood were below the 0.01 μg/L detection limit for about 90% of the people sampled. Assuming that nondetects equal half of the detection limit, the mean concentration was 0.015 μg/L. (Wu and Schaum 2000). The Agency for Toxic Substances and Disease Registry (ATSDR) is developing information on

potential public exposure to TCE and possible long-term health consequences in a subregistry to the National Exposure Registry for hazardous waste sites. The TCE subregistry includes three sites in Michigan, four sites in Indiana, six sites in Illinois, and one site each in Pennsylvania and Arizona. Environmental and tissue data will serve as the basis for estimating exposure (Gist *et al.* 1994).

2.6.1 Air

Air is the primary route of potential environmental exposure to TCE. Mean TCE background levels in air range from 0.03 ppb (0.16 μg/m^3) in rural areas to 0.46 ppb (2.5 μg/m^3) in urban and suburban areas. Areas near emission sources have TCE in the air at concentrations of up to 1.2 ppb (6.4 μg/m^3) (ATSDR 1995).

TCE is one of the volatile organic compounds (VOCs) measured in U.S. Environmental Protection Agency's (EPA's) large-scale Total Exposure Assessment Methodology studies (Wallace *et al.* 1996). In studies in Maryland, New Jersey, and California from 1981 through 1987, determination of TCE exposure via personal air monitors carried by 750 persons for two consecutive 12-hour periods revealed TCE median personal air concentrations of 0.3 to 3.0 μg/m^3. Breath samples taken in the evenings after several hours at home from 50 to 350 persons in two New Jersey cities in 1981 to 1983 and 75 persons in two California towns in 1984 contained TCE at concentrations of 0.1 to 0.9 μg/m^3 (median personal air concentrations of 1.7 to 3.0 μg/m^3). However, TCE was not detected in the breath of 140 persons in Los Angeles, CA (with TCE personal air levels of 0.3 to 1.2 μg/m^3 in 1984 or 1987), nor in the breath of 75 persons in Baltimore, MD (with TCE personal air levels of 1.1 μg/m^3) in 1987.

Ambient air measurements for TCE were obtained from various state and local environmental agencies from 1985 to 1998 by the Aerometric Information Retrieval System (AIRS). These data represent 1,200 measurements from 25 states. The 1998 air levels come from 115 monitors located in 14 states (mean 0.01 to 3.9 μg/m^3, mean = 0.88 μg/m^3). Using this mean and an inhalation rate of 20 m^3 air/day, the estimated inhalation exposure to TCE is 18 μg/day (Wu and Schaum 2000). Table 2-1 summarizes TCE air levels across monitors by year. Table 2-2 summarizes TCE air levels across monitors by land setting and use.

Table 2-1. Mean TCE air levels by year

Year	N	Mean concentration (μg/m^3)	Mean concentration (ppb)
1985	11	1.4	0.26
1986	21	1.39	0.26
1987	53	1.68	0.31
1988	57	4.87	0.91
1989	96	1.69	0.32
1990	59	1.84	0.34
1991	70	2.86	0.53
1992	76	1.37	0.26

Year	N	Mean concentration ($\mu g/m^3$)	Mean concentration (ppb)
1993	84	1.12	0.21
1994	89	0.95	0.18
1995	146	0.78	0.15
1996	150	0.65	0.12
1997	129	0.74	0.14
1998	115	0.88	0.16

Source: Wu and Schaum (2000)

Table 2-2. Mean TCE air levels by land setting and use

Year	N	Mean concentration ($\mu g/m^3$)	Mean concentration (ppb)
Rural	93	0.42	0.08
Suburban	500	1.26	0.24
Urban	558	1.61	0.30
Agricultural	31	1.08	0.20
Commercial	430	1.84	0.34
Forest	17	0.1	0.02
Industrial	186	1.54	0.29
Mobile	39	1.5	0.28
Residential	450	0.89	0.17

Source: Wu and Schaum (2000)

2.6.2 Water

TCE background levels in large bodies of water range from 0.001 to 0.007 ppb (µg/L), while values reported for rainwater and snow are 0.0008 to 0.039 ppb (µg/L) (Gist and Burg 1995). In the U.S. EPA's Contract Laboratory Program Statistical Database, TCE was noted as occurring in approximately 3% of surface-water samples at a geometric mean concentration of 40.2 ppb (individual sample values ranged from 0.0001 to 120 ppb) and in approximately 19% of ground water samples at a geometric mean concentration of 27.3 ppb (individual sample values ranged from < 0.1 to ≤ 27,300 ppb) (U.S. EPA 1989, cited in IARC 1995e). The California survey of large water utilities in 1984 found a median concentration of 3.0 µg/L. Using this median and the average water consumption rate of 2 L/day yields an estimate of 6 µg/day TCE exposure through drinking water (Wu and Schaum 2000). This is consistent with the ATSDR estimated average daily water intake for the general population of 2 to 20 µg/day (ATSDR 1995). Table 2-3 summarizes TCE measurements in rain, surface waters, groundwater, drinking water, and sea water in the United States.

Table 2-3. TCE levels in water

Water type	Location	Year	Mean	Median	Range (µg/L)	Number of samples	Reference
Industrial effluent	US	1983	--	0.5	--	--	IARC 1995e
Surface waters	US	1983	--	0.1	--	--	IARC 1995e
Rainwater	Portland, OR	1984	0.006	--	0.002-0.02	--	Ligocki *et al.* 1985
Groundwater	MN	1983	--	--	0.2-144	--	Sabel and Clark 1984
	NJ	1976	--	--	<1,530	--	Burmaster 1982
	NY	1980	--	--	<3,800	--	Burmaster 1982
	PA	1980	--	--	<27,300	--	Burmaster 1982
	AZ		--	--	8.9-29	--	IARC 1995e
	MA	1976	--	--	<900	--	Burmaster 1982
Drinking water	US	1976	--	--	0.2-49	--	IARC 1995e
	US	1977	--	--	0-53	--	IARC 1995e
	US	1978	--	--	0.5-210	--	IARC 1995e
	NJ	1984 -85	23.4	--	maximum 67	1,130	Cohn *et al.* 1994
	CA	1984	--	--	8-12	486	U.S. EPA 1987
	CA	1984	66	--	--	486	U.S. EPA 1987
	NC	1984	5	--	--	48	U.S. EPA 1987
	ND	1984	5	--	--	48	U.S. EPA 1987
	MA		--	--	maximum 267	48	U.S. EPA 1987

Source: Wu and Schaum (2000)

2.6.3 Consumer products

TCE is present in typewriter correction fluids, paint removers, strippers, adhesives, spot removers, and rug-cleaning fluids (Gist and Burg 1995). Uses of TCE as an extraction solvent for cosmetics and drug products and as a dry-cleaning agent have been discontinued (IARC 1995e).

2.6.4 Food

TCE has been found in a variety of foods with the highest levels found in meats, at 12 to16 ppb (0.09 to 0.12 μmol/kg), and U.S. margarine, at 440 to 3,600 ppb (3.35 to 27.4 μmol/kg) (ATSDR 1995). TCE had been used as a solvent for extraction of natural fats and oils, spices, hops, and caffeine (from coffee), but the U.S. Food and Drug Administration (FDA) banned these uses in 1977 (IARC 1995e).

2.7 Occupational exposure

According to the U.S. National Institute for Occupational Safety and Health (NIOSH) National Occupational Exposure Survey (NIOSH 1990), 401,373 employees in 23,225 plants in the United States were potentially exposed to TCE from 1980 to 1983 (Table 2-4).

Table 2-4. Numbers of U.S. workers (total and female) potentially exposed to TCE from 1980 to 1983, by industry

Industry	Plants	Total workers	Female workers
Agricultural services	339	1,695	1,695
General building contractors	661	5,463	3,106
Heavy construction contractors	65	5,420	5,306
Special trade contractors	834	1,879	1,287
Food and kindred products	190	2,062	604
Tobacco manufactures	43	517	-
Textile mill products	214	26,846	21,509
Apparel and other textile products	207	1,226	1,188
Lumber and wood products	505	4,932	1,189
Furniture and fixtures	184	1,352	-
Paper and allied products	167	4,331	1,846
Printing and publishing	2,372	26,317	10,227
Chemicals and allied products	236	10,277	3,151
Petroleum and coal products	256	2,020	-
Rubber and miscellaneous plastic products	862	15,772	2,381
Leather and leather products	33	65	-
Stone, clay, and glass products	275	1,494	1,341
Primary metal industries	379	5,047	417
Fabricated metal products	2,196	49,046	30,065
Machinery, except electrical	1,871	22,210	2,786
Electric and electronic equipment	1,197	97,000	47,714
Transportation equipment	207	9,305	559
Instruments and related products	984	16,293	5,032

Industry	Plants	Total workers	Female workers
Miscellaneous manufacturing industries	803	6,261	2,938
Railroad transportation	22	262	-
Trucking and warehousing	989	5,852	5,072
Transportation by air	481	15,216	3,782
Communication	603	8,776	1,802
Electric, gas, and sanitary services	117	4,336	429
Wholesale trade - durable goods	960	3,735	2,260
Wholesale trade - nondurable goods	352	704	-
Personal services	277	1,044	70
Business services	716	12,973	3,475
Auto repair, services, and garages	1,295	11,197	4,861
Miscellaneous repair services	406	812	-
Health services	569	11,302	9,059
Museums, botanical, zoological gardens	82	1,643	164
TOTAL	23,225	401,373	175,316

Source: National Occupational Survey (NIOSH 1990)

2.8 Regulations and criteria

FDA regulations govern the presence of TCE in color additives, in bottled water, in food as extraction solvent residues and as indirect additives as migrants from adhesives and other materials used in food packaging.

The Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) for time-weighted-average (TWA) exposure to TCE in workroom air in a 40-hour work week is 100 ppm (537 mg/m^3), with a ceiling value of 200 ppm (1,070 mg/m^3) (29 CFR 1910.1000 1996 [CHEMLIST 1997]). The NIOSH considers TCE to be a potential occupational carcinogen, recommending that exposure be limited to the lowest feasible concentration. The NIOSH recommended exposure level (REL) is 2 ppm (11 mg/m^3) during use of TCE as an anesthetic and a 10-hour TWA of 25 ppm (130 mg/m^3) during all other exposures (Ludwig 1994). The threshold limit value (TLV) recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) (1992, 1996) is 50 ppm (269 mg/m^3), and the recommended short-term exposure limit or ceiling is 100 ppm (537 mg/m^3). The ACGIH (1996) classified TCE as A5 (*not suspected as a human carcinogen*).

The U.S. EPA regulates TCE as a hazardous air pollutant under the Clean Air Act (CAA) 1990 Amendments and as a VOC subject to emission standards under CAA Section 111 (40 CFR Part 60 1995) (CHEMLIST 1997).

Under the Safe Drinking Water Act (SDWA), the maximum contaminant level (MCL) for community and nontransient, noncommunity water systems is 0.005 mg/L (40 CFR Part 141 1996) (CHEMLIST 1997). The World Health Organization (WHO) recommended a provisional

guideline value for TCE in drinking water of 0.070 mg/L (WHO 1993). Based on a 1985 study by Buben and O'Flaherty, the WHO (1993) calculated a total daily intake of 0.0238 mg/kg body weight (b.w.) by applying an uncertainty factor of 3,000 to the study's lowest observable adverse effect level of 100 mg/kg b.w. per day when mice were exposed five days a week for six weeks. The observed adverse effects were minor effects on relative liver weight. For derivation of the provisional guidance value of 70 µg/L, 10% of the total daily intake was allocated to drinking water.

TCE is regulated under Resource Conservation and Recovery Act (RCRA) as a halogenated organic compound and under the Land Disposal Restrictions. Under the latter regulation, hazardous wastes that contain total concentrations of halogenated organic compounds of at least 1,000 mg/L (liquids) or 1,000 mg/kg (nonliquids) are prohibited from land disposal. Under 40 CFR 268.40 and 268.48, treatment standards are given for wastewater and non-wastewater extract concentrations, or the applicable Technology Code (40 CFR 268.42) is given (CHEMLIST 1997).

TCE is regulated under Sections 110 and 313 of the Superfund Amendment Reauthorization Act (SARA). Priority data needs that were established under Section 110 include exposure levels in humans living near hazardous waste sites and other populations and epidemiological studies on health effects, including carcinogenicity. Under SARA Section 313, the Emergency Planning and Community Right-to-Know Act, and the TRI, 40 CFR Part 372 Subpart D (1992), TCE is one of the 19 substances for which the *de minimus* for reporting was changed from 1.0% to 0.1%. Under the TRI, since 1989, manufacturers of at least 25,000 lb/yr (11,350 kg/yr) and other handlers of at least 10,000 lb/yr (4,540 kg/yr) must report releases of TCE to any environmental medium. Under 40 CFR Part 302 Table 302.4, TCE is on the Comprehensive Environmental Responsibility Compensation and Liability Act (CERCLA) List of Hazardous Substances, with a reportable quantity (RQ) for releases set at 100 lb (45.4 kg) (CHEMLIST 1997).

TCE is regulated under the Clean Water Act (CWA) Sections 301, 307, and 311 (40 CFR Part 423 1996; 40 CFR Parts 116 and 117 1996). TCE is a priority pollutant in final discharges resulting from steam electric power generation. It is designated a hazardous substance if discharged to navigable waters. The RQ for notification is 100 lb (45.4 kg) (CHEMLIST 1997).

Table 2-5 summarizes EPA regulations that affect TCE. Table 2-6 summarizes FDA regulations. Table 2-7 summarizes OSHA regulations.

Table 2-5. U.S. EPA regulations

EPA Regulations	
Regulatory action	**Effect of regulation and other comments**
40 CFR 60—PART 60—STANDARDS OF PERFORMANCE FOR NEW STATIONARY SOURCES. Promulgated: 48 FR 48335, 10/18/83.	The provisions of this part apply to the owner oroperator of any stationary source that contains an affected facility (a stationary source with an apparatus to which a standard is applicable).
40 CFR 60.480 ff.—Subpart B—Standards of Performance for Equipment Leaks of VOC in the Synthetic Organic Chemicals Manufacturing Industry.	Each owner or operator of facilities producing TCE as an intermediate or final product must demonstrate compliance with the provisions of this subpart.
40 CFR 60.660 ff.—Subpart NNN—Standards of Performance for Volatile Organic Compound (VOC) Emissions from Synthetic Organic Chemical Manufacturing Industry (SOCMI) Distillation Operations. Promulgated: 55 FR 26942, 06/29/90.	This subpart affects distillation units not discharging their vent steams into a recovery system, or a combination of two or more distillation units and a common recovery system into which their vent steams are discharged, which use, contain, or produce TCE. Specific standards, monitoring, and recordkeeping requirements apply.
40 CFR 60.700 ff.—Subpart RRR—Standards of Performance for Volatile Organic Compounds Emissions from Synthetic Organic Chemical Manufacturing Industry (SOCMI) Reactor Processes. Promulgated: 58 FR 45962, 08/31/93.	This subpart affects reactor units not discharging their vent steams into a recovery system, or a combination of two or more reactor units and a common recovery system into which their vent steams are discharged, which use, contain or produce TCE. Specific standards, monitoring, and recordkeeping requirements apply.
40 CFR 61—PART 61—NATIONAL EMISSION STANDARDS FOR HAZARDOUS AIR POLLUTANTS. Promulgated: 38 FR 8826, 04/06/73. U.S. Codes: 42 U.S.C. 7401, 7412, 7414, 7416, 7601.	This part lists substances that, pursuant to section 112 of the CAA, have been designated as hazardous air pollutants, and applies to the owner or operator of any stationary source for which a standard is prescribed under this part. As of 50 FR 52422, 12/23/95, TCE was listed because of the serious health effects, including cancer, from ambient air exposure.
40 CFR 63—PART 63—NATIONAL EMISSION STANDARDS FOR HAZARDOUS AIR POLLUTANTS FOR SOURCE CATEGORIES. Promulgated: 57 FR 61992, 12/29/92. U.S. Codes: 42 U.S.C. 7401 et seq.	Standards that regulate specific categories of stationary sources that emit (or have potential to emit) one or more hazardous air pollutants are listed in this part pursuant to section 112(b) of the CAA.
40 CFR 63.100 ff.—Subpart F—National Emission Standards for Organic Hazardous Air Pollutants From the Synthetic Organic Chemical Manufacturing Industry. Promulgated: 59 FR 19454, 04/22/94.	This subpart applies to chemical manufacturing process units that manufacture TCE and are located at a plant site that is a major source as defined in section112(a) of the CAA. Owners and operators of sources subject to this subpart shall comply with the requirements of subparts G and H of this part.
40 CFR 63.110 ff.—Subpart G—National Emission Standards for Organic Hazardous Air Pollutants From the Synthetic Organic Chemical Manufacturing Industry for Process Vents. Promulgated: 59 FR 19468, 4/22/94.	The provisions of this subpart apply to all process vents, storage vessels, transfer racks, and wastewater streams within a source subject to subpart F of this part. Emission standard: Emissions of TCE shall be controlled to the level represented by a given equation (see 40 CFR 63.112[a]). Specific process vent and methods and procedures provisions apply.

EPA Regulations	
Regulatory action	**Effect of regulation and other comments**
40 CFR 63.460 ff.—Subpart T—National Emission Standards for Halogenated Solvent Cleaning. Promulgated: 59 FR 61805, 12/2/94.	Individual batch vapor, in-line vapor, in-line cold, and batch cold solvent cleaning machines that use TCE alone or in a mixture with other hazardous air pollutants listed in a total concentration greater than 5%. Specific batch cold cleaning, vapor, in-line, and alternative standards and monitoring and recordkeeping requirements apply.
40 CFR 63.680 ff.—Subpart DD—Applicability and designation of affected sources. Promulgated: 61 FR 34158, 07/01/96.	The provisions of this subpart apply to plant sites at which a major source of TCE emissions occurs as defined in 40 CFR 63.2, or at which is located one or more operations that receive offsite materials as specified in 40 CFR 63.680(b).
40 CFR 63.800 ff.—Subpart JJ—National Emission Standards for Wood Furniture Manufacturing Operations. Promulgated: 60 FR 62936, 12/7/95.	The provisions of this subpart apply to each facility that is engaged in the manufacture of wood furniture or wood furniture components and that is a major source as defined in 40 CFR 63.2. TCE is excluded from use in cleaning and washoff solvents.
40 CFR 116—PART 116—DESIGNATION OF HAZARDOUS SUBSTANCES. Promulgated: 43 FR 10474, 03/13/1978. U.S. Codes: 33 U.S.C. 1251 et seq.	This regulation designates TCE as a hazardous substance under section 311(b)(2)(a) of the Federal Water Pollution Control Act (FWPCA). The regulation applies to discharge of the substances identified in table 116.4 to surface waters.
40 CFR 117—PART 117—DETERMINATION OF REPORTABLE QUANTITIES FOR HAZARDOUS SUBSTANCES. Promulgated 44 FR 50776, 08/29/79. U.S. Codes: FWPCA 311(b)(2)(A) and 501(a) as amended by the CWA of 1977.	Discharges to water of amounts equal to or greater than the RQ must be reported to the EPA. The RQ for environmental releases of TCE to water is 100 lb (45.4 kg).
40 CFR 132—PART 132—WATER QUALITY GUIDANCE FOR THE GREAT LAKES SYSTEM. Promulgated: 60 FR 15387, 03/23/95. U.S. Codes: 33 U.S.C. 1251 et seq.	Water criteria for protection of human health are provided. The limits for TCE are 0.29 g/L in drinking water and 0.037 g/L in non-drinking water.
40 CFR 141—PART 141—NATIONAL PRIMARY DRINKING WATER REGULATIONS. Promulgated: 40 FR 59570, 12/24/75. U.S. Codes: Public Health Service Act sections 1413-1416, 1445, and 1450 as amended by 1974 SDWA. U.S.C. 300.	To protect a safe drinking water supply, community and non-transient, non-community water systems must monitor for certain compounds listed.
40 CFR 141 ff.—Subpart D—Reporting, Public Notification and Record keeping. Promulgated: 60 FR 33932, 06/29/95.	The EPA has set forth an enforceable drinking water standard to limit TCE levels to 0.005 ppm to reduce the risk of cancer or other adverse health effects that have been observed in laboratories.
40 CFR 141.50 ff.—Subpart F—Maximum Contaminant Level Goals. Promulgated: 50 FR 46901, 11/13/85, and others.	The maximum contaminant level goal for TCEin primary drinking water is zero.
40 CFR 141.60 ff.—Subpart G—National Revised Primary Drinking Water Regulations: Maximum Contaminant Levels. Promulgated: 52 FR 25716, 07/08/87.	The maximum contaminant levels apply to community water systems and non-transient, non-community water systems based on the best available technology treatment techniques. The MCL for TCE is 0.002 mg/L.

EPA Regulations	
Regulatory action	**Effect of regulation and other comments**
40 CFR 148—PART 148—HAZARDOUS WASTE INJECTION RESTRICTIONS. Promulgated: 53 FR 28154, 06/26/88.	TCE is identified as a hazardous waste to be restricted from EPA Class I hazardous waste injection wells.
40 CFR 257—PART 257—CRITERIA FOR CLASSIFICATION OF SOLID WASTE DISPOSAL FACILITIES AND PRACTICES. Promulgated: 56 FR 51016 10/9/91. U.S. Codes: 42 U.S.C. 6907(a)(3) and 6944(a); 33 U.S.C. 1345(d).	The maximum TCE contaminant level in groundwater for solid waste disposal facilities is 0.005 mg/L. The RQ of TCE is 100 lb (45.4 kg). Label, packaging, and shipping codes also are listed in the Hazardous Materials Table.
40 CFR 258—PART 258—CRITERIA FOR MUNICIPAL SOLID WASTE LANDFILLS. Promulgated: 56 FR 51016, 10/09/91. U.S. Codes: 33 U.S.C. 1345(d) and (e); 42 U.S.C. 6907(a)(3), 6912(a), 6944(a) and 6949a(c).	The provisions of this part establish minimum national criteria under RCRA, as amended, for all municipal solid waste landfill (MSWLF) units and under the CWA, as amended, for MSWLF units that are used to dispose of sewage sludge. The criteria ensure the protection of human health and the environment. The maximum contaminant level for TCE is 0.005 mg/L.
40 CFR 261—PART 261—IDENTIFICATION AND LISTING OF HAZARDOUS WASTES. Appendix VIII—Basis for Listing Hazardous Waste. Promulgated: 45 FR 33119, 05/19/80; 53 FR 13388, 04/22/88. U.S. Codes: 42 U.S.C. 6905, 6912(a), 6921, 6922, and 6938.	Appendix VIII is a consolidated list of hazardous constituents identified in this part. Solid wastes containing these constituents are subject to notification requirements of RCRA section 3010 and must be disposed of in RCRA-permitted facilities. For TCE, the regulatory level is 0.5 mg/L; its hazardous waste number is D040.
40 CFR 264—PART 264—STANDARDS FOR OWNERS AND OPERATORS OF HAZARDOUS WASTE TREATMENT, STORAGE, AND DISPOSAL FACILITIES, Appendix IX. List (Phase 1) of Hazardous Constituents for Ground-Water Monitoring. Promulgated: 45 FR 33221, 05/19/80. U.S. Codes: 42 U.S.C. 6905, 6912(a), 6924, and 6925.	The provisions of this part establish minimum national standards which define the acceptable management of hazardous waste, and apply to owners and operators of all facilities that treat, store, or dispose of hazardous waste; exceptions do exist. TCE has a practical quantitation limit of 1 μg/L.
40 CFR 266—PART 266—STANDARDS FOR THE MANAGEMENT OF SPECIFIC HAZARDOUS WASTES AND SPECIFIC TYPES OF HAZARDOUS WASTE MANAGEMENT FACILITIES. Promulgated: 50 FR 666, 1/4/85. U.S. Codes: 42 U.S.C. 6905, 6912(a), 6924, and 6934.	Standards to control emissions are promulgated for generators, transporters, and users of materials used in a manner that constitutes disposal. Affected compounds are listed in 40 CFR 266.40.
40 CFR 266.100 ff.—Subpart H—Hazardous Waste Burned in Boilers and Industrial Furnaces. Promulgated: 56 FR 7208, 02/21/91.	Hazardous waste burned or processed in a boiler or industrial furnaces is regulated by this subsection to limit release into the environment. The maximum concentration limit for TCE in residues is 0.005 mg/kg. The maximum allowable wastewater concentration is 6.6 ppm, and the maximum allowable concentration in solid waste is 0.05 ppm.
40 CFR 302—PART 302—DESIGNATION, REPORTABLE QUANTITIES, AND NOTIFICATION. Promulgated: 50 FR 13474, 04/04/85. U.S. Codes: 42 U.S.C. 9602, 9603, and 9604; 33 U.S.C. 1321 and 1361.	This part designates under section 102(a) of CERCLA 1980 those substances in the statutes referred to in section 101(14) of CERCLA, identifies reportable quantities for these substances, and sets forth the notification requirements for releases of these substances. This part also sets forth reportable quantities for hazardous substances designated under section 311(b)(2)(A) of the CWA.

EPA Regulations	
Regulatory action	**Effect of regulation and other comments**
40 CFR 302.4—Sec. 302.4 Designation of hazardous substances. Superfund (CERCLA, SARA) reportable quantity (RQ) is 100 lb (45.4 kg).	The EPA designated as hazardous those substances that when released into the environment may present substantial danger to the public health or welfare or the environment. Notification of the EPA is required if the RQ is released to the environment.
40 CFR 372—PART 372—TOXIC CHEMICAL RELEASE REPORTING: COMMUNITY RIGHT-TO-KNOW. Promulgated: 53 FR 4525, 02/16/88. U.S. Codes: 42 U.S.C. 11013, 11028.	This part sets forth requirements for the submission of information relating to the release of toxic chemicals under section 313 of Title III of SARA (1986). Information collected under this part is intended to inform the general public and the communities surrounding covered facilities about releases of toxic chemicals, to assist research, and to aid in the development of regulations, guidelines, and standards. TCE is listed under the specific toxic chemical listings, with 1/1/87 as the effective date for reporting.
40 CFR 401—PART 401—GENERAL PROVISIONS. Promulgated: 39 FR 4532, 02/01/74, as amended at 47 FR 24537, 06/04/82. U.S. Codes: 33 U.S.C. 1251 et seq.	The provisions of this part set forth the legal authority and general definitions that will apply to all regulations issued concerning specific classes and categories of point sources of industrial effluents under parts 402 through 699. TCE is listed as a toxic pollutant.
40 CFR 413—PART 413—ELECTROPLATING POINT SOURCE CATEGORY. Promulgated: 46 FR 9467, 01/28/81. U.S. Codes: 33 U.S.C. 1251 et seq., as amended by the CWA of 1977 (Public Law 95-217).	This part regulates discharge of waste streams from several categories of industrial processes that involve electroplating or electroless plating. The concentration limit of TCE is 0.01 mg/L.
40 CFR 414—PART 414—ORGANIC CHEMICALS, PLASTICS, AND SYNTHETIC FIBERS. Promulgated: 52 FR 42568, 11/5/87. U.S. Codes: 33 U.S.C. 1311, 1314, 1316, 1317, and 1361.	The EPA gives pretreatment standards for existing sources for metals and organics in effluents from several manufacturing categories. Limitations represent the degree of effluent reduction attainable by application of best available technology.
40 CFR 414.91 ff.—Subpart I—Direct Discharge Point Sources That Use End-of-Pipe Biological Treatment.	The effluent limitation for TCE maximum concentrations for any one day is 54 μg/L and for any monthly average is 21 μg/L.
40 CFR 414.101 ff.—Subpart J—Direct Discharge Point Sources That Do Not Use End-of-Pipe Biological Treatment	The effluent limitation for TCE maximum concentrations for any one day is 69 μg/L and for any monthly average is 26 μg/L.
40 CFR 414.110 ff.—Subpart K—Indirect Discharge Point Sources.	The effluent limitation for TCE maximum concentrations for any one day is 69 μg/L and for any monthly average is 26 μg/L.
40 CFR 423—PART 423—STEAM ELECTRIC POWER GENERATING POINT SOURCE CATEGORY. Promulgated: 47 FR 52304, 11/19/82. U.S. Codes: 33 U.S.C. 1311; 1314(b), (c), (e), and (g); 1316(b) and (c); 1317 (b) and (c); and 1361.	The provisions of this part apply to TCE discharges resulting from the operation of a generating unit by an establishment generating electricity for distribution and sale, which results from a process utilizing fossil-type or nuclear fuel in conjunction with a thermal cycle that uses the steam water system as the thermodynamic medium.

EPA Regulations	
Regulatory action	**Effect of regulation and other comments**
40 CFR 433—PART 433— METAL FINISHING POINT SOURCE CATEGORY. Promulgated: 48 FR 32485, 07/15/83. U.S. Codes: 33 U.S.C. 1311, 1314(b)(c), (e), and (g), 1316(b) and (c), 1317(b) and (c), 1318, and 1361.	The provisions of this subpart apply to plants which discharge TCE while performing any of the following six metal finishing operations on any base material: electroplating, electroless plating, anodizing, coating (chromating, phosphating, and coloring), chemical etching and milling, and printed circuit board manufacture.
40 CFR 464—PART 464—METAL MOLDING AND CASTING POINT SOURCE CATEGORY. Promulgated: 50 FR 45247, 10/30/85. U.S. Codes: 33 U.S.C. 1311, 1314(b), (c), (e), and (g), 1316(b) and (c), 1317(b) and (c), 1318, and 1361.	The provisions of subparts A through D apply to metal molding and casting facilities that discharge or may discharge TCE to waters of the United States or that introduce TCE into a publicly owned treatment works (POTW).
40 CFR 467—PART 467—ALUMINUM FORMING POINT SOURCE CATEGORY. Promulgated: 48 FR 49149, 10/24/83. U.S. Codes: 33 U.S.C. 1311, 1314(b), (c), (e), and (g), 1316(b) and (c), 1317(b) and (c), 1318, and 1361.	This part applies to any aluminum forming facility that discharges or may discharge TCE to U.S. waters or that introduces or may introduce TCE into a POTW.
40 CFR 468— PART 468—COPPER FORMING POINT SOURCE CATEGORY. Promulgated: 48 FR 36957,08/15/83. U.S. Codes: 33 U.S.C. 1311, 1314(b), (c), (e), and (g), 1316(b) and (c), 1317(b) and (c), and 1361.	The provisions of this part apply to discharges of TCE resulting from the manufacture of formed copper and copper alloy products.
40 CFR 469—PART 469—ELECTRICAL AND ELECTRONIC COMPONENTS POINT SOURCE CATEGORY. Promulgated: 48 FR 15394, 04/08/83. U.S. Codes: 33 U.S.C. 1311, 1314, 1316, 1317, 1318, and 1361.	The provisions of subparts B through D are applicable to discharges of TCE resulting from the manufacture of electronic crystals, cathode ray tubes, and luminescent materials.

Source: The regulations in this table have been updated through 62 Federal Register 37448, 11 July 1997.

Table 2-6. FDA regulations

FDA regulations	
Regulatory action	**Effect of regulation and other comments**
21 CFR 73—PART 73—LISTING OF COLOR ADDITIVES EXEMPT FROM CERTIFICATION. Promulgated: 42 FR 15643 03/22/77. S. Code: 21 U.S.C. 321, 341, 342, 343, 348, 351, 352, 355, 361, 362, 371, 379e	This part lists color additives that are exempt from certification in foods, drugs, cosmetics, and medical devices.
21 CFR 73.30—Sec. 73.30 Annatto extract.	TCE may be safely used in the color additive Annatto extract, including pigments precipitated therefrom.
21 CFR 103—PART 103—QUALITY STANDARDS FOR FOODS WITH NO IDENTITY STANDARDS. Promulgated: 42 FR 14325 03/15/77. U.S. Code: 21 U.S.C. 321, 341, 343, 348, 349, 371, 379e.	The label of a food is required to state its quality based on, but not limited to, levels of microorganisms and such physical characteristics as turbidity, color, flavor, and odor.
21 CFR 103.35—Sec. 103.35 Bottled Water. Promulgated: 60 FR 57123 11/13/95 [Sec. 103.35 was removed 6/13/96.] U.S. Code: 21 U.S.C. 321, 341, 343, 3348, 349, 371, 379e.	The allowable level for VOC TCE in bottled water is 0.005 mg/L.

FDA regulations	
Regulatory action	**Effect of regulation and other comments**
21 CFR 165.110 ff—Subpart B—Requirements for Specific Standardized Beverages—Bottled water. Promulgated: 60 FR 57124 11/13/95. U.S. Code: 21 U.S.C. 321, 341, 343, 343A, 348, 349, 371, 379e.	The regulations in subparts A and B govern the labeling and effective chemical substance limits for specific standardized beverages. The allowable level for VOC TCE in bottled water is 0.005 mg/L.
21 CFR 172—PART 172—FOOD ADDITIVES PERMITTED FOR DIRECT ADDITION TO FOOD FOR HUMAN CONSUMPTION. Promulgated: 42 FR 14491 03/15/77. U.S. Code: 21 U.S.C. 321, 341, 342, 348, 371, 379e.	The regulations in subparts A through I govern the amount of food additives allowed for human consumption.
21 CFR 172.560—Sec. 172.560 Modified hop extract.	The residues of the modified hop extract, manufactured from hops by initial extraction and fractionation, may not contain TCE at more than 150 ppm.
21 CFR 173—PART 173—SECONDARY DIRECT FOOD ADDITIVES PERMITTED IN FOOD FOR HUMAN CONSUMPTION. Promulgated: 42 FR 14526 03/15/77. U.S. Code: 21 U.S.C. 321, 342, 348.	The subparts A through D govern which polymer substances, polymer adjuvants for food treatments, enzyme preparations, microorganisms, solvents, lubricants, release agents, and related substances may be used in food for human consumption.
21 CFR 173.290—Sec. 173.290 TCE.	Tolerances are established for residues of TCE resulting from its use as a solvent in the manufacture of foods: 25 ppm in decaffeinated ground coffee, 10 ppm in decaffeinated soluble (instant) coffee extract, and 30 ppm in spice oleoresins.
21 CFR 175— PART 175—INDIRECT FOOD ADDITIVES: ADHESIVES AND COMPONENTS OF COATINGS. Promulgated: 42 FR 14534 03/15/77. U.S. Code: 21 U.S.C. 321, 342, 348, 379e.	The subparts A through C deal with components of adhesives and of coatings that may migrate into food from packaging.
21 CFR 175.105— Sec. 175.105 Adhesives.	TCE may be safely used in adhesives intended for use as components of articles intended for use in packaging, transporting, or holding food.

Source: The regulations in this table have been updated through 62 Federal Register 37448, 11 July 1997.

Table 2-7. OSHA regulations

OSHA regulations	
Regulatory action	**Effect of regulation and other comments**
1/78. Special Occupational Hazard review of TCE. DHEW Pub. No. (NIOSH) 78-130, NTIS No. PB8-1226987. 3/77. Criteria for a Recommended Standard….Occupational Exposure to Waste Anesthetic Gases and Vapors. Pub. No. 77-140, NTIS No. PB274 238.	TheNIOSH recommends that TCE be treated as a potential occupational carcinogen. Summary of the NIOSH recommendation: recommended exposure limit, 25 ppm TWA; 2 ppm ceiling limit (1 h) as a waste anesthetic gas.
6/6/75. Current Intelligence Bulletin #2—TCE (TCE). In: NIOSH Current Intelligence Bulletin Reprints-Bulletins 1 through 18 (1975-1977). Pub. No. 78-127, NTIS No. PB83-105080.	

OSHA regulations	
Regulatory action	**Effect of regulation and other comments**
1973. Criteria for a Recommended Standard….Occupational Exposure to TCE. DHEW (NIOSH) Pub. No. 73-11025, NTIS No. PB 222 222.	
29 CFR 1910—PART 1910—OCCUPATIONAL SAFETY AND HEALTH STANDARDS. Promulgated: 39 FR 23502, 06/27/74. U.S. Code: 29 U.S.C. 653, 655, and 657.	
29 CFR 1910—Subpart Z—Toxic and Hazardous Substances.	
29 CFR 1910.1000—Sec. 1910.1000 Air contaminants. Promulgated: 58 FR 40191, 07/27/93. U.S. Code: also includes 5 U.S.C. 553.	PEL $\leq$100 ppm (546 mg/m^3) 8-h TWA. Ceiling 2,000 ppm (1,090 mg/m^3)
20 CFR 1926—PART 1926—SAFETY AND HEALTH REGULATIONS FOR CONSTRUCTION. Promulgated: 44 FE 8577, 02/09/79; 44 FR 20940, 04/06/79. U.S. Code: 29 U.S.C. 653, 655, and 657.	
29 CFR 1926—Subpart D—Occupational Health and Environmental Controls.	
29 CFR 1926.55—Sec. 1926.55 Gases, vapors, fumes, dusts and mists. Promulgated: 61 FR 9249, 9250 03/07/96. U.S. Code: 40 U.S.C. 333; 29 U.S.C. 653, 655, and 657.	PEL $\leq$100 ppm (546 mg/m^3) 8-h TWA.

Source: The regulations in this table have been updated through 62 Federal Register 37448, 11 July 1997.

3 Human Cancer Studies

Trichloroethylene is classified by the IARC (1995e) as *probably carcinogenic to humans* (Group 2A) based on sufficient evidence of carcinogenicity in experimental animal and limited evidence of carcinogenicity in humans. A number of TCE studies have been published since the IARC review. These include three major cohort studies (Boice *et al*. 1999, Morgan *et al*. 1998, Ritz 1999), one cohort study update (Blair *et al.* 1998), two kidney cancer case control studies (Dosemeci *et al*. 1999, Vamvakas *et al.* 1998), one melanoma case control study (Fritschi and Siemiatycki 1996), and one case-control study examining cancer at many anatomical sites (Greenland *et al.* 1994). These studies, along with new reviews (Weiss 1996, McLaughlin and Blot 1997, Wartenberg *et al.* 2000) and commentaries (Henschler *et al.* 1995, Bloemen and Tomenson 1995, Swaen 1995, Green and Lash 1999, Vamvakas *et al.* 2000), add greatly to the richness of the data available for evaluation of the carcinogenicity of TCE to humans. Although they are limited by a paucity of directly measured exposures and the generally small numbers of site-specific cancers within studies, these studies overall represent a large and generally consistent body of evidence indicating that TCE is a human carcinogen.

Below is a brief review of the IARC (1995e) findings followed by more detailed evaluations of the most recent studies.

3.1 IARC evaluation

The IARC (1995e) considered case reports, descriptive studies, cohort studies, case-control studies, and drinking-water studies in its comprehensive assessment and review of the possible carcinogenicity of TCE to humans. The case reports and descriptive studies discuss individuals exposed to TCE and other solvents who subsequently developed cancer. Given the number and quality of cohort and case-control studies, the case reports and descriptive studies did not play an important role in the IARC evaluation.

To conduct its assessment, the IARC divided the cohort studies into three occupational groups: (1) dry cleaners, (2) workers who had undergone biological monitoring for exposure to TCE, and (3) workers employed in miscellaneous manufacturing industries.

The cohort studies of dry cleaners were considered least important. These workers likely had only limited exposure to TCE because it was used mainly for spot removal. They also had exposure to other solvents, particularly tetrachloroethylene (also known as perchloroethylene, or PERC), another suspected carcinogen, making chemical-specific inferences difficult.

Two cohort studies evaluated TCE exposure by biological monitoring of urine samples. Axelson *et al.* (1994) followed Swedish workers in a TCE production facility for 32 years. Overall cancer incidence and mortality were not unusual (standardized incidence and mortality ratios [SIRS and SMRs] both were1.0). However, for men only, there was a statistically significant excess of skin cancer (SIR = 2.4, 95% CI 1.0 to 4.7; n = 8) and non-significant excesses of non-Hodgkin's lymphoma (SIR = 1.6, 95% CI 0.5 to 3.6; n = 5) and liver and biliary cancer (SIR = 1.4, 95% CI 0.4 to 3.6;n = 4). In addition, an exposure-response relationship was seen for all cancers combined. The other biomonitoring study (Anttila *et al.* 1995) measured Finnish workers' exposure to three halogenated hydrocarbons (TCE, PERC, and 1,1,1 trichloroethane) and had a

26-year follow-up period. Among those exposed to TCE, there was a slight overall excess incidence of cancer (SIR = 1.1, 95% CI 0.9 to1.2; n = 208); a significant excess of cervical cancer (SIR = 2.4, 95% CI 1.1 to 4.8; n = 8), particularly among those more highly exposed (SIR = 4.4, 95% CI 1.4 to10.0; n = 5); a non-significant excess of liver cancer (SIR = 2.3, 95% CI 0.7 to 5.3; n = 5), particularly among those more highly exposed (SIR = 2.7, 95% CI 0.3 to 9.9; n = 2); and a non-significant excess of non-Hodgkin's lymphoma (SIR = 1.8, 95% CI 0.8 to 3.6; n = 8).

Among the manufacturing studies reviewed by the IARC, one large study looked at aircraft manufacturing (Garabrant *et al.* 1988), another at aircraft maintenance workers (Spirtas *et al.* 1991), and another at cardboard manufacturing plant workers (Henschler *et al.* 1995a). In all of these studies, exposure to TCE was principally from its use as a degreasing agent. In Garabrant *et al.* (1988), only one-third of the jobs involved TCE exposure and individual workers could not be classified as exposed or not. The overall mortality rate was less than expected (SMR = 0.8, 95% CI 0.7 to 0.8; n = 1,804), as was the cancer mortality rate (SMR = 0.8, 95% CI 0.8 to 0.9; n = 453). None of the SMRs for the individual cancer sites were significantly elevated. In Spirtas *et al.* (1991) the highest excess cancer mortalities were observed for liver and biliary, bone, and cervical cancer and non-Hodgkin's lymphoma. The results of this study were updated with longer follow-up by Blair *et al.* (1998, see Section 3.2). Henschler *et al.* (1995), in a relatively small study, identified exposed (n = 169) and unexposed (n = 190) workers from a single factory and developed 34 years of follow-up data. Exposure of the workers was believed to be particularly high as TCE was kept in open barrels and used biweekly to soak rags for cleaning machinery. There were anecdotal reports of acute TCE toxicity among workers. Henschler *et al.* (1995) reported four incident cases of renal-cell carcinoma and one of renal pelvic cancer among the exposed (SIR = 8.0, 95% CI 2.6 to18.6; n = 5). No renal-cell or renal pelvic cancer was observed among the unexposed. Concerns were raised about this study because it was conducted in response to an apparent cancer cluster and because cases identified by abdominal sonography of the entire workforce were compared with registry cases identified by more traditional diagnostic procedures (IARC 1995e, Swaen 1995, Bloemen and Tomenson 1995, Henschler *et al.* 1995). Other manufacturing studies were considered less important.

The IARC reviewed case-control studies of liver cancer, lymphoma, Hodgkin's disease, renal-cell carcinoma, colon cancer, brain tumors, childhood leukemia, and childhood brain tumors, as well as one multisite study. These studies were limited because they generally did not provide TCE-specific risk estimates. Nonetheless, most of these studies showed excess cancer at the same anatomical sites as the cohort studies.

Several cross-sectional studies of TCE-contaminated drinking water also were considered. In each of these, however, exposure was not well quantified, was to several contaminants simultaneously, and was at the community level rather than that of the individual. Two studies showed a weak association between contamination and the incidence of leukemia, and two showed a marginal increase in non-Hodgkin's lymphoma associated with contamination.

In its summary, the IARC pooled data from the cohort studies of Axelson *et al.* (1994), Anttila *et al.* (1995) and Spirtas *et al.* (1991) and reported excess liver and biliary cancer (23 observed, 12.87 expected) and non-Hodgkin's lymphoma (27 observed, 18.9 expected). It also reported a doubled incidence of cervical cancer based on two of these studies.

3.2 Recent cohort studies

Blair *et al.* (1998) extended the follow-up of the Spirtas *et al.* (1991) study of 14,457 aircraft maintenance workers to include an additional eight years (1982 to 1990). The main solvent used was TCE, but several other solvents also were used. TCE was used in bench-top work until 1968 and for vapor degreasing until 1978. Mortality patterns were largely unchanged with the additional follow-up, showing excesses for liver, kidney, bone, breast, cervical, colon, and esophageal cancer and non-Hodgkin's lymphoma and multiple myeloma. There were differences among males and females, and no clear exposure-response patterns were identified. Incidence data were reported for four exposure levels, rather than summarized overall. At the highest exposure level, excess mortality was observed for liver cancer, colon cancer, kidney cancer, and multiple myeloma in men and for kidney cancer in women. Workers with exposure to solvents other than TCE often had relative risks as high as those reported for TCE-exposed workers, particularly for non-Hodgkin's lymphoma, multiple myeloma, and female breast cancer, complicating interpretation. Information on lifestyle factors (e.g., tobacco and alcohol use and diet) and nonoccupational exposures was not available. However, smoking was unlikely to have been a confounder, because it is not known to be associated with most of the cancers found in excess, and two of the three cancers typically associated with smoking (lung and bladder) were not found in excess. Alcohol use and diet were possible but unlikely confounders for the effects reported.

Morgan *et al.* (1998) updated the work of Wong and Morgan (1990) on the mortality experience of 20,508 workers at a Hughes Aircraft manufacturing facility. TCE was used in vapor degreasing units. To categorize exposure, workers employed for at least 30 years were asked to rank the TCE exposure in each job classification. These rankings were combined by industrial hygienists into a four-category exposure scale. The highest exposure category was thought to correspond to a TCE level of about 50 ppm. In the TCE-exposed subcohort, small excesses in mortality were reported for kidney, bladder, ovary, and prostate cancers. The SMRs for these sites in the total cohort were lower. In an analysis by low or high cumulative TCE exposure using a Cox proportional hazards model, the relative risk for each of these four cancers increased with higher exposure. This study was limited by small numbers of cases and lack of direct exposure measurements, information on other occupational or nonoccupational exposures, or lifestyle factors.

Boice *et al.* (1999) studied another cohort of aircraft manufacturing workers, 77,965 employees of Lockheed Martin working at six separate facilities. Exposures included TCE, chromate, PERC, and other solvents. Subjects were enrolled in the study if they were working in 1960. TCE was replaced by PERC in 1966. Exposure assessment was conducted through the development of a job exposure matrix based on "walk throughs" of comparable facilities, interviews with longtime employees, industrial hygiene files, and job descriptions. Exposures were classified as not likely, intermittent, or routine for each of TCE, PERC, chromate, and mixed solvents. Duration of employment also was assessed. Elevated mortality rates were reported for non-Hodgkin's lymphoma, Hodgkin's disease, and cancers of connective tissue, stomach, rectum, and breast. No sites investigated for exposure-response patterns showed significantly positive effects. Results for workers exposed to TCE (but not PERC), PERC (but not TCE), both, or neither (but to other solvents) were not reported, but were said to show the same patterns as the results reported. This common response to all solvents makes it more difficult to infer exposure-specific causation from this study.

Ritz (1999) conducted a study of 3,814 uranium processing workers employed at the Fernald Feed Materials Production Center, using the Comprehensive Epidemiology Data Resource, a public-access database maintained by the U.S. Department of Energy. Exposure was assessed through the development of a job exposure matrix based on ratings provided by a panel of plant experts who had been employed for at least 20 years. Workers were classified into one of four exposure levels. Mortality was evaluated from these data and data on duration of employment. For internal comparisons only, adjustments were made for ionizing radiation dose. External comparisons were made with national, rather than regional, mortality data. Data available for a small subset of workers (those hired after 1967) showed that cigarette smoking rates among workers were less than those for the U.S. population and were not associated with chemical exposures. Thus, smoking was unlikely to have been a confounder. Salary status (hourly vs. salaried) was available as a crude measure of socioeconomic status. Many workers had joint low-level exposures to the three agents assessed (TCE, cutting fluids, and kerosene). Workers also were exposed to respiratory irritants. Moderate cutting fluid exposure always occurred with moderate TCE exposure. No workers had high TCE exposure. At sites for which TCE exposure effects were assessed, mortality rates were elevated for hematopoietic and lymphopoietic, liver, and brain cancer at both low and moderate exposure levels. No brain cancer excess was seen when the data were adjusted for cutting-fluid exposure. Liver cancer showed a strong exposure-response relationship and increased with exposure duration. Mortality rates for esophagus, stomach, liver, pancreas, prostate, brain, and lymphopoietic cancer and Hodgkin's disease were elevated for all workers, compared with U.S. rates.

3.3 Recent case-control studies

Vamvakas *et al.* (1998) conducted a hospital-based case-control study of renal-cell carcinoma. They selected 58 patients with renal-cell carcinoma diagnosed from December 1987 to May 1992 (79% of those identified) and 84 accident patients treated in 1993 from three hospitals within 20 miles of the hospital from which the cancer cases were drawn (75% of those identified). There was no matching, and the cancer patients were older, heavier, and more hypertensive than the controls. Fewer cancer patients smoked, and more took diuretics and did so for longer periods. This disparity in subject selection may have led to control selection bias, although it is not clear whether the differences were related to TCE exposure. The primary exposure to TCE was through metal degreasing. Exposure assessment was through an unblinded interview with a single physician (occasionally assisted). Specific exposure to any of a variety of chemicals was documented with a specially designed questionnaire. Each subject was assigned to one of three exposure classes based on a cumulative exposure calculation.

The authors suggested that exposure was higher than in most previous studies. An elevated odds ratio was reported for any exposure to TCE, adjusted for age, gender, smoking, body mass index, blood pressure, and intake of diuretics. In an analysis stratified by age, the odds ratio also was elevated in each 10-year age stratum, and the age-specific odds ratios were homogeneous ($P = 0.7$). There was a marginally significant exposure-response relationship. Green and Lash (1999) criticized the Vamvakas *et al.* (1998) study, citing possible selection bias and suggesting that the controls had less opportunity for high TCE exposure because they were younger and were selected later. They further argued that the study was suspect because the risk was so large (and greater than in previous studies). Vamvakas *et al.* (2000) countered that selection bias was unlikely, noting that exposure was higher than in many other studies, and cited supporting experimental data showing an association between TCE exposure and renal-cell carcinoma.

Dosemeci *et al.* (1999) evaluated the risk of renal-cell carcinoma among men and women exposed to organic solvents in a population-based case-control study in Minnesota (Chow *et al.* 1994). Cases were excluded if they failed to complete an interview or died (to avoid use of next-of-kin interviews); as a result, only 55% of originally identified cases were included in the analysis. In contrast, 97% of controls were included, raising concerns about possible selection bias. Exposure assessment was conducted through the use of a job exposure matrix developed through extensive work at the National Cancer Institute (Dosemeci *et al.* 1994, Gomez *et al.* 1994). An elevated odds ratio was found for all subjects together; it was statistically significant for women but not men. As noted by Dosemeci *et al.* (1999), this gender difference was seen in several previous studies. Results were adjusted for age, smoking, hypertension status, use of diuretics and anti-hypertension drugs, and body mass index; however, concerns about other occupational and nonoccupational exposures and lifestyle differences remain.

As part of a large case-control study of cancer at 19 anatomical sites (Siemiatycki 1991), Fritschi and Siemiatycki (1996) evaluated the risk of melanoma by comparing 103 newly diagnosed men in Montreal, Canada, aged 35 to 70 (83% response rate) with two control groups, one population-based (randomly selected from electoral lists and random-digit dialing, n = 533) and one of other cancer patients from their larger study (n = 533). Data collected by questionnaire included age, ethnicity, residence for the first 15 years of life, height, weight, education, income, and recent hobbies. A semistructured, probing interview was used to elicit detailed job history information, which a team of chemists and industrial hygienists later transformed into potential exposures to specific substances. Melanoma patients were younger, had higher incomes, and were better educated than controls. They participated in more outdoor sports and did more gardening. Adjustment for age, ethnicity, and years of education controlled for all of the measured confounders. Significantly elevated odds ratios were reported for insubstantial, substantial, and any exposure to TCE, based on comparison with a pooled control group of 1,066 subjects. Although the authors had information on many confounding variables, these data were highly correlated and thus difficult to assess individually for the small number of cases observed. Notably missing was information on exposure to the sun, complexion, and number of nevi observed.

Greenland *et al.* (1994) studied a cohort of white male workers at a General Electric transformer assembly plant in Massachusetts. A series of nested case-control analyses were conducted comparing 512 cancer deaths (from disease at a variety of anatomical sites) and 1,202 noncancer deaths. Workers were included if they were employed before 1985, died between 1969 and 1984, had their death reported to and recorded by the company pension office, and had a useable job history record. Information from 18 long-term, knowledgeable employees and industrial hygienists was used to select seven substances for job exposure ratings. A job exposure matrix was developed and combined with data on job title, department, and building to assign each worker to one of four exposure categories. For TCE, these were later reduced to two categories, exposed or unexposed. No statistically significant odds ratios were reported, but odds ratios were elevated for pancreatic cancer (OR = 1.6, 95% CI 0.8 to 3.3; n = 33) and oral, pharyngeal, and laryngeal cancer (OR = 1.3, 95% CI 0.5 to 3.1; n = 21). Odds ratios for esophageal, stomach, colon, rectum, liver and biliary, lung, prostate, bladder, kidney, and brain cancer, lymphoma, and leukemia all were < 1.2. Limitations of the study included selection bias, exposure misclassification, loss to follow-up, and uncontrolled confounding.

3.4 Reviews

As noted above, the IARC (1995e) reviewed the carcinogenicity of TCE. Its summary focused on three cohort studies (Axelson *et al.* 1994, Anttila *et al.* 1995, Spirtas *et al.* 1991), noting limitations of the exposure assessments and the likelihood of uncontrolled confounding. Nonetheless, the findings were summarized as showing 23 cases of liver and biliary cancer where 12.87 were expected, 27 cases of non-Hodgkin's lymphoma where 18.9 were expected, and a doubled risk of cervical cancer. The risk of kidney cancer was not elevated except in the study of Henschler *et al*. (1995), but this result was considered less relevant, because the study was initiated in response to observation of a cancer cluster. The risk for bladder cancer was not increased in the two Scandinavian cohort studies, but was elevated in the two U.S. cohort studies. Generally, case-control studies did not add substantially to this assessment, but were consistent with the findings of the cohort studies. A weak association was reported between TCE in groundwater and leukemia incidence, as well as a marginal increase in the incidence of non-Hodgkin's lymphoma. The two most important findings were considered to be elevated risks of liver and biliary cancer and of non-Hodgkin's lymphoma.

Weiss (1996), in assessing the carcinogenicity of TCE, reviewed the three cohort studies emphasized by the IARC (1995), along with an unpublished study of Hughes Aircraft workers (Wong and Morgan 1990) and several case-control studies. Liver cancer showed a small excess in all four cohort studies (16 cases where 9.5 were expected). Although biliary cancer was reported in only two of the four studies, they showed a doubling of risk (12 cases where 6.2 were expected). The risk of Hodgkin's disease was not elevated, but the risk of non-Hodgkin's lymphoma showed an exposure-response relationship, and the elevated risk was supported by a case-control study (Hardell *et al.* 1981). Weiss reported that the cohort studies did not show excess bladder or kidney cancer, overall. He noted the excess of these cancers reported by Henschler *et al.* (1995), but questioned its relevance because this study appeared to have been initiated in response to a cluster report. He reported no excess of oral, esophageal, colon, rectal, or pancreatic cancer, but one case-control study showed an association of TCE exposure with colon cancer (Fredriksson *et al.* 1989). No evidence of excess lung or brain cancer was found, although the incidence of prostate cancer was slightly elevated. Weiss concluded that the only plausible excesses suggested by the data were for liver cancer, biliary tract and kidney cancer, and non-Hodgkin's lymphoma. He argued that the absence of excess lung cancer in these studies, in contrast to the animal studies, was attributable to biological differences. Overall, he viewed the data as weak, because of the rarity of disease, the relatively small relative risks, and the lack of clear exposure-response patterns.

McLaughlin and Blot (1997) reviewed the possible association between TCE or PERC exposure and renal-cell cancer. They noted that known risk factors included cigarette smoking, high body weight, analgesic and diuretic use, high blood pressure, and, perhaps, a high-protein diet. They reviewed the same five cohort studies emphasized by the IARC (1995e) and Weiss (1996), plus studies by Garabrant *et al.* (1988) and Shindell and Ulrich (1985). In the latter study, McLaughlin and Blot (1997) inferred the absence of excess kidney cancer, even though it was not reported specifically, because both observed total cancer and observed nonrespiratory cancer were much lower than expected. They were critical of the Henschler study because it appeared to have been initiated in response to a cluster observation and because of methodological concerns, including the use of different diagnostic procedures with exposed and unexposed individuals. In summarizing this set of cohort studies, they noted that only Henschler *et al.* (1995) showed an

association between TCE and kidney cancer risk. They noted that Spirtas *et al.* (1991) was the most informative study, because of good exposure assessment and statistical power and long-term follow-up, and that it showed virtually no excess in cancer mortality.

McLaughlin and Blot (1997) also reviewed six case-control studies of kidney cancer (Asal *et al.* 1988, Harrington *et al.* 1989, Sharpe *et al.* 1989, Partanen *et al.* 1991, Siemiatycki 1991, Greenland *et al.* 1994). They reported that although these studies addressed solvent exposures, exposure to TCE was unlikely in some of the studies. Two studies showed elevated odds ratios, but McLaughlin and Blot (1997) argued that these studies provided little support for a causal association. They concluded that there was "no credible evidence of an association between risk of renal-cell cancer and TCE". An important limitation of this review was that the authors failed to distinguish between males and females or incidence and mortality in their evaluations.

A review by Wartenberg *et al.* (2000) added to previous reviews by including updates of two of the major cohorts (Blair *et al.* 1998, Morgan *et al.* 1998), two new cohort studies (Boice *et al.* 1999, Ritz 1999), and several new case-control studies. Several of these showed positive results for some anatomical sites. Wartenberg *et al.* (2000) divided the cohort studies into three tiers: Tier I, in which TCE exposure was inferred for individual study subjects and was best characterized; Tier II, in which there was putative TCE exposure, but it was less well characterized; and Tier III, studies of dry cleaners and laundry workers. Cohort results for both incidence and mortality were tabulated for each cancer site reported and were summarized as an average relative risk. Some of the averages were calculated on sets of studies that were heterogeneous as assessed by the Q statistic (e.g., kidney cancer, $P < 0.01$), but it was beyond the scope of the review to address possible explanations for that heterogeneity. Case-control studies were tabulated for kidney cancer, liver cancer, and lymphoma (both Hodgkin's disease and non-Hodgkin's lymphoma). Community-based (groundwater) studies also were reviewed.

The Tier I studies (10 articles representing seven cohorts) showed elevated average relative risks (RR > 1.2) for incidence of the following cancers:

- cervical cancer (RR = 2.4, 95% CI 1.2 to 4.8; n = 8)
- skin cancer (RR = 2.4, 95% CI 1.2 to 4.7; n = 8)
- liver cancer (RR = 1.9, 95% CI 1.0 to 3.4; n = 12)
- kidney cancer (RR = 1.7, 95% CI 1.1 to 2.7; n = 21)
- rectal cancer (RR = 1.7, 95% CI 1.0 to 3.0; n = 12)
- non-Hodgkin's lymphoma (RR = 1.5, 95% CI 0.9 to 2.3; n = 22)
- Hodgkin's disease (RR = 1.5, 95% CI 0.6 to 3.7; n = 4)
- multiple myeloma (RR = 1.5, 95% CI 0.7 to 3.3; n = 10)
- lymphohematopoietic cancer (RR = 1.4, 95% CI 1.0 to 2.0; n = 40)
- larynx cancer (RR = 1.4, 95% CI 0.4 to 5.0; n = 2)
- prostate cancer (RR = 1.3, 95% CI 1.0 to 1.6; n = 95)

The Tier I studies showed elevated average relative risks for mortality for the following cancers:

- Hodgkin's disease (RR = 2.0, 95% CI 1.1 to 3.4; n = 16)

- multiple myeloma (RR = 1.9, 95% CI 1.0 to 3.7; n = 18)
- cervical cancer (RR = 1.8, 95% CI 0.5 to 6.5; n = 5)
- liver cancer (RR = 1.7, 95% CI 0.2 = 16.2; n = 4)

In addition, the relative risk of kidney cancer mortality was slightly elevated (RR = 1.2).

Although incidence data for Tier II studies were relatively sparse, one study (Sinks *et al.* 1992), initiated in response to a cluster report, showed a large excess of kidney cancer (RR = 3.7, 95% CI 1.7 to 8.1; n = 6). Among the Tier II studies, the only average relative risks for mortality > 1.2 were for liver cancer (RR = 2.0, 95% CI 1.3 to 3.3; n = 15) and kidney cancer (RR = 1.3, 95% CI 0.9 to 1.7; n = 41). The liver cancer mortality studies were heterogeneous, but the kidney cancer mortality studies were not. Tier III studies were more difficult to interpret, as exposure to TCE was not well characterized. The case-control studies were plagued by poor exposure characterization. Nonetheless, several showed results supporting the cohort studies (kidney, Dosemeci *et al.* 1999, Sinks *et al.* 1992, Vamvakas *et al.* 1998; non-Hodgkin's lymphoma, Hardell *et al.* 1981, 1984, Persson *et al.* 1989). The community-based studies also were difficult to interpret, because the exposure was not specific to the individual and generally was to several solvents and contaminants simultaneously. Nonetheless, elevated risks were reported for leukemia, non-Hodgkin's lymphoma, multiple myeloma, and bladder cancer.

Wartenberg *et al.* (2000) summarized their view as consistent with that of the IARC (1995e) and Weiss (1996), but they argued that the evidence more strongly suggested an association of TCE exposure with liver and kidney cancer and provided some support for associations with non-Hodgkin's lymphoma and Hodgkin's disease. Further, they argued that there was some evidence for association of TCE exposure with cervical cancer and possibly with multiple myeloma and prostate, laryngeal, and colon cancer.

3.5 Discussion

There is a large body of evidence assessing the possible carcinogenicity of TCE in humans. These studies were conducted in a variety of countries and in many different types of workplaces. Overall, the cohort studies showed some consistency in the reporting of elevated rates of both incidence and mortality, particularly for liver cancer, kidney cancer, non-Hodgkin's lymphoma, multiple myeloma, and prostate cancer. Sparser data showed elevated risks for esophageal, cervical, pancreatic, laryngeal, and colon cancer and Hodgkin's disease.

A strength of the cohort studies, in general, was their size and long follow-up periods. The seven best-characterized cohort studies included over 120,000 workers and followed them for an average of over 30 years. However, a limitation of these studies was the small number of site-specific cancers observed. For example, for the sites of greatest concern—liver and biliary cancer, kidney cancer, non-Hodgkin's lymphoma, Hodgkin's disease, and cervical cancer—the reported incidences were 12, 21, 22, 4, and 8, respectively, and the reported deaths were 33, 37, 56, 16, and 5. (The deficit in cases relative to deaths existed because most studies reported mortality only.) An additional strength of the cohort studies was that two of the four most recent studies used state death certificates as the comparison population, rather than national death certificates, removing some of the concern about regional variation in mortality rates.

Another strength of the cohort studies was the extensive work done to characterize exposures. Two studies measured exposures, although not on a repeated basis. In other studies, exposures were inferred from interviews with longtime employees, industrial hygiene assessments, and occasional area monitors. The use of job exposure matrices, particularly when supplemented with facility-specific information, has been shown to provide moderately good exposure classification relative to classification by exposure measured with passive dosimeters (Tielemans *et al*. 1999). However, this approach makes comparison across studies difficult. More comprehensive exposure measurement schemes could be designed, but they would be expensive to implement. Because most analyses condensed the exposure classifications into two groups, exposure misclassification would most likely bias results towards the null. In addition, some of the studies reported results for entire cohorts even though only a portion of the cohort was exposed to TCE, which likely resulted in underestimation of the actual risks of disease.

A related issue is that most exposures, despite being occupational, were relatively low, limiting the sensitivity of the studies. In addition, TCE use was phased out of many of the workplaces between 1960 and 1980. For such workplaces, cumulative exposure would depend on jobs held, date of first employment, and duration of employment. In studies with later start dates (e.g., Boice *et al.* 1999 started enrollment nearly 10 years later than the other recent cohort studies), workers likely had lower cumulative exposures; however, this cannot be assessed from the published information.

A complication in interpreting the results based on external comparison populations is that there was a moderate “healthy worker” effect in most of these studies. That is, total SMRs and total cancer SMRs tended to be < 1.0, often around 0.8. Use of these levels as baselines for comparisons would result in null to slightly elevated SMRs for specific cancer sites among exposed workers in studies where risk actually was elevated over that for similar non-exposed working populations. However, the latter comparison typically was not made. Internal comparisons would provide better exposure-specific evaluations.

Finally, confounding is a concern for all epidemiologic studies. The absence of data on personal characteristics (such as hypertension and high body weight), lifestyle factors (such as tobacco use, alcohol consumption, and diet), and nonoccupational exposures (such as residential exposures and hobbies) makes confounding difficult to assess. However, for confounding to be a major concern, the factors would have had to be associated with both exposure and disease. Such associations are unlikely but cannot be ruled out, because no data were presented to allow direct evaluation of confounding.

The case-control studies are of limited use, as most did not report TCE-specific risks. However, those that did generally supported the findings of the cohort studies. A concern about the studies discussed in detail above is the possibility of control selection bias, as there were documented differences between the cases and controls; however, the studies did not directly assess the likelihood of bias. The community-based studies were even more difficult to interpret, because they lacked quantitative chemical-specific exposure information for individuals.

3.6 Summary

The number and sophistication of studies assessing the possible carcinogenicity of TCE is impressive. Although the studies are not perfectly consistent, strong patterns emerge. In

particular, associations with TCE exposure generally were observed for kidney cancer, liver cancer, non-Hodgkin's lymphoma, multiple myeloma, and prostate cancer. Particular aspects of design or implementation may limit the usefulness or interpretation of individual studies, but, by and large, these studies were well designed and executed. Viewed from the perspective of Hill's aspects of causation (Hill 1965), several of the criteria are fulfilled. Moderately strong associations were observed for some outcomes, with limited positive biological gradient (exposure-response) data. TCE was not specific as evidenced by the multiple cancers observed. However, the results did show temporality (at least in the cohort studies), coherence, and were supported by animal studies. Based on Hill's aspects, there are strong data supporting a causal relationship between TCE exposure and human cancer.

Table 3-1. Recent cohort studies (including all SMRs or RRs > 1.2 and based on more than one death)

Reference	Study design	Population	Exposure	Effects	Potential confounders
Blair *et al.* 1998 U.S.	historical cohort	14,457 aircraft maintenance workers employed ≥ 1 yr between 1952 and 1956 and followed through 1990. Comparison population was unexposed workers for incidence analyses and Utah death certificates for mortality analyses.	Exposure was assessed with a job exposure matrix based on industrial hygiene walk throughs, interviews, historical records, monitoring data, job descriptions, and two surveys of vapor degreasers (Stewart *et al.* 1991). Each job was scored on exposure intensity, frequency, and duration. Exposures were to multiple solvents, although TCE was the main solvent used historically, through 1978.	Workers exposed to TCE showed nonsignificant mortality excesses for esophageal cancer (RR = 5.6, 95% CI 0.7–44.5; n = 10), bone cancer (RR = 2.1, 95% CI 0.2–18.8; n = 5), non-Hodgkin's lymphoma (RR = 2.0, 95% CI 0.9–4.6; n = 28), cervical cancer (RR = 1.8, 95% CI 0.5–6.5; n = 5), breast cancer (RR = 1.8, 95% CI 0.9–3.3; n = 20), kidney cancer (RR = 1.6, 95% CI 0.5–5.1; n = 15), buccal cavity or pharyngeal cancer (RR = 1.4, 95% CI 0.4–5.2; n = 9), colon cancer (RR = 1.4, 95% CI 0.8–2.4; n = 54), Hodgkin's disease (RR = 1.4, 95% CI 0.2–12.0; n = 5), liver and biliary cancer (RR = 1.3, 95% CI = 0.5–3.4; n = 15), multiple myeloma (RR = 1.3, 95% CI 0.5–3.4; n = 14), pancreatic cancer (RR = 1.2, 95% CI 0.6–2.3; n = 33), and bladder cancer (RR = 1.2, 95% CI 0.5–2.9; n = 17). Dose-response assessments were inconclusive.	Exposures to solvents were not mutually exclusive, making attribution to a single agent difficult. Information on lifestyle factors and non-occupational exposures was not available.

Reference	Study design	Population	Exposure	Effects	Potential confounders
Morgan *et al.* 1998 U.S.	historical cohort	20,508 aircraft manufacturing employees employed ≥ 6 mo between 1950 and 1985 and followed through 1993, of whom 4,733 were TCE-exposed. For internal cohort analyses, the comparison population was the unexposed workers. For overall SMRs, comparison population was the U.S.	Exposure was assessed with a job exposure matrix based on exposure ranking provided by workers employed for 30 or more years	Elevated mortality rates among those exposed to TCE were found for cancer of the bladder (SMR = 1.4, 95% CI 0.6–2.7; n = 8), kidney (SMR = 1.3, 95% CI 0.6–2.6; n = 8), ovary (SMR = 1.2, 95% CI 0.5–2.4; n = 8), and prostate (SMR = 1.2, 95% CI 0.7–1.8; n = 21).	There was no discussion of other occupational exposure, including exposure to other solvents. Information on lifestyle factors and non occupational exposure was not available.
Boice *et al.* 1999 U.S.	historical cohort	77,965 aircraft manufacturing employees employed ≥ 1 yr in or after 1960 and followed through 1996. Comparison population was the California population.	Exposure was assessed with a job exposure matrix based on walk throughs of comparable facilities, interviews of longtime employees, industrial hygiene files, and job descriptions. TCE was used through 1966.	Elevated mortality rates were reported for Hodgkin's disease (SMR = 2.8, 95% CI 0.8–7.1; n = 4); cancer of the connective tissue (SMR = 1.9, 95% CI 0.4–5.7; n = 3), stomach (SMR = 1.3, 95% CI 0.8–2.1; n = 17), breast (SMR = 1.3, 95% CI 0.5–2.7; n = 7), and rectum (SMR = 1.3, 95% CI 0.6–2.5; n = 9); and non-Hodgkin's lymphoma (SMR = 1.2, 95% CI 0.7–2.0; n = 14)	Exposures to solvents were not mutually exclusive, making attribution to a single agent difficult. Information on lifestyle factors and other occupational and non-occupational exposure was not available.

Reference	Study design	Population	Exposure	Effects	Potential confounders
Ritz (1999) U.S.	historical cohort	3,814 uranium processing workers employed ≥ 3 mo between 1951 and 1972 and followed through 1991. For internal cohort analyses, comparison population was the unexposed workers. For overall SMRs, comparison population was the U.S.	Exposure was assessed with a job exposure matrix based on ratings provided by a panel of plant experts who had been employed at least 20 yr.	For exposure duration > 5 yr and an exposure lag of 15 yr, elevated mortality rates were reported for brain cancer (RR = 5.4, 95% CI 0.9–33.9; n = 3), liver cancer (RR = 2.9, 95% CI 0.5–17.3; n = 3), and hematopoietic and lymphopoietic cancer (RR = 1.8, 95% CI 0.8–4.1; n = 12).	Limited data were available to assess the effects of smoking. Salary status (hourly vs. salaried) could be used as a crude measure of socioeconomic status. For internal comparisons, adjustments were made for ionizing radiation dose. Many TCE exposures were confounded by exposure to cutting fluid, kerosene, and respiratory irritants.

Table 3-2. Recent case-control studies

Reference	Study design	Population	Exposure	Effects	Potential confounders
Vamvakas *et al.* 1998. Germany	case-control	58 cases of renal-cell carcinoma diagnosed in a hospital between 12/1/87 and 5/31/92 and 84 accident patients from three nearby hospitals.	Exposure assessment was by unblinded interview using a questionnaire.	OR for renal-cell carcinoma was elevated (OR = 10.8, 95% CI 3.4–34.8; n = 19). The effect increased with exposure intensity.	There are concerns about selection bias, other occupational and non-occupational exposure, and lifestyle factors (other than smoking).
Dosemeci *et al.* 1999 U.S.	case-control	438 cases of renal-cell carcinoma identified through the Minnesota Cancer Surveillance System and 687 age- and gender-stratified controls identified through random-digit dialing (ages 20–64) or the Health Care Financing Administration (ages 65 and over).	Exposure assessment was based on an extensive job exposure matrix developed previously at the NCI and on reported work histories.	OR for renal-cell carcinoma was elevated for all subjects (OR = 1.3, 95% CI 0.9–1.9; n = 55) and was statistically significant for women (OR = 2.0, 95% CI 1.0–4.0; n = 22), but not for men.	Possible selection bias (55% of originally identified cases and 97% of controls were included). Data on other occupational and nonoccupational exposure and lifestyle factors (except smoking) were not available.
Fritschi and Siemiatycki (1996) Canada	case-control	103 newly diagnosed cases of melanoma among men aged 35–70 and two sets of controls: 533 population-based randomly selected controls and 533 cancer controls.	A structured questionnaire was used for general demographic and risk-factor information, followed by a semistructured, probing interview for detailed information about all jobs. These data were translated into a list of potential exposures to several substances.	OR for melanoma was significantly elevated for any exposure to TCE (OR = 3.6, 95% CI 1.5–9.1; n = 8).	This study had extensive confounder information but no data on exposure to the sun, complexion, or number of nevi.

Reference	Study design	Population	Exposure	Effects	Potential confounders
Greenland *et al.* 1994 U.S.	nested case-control	512 cancer deaths as cases (at a variety of anatomical sites) and 1,202 noncancer deaths as controls, all white males employed at a transformer production facility before 1985 and deceased between 1969 and 1984.	Exposure was assessed with a job exposure matrix based on job title, department, and building. Based on information from 18 longtime, knowledgeable employees and industrial hygienists, exposure to seven substances was characterized.	No elevated ORs were statistically significant, but elevated ORs were reported for pancreatic cancer (OR = 1.6, 95% CI 0.8–3.3, n = 33) and oral, pharyngeal, and laryngeal cancer (OR = 1.3, 95% CI 0.5–3.1; n = 21).	Exposures to substances other than TCE were not mutually exclusive, making attribution to a single agent difficult. Information on lifestyle factors and other occupational and nonoccupational exposure was not available.

4 Studies of Cancer in Experimental Animals

4.1 Experimental carcinogenesis

The carcinogenicity of TCE was investigated by the National Cancer Institute (NCI 1976) and the NTP (1988, 1990). These and a few other studies conducted before 1995 were reviewed by the IARC (1995e, pp. 105-109; see Appendix A). More recent experimental carcinogenicity studies were not located. The principal findings of the carcinogenicity studies are summarized below. Based on the studies reviewed, the IARC (1995e) concluded that there was sufficient evidence of carcinogenesis in experimental animals for TCE.

4.1.1 Gavage studies

The NCI (1976) tested industrial grade (> 99% pure; containing 0.19% epoxybutane and 0.09% epichlorohydrin) TCE in Osborne-Mendel rats and B6C3F_1 mice. Groups of 50 animals of each species and sex were tested at the maximum tolerated dose (MTD) and one half the MTD. Twenty animals of each species and sex were used as controls. Carbon tetrachloride was used as a positive control. Rats were exposed to time-weighted average doses of 549 and 1,097 mg/kg, male mice were exposed to 1,169 and 2,339 mg/kg, and female mice were exposed to 869 and 1,739 mg/kg. All animals were exposed by oral gavage five days/week for 78 weeks. Animals were observed until terminal sacrifice at 110 weeks for rats and 90 weeks for mice.

Mortality in treated and control rats was high with only 3/20, 8/50, and 3/50 male rats and 8/20, 13/48, 13/50 female rats surviving in the control, low-dose, and high-dose group, respectively. No significant differences in tumor incidence were observed in rats. However, there was a highly significant, dose-related increase in hepatocellular carcinomas in both male and female mice (Table 4-1).

Table 4-1. Summary of tumors and their incidences in B6C3F$_1$ mice administered TCE by gavage for two years

Mouse sex/tumor type	TCE gavage doses			
	Colony controls	Vehicle control	869 mg/kg (female) 1,169 mg/kg (male)	1,739 mg/kg (female) 2,339 mg/kg (male)
	Survival adjusted tumor incidence/Number examined			
Males				
Liver				
Hepatocellular carcinoma	5/77	1/20	26/50**	31/48**
Forestomach papilloma	0/77	0/20	0/50	1/48
Females				
Liver				
Hepatocellular carcinoma	1/80	0/20	4/50	11/47**

Source: NCI 1976.

**P ≤ 0.01, statistically different from vehicle controls based on the survival-adjusted Cox and Tarone test.

The NTP (1988) conducted a two-year carcinogenicity study to compare the differences in sensitivity to TCE administered by gavage among four strains of rats (ACI, August, Marshall, and Osborne-Mendel). TCE, stabilized with diisopropylamine (8 ppm) and containing no epichlorohydrin, was administered in corn oil five days per week at concentrations of 500 or 1,000 mg/kg for 103 weeks. Both sexes of all four rat strains were observed to be susceptible to the nephrotoxic effects of TCE. Tubular cell cytomegaly and toxic nephropathy occurred in 82% to 100% and 17% to 80% of the treated animals, respectively. These effects were not observed in controls. However, the NTP concluded that this study was inadequate because of chemically induced toxicity, reduced survival, and incomplete data documentation. Despite these limitations, the incidence of renal tubular-cell adenoma was significantly increased in male Osborne-Mendel rats, and interstitial-cell neoplasms of the testis were observed in Marshall rats. No statistically significant increases in tumors of any type were observed in ACI or August rats; however, interstitial cell tumors of the testis occurred with a positive trend in ACI rats. The tumor data for male and female Osborne-Mendel and Marshall rats are summarized in Table 4-2.

Table 4-2. Tumor incidences in Osborne-Mendel and Marshall rats administered TCE by gavage for two years

Rat strain/tumor type	TCE gavage doses			
	Untreated controls	Vehicle control	500 mg/kg	1,000 mg/kg
	Tumor response/Number examined			
Osborne-Mendel				
Kidney tubular cell adenoma				
Males	0/50	0/50	6/50**	1/50
Females	1/50	0/50	0/50	1/49
Marshall				
Kidney tubular cell adenoma				
Males	2/49	0/49	1/50	0/47
Females	1/49	1/50	1/48	0/44
Testicular tumors				
Interstitial cell tumor	16/46	17/46	21/48	31/48**
Interstitial cell tumor or malignant interstitial cell tumor	16/46	17/46	21/48	32/48**

Source: NTP 1988.

**P ≤ 0.01, statistically different from vehicle controls based on the survival adjusted incidental tumor test

Subsequently, the NTP (1990) evaluated the carcinogenicity of epichlorohydrin-free TCE administered by gavage to F344/N rats and B6C3F_1 mice (Appendix B). Survival of treated male rats and male mice was significantly reduced compared to vehicle controls in these experiments. Male F344/N rats exposed to TCE had an increased incidence of renal tubular-cell neoplasms at the high dose and mesotheliomas at the low dose (Table 4-3); however, these results were considered inadequate for evaluating the presence or absence of a carcinogenic response because of poor survival. TCE was not carcinogenic in female rats. TCE was carcinogenic in B6C3F_1 mice, inducing a significant increase in the incidence of hepatocellular carcinomas and adenomas in both sexes; and malignant lymphoma, lymphoma or leukemia, and alveolar or bronchiolar adenoma in females. However, the increased incidence of malignant lymphoma, lymphoma or leukemia, and alveolar or bronchiolar adenoma were not considered to be related to TCE exposure for several reasons. The incidence of malignant lymphoma and leukemia were within the historical control ranges and the combined incidence of alveolar or bronchiolar adenoma and carcinoma was not significant. Primary tumor data for mice are summarized in Table 4-4.

Table 4-3. Primary tumor incidences in male F344/N rats administered TCE by gavage for two years

Tumor type	TCE gavage doses			
	Untreated controls	Vehicle control	500 mg/kg	1,000 mg/kg
	Tumor response/Number examined			
Males				
Kidney				
Tubular cell adenocarcinoma	0/49	0/48	0/49	3/49*
Tubular cell adenoma or carcinoma	0/49	0/48	2/49	3/49*
Peritoneum				
Malignant mesothelioma	1/50	1/50	5/50**	0/49
All mesothelioma	1/50	1/50	5/50**	1/49

Source: NTP 1990

*P ≤ 0.05, statistically different from vehicle controls by the incidental tumor test.

**P ≤ 0.05, statistically different from vehicle controls by life table analysis (not significant by incidental tumor test).

Table 4-4. Primary tumor incidences in B6C3F$_1$ mice administered TCE by gavage for two years

Tumor type	TCE gavage doses	
	Vehicle control	1,000 mg/kg/day
	Tumor response/Number examined	
Males		
Liver		
Adenoma	7/48	14/50**
Carcinoma	8/48	31/50**
Adenoma or carcinoma	14/48	39/50**
Females		
Lungs		
Alveolar or bronchiolar adenoma	0/48	4/48*[a]
Adenoma or carcinoma	1/48	4/48
Hemapoietic system		
All malignant lymphoma	7/48	13/49* [a]
Lymphoma or leukemia	7/48	14/49* [a]
Liver		
Adenoma	4/48	16/49**
Carcinoma	2/48	13/49**
Adenoma or carcinoma	6/48	22/49**

Source: NTP 1990

*P ≤ 0.05, statistically different from vehicle controls by life table analysis.

**P ≤ 0.01, statistically different from vehicle controls by life table analysis.

[a] Results were not significant based on the incidental tumor test.

The IARC (1995e) reviewed two other studies where TCE was administered by gavage. Van Duuren *et al.* (1979) administered 0.5 mg TCE once per week for 74 weeks to 30 male and female ICR:Ha Swiss mice. Forestomach tumors were not increased compared to vehicle controls. Tumor data for other sites were not reported. Maltoni *et al.* (1986) administered 50 or 250 mg/kg TCE four to five days per week for 52 weeks to groups of 30 male and female Sprague-Dawley rats. A nonsignificant increase in leukemias was observed in the male rats.

4.1.2 Inhalation studies

The IARC (1995e) reviewed several inhalation studies and noted that TCE induced an increased incidence of lymphomas in female NMRI mice (Henschler *et al.* 1980), liver tumors in male Swiss mice (Maltoni *et al.* 1986, 1988), and lung tumors in female ICR, male Swiss, and female $B6C3F_1$ mice (Fukuda *et al.* 1983, Maltoni *et al.* 1986, 1988). Henschler *et al.* (1980) did not find an increase in tumors in groups of 30 male and female Wistar rats or Syrian hamsters exposed 6 hours/day, five days/week for 18 months to air containing TCE at 100 or 500 ppm. Sprague-Dawley rats exposed to air containing TCE at concentrations of 50 to 450 ppm did not show an increased incidence of tumors (Fukuda *et al.* 1983). Inhalation exposure to TCE was carcinogenic to Sprague-Dawley rats, inducing dose-related Leydig cell tumors of the testis (23.8% at 3,240 mg/m^3), renal tubular adenocarcinoma (3.1% at 3,240 mg/m^3) and cytokaryomegaly (77.7% at 3,240 mg/m^3) in male rats (Maltoni *et al.* 1986, 1988).

4.2 Summary

Exposure to TCE resulted in tumors at multiple sites (liver, kidney, lung, testis, and hematopoietic system) in experimental animals depending of the species, strain, sex, and route of administration. TCE administerd by gavage produced liver tumors in both sexes of mice ($B6C3F_1$) but not in rats and kidney tumors in male rats (Osborne-Mendal and F344/N) but not in mice. Other tumors produced by gavage administration included testicular tumors in Marshall rats and mesotheliomas (low dose) in male F344/N rats. Inhalation exposure to TCE induced lung tumors in male Swiss and female ICR and $B6C3F_1$ mice but not in rats or hamsters. Other tumors observed from inhalation of TCE included lymphomas in female NMRI mice, liver tumors in male Swiss mice, and testicular and kidney tumors in Sprague-Dawley rats.

5 Genotoxicity

5.1 Genotoxicity studies reviewed in IARC (1995e)

Genotoxicity studies reported before 1995 were reviewed by the IARC (1995e, pp. 122-133; see Appendix A). In general, TCE was not genotoxic in a broad range of bacterial, lower eukaryotic, and *in vitro* and *in vivo* mammalian cell assays. It has been suggested that the few positive responses observed could have been due to impurities in TCE and/or the presence of potentially mutagenic stabilizers (Goeptar *et al.* 1995). The following is summarized from IARC (1995e).

In prokaryotic systems, pure TCE usually did not induce gene mutations or DNA damage, whereas TCE preparations containing epoxide stabilizers were mutagenic. In lower eukaryotic systems, TCE did not induce gene conversion or reverse mutations in *Saccharomyces cerevisiae* (with or without metabolic activation), forward mutations in *Schizosaccharomyces pombe* (with or without metabolic activation), mitotic crossing over in *Aspergillus nidulans* (without metabolic activation), or sex-linked recessive lethal mutations in *Drosophila melanogaster* exposed via injection. TCE induced forward mutations in *A. nidulans* (without metabolic activation) and gave equivocal results for sex-linked recessive lethal mutations in *D. melanogaster* exposed via feed.

In *in vitro* studies with mammalian cells, TCE did not induce unscheduled DNA synthesis (UDS) in primary rat hepatocytes, gene mutations in human lymphoblastoid cells (with or without metabolic activation), chromosomal aberrations in Chinese hamster ovary (CHO) cells (with or without metabolic activation), or inhibition of intercellular communication in rat hepatocytes. In contrast, TCE gave positive results *in vitro* for covalent binding to calf thymus and salmon sperm DNA (with metabolic activation only) and primary mouse and rat hepatocyte DNA, sister chromatid exchanges (SCE) in CHO cells (with or without metabolic activation), gene mutations in mouse lymphoma cells (with metabolic activation only), inhibition of intercellular communication in primary mouse hepatocytes, and morphological transformation in rat embryo cells.

In vivo, TCE administered orally or by intraperitoneal (i.p.) injection gave equivocal results for covalent binding to mouse and rat liver DNA and negative results for binding to mouse spleen, pancreas, lung, testis, kidney, or brain DNA. When administered orally or by inhalation, TCE did not induce UDS in mouse hepatocytes, SCE in mouse splenocytes or rat lymphocytes, dominant lethal mutations in mice, or chromosomal aberrations in mouse splenocytes or rat lymphocytes. TCE did not induce micronuclei in mouse bone marrow when administered i.p. or in mouse splenocytes, mouse spermatocytes, or rat lymphocytes when administered by inhalation. TCE gave both negative and positive results for DNA single-strand breaks or alkali-labile sites in mouse liver (administered i.p. or orally) and positive results for micronucleated polychromatic erythrocytes in mice exposed orally and in rats exposed by inhalation.

Genotoxic effects in occupationally exposed humans were evaluated in several studies. TCE exposure did not increase the frequency of sperm-head abnormalities. One study evaluating the frequency of SCE in mitogen-stimulated lymphocytes reported no

increase, whereas a second study reported an increase among TCE-exposed smokers but not among nonsmokers. Two studies detected a significant increase in chromosomal damage in mitogen-stimulated lymphocytes.

5.2 Genotoxicity studies published after the IARC (1995e) review

The mutagenicity of TCE and its metabolites chloral hydrate (CH), dichloroacetic acid (DCA), trichloroacetic acid (TCA) trichloroethanol, S-(1,2-dichlorovinyl)-1-cysteine (DCVC), and S-(1,2-dichlorovinyl) glutathione (DCVG) was recently reviewed by Moore and Harrington-Brock (2000). These researchers concluded that TCE and its metabolites CH, DCA, and TCA are unlikely to induce tumors in humans because very high doses are required for genotoxicity. There was not enough information to assess the mutagenicity of trichloroethanol and the two TCE conjugates, DCVC and DCVG; although, there was some evidence that DCVC is a more potent mutagen than CH, DCA, or TCA (see Section 5.4 for discussion of genotoxicity studies of TCE metabolites).

TCE did not induce mitotic recombination (as measured by the eye mosaic test) in *D. melanogaster* exposed via inhalation (Vogel and Nivard 1993), chromosomal aberrations in Chinese hamster lung cells (with or without metabolic activation) (Matsuoka *et al.* 1996), or UDS in hepatocytes of $B6C3F_1$ mice exposed orally (Miyagawa *et al.* 1995).

The ability of TCE to modulate DNA methylation and the expression of immediate-early protooncogenes was evaluated by Tao *et al.* (1999). Female $B6C3F_1$ mice were administered TCE by gavage (1000 mg/kg TCE) five days per week. The mice were sacrificed after 5, 12, or 33 days of administration. TCE decreased methylation both of the total DNA and the promoters for c-*jun* and c-*myc* genes and increased the expression of their mRNA.

TCE's ability to induce gene mutations and small deletions was tested in *lac*Z transgenic mice. Male and female mice were exposed by inhalation to 0; 203; 1,153; and 3,141 ppm TCE, six hours per day for 12 days. Animals were sacrificed 14 and 60 days following last exposure and the mutation frequency in bone marrow, spleen, kidney, liver, lung, and testicular germ cells determined. The researchers observed that TCE did not induce base-change or small-deletion mutations in any of the tissues examined (Douglas *et al.* 1999).

These studies are summarized in greater detail in Table 5-1.

Table 5-1. Genotoxic effect of TCE in recent studies[a]

System	Effect	Metabolic Activation	Form and purity	Exposure level	Response (+/-) activation	Comments	Reference
Lower eukaryotes							
Drosophila melanogaster strain C-1	mitotic recombination (eye mosaic assay)	NA	NG	280 to 4000 ppm via inhalation for 17 h	negative	genetic principle involves loss of heterozygosity for the wild type and white-eye color gene resulting from mitotic recombination between two X chromosomes	Vogel and Nivard 1993
Mammalian systems *in vitro*							
Chinese hamster lung cell line CHL/Ius	chromosomal aberrations	-/+	NG	0.33, 0.66, and 1.31 mg/mL for 6, 24, and 48 h -S9 and 6 h +S9	negative/ negative	no increase in chromosomal aberrations at any exposure period with or without S9	Matsuoka *et al.* 1996
Mammalian systems *in vivo*							
$B6C3F_1$ mouse hepatocytes	UDS	NA	NG	600 and 1200 mg/kg (single gavage treatment)	negative	hepatocytes were collected 24, 39, and 48 h after treatment and exposed to [^{3}H]thymidine for 4 h	Miyagawa *et al.* 1995
*Lac*Z transgenic mice (male and female)	base-change and small-deletion mutation	NA	NG	0, 203, 1153, 3141 mg/kg (inhalation)	negative	mutation frequency in bone marrow, kidney spleen, liver, lung, and testicular germ cells determined	Douglas *et al*. 1999
$B6C3F_1$ mice (female)	DNA modulation and expression of immediate-early protooncogenes	NA	NG	1000 mg/kg by gavage five days/week	positive	TCE decreased methylation of DNA and the promoters for *c-jun* and *c-myc* genes and increased the expression of their mRNA	Tao *et al.* 1999

[a] NA = not applicable; NG = not given; UDS = unscheduled DNA synthesis

5.3 Genotoxicity studies of structural analogues

A knowledge-based structure-activity approach (MULTICASE) was used to examine the structural basis for, among other things, the induction of aneuploidy in *A. nidulans* by chlorinated alkanes and alkenes (Rosenkranz and Klopman 1996). Data on induction of aneuploidy by 35 chlorinated alkanes or alkenes came from Crebelli *et al.* (1992, cited in Rosenkranz and Klopman 1996). Compared with inactive compounds, compounds inducing aneuploidy had significantly higher water solubilities (1.157 vs. 0.764, $P = 0.02$) and significantly lower octanol-water partition coefficient values (log P = 1.988 vs. 2.424, $P = 0.009$). Nine structural fragments were found to correlate with the ability to induce aneuploidy in 11 of 12 chemicals. One of these fragments, Cl-CH=C-Cl, is found in TCE.

5.3.1 Vinyl chloride

Green (1990) observed that vinyl chloride's wide range of effects in many species was characteristic of a genotoxic carcinogen. As reviewed in IARC (1979), vinyl chloride induced UDS in primary rat hepatocytes, gene mutation in cultured Chinese hamster lung cells, gene conversion in yeast, and DNA damage and mutation in bacteria. It also induced sex-linked recessive lethal mutation in *D. melanogaster* and was mutagenic in plants and *S. pombe* (but not other fungi). In mice and rats exposed *in vivo*, vinyl chloride induced chromosomal aberrations, SCE, and micronuclei in bone marrow cells and alkylated DNA in various tissues. Workers exposed to vinyl chloride vapor showed induction of chromosomal aberrations in peripheral blood lymphocytes. Two additional studies of exposed workers indicated negative results for SCE, while one study indicated a weakly positive response. Green (1990) suggested that vinyl chloride's carcinogenic activity resulted from its metabolism by microsomal mixed-function oxidases to chloro-oxirane (chloroethylene oxide) and chloroacetaldehyde, two mutagenic metabolites, and concluded that vinyl chloride was a classical genotoxin causing cancer by somatic mutation.

5.3.2 Vinylidene chloride

As reviewed in the IARC (1987b), vinylidene chloride was mutagenic in plant cells and bacteria and induced mutation and gene conversion in yeast. Although it was not mutagenic or clastogenic in cultured Chinese hamster lung cells, it did induce UDS in primary rat hepatocytes. *In vivo*, vinylidene chloride did not induce dominant lethal mutations in mice or rats or chromosomal aberrations in bone marrow cells of rats, but did induce UDS in mice.

5.3.3 Tetrachloroethylene

Tetrachloroethylene has generally given negative results in most genetic toxicology assays (IARC 1995c). Tetrachloroethylene was not active in the SOS chromotest with *Escherichia coli* and was not mutagenic in bacteria in the absence of metabolic activation. Purified tetrachloroethylene was not mutagenic in *Salmonella typhimurium* or *E. coli* in the presence of rat liver S9. However, purified tetrachloroethylene was mutagenic in *S. typhimurium* TA100 in the presence of rat liver glutathione *S*-transferase, glutathione, and rat kidney microsomes (Vamvakas *et al.* 1989). In stationary-phase

yeast, it did not induce gene conversion, mitotic recombination, or reverse mutation, but conflicting data were obtained for cells in logarithmic growth.

Tetrachloroethylene did not induce sex-linked recessive lethal mutation in *D. melanogaster*, UDS in rat primary hepatocytes, chromosomal aberrations or SCE in cultured Chinese hamster lung cells (with or without metabolic activation), or mutation in mouse lymphoma cells (with or without metabolic activation). However, DNA binding of radioactively labeled tetrachloroethylene to calf thymus DNA *in vitro* in the presence of metabolic activation and to DNA and proteins of mouse and rat liver, kidney, and stomach *in vivo* were reported. Tetrachloroethylene also induced cell transformation in Fischer rat embryo cells but not in mouse BALB/c-3T3 cells. In *in vivo* studies, the frequencies of gene conversion and reverse mutation were not increased in a host-mediated assay using yeast recovered from the liver, lungs, and kidneys of mice treated with tetrachloroethylene. Exposure to tetrachloroethylene significantly increased DNA damage (strand breaks or alkali-labile sites) in mouse liver and kidney, but not lung.

The IARC (1995c) noted that two studies of workers occupationally exposed to tetrachloroethylene reported small increases in peripheral lymphocytes showing numerical chromosome abnormalities (Ikeda *et al.* 1980) and SCE frequency in subjects who smoked (Seiji *et al*. 1990). Neither study controlled for the possible confounding effects of smoking.

5.4 Genotoxicity studies of metabolites

5.4.1 Chloral hydrate

Chloral hydrate has been extensively evaluated for its ability to induce aneuploidy in various test systems (IARC 1995a). It induced aneuploidy in the absence of metabolic activation in fungi, human lymphocytes *in vitro*, secondary spermatocytes of mice exposed *in vivo* (in three of four studies), and bone marrow cells of mice exposed *in vivo*. Chloral hydrate significantly increased the frequency of micronuclei in Chinese hamster cell lines and human lymphocytes *in vitro* and in bone marrow erythrocytes (in two of four studies) and spermatids (in two of three studies) of mice exposed *in vivo*. Where evaluated, the micronuclei most frequently were induced by numerical rather than structural chromosomal damage.

The IARC (1995a) noted conflicting results for DNA damage by chloral hydrate. It was mutagenic, with or without metabolic activation, in *S. typhimurium* TA100 (two of four studies) and in TA104 (one study) but not in TA1535, TA1538, or TA98. It gave negative results for mitotic crossing over in *A. nidulans* in the absence of metabolic activation, but weakly positive results for meiotic recombination and gene conversion (but not reverse mutation) in *S. cerevisiae* in the presence and absence of metabolic activation, respectively. It induced somatic mutation in *D. melanogaster*, but not DNA-protein cross-links in rat liver nuclei or DNA single-strand breaks or alkali-labile sites in primary rat hepatocytes. Chloral hydrate was a weak inducer of SCE in cultured human lymphocytes. *In vivo*, it induced mitotic gene conversion in a host-mediated assay with *S. cerevisiae* recovered from mouse lungs. One laboratory reported a significant increase in strand breaks in liver DNA of exposed rats and mice, whereas another laboratory reported

negative results. Chloral hydrate did not induce chromosomal aberrations in mouse bone marrow cells, spermatogonia, spermatocytes, or oocytes. However, one study reported a significant increase in chromosomal aberrations in mouse secondary spermatocytes. CH induced mutations at the Tk locus in a mouse lymphoma assay (Harrington-Brock *et al.* 1998, cited in Moore and Harrington-Brock 2000). The predominantly small colony Tk mutants indicated that most CH-induced mutants resulted from chromosomal mutations rather than point mutations.

5.4.2 Dichloroacetic acid

The results for genetic toxicity of DCA in prokaryotic and animal cells are inconsistent (IARC 1995b). In *S. typhimurium*, DCA did not induce differential toxicity in DNA-repair-deficient strains but was mutagenic. DCA did not induce λ prophage in *E. coli*, nor did it induce DNA strand breaks in cultured human CCRF-CEM cells (without metabolic activation) or in mouse or rat hepatic cells. *In vivo*, acute administration of DCA induced DNA strand breaks in liver cells of rats and mice in one laboratory, but another laboratory using higher doses reported no DNA strand breakage in rat or mouse hepatic cells after single or repeated administrations, or in epithelial cells from mouse spleen, stomach, and duodenum after a single administration.

5.4.3 Trichloroacetic acid

As reviewed in IARC (1995d), TCA was not mutagenic to *S. typhimurium*, nor did it induce λ prophage in *E. coli*, with or without metabolic activation. TCA, neutralized to avoid the effects of low pH, was not clastogenic in cultured human lymphocytes. TCA did not induce DNA strand breaks in cultured or human CCRF-CEM cells (without metabolic activation) or in mouse or rat hepatic cells. *In vivo*, TCA administered acutely induced DNA strand breaks in liver cells of rats and mice in one laboratory, but another laboratory using higher doses reported no increase in DNA strand breaks in rat or mouse hepatic cells or in mouse epithelial cells from the stomach or duodenum. TCA injected into Swiss mice induced abnormal sperm morphology and micronuclei and chromosomal aberrations in bone marrow cells, but in C57BL/JfBL/Alpk mice, TCA did not induce micronuclei at a 10-fold higher dose.

5.4.4 Trichloroethanol

Trichloroethanol was found to be negative in the *Salmonella* assays (DeMarini *et al.* 1994, cited in Moore and Harrington-Brock 2000). It has not been evaluated by other assays.

5.4.5 Trichloroethylene conjugates (DCVC and DCVG)

Both DCVC and DCVG are capable of inducing point mutations as evidenced by their mutagenicity in bacteria (Vamvakas *et al.* 1988 and DeMarini *et al.* 1994, cited in Moore and Harrington-Brock 2000). DCVC and DCVG were found to be mutagenic based on *Salmonella* assays. There is some indication that DCVC can induce primary DNA damage in mammalian cells *in vitro* and *in vivo* (Jaffe *et al.* 1985 and Vamvakas *et al.* 1989, cited in Moore and Harrington-Brock 2000). DNA damage in kidney tubules was induced *in vivo* and *in vitro* by DCVC, and double-strand breaks were found in LLC-PK_1

cells (Jaffe *et al.* 1985, Vamvakas *et al.* 1992, both cited in Vamvakas *et al.* 1993). Addition of radiolabeled cysteine conjugates to bacterial and renal cells resulted in covalent binding to DNA (Bhattacharya and Schultze, 1972, 1973a,b, Vamvakas *et al.* 1988, both cited in Vamvakas *et al.* 1993). Pyridine nucleotide oxidation was induced by DCVC incubated with kidney mitochondria (Meadows *et al.* 1988, Vamvakas *et al.* 1992, both cited in Vamvakas *et al.* 1993). Vamvakas and Koster (1993, cited in Moore and Harrington-Brock 2000) observed that DCVC can induce the expression of two protooncogenes, c-*fos* and c-*myc*, but their involvement in tumor induction is unknown.

5.5 Summary

The available data indicate that TCE and its metabolites are not potent genotoxic chemicals. Most studies, in a broad range of test systems, were negative or equivocal. In general, high doses were required to induce a positive response. TCE did not induce gene mutations in human cells and studies of chromosomal aberrations, aneuploidy, and SCE in peripheral lymphocytes of workers exposed to TCE were considered inconclusive. Limited data for DCVC and DCVG indicate that these metabolites can induce point mutations and that DCVC may induce DNA damage in mammalian cells; however, the relevance to human tumors is uncertain.

6 Other Relevant Data

6.1 Absorption, distribution, metabolism, and excretion

Sex-, species-, and strain-dependent differences in absorption, distribution, metabolism, and excretion of TCE are important for understanding differences in susceptibility and in determining which metabolites are associated with toxicity. TCE in vapor or liquid form is readily absorbed through the lungs and gastrointestinal tract and distributed throughout the body via the circulatory system. Because the blood/gas partition coefficient is about 1.5 to 2.5 times lower in humans than in rats and mice, absorption and distribution of inhaled TCE is not as efficient in humans as in rodents. Dermal absorption of the vapor is negligible; however, significant dermal absorption may occur following direct skin contact with the liquid or with aqueous solutions of TCE (Lash *et al.* 2000a). Several studies in rats and mice reviewed by the IARC (1995e) showed rapid absorption of TCE through the lungs and from the gastrointestinal tract (IARC 1995e). Mean blood TCE concentrations after four hours of exposure to TCE in air were 35.5 µg/mL (0.27 µmol/mL) in male Fischer 344 rats exposed at a concentration of 529 ppm (2,840 mg/m^3, 21.6 mmol/m^3) and 25.8 µg/mL (0.196 µmol/mL) in females exposed at 600 ppm (3,220 mg/m^3, 24.5 mmol/m^3) (Fisher *et al.* 1991). Male and female B6C3F$_1$ mice were exposed for four hours to TCE at concentrations of 110 to 748 ppm (591 to 4,020 mg/m^3, 4.50 to 30.6 mmol/m^3) and 42 to 889 ppm (226 to 4,780 mg/m^3, 1.72 to 36.4 mmol/m^3), respectively. The highest mean blood concentration in males was 7.3 µg/mL (0.056 µmol/mL) after exposure at 748 ppm (4,020 mg/m^3, 30.6 mmol/m^3) and in females was 6.3 µg/mL (0.048 µmol/mL) after exposure at 368 ppm (1,980 mg/m^3, 15.1 mmol/m^3) (Fisher *et al.* 1991).

Following absorption, TCE is distributed to three major compartmental tissue groups: richly perfused tissues (e.g., liver, kidneys, lungs), poorly perfused tissues (e.g., muscle, skin), and adipose tissue (Lash *et al.* 2000a). Mice given 280 mg/kg b.w. (2.13 mmol/kg) of radiolabeled TCE in a 10-minute inhalation exposure were studied by whole body autoradiography (Bergman 1983). TCE was found throughout the body in well-perfused organs; redistribution to adipose tissue occurred after 30 minutes. With an oil-water partition coefficient of 900:1, TCE is concentrated in lipophilic organs such as liver or brain (Müller *et al.* 1975, Kilburn and Warshaw 1993, both cited in Gist and Burg 1995). Other tissues in which TCE concentrates are ovaries (Manson *et al.* 1984, cited in Gist and Burg 1995) and spermatocytes (Land *et al.* 1979, cited in Gist and Burg 1995).

The amount of TCE available for conversion to toxic metabolites is determined largely by blood flow and metabolic rate. TCE is metabolized via two major pathways: oxidation by cytochrome P-450 and conjugation with glutathione (Byington and Leibman 1965, Leibman 1965, Dekant *et al.* 1986, 1990, Commandeur and Vermeulen 1990, Goeptar *et al.* 1995, all cited in Bernauer *et al.* 1996; Lash *et al.* 2000a) (see Figure 6-1). Chloral hydrate, dichloroacetic acid (DCA), and trichloroacetic acid (TCA) are the metabolites most often associated with liver and lung damage whereas 1,2-dichlorovinylcysteine (DCVC), a metabolite produced through a glutathione pathway has been associated with kidney toxicity (Clewell *et al.* 2000, Lash *et al.* 2000a). The data indicate that mice

metabolize and eliminate TCE faster than rats. This is consistent with the observation that mice are more susceptible than rats to liver injury and carcinogenesis (Lash *et al.* 2000a).

More than 99% of urinary TCE metabolites stem from reactions catalyzed by cytochrome P-450 (Dekant *et al.* 1984,). One such reaction is the oxidation of TCE to chloral, which may proceed through rearrangement of the putative epoxide intermediate 1,1,2-trichlorooxirane (Powell 1945, Bonse *et al.* 1975, both cited in Vamvakas *et al.* 1993), or by rearrangement of a non-epoxide intermediate (Miller and Guengerich, 1982, cited in Vamvakas *et al.* 1993). Upon contact with aqueous solutions, chloral is rapidly converted to its hydrate (IARC, 1995a). Reduction of chloral hydrate yields trichloroethanol and trichloroethanol glucuronide while oxidation results in TCA (Butler 1949, Daniel 1963, Kimmerle and Eben 1973). TCA glucuronide has been found in the urine of non-human primates administered TCE by intramuscular injection (Müller *et al.* 1982).

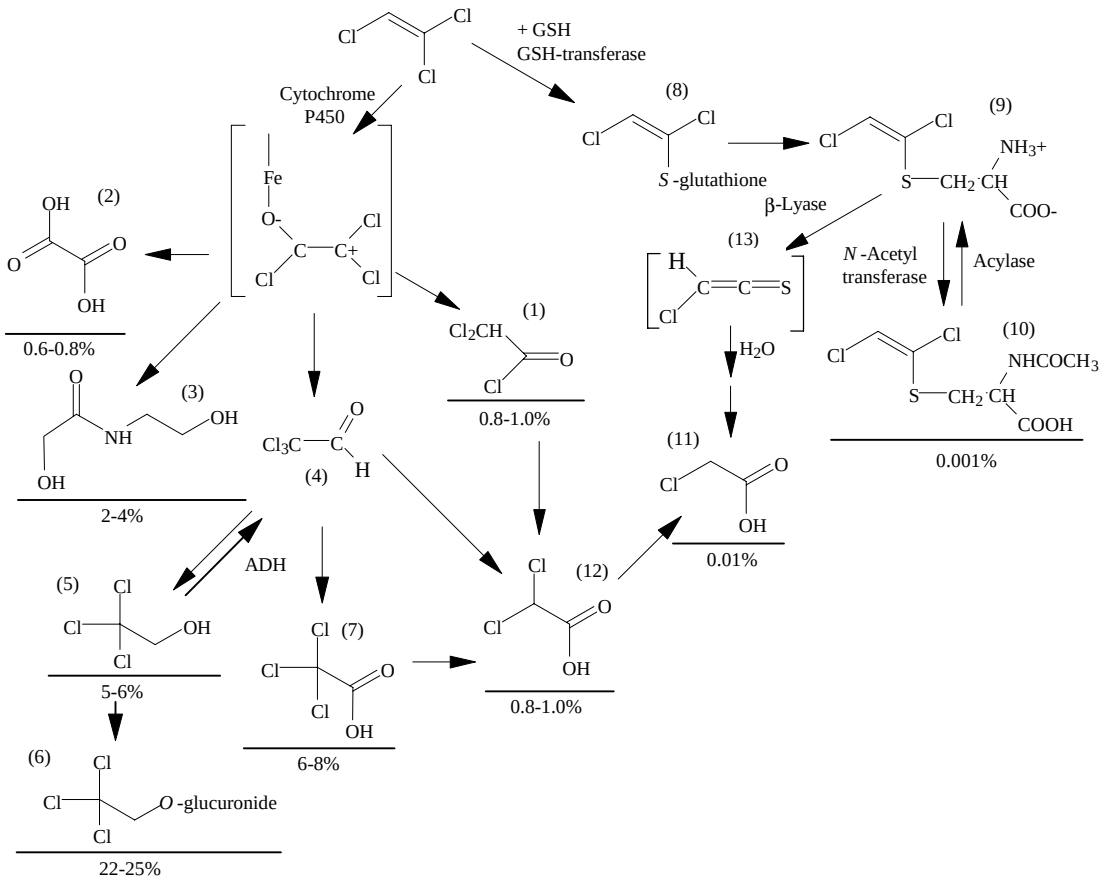

Figure 6-1. Proposed metabolism of TCE in rats

Source: IARC 1995e.

Underlined compounds are identified urinary metabolites (IARC 1995e). (1) dichloroacetyl chloride, (2) oxalic acid, (3) *N*-(hydroxyacetyl)aminoethanol, (4) chloral, (5) trichloroethanol, (6) trichloroethanol glucuronide, (7) trichloroacetic acid, (8) dichlorovinylglutathione, (9) *S*-1,2-dichlorovinylcysteine, (10) *S*-1,2-dichlorovinyl-*N*-acetylcysteine, (11) monochloroacetic acid, (12) dichloroacetic acid, (13) chlorothiketene. Percentages were determined after an oral dose of 200 mg/kg. Compounds shown in brackets are postulated intermediates.

DCA may be formed by a rearrangement of the putative epoxide intermediate 1,1,2-trichlorooxirane and subsequent hydrolysis (Hathway 1980) or by biotransformation of choral hydrate or TCA (Larson and Bull 1992). Oxalic acid may be formed by oxidation of DCA (Larson and Bull 1992) or by cleavage, either enzymatic or non-enzymatic, of 1,1,2-trichlorooxirane followed by spontaneous elimination of two equivalents of hydrochloric acid, reaction with water, and oxidation (Dekant *et al.* 1984). *N*-Hydroxyaminoacetylethanol is thought to be formed by the reaction of TCE oxidized intermediates with aminoethanol or phosphatidylethanol with subsequent hydrolysis of the acylated lipid (Dekant *et al.* 1984). There are significant quantitative differences between rats and mice in the metabolism of TCE to TCA and DCA (Green 1990). TCE

metabolism in the mouse is linear over a wide range of dose levels, while metabolism becomes saturated in the rat at relatively low dose levels.

In the less common of the two metabolic pathways, TCE is conjugated to glutathione. The result is dichlorovinylglutathione (DCVG), which can be converted to *S*-1,2-dichlorovinylcysteine (DCVC). DCVC can then follow one of two paths, forming either monochloroacetic acid or *N*-acetyldichlorovinylcysteine, which has been found to be excreted in a dose-dependent manner after experimental exposure of rats and human volunteers to TCE (Bernauer *et al.* 1996).

TCE metabolism in humans and laboratory animals is qualitatively similar. Most TCE metabolites found in experimental animals also have been found in humans (see Table 6-1); however, rodents have a much higher capacity to metabolize TCE than humans (IARC 1995e, Fisher 2000, Lash *et al.* 2000a). Based on *in vitro* metabolism studies with 23 human hepatic microsomal samples, Lipscomb *et al.* (1997) concluded that the CYP2E1 form of cytochrome P-450 was predominantly responsible for the microsome-mediated metabolism of TCE. Furthermore, the lack of uniformity among these samples in their capacity to metabolize TCE suggested to these investigators that subpopulations of individuals may exist with increased susceptibility to TCE.

After TCE is absorbed from the gastrointestinal tract, first-pass elimination by the liver and lungs play a major role in clearing TCE. When male Sprague-Dawley rats were injected with 0.17 mg/kg (1.3 μmol/kg) of TCE in a 5% aqueous Alkamuls emulsion, the liver eliminated 10 times more chemical as did the lungs on the first pass (Lee *et al.* 1996). As the dose increased beyond 1 to 2 mg/kg (8 to 15 μmol/kg), hepatic first-pass elimination diminished. In human subjects exposed by inhalation to TCE at concentrations up to 315 ppm (1,690 mg/m^3; 12.9 mmol/m^3) for three hours, metabolism was not saturated (Ikeda 1977, Nomiyama and Nomiyama 1977, both cited in Lee *et al.* 1996). This finding led the authors to hypothesize that a single pass through the liver was sufficient to remove TCE from the blood completely. Based on these data, Lee *et al.* (1996) concluded that because metabolism should not be saturated by the daily doses of TCE to which humans are exposed, first-pass elimination should remove a substantial portion of the TCE from the blood before it reaches extra-hepatic organs.

A substantial delay between elimination of TCE from blood and appearance of the metabolite TCA in blood was reported in Templin *et al.* (1993, cited in Stenner *et al.* 1997). Stenner *et al.* (1997) performed a study to determine whether enterohepatic recirculation of trichloroethanol and TCA could explain the TCA concentrations seen in blood following administration of TCE. Male Fischer F344 rats with and without intact enterohepatic recirculation were given trichloroethanol at an intravenous (i.v.) dose of 100 mg/kg (0.669 mmol/kg). The results demonstrated that roughly 36% of the trichloroethanol and 76% of the TCA in systemic blood were due to enterohepatic recirculation. Urinary excretion of TCA following i.v. administration of trichloroethanol was decreased by 80% in rats lacking enterohepatic recirculation (Stenner *et al.* 1997). Using these and previous findings, the authors concluded that enterohepatic recirculation could account for the delayed appearance of TCA in the blood after oral administration of TCE.

Table 6-1. Metabolites of TCE by species

Metabolite[a]	References
Rats	
N-acetyl-*S*-(1,2-dichlorovinyl)-*L*-cysteine (10)	Dekant *et al.* 1986, 1990, Commandeur and Vermeulen 1990)
N-acetyl-*S*-(2,2-dichlorovinyl)-*L*-cysteine (isomer of 10)	Dekant *et al.* 1986, 1990, Commandeur and Vermeulen 1990)
chloroacetic acid (11)	Green and Prout 1985
dichloroacetic acid (12)	IARC 1995e
N-(hydroxyacetyl)aminoethanol (3)	IARC 1995e
oxalic acid (2)	IARC 1995e
trichloroacetic acid (7)	Kimmerle and Eben 1973
trichloroethanol (5)	Kimmerle and Eben 1973
trichloroethanol glucuronide (6)	IARC 1995e
Chimpanzees, baboons, and rhesus monkeys	
trichloroacetic acid glucuronide (formed from 7)	Müller *et al.* 1982
Humans	
N-acetyl-*S*-(1,2-dichlorovinyl)-*L*-cysteine (10)	Birner *et al.* 1993, Bernauer *et al.* (1996)
N-acetyl-*S*-(2,2-dichlorovinyl)-*L*-cysteine (isomer of 10)	Birner *et al.* 1993, Bernauer *et al.* (1996)
chloral hydrate (formed from 4)	Cole *et al.* 1975
N-(hydroxyacetyl)aminoethanol (3)	Dekant *et al.* 1984
oxalic acid (2)	Dekant *et al.* 1984
trichloroacetic acid (7)	Cole *et al.* 1975
trichloroethanol (5)	Cole *et al.* 1975
trichloroethanol glucuronide (6)	Cole *et al.* 1975

[a]Numbers in parentheses correspond to the numbers in Figure 6-1.

TCE metabolites are excreted primarily in the urine; however, up to 30% can be eliminated by excretion in the bile (Gist and Burg 1995). The major metabolites found in human urine are trichloroethanol, trichloroethanol glucuronide, and TCA (Cole *et al.* 1975, Clewell *et al.* 2000). However, Nomiyama and Nomiyama (1971, cited in Clewell *et al.* 2000) demonstrated that the relative amounts of TCA and trichloroethanol excreted by men and women differed; women excreted more TCA and less trichloroethanol than men.

6.2 Pharmacokinetics

The maximum metabolic rate (V_{max}) for TCE in rats is 6.04 mg/h (0.046 mmol/h) with a Michaelis constant of 5.05 µg/mL (0.038 µmol/mL). Absorption by organs occurs with the following organ-to-blood partition coefficients: gastrointestinal tract, 1.35; liver, 2.17; spleen 0.63 (Varkonyi *et al.* 1995).

Another study calculated the V_{max} in humans to be 215.0 mg/h (1.636 mmol/h) based on the V_{max} found in rats using the allometric relationship (human V_{max}) = (Rat V_{max}) $[70/(\text{rat wt., kg})]^{0.7}$ (Gargas *et al.* 1986, cited in Rappaport 1993). Of the absorbed dose of TCE, 0.75 is the fraction metabolized based on estimated human clearance rates (Sato and Nakajima, 1987, cited in Rappaport 1993). Using these two values, Rappaport (1993) calculated that 178.3 mg/m^3 (1,357 mmol/m^3) was the highest mean TCE concentration to which a person could be exposed while maintaining linear kinetics. This is slightly lower than the occupational threshold limit value of 269.0 mg/m^3 (50 ppm; 2.047 mmol/m^3) (ACGIH 1996).

The urine of three male volunteers exposed to TCE by inhalation at a concentration of 40, 80, or 160 ppm (217 to 869 mg/m3) for six hours was examined for the presence of TCE metabolites (Bernauer *et al.* 1996). After inhalation of TCE at 160 ppm, excretion of the mercapturate metabolites, *N*-acetyl-*S*-(1,2-dichlorovinyl)-*L*-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-*L*-cysteine, seemed to become saturated, while excretion of oxidatively formed metabolites increased linearly. Elimination of mercapturates in humans exhibited a biphasic nature and was slower than elimination of the oxidatively formed metabolites. When five male volunteers were exposed to TCE at 70 ppm (380 mg/m^3, 2.9 mmol/m^3) four hours per day for five days, trichloroethanol concentrations in urine rose rapidly, then stabilized and remained high for the duration of the five days of exposure (Monster *et al.* 1979). Urinary excretion of TCA continued to rise until the end of exposure. The major metabolites of TCE are cleared from humans at very different rates. TCA is eliminated slowly in urine with a half-life of about 52 hours, but trichloroethanol and trichloroethanol glucuronide are eliminated rapidly with half-lives of about 10 hours (Müller *et al.* 1972, 1974). The difference is even more marked in rats; TCA is present in the blood at high levels for up to 30 hours in contrast to trichloroethanol and chloral hydrate, which are cleared from the blood with a half-life of 1 to 2 hours (Kimmerle and Eben 1973).

6.3 Metabolites

DCA, TCA, and chloral hydrate have been evaluated for carcinogenicity by the IARC; however, DCVC has not been classified. The IARC findings and other relevant data are briefly discussed below. Genotoxicity data for these metabolites were discussed in Section 5.

6.3.1 Dichloroacetic Acid and Trichloroacetic Acid

The IARC (1995b) concluded that the evidence for the carcinogenicity of DCA was inadequate in humans and limited in experimental animals (Group 3). In four studies, oral administration of pH-neutralized DCA to male B6C3F_1 mice resulted in an increased incidence of hepatocellular adenoma and carcinoma. In hepatic carcinomas sampled from DCA-exposed mice, expression of c-*myc* and c-H-*ras* was increased approximately 3-fold and 4-fold, respectively. Although the frequency of mutations at codon 61 of H-*ras* was not significantly different between liver tumors in exposed and control mice, the spectra of the mutations in DCA-exposed mice showed a significant increase in CTA and a corresponding decrease in AAA.

The IARC (1995d) concluded that there was inadequate evidence in humans and limited evidence in experimental animals for the carcinogenicity of TCA (Group 3). TCA significantly increased the incidence of hepatocellular adenoma and carcinoma in two drinking-water studies with B6C3F_1 male mice (IARC 1995d). In hepatic carcinomas sampled from TCA-exposed mice, expression of c-*myc* and c-H-*ras* was increased approximately 6-fold and 4-fold, respectively.

6.3.2 Chloral hydrate

IARC evaluated chloral and chloral hydrate as not classifiable as to their carcinogenicity in humans (Group 3), citing inadequate evidence in humans and limited evidence in animals (IARC 1995a). In an experimental carcinogenicity study that the IARC deemed adequate, male mice given chloral hydrate by gavage showed a significantly increased incidence of hepatic adenomas and carcinomas.

The NTP has issued a draft report on the toxicology and carcinogenicity of chloral hydrate (NTP 2000b). Groups of female B6C3F_1 mice received chloral hydrate in distilled water by gavage at concentrations of 0, 10, 25, 50, or 100 mg/kg. Some mice were exposed to a single dose and held for 105 weeks while others were exposed 5 days/week. Male mice were only exposed to a single dose of chloral hydrate at 10, 25, or 50 mg/kg. There was equivocal evidence of carcinogenic activity in female mice treated continuously based on increased incidences of pituitary gland pars distalis adenoma. No tumors were increased in female or male mice receiving a single dose of chloral hydrate.

6.3.3 Dichlorovinylcysteine

The IARC has not yet determined a classification for DCVC. DCVC was mutagenic in the Ames test (see Section 5) and highly nephrotoxic (Vamvakas *et al.* 1993, Clewell *et al.* 1995). At concentrations producing small or undetected decreases in cell growth, DCVC induced Ca^{2+}-dependent DNA damage. Following this DNA fragmentation, an increase was seen in adenosine diphosphate ribosylation of nuclear proteins (Vamvakas

et al. 1992, cited in Vamvakas *et al.* 1993), which, if moderate, was shown in mouse fibroblasts to be associated with increased cell proliferation (Muehlematter *et al.* 1988, cited in Vamvakas *et al.* 1993). Before collapse of the mitochondrial membrane potential, cytosolic Ca^{2+} concentrations were increased by DCVC in renal cells (Vamvakas *et al.* 1990, cited in Vamvakas *et al.* 1993). Tumor promoters that induce oxidative stress commonly produce such an effect (Vamvakas *et al.* 1993).

6.4 Immune suppression

Sprague-Dawley rats and $B6C3F_1$ mice given TCE by i.p. injection showed inhibition of immune system activity, as demonstrated by decreases in splenocyte viability, inhibition of lipopolysaccharide-stimulated mitogenesis, inhibition of hepatic natural killer cells, and inhibition of natural cytotoxic cell activities in all groups of effector cells (IARC 1995e).

6.5 Molecular changes in human tumors

Brüning *et al.* (1997) examined tissues from 23 patients with renal-cell carcinoma and high occupational exposure to TCE. All 23 patients had aberrations of the von Hippel-Lindau (VHL) tumor suppressor gene compared to mutation frequencies of 33% to 55% in patients with renal-cell carcinoma but without known occupational exposure to TCE. In a similar study, Brauch *et al.* (1999) reported evidence for a linkage between TCE exposure and somatic mutation of the von Hippel-Lindau (VHL) tumor suppressor gene, a known molecular-genetic cause for renal-cell carcinoma (Gnarra *et al.* 1994). The cases were 44 renal-cell carcinoma patients who were registered as former employees of metal-processing factories in Germany with histories of high cumulative TCE exposure. TCE exposure was ranked as one of three levels (high, medium, or low) by a defined scoring system that integrated total exposure time as well as frequency and duration of acute adverse effects. For evaluation of the effects of TCE exposure, 107 controls were recruited who had renal-cell carcinoma but were not registered as having been exposed to TCE. Of these controls, 34 were from the same geographic location as the TCE-exposed patients, and 73 were from other regions in Germany. DNA was isolated from tumor tissue of both the TCE-exposed and control patients by microdissection. DNA also was isolated from the adjacent non-neoplastic kidney parenchyma of the 44 TCE-exposed renal-cell carcinoma cases.

Controls for the analysis of germline VHL gene status included the lymphocyte DNA from the 44 TCE-exposed renal-cell carcinoma patients and 97 nondiseased individuals, 47 of whom had been exposed to TCE and 50 of whom were population controls without any history of TCE exposure. DNA was isolated from lymphocytes of these individuals.

Exons 1, 2, and 3 of the VHL gene from the tumor DNA of the 44 TCE-exposed renal-cell carcinoma patients were amplified by polymerase chain reaction (PCR) and analyzed by single-strand conformation polymorphism and sequencing. *VHL* mutations were identified in 33 (75%) of the samples, and 14 (42%) had multiple mutations. Most of the mutations were located in exon 1 (52%), with 20% occurring in exon 2 and 28% in exon 3. The majority of the mutations (54%) were missense mutations, of which 89% involved a cytosine change; 19 samples had a C to T change, and 5 had a C to G change. Of the 33 samples with *VHL* mutations, 13 (39%) had a C to T change at nucleotide (nt) 454 (in

exon 1), which codes for a Pro to Ser change at codon 81. This nt 454 mutation occurred at a greater frequency (41%) in those patients classified as having a high severity of exposure than those with either a medium (25%) or low (0%) severity of exposure.

DNA from tumors of the 107 renal-cell carcinoma controls and lymphocyte DNA from the germline *VHL* status controls were screened for the nt 454 mutation with a PCR–restriction fragment length polymorphism assay. None of these samples had the nt 454 mutation, suggesting that this mutation was both specific for TCE exposure (as evident from the non-TCE-exposed renal-cell carcinoma controls) and disease-related (as evident from the germline controls). The nt 454 mutation was found in the adjacent non-neoplastic kidney parenchyma in four TCE-exposed renal-cell carcinoma patients.

In contrast, Schraml *et al.* (1999) analyzed DNA from normal and renal-cell carcinoma tissues from 12 patients with occupational exposure to varying levels of TCE and other solvents. Comparison of these results with data obtained from sporadic renal-cell carcinoma failed to suggest a unique mutation pattern in the VHL gene of renal tumors associated with exposure to TCE. However, TCE exposure was not as severe as in the study reported by Brauch *et al.* (1999). Subjects in this study were exposed to varying doses of TCE; whereas, the Brauch *et al.* (1999) study included subjects with prenarcotic symptoms (e.g., dizziness, nausea, equilibrium disorders).

6.6 Mechanisms of carcinogenesis

6.6.1 Liver cancer

Hepatocellular carcinomas have been reported in some strains of mice but not in rats exposed to TCE (see Section 4), and species and strain differences in metabolism of TCE to TCA and DCA are thought to account for this difference (Goeptar *et al.* 1995, Maronpot *et al.* 1995, Clewell *et al.* 1995). Channel *et al.* (1998) demonstrated that TCE given in drinking water to $B6C3F_1$ mice induced lipid peroxidation, peroxisome proliferation, and mitogenesis without an increase in apoptosis. This initial cellular response may be characteristic of the mouse liver and may provide further explanation of the species and strain differences. TCA is a peroxisome proliferator at doses that induce liver cancer in mice; however, the actual mechanisms of carcinogenesis may be only loosely associated with peroxisome proliferation.

Data suggest that the level of expression of the peroxisome proliferator activated receptor alpha ($PPAR_\alpha$) may be involved in carcinogenesis. Mice with a targeted disruption of the $PPAR_\alpha$ gene did not develop tumors when exposed to a potent peroxisome proliferator while mice of the same strain with an intact gene did develop tumors (Bull 2000). TCE-induced peroxisome proliferation has not been demonstrated in rats (Elcombe 1985). Goeptar *et al.* (1995) concluded that the species difference in TCE-induced peroxisome proliferation was most likely due to saturation of the oxidative metabolism of TCE in the rat, thereby limiting the maximal levels of TCE to below those required to induce this effect.

The relevance of peroxisome proliferation to carcinogenesis in humans is controversial. Human cells and mouse cells respond differently to peroxisome proliferators (Bull 2000).

Exposure to peroxisome proliferators induces a much weaker response in humans compared to mice. This may be explained by the relatively low levels of $PPAR_\alpha$ expressed in human liver (Maloney and Waxman 1999, Bull 2000). Maloney and Waxman (1999) conducted cell transfection studies to investigate the interactions of peroxisome proliferators with cloned human and mouse $PPAR_\alpha$ and $PPAR_\gamma$, an isoform of $PPAR_\alpha$. $PPAR_\gamma$ is highly expressed in many human tissues, including colon, heart, liver, testis, spleen, hematopoietic cells, and fat. Their data showed that human $PPAR_\alpha$ was less sensitive than mouse $PPAR_\alpha$ to some but not all peroxisome proliferators. Human and mouse $PPAR_\alpha$ showed similar sensitivity to TCA and DCA. $PPAR_\gamma$ was not activated by TCA or DCA.

Both TCA and DCA induced hepatocellular adenomas and carcinomas in mice (see Section 6.3.3.1), possibly mediated through the induction of peroxisome proliferation, cytotoxicity, and reparative hyperplasia (Maronpot *et al.* 1995). However, Barton *et al.* (1999) questioned the role of DCA in TCE-induced liver cancer. Their data suggested that the low concentrations of DCA present in the liver would be insufficient to contribute significantly to TCE-induced liver cancer. The involvement of TCA and DCA in TCE-induced hepatocellular carcinoma in mice is supported by studies in which strain differences in the incidence of liver tumors correlated with differences in the oxidative metabolism of TCE. For example, TCE induced liver tumors in Swiss and $B6C3F_1$ mice (NTP 1986; Maltoni *et al.* 1986, cited in Goeptar *et al.* 1995) but not NMRI mice (Henschler *et al.* 1980, cited in Goeptar *et al.* 1995). In Swiss and $B6C3F_1$ mice, TCA and DCA accounted for 7% to 12% and 2% of the administered TCE, respectively (Green and Prout 1985, cited in Goeptar *et al.* 1995), but in the NMRI mice, TCA and DCA each accounted for only 0.1% of the TCE dose (Dekant *et al.* 1984, cited in Goeptar *et al.* 1995). By this line of reasoning, the ability of TCE to induce liver tumors in humans would depend on the rate of formation of TCA and DCA and on the induction of peroxisome proliferation in liver cells. Although humans appear more similar to mice than to rats in their ability to oxidatively metabolize TCE, they metabolize approximately 60 times less TCE on a body-weight basis than mice at similar exposure levels, and TCA does not appear to induce peroxisome proliferation in human hepatocytes (Goeptar *et al.* 1995).

Bull (2000) reviewed four possible modes of action for TCE-induced liver cancer: somatic mutation, modification of cell signal pathways, cell death and reparative hyperplasia, and hepatomegaly and cytomegaly. He concluded that there is no evidence that clearly associates a genotoxic effect of TCE or its metabolites with liver cancer. The data suggested that TCE induced liver tumors by modifying cell-signaling systems involved in cell division and death and indicated that DCA and TCA induce tumors by separate mechanisms. DCA differentially inhibits normal cell replication at low doses but stimulates marked cell replication within liver tumors at high doses resulting in a nonlinear dose-response relationship. Only the differential inhibition of normal cell replication was observed with TCA. TCA appears to induce tumors with a higher rate of replication than those induced by DCA. At high doses, DCA increases the growth rate of tumors with a less malignant phenotype.

6.6.2 Lung cancer

TCE administered by inhalation significantly increased the incidence of lung tumors (adenoma and carcinoma) in female (but not male) B6C3F_1 mice and male (but not female) Swiss mice. TCE is not carcinogenic in the rat lung. Mechanistic studies on mouse lung tumor formation suggest that chloral formation in Clara cells may explain the sex and species differences (Goeptar *et al.* 1995, Green *et al.* 1997). High cytochrome P-450 activity and impaired metabolism of chloral in Clara cells are believed to be responsible for the toxic and carcinogenic effects observed in the mouse lung (Green 2000). TCE metabolism in the human lung was reported to be about 600 times less than in the mouse lung (Green 2000).

Mouse Clara cells studied *in vitro* were found to have relatively high cytochrome P-450 activity and relatively low activities of alcohol dehydrogenase, the enzyme that converts chloral to trichloroethanol, and uridine diphosphate glucuronosyl transferase, the enzyme responsible for the glucuronidation of trichloroethanol (Odum *et al.* 1992, cited in Clewell *et al.* 1995). Thus, chloral would be anticipated to accumulate in mouse Clara cells, which may explain the formation of lung tumors in mice. Consistent with this hypothesis, exposure of mice to chloral resulted in lesions in lung Clara cells similar to those caused by a 10-fold higher concentration of TCE (Odum *et al.* 1992, cited in Clewell *et al.* 1995). Goeptar *et al.* (1995) hypothesized that the absence of smooth endoplasmic reticulum in human lung Clara cells (Smith *et al.* 1979, cited in Goeptar *et al.* 1995) implied a lack of cytochrome P-450 activity and a corresponding lack of risk for chloral accumulation.

6.6.3 Kidney cancer

In contrast to tumors of the lung and liver, kidney tumors were found in rats but not in mice, and at doses associated with a high incidence of nephrotoxicity (Goeptar *et al.* 1995, Clewell *et al.* 1995). Goldsworthy *et al.* (1988) did not find evidence that TCE induced kidney cancer in male rats by $\alpha_{2\mu}$-globulin protein droplet accumulation; however, experiments with tetrachloroethylene indicated that protein droplet accumulation did occur with this chemical. With TCE, nephrotoxicity has most often been associated with toxic metabolites; however, the relative importance of various metabolic pathways and species differences in toxic responses is controversial.

In a minor mercapturic metabolic pathway, TCE is conjugated to glutathione in the liver. The conjugated TCE is further metabolized in the kidney to the cysteine conjugate DCVC and then to a reactive intermediate (Birner *et al.* 1993, cited in Clewell *et al.* 1995). The mutagenic and nephrotoxic properties of the *S*-1,2 isomer of DCVC are described in Sections 5.4.5 and 6.3.3, respectively. The question is not whether or not DCVC is nephrotoxic but whether or not sufficient amounts of TCE or DCVG reach the kidneys to produce enough reactive metabolites to cause toxicity (Lash *et al.* 2000b). The data are mixed on this question. Lash *et al.* (2000b) reported that oxidative or glutathione-derived metabolites from TCE can form within the kidneys at appreciable rates. On the other hand, Green *et al.* (1997b) questioned the role of glutathione conjugation and DCVC in renal toxicity and carcinogenicity. They conducted studies with male Fischer 344 rats and male B6C3F_1 mice given TCE (1, 10, or 42 days) or

DCVC (1 or 10 days) by gavage. In this study, the glutathione conjugation pathway accounted for less than 0.01% of the dose and was higher in the mouse than the rat. DCVC was 5 to 10 times more nephrotoxic to mice compared to rats and the nephrotoxic dose in rats was three orders of magnitude higher than the level of DCVC formed from TCE *in vivo*. They also noted the lack of correlation between metabolism of TCE to DCVC and renal cancer in both rats and mice.

Dow and Green (2000) suggested that increased excretion of formic acid may play a role in TCE kidney cancer. Male Fischer 344 rats exposed to metabolites of TCE (TCA and trichloroethanol) excreted higher amounts of formic acid in their urine. Rats receiving chloral or DCVC did not excrete higher amounts of formic acid. Formic acid is not a product of TCE metabolism; however, metabolites of TCE may interact with vitamin B12 and produce a folate deficiency. Folate deficiency affects formate metabolism resulting in increased formic acid excretion. Sustained excretion of high levels of formic acid may result in renal toxicity.

Lash *et al.* (2000b) reviewed evidence for four possible modes of action of TCE-induced kidney cancer: (1) peroxisome proliferation, (2) accumulation of the male rat-specific protein α_{2u}-globulin, (3) direct genotoxicity, and (4) acute and chronic toxicity. These authors concluded that there was little evidence that the first two mechanisms are important for TCE-induced kidney cancer; however, the data do suggest that different modes or combinations of several modes of action may be important. These researchers also questioned the role of formic acid excretion proposed by Dow and Green (2000) because there is no evidence that formic acid produces renal tumors. Renal effects mediated through the glutathione conjugation pathway include cytotoxicity, induction of DNA repair, and proliferative responses. High doses of DCVC may produce oxidative stress, protein and DNA alkylation, and mitochondrial dysfunction followed by inhibition of active transport, ATP depletion, cytotoxicity, and acute tubular necrosis. At lower doses, less severe effects on mitochondrial function, oxidative stress, and selective alkylation of protein and DNA may lead to altered gene expression and cell growth.

Goeptar *et al.* (1995) concluded that it seemed improbable that the oxidative pathway would become saturated in humans at likely exposure levels of TCE. However, urinary excretion of *N*-acetylated DCVC (a detoxification product of DCVC) has been detected in humans occupationally exposed to TCE (Birner *et al.* 1993, cited in Clewell *et al.* 1995).

Furthermore, one human study strongly suggested that kidney damage was associated with exposure to TCE. Brüning *et al.* (1996) compared the urinary protein patterns of 17 patients diagnosed with renal-cell cancer after many years of high-level occupational exposure to TCE with those of 35 renal-cell cancer patients not exposed to TCE. Exposure to TCE was associated with degreasing, production of rubber boxes, and cleaning of cardboard-making machines. These exposures were without protection from hoods, ventilating systems, or the use of gloves. The average year of initial exposure was 1959, mean exposure duration was 15.2 years, mean time of diagnosis for renal-cell carcinoma was 1990, and mean latency period was 30.4 years. Symptoms of exposure

included dizziness, headache, a sense of drunkenness, and drowsiness. The postoperative period for the unexposed patients was similar to that for the TCE-exposed patients.

In all 17 exposed patients, protein excretion patterns indicated tubule damage in their remaining kidney. Among the 35 non-exposed patients, 12 had tubule damage, 4 had glomerular and tubule damage, and 1 had glomerular damage. Brüning *et al.* (1996) concluded that although their data were limited, the findings supported the likelihood that chronic tubule damage contributed to the induction of renal-cell cancer by TCE. This finding is consistent with the involvement of the mercapturic metabolic pathway in the metabolism of TCE in humans (Brüning *et al.* 1996).

It is biologically plausible that the observed kidney tumors were related to TCE exposure, for four reasons: (1) the site and histopathological characteristics of the tumors observed in patients and experimental animals were identical (Vamvakas *et al.* 1993), (2) the molecular mechanism of this type of nephrocarcinogenicity has been elucidated (Dekant *et al.* 1986, cited in IARC 1995e), (3) the metabolites derived from the likely ultimate electrophilic intermediates of the bioactivation of TCE were identical in humans and in experimental animals (Birner *et al.* 1993, cited in Clewell *et al.* 1995), and (4) taking the key urinary metabolites (mercapturic acids) as an indicator of the bioactivation of TCE (Birner *et al.* 1993, cited in Clewell *et al.* 1995), humans seem to be more sensitive than rats in developing the primary biochemical lesion leading to the induction of renal cancer.

6.6.4 Structural analogues

Structural analogs of TCE include vinyl chloride (chloroethylene), vinylidene chloride (1,1-dichloroethylene), and tetrachloroethylene. Tetrachloroethylene appears to be the most similar to TCE in the sites of tumor formation. The genotoxic effects of these structural analogues were discussed in Section 5.

6.6.4.1 Vinyl chloride

Based on human epidemiological studies and case reports and rodent carcinogenicity data, the IARC (1979) concluded that there was sufficient evidence for the carcinogenicity of vinyl chloride in humans and experimental animals. The IARC (1987a) reaffirmed vinyl chloride's evaluation as a human carcinogen (Group 1), citing several additional epidemiological studies and case reports. Occupational exposure to vinyl chloride was associated with increased risks of angiosarcoma of the liver, hepatocellular carcinoma, brain and lung tumors, and malignancy of the hematopoietic and lymphatic system. Some studies indicated a possibility of increased risk of gastric and gastrointestinal cancer (other than liver) (IARC 1987a). Green (1990) noted that workers in vinyl chloride manufacturing also experienced increases in tumors of the skin and thyroid, although a causal relationship was not established. One study indicated excess fetal mortality among wives of workers exposed to vinyl chloride, and several other studies reported increased rates of birth defects in children whose parents lived in communities with vinyl chloride–poly(vinyl chloride) or other chemical processing facilities (IARC 1979).

Vinyl chloride has been extensively tested in rats, hamsters, and mice via inhalation exposure and oral, subcutaneous, and i.p. administration. Oral administration or inhalation of vinyl chloride induced Zymbal gland tumors in rats and hamsters, nephroblastoma in rats, forestomach papilloma and melanoma in hamsters, and pulmonary and mammary gland tumors in mice (IARC 1979, 1987a). In all three species, exposure to vinyl chloride induced hemangiosarcoma of the liver (IARC 1979, 1987a, Green 1990). Vinyl chloride was carcinogenic in rats exposed prenatally (IARC 1979).

6.6.4.2 Vinylidene chloride

The IARC (1999) concluded that vinylidene chloride was not classifiable as to its carcinogenicity in humans because of inadequate evidence (Group 3) and considered the evidence for its carcinogenicity in animals to be limited. No data were available on its genetic and related effects in humans. Green (1990) stated that the question of vinylidene chloride's carcinogenicity had never been resolved, although, as the closest analog of the well-established carcinogen vinyl chloride, it might be expected to be carcinogenic.

Carcinogenicity of vinylidene chloride has been tested via inhalation and oral administration in mice and rats, via topical application and subcutaneous administration to mice, and via inhalation in hamsters (IARC 1987b). Oral administration produced negative results in mice and rats. Inhalation produced no exposure-related neoplasms in rats and hamsters; in mice, however, males showed an exposure-related increase in the incidence of kidney adenocarcinoma, females showed an increase in the incidence of mammary carcinoma, and both males and females showed an increase in pulmonary adenoma. Mice given several subcutaneous administrations showed no tumors at injection sites. In Swiss mice exposed to vinylidene chloride at high doses, Maltoni *et al.* (1984a,b, cited in Green 1990) found severe nephrotoxicity, and tumors in only 2 of 18 surviving mice. Male Swiss mice were more susceptible to nephrotoxic effects than were other mouse strains, rats, or hamsters. Green (1990) suggested that kidney damage in Swiss mice may have facilitated expression of the weak genotoxic potential of vinylidene chloride's metabolites.

6.6.4.3 Tetrachloroethylene

The IARC (1995c) evaluated tetrachloroethylene as probably carcinogenic to humans (Group 2A), based on limited evidence in humans and sufficient evidence in experimental animals. In epidemiological studies, occupational exposure to tetrachloroethylene presented increased risk for esophageal cancer, non-Hodgkin's lymphoma, and cervical cancer. In experimental carcinogenicity studies, mice given tetrachloroethylene by gavage showed an increased incidence of hepatocellular carcinoma (IARC 1995c). Mice exposed to high doses by inhalation showed exposure-related increases in hepatocellular adenoma and carcinoma (NTP 1986). Rats exposed to high doses by inhalation showed a dose-related increase of mononuclear-cell leukemia; also observed but not statistically significant was an increase in the incidence of renal tubular-cell adenoma and adenocarcinoma in male rats (NTP 1986). In a study by Anna *et al.* (1994), the liver tumors induced in mice treated chronically with tetrachloroethylene for up to 76 weeks exhibited a decreased frequency of H-*ras*

mutations and an increased frequency of K-*ras* mutations, compared with liver tumors from concurrent and historical control animals.

6.7 Summary

TCE is rapidly absorbed from the gastrointestinal tract and lungs. The liquid can be absorbed through the skin; however, dermal absorption of the vapor is negligible. Following absorption, TCE is distributed throughout the body and concentrates in lipophilic tissues (e.g., liver, brain, fat). Oxidation by cytochrome P-450 and conjugation with glutathione are the primary metabolic pathways. Chloral hydrate, DCA, and TCA are the primary toxic metabolites produced by the P-450 pathway and have been associated with liver and lung toxicity. DCVC is a metabolite of the glutathione pathway and has been associated with kidney toxicity. Although TCE metabolism in mice, rats, and humans are qualitatively similar, there are significant quantitative species and sex differences. Mice metabolize TCE faster than rats and rats metabolize TCE faster than humans. Human studies have shown that women excrete more TCA and less trichloroethanol than men.

Several structural analogues (vinyl chloride, vinylidene chloride, and tetrachloroethylene) and metabolites (chloral hydrate, DCA, and TCA) of TCE have been tested for carcinogenicity. Most of these chemicals have induced liver tumors in mice.

TCE induces liver cancer in mice but not in rats. Species and strain differences in metabolism of TCE to TCA and DCA may provide an explanation for these susceptibility differences. These metabolites may induce liver cancer through peroxisome proliferation, cytotoxicity, and mitogenesis. Strain differences in oxidative metabolism show some correlation with liver tumor incidence. Humans metabolize about 60 times less TCE on a body-weight basis than mice at similar exposure levels and TCA does not appear to induce peroxisome proliferation in human hepatocytes.

Lung cancer has been induced in female $B6C3F_1$ mice and male Swiss mice but not in rats following inhalation exposure to TCE. High cytochrome P-450 activity and impaired metabolism of chloral in Clara cells are believed to be responsible for the carcinogenic effects. Chloral formation in Clara cells may explain the sex and species differences.

Unlike liver and lung tumors, kidney tumors are induced in rats but not in mice exposed to TCE. Glutathione conjugation of TCE in the liver and further metabolism to DCVC in the kidney may be related to kidney carcinogenicity; however, there are data that question this. It is likely, that several different modes of action are involved and include cytotoxic, repair, and proliferative responses.

Mechanistically, renal-cell carcinomas from workers occupationally exposed to high levels of TCE exhibited somatic mutations of the VHL tumor suppressor gene, a gene that has been associated with renal cell carcinomas (Brauch *et al.* 1999). Moreover, this mutation was found to be both disease-related and specific for TCE exposure. It is biologically plausible that kidney tumors observed in humans may be related to TCE exposure. The site and histopathological characteristics of the tumors observed in patients and the metabolites derived from the likely ultimate electrophilic intermediates of the

bioactivation of TCE were identical in humans and in experimental animals. Furthermore, humans seem to be more sensitive than rats in developing the primary biochemical lesion leading to the induction of renal cancer.

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Appendix A: Excerpts from the IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans Volume 63 (Dry Cleaning, Some Chlorinated Solvents and Other Industrial Chemicals) Trichloroethylene pp. 75-158 (1995). A-1 - A-84.

TRICHLOROETHYLENE

This substance was considered by previous working groups, in June 1978 and March 1987 (IARC, 1979, 1987a). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

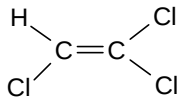
1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 79-01-6
Deleted CAS Reg. No.: 52037-46-4
Chem. Abstr. Name: Trichloroethene
IUPAC Systematic Name: Trichloroethylene
Synonyms: Ethinyl trichloride; ethylene trichloride; TCE; 1,1,2-trichlorethylene

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

(*a*) *Description*: Mobile liquid with chloroform-like odour (Budavari, 1989)

(*b*) *Boiling-point*: 87 °C (Lide, 1993)

(*c*) *Melting-point*: –73 °C (Lide, 1993)

(*d*) *Density*: 1.4642 at 20 °C/4 °C (Lide, 1993)

(*e*) *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).

(*f*) *Solubility*: Slightly soluble in water (1.1 g/L at 25 °C); soluble in ethanol, diethyl ether, acetone and chloroform (Lide, 1993; PPG Industries, Inc., 1994)

(*g*) *Volatility*: Vapour pressure, 100 mm Hg [13.3 kPa] at 31.4 °C (Lide, 1993); relative vapour density (air = 1.0), 4.53 (Budavari, 1989)

(*h*) *Stability*: Photo-oxidized in air by sunlight (half-time, five days) giving phosgene and dichloroacetyl chloride (United States Environmental Protection Agency, 1985)

(*i*) *Reactivity*: Incompatible with strong caustics and alkalis and with chemically active metals such as barium, lithium, sodium, magnesium, titanium and beryllium (United States National Institute for Occupational Safety and Health, 1994a)

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

(*k*) *Conversion factor*: $mg/m^3 = 5.37 \times ppm$[1]

1.1.4 Technical products and impurities

Commercial grades of trichloroethylene, formulated to meet use requirements, differ in the amount and type of added inhibitor. Typical grades contain > 99% trichloroethylene; they include a neutrally inhibited vapour-degreasing grade and a technical grade for use in formulations. Stabilizers that have been used in formulations of trichloroethylene include neutral inhibitors and free-radical scavengers, amyl alcohol, *n*-propanol, isobutanol, 2-pentanol, diethylamine, triethylamine, dipropylamine, diisopropylamine, diethanolamine, triethanolamine, morpholine (see IARC, 1989a), *N*-methylmorpholine, aniline (see IARC, 1987b), acetone, ethyl acetate, borate esters, ethylene oxide (see IARC, 1994a), propylene oxide (see IARC, 1994b), 1,2-epoxybutane (see IARC, 1989b), cyclohexene oxide, butadiene dioxide, styrene oxide (see IARC, 1994c), pentene oxide, 2,3-epoxy-1-propenol, 3-methoxy-1,2-epoxypropane, stearates, 2,2,4-trimethyl-1-pentene, 2-methyl-1,2-epoxypropanol, epoxycyclopentanol, epichlorohydrin (see IARC, 1987c), tetrahydrofuran, tetrahydropyran, 1,4-dioxane (see IARC, 1987d), dioxalane, trioxane, alkoxyaldehyde hydrazones, methyl ethyl ketone, nitromethanes, nitropropanes, phenol (see IARC, 1989c), *ortho*-cresol, thymol, *para*-*tert*-butylphenol, *para*-*tert*-amylphenol, isoeugenol, pyrrole, *N*-methylpyrrole, *N*-ethylpyrrole, (2-pyrryl)trimethylsilane, glycidyl acetate, isocyanates and thiazoles (United States Environmental Protection Agency, 1985; WHO, 1985).

Apart from added stabilizers, commercial grades of trichloroethylene should not contain more than the following amounts of impurities: water, 100 ppm [mg/L]; acidity (as HCl), 5 ppm; insoluble residue, 10 ppm (Mertens, 1993). Free chlorine should not be detectable (PPG Industries, Inc., 1994). Impurities that have been found in commercial trichloroethylene products include: carbon tetrachloride (see IARC, 1987e), chloroform (see IARC, 1987f), 1,2-dichloroethane (see IARC, 1987g), *trans*-1,2-dichloroethylene, *cis*-1,2-dichloroethylene, pentachloroethane (see IARC, 1987h), 1,1,1,2-tetrachloroethane (see IARC, 1987i), 1,1,2,2-tetrachloroethane (see IARC, 1987j), 1,1,1-trichloroethane (see IARC, 1987k), 1,1,2-trichloroethane (see IARC, 1991), 1,1-dichloroethylene, tetrachloroethylene (see monograph, this volume), bromodichloromethane, bromodichloroethylene and benzene (see IARC, 1987l) (WHO, 1985; Mertens, 1993).

Trade names for trichloroethylene include: Algylen, Anamenth, Chlorilen, Chlorylen, Densinfluat, Fluate, Germalgene, Narcogen, Narkosoid, Threthylen, Threthylene, Trethylene,

[1] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming normal temperature (25 °C) and pressure (101 kPa)

Tri, Trichloran, Trichloren, Triclene, Trielene, Trielin, Trieline, Trilen, Trilene, Trimar and Westrosol.

1.1.5 Analysis

Selected methods for the analysis of trichloroethylene in various matrices are identified in Table 1.

Table 1. Methods for the analysis of trichloroethylene

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Adsorb on charcoal; desorb with carbon disulfide	GC/FID	0.01 mg/sample	Eller (1994); US Occupational Safety and Health Administration (1990)
	Draw air into sample bag; inject aliquot into gas chromatograph	GC/PID	0.25 ng/sample	Eller (1994)
	Draw air through Tenax sample tube; heat; desorb on cold trap	GC/MS	20 ng	US Environmental Protection Agency (1988a)
	Draw air into cryogenically cooled trap; heat	GC/FID and/or GC/EC	1–5 ng	US Environmental Protection Agency (1988a)
	Draw air into SUMMA® passivated stainless-steel canister; desorb on cold trap	GC/MS or GC/EC-FID-PID	NR	US Environmental Protection Agency (1988a)
Coffee	Isolate sample by closed-system vacuum distillation with toluene	GC/EC or GC/ECD	NR	US Food and Drug Administration (1983)
Grain	Add sample to acetone; store 48 h in the dark; add sodium chloride; add calcium chloride	GC/ECD	NR	Sawyer *et al.* (1990)
Spice oleoresins	Add sample to absolute alcohol/ 1,2-dichloropropane mixture; dilute with absolute alcohol and shake	GC	NR	Fazio (1990)
	Isolate sample by closed-system vacuum distillation with toluene	GC/EC	NR	US Food and Drug Administration (1983)
Water	Purge (inert gas); trap on suitable sorbent material; desorb as vapour onto packed gas chromatographic column	GC/ECD or GC/MCD	0.001 and 0.12 µg/L	US Environmental Protection Agency (1988b, 1994)
		GC/MS	0.4 and 1.9 µg/L	US Environmental Protection Agency (1988b, 1994)

Table 1 (contd)

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Water (contd)	Purge and trap as above; desorb as vapour onto capillary gas chromatographic column	GC/PID-ECD GC/PID	0.01–0.06 μg/L 0.02–0.19 μg/L	US Environmental Protection Agency (1988b, 1994)
	Purge (inert gas); trap on suitable sorbent material; desorb as vapour onto gas chromatographic column	GC/PID	0.01 μg/L	US Environmental Protection Agency (1988b, 1994)
	Add internal standard (isotope-labelled trichloroethylene); purge, trap and desorb as above	GC/MS	10 μg/L	US Environmental Protection Agency (1994)
Liquid and solid wastes	Purge (inert gas); trap on suitable sorbent material; desorb as vapour onto packed gas chromatographic column	GC/ECD	0.12 μg/L	US Environmental Protection Agency (1986a)
		GC/MS	PQL	US Environmental Protection Agency (1986b)

GC, gas chromatography; FID, flame ionization detection; PID, photoionization detection; MS, mass spectrometry; NR, not reported; EC, electron capture detection; ECD, electrolytic conductivity detection; MCD, microcoulometric detection; PQL, practical quantification limit: 5 μg/L for groundwater; 5 μg/kg for soil and sediment samples; 250–2500 μg/kg for liquid wastes

Three gas chromatography/mass spectrometry (GC/MS) and three purge-and-trap GC methods for purgeable organic compounds, including trichloroethylene, are usually used for analysing aqueous samples (see also Table 1). The first method (EPA Method 624 and APHA/AWWA/WEF Method 6210B) is a packed-column method useful for the determination of trichloroethylene in municipal and industrial wastes. A similar purge-and-trap method (EPA Method 503.1 and APHA/AWWA/WEF Method 6220C), which includes photoionization detection, is applicable for the determination of trichloroethylene in drinking-water and raw source water. The second GC/MS method (EPA Method 524.1 and APHA/ AWWA/WEF Method 6210C), also involving a packed column, is also applicable for the determination of trichloroethylene in drinking-water and raw source water. Similar purge-and-trap methods (EPA Methods 601 and 502.1 and APHA/AWWA/WEF Methods 6230B and 6230C), including electrolytic conductivity and microcoulometric detection, are applicable for the determination of trichloroethylene in municipal and industrial discharges (6230B) and in drinking-water and raw source water (6230C). The third group of GC/MS and purge-and-trap methods (EPA Method 524.2 and APHA/AWWA/WEF Method 6210D; EPA Method 502.2 and APHA/AWWA/WEF Method 6230D) are identical to the previous ones except that a capillary column is used. The second and third methods are intended primarily for the detection of large numbers of contaminants at very low concentrations, which are not detectable with the first method (Greenberg *et al*., 1992).

Trichloroethylene can also be determined by colorimetry in the Fujiwara test, in which it is treated with pyridine in an alkaline environment. Solution absorbency is then determined at 535 or 470 nm, with a sensitivity of about 1 mg/kg. Trichloroethylene can also be determined by infrared spectroscopy. Gaseous compound is measured from the optical density of the mixture at a wavelength of 11.8 μm (detection sensitivity, ≥ 0.5 μg/L). High-resolution GC with electron capture detection has been used for determining trichloroethylene in soil. High-resolution GC with MS have been used for confirmation, with a detection threshold of about 10 mg/kg. Similar methods can be used to determine trichloroethylene and its major metabolites, trichloroacetic acid and trichloroethanol, in human tissues and fluids (WHO, 1985).

1.2 Production and use

1.2.1 Production

Trichloroethylene was first prepared in 1864 by Fischer in experiments on the reduction of hexachloroethane with hydrogen (Hardie, 1964). Commercial production of trichloroethylene began in Germany in 1920 and in the United States of America in 1925 (Mertens, 1993).

Until 1968, about 85% of United States production capacity of trichloroethylene was based on acetylene. The acetylene-based process consists of two steps: acetylene is first chlorinated to 1,1,2,2-tetrachloroethane, with a ferric chloride, phosphorus chloride or antimony chloride catalyst, and the product is then dehydrohalogenated to trichloroethylene (Mertens, 1993). The current method of manufacture is from ethylene or 1,2-dichloroethane. In a process used by one plant in the United States, trichloroethylene is produced by noncatalytic chlorination of ethylene dichloride or other C_2 chlorinated hydrocarbons. Another method is to react ethylene dichloride and other C_2 hydrocarbons with a mixture of oxygen and chlorine or hydrogen chloride (Linak *et al*., 1992).

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 in Japan and is used there (Linak *et al*., 1992).

Table 2 shows the production of trichloroethylene in selected countries between 1941 and 1990. Production has declined in recent years. Trichloroethylene is manufactured by one company each in Austria (with an annual capacity of 6000 tonnes), Germany (10 000 tonnes), Italy (15 000 tonnes) and Spain (29 000 tonnes). Two companies manufacture trichloroethylene in France (90 000 tonnes) and the United States (145 000 tonnes). Three companies in Japan produce trichloroethylene, with an estimated annual capacity of 85 000 tonnes (Linak *et al*., 1992). Two companies in Canada were the only domestic manufacturers of trichloroethylene. In 1976, the total capacity of these plants was 38 000 tonnes, and 22 500 tonnes were produced. One plant closed in 1985, and imports have increased as a result (Moore *et al*., 1991).

Trichloroethylene is also produced in Argentina, Australia, Belgium, China, India, Macedonia, Poland, Romania, the Russian Federation, Slovakia, South Africa and the United Kingdom (Chemical Information Services Ltd, 1994).

Table 2. Production of trichloroethylene in selected countries (thousand tonnes)

Year	Western Europe	Japan	USA[a]
1941			25
1945			84
1955			143[b]
1960			160
1965			197
1970			277
1975		85	133
1980	210	82	121
1981	205	74	177
1982	210	67	86
1983	200	67	91
1984	215	74	91
1985	205	73	79
1986	183	71	77
1987	166	64	88
1988	169	70	82
1989	154	65	79
1990	131	57	79

From Linak *et al.* (1992)

[a] The US International Trade Commission stopped reporting trichloroethylene production and sales in 1982. The data for 1983–90 are estimates from the *Chemical Economics Handbook* (Linak *et al.*, 1992).

[b] From Su & Goldberg (1976)

1.2.2 Use

Trichloroethylene was used earlier as an extraction solvent for natural fats and oils, such as palm, coconut and soya bean oils. It was also an extraction solvent for spices, hops and the decaffeination of coffee (Linak *et al.*, 1992). The United States Food and Drug Administration (1977) banned these uses of trichloroethylene because of its toxicity; its use in cosmetic and drug products was also discontinued (Mertens, 1993).

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, but trichloroethylene was replaced in dry cleaning by tetrachloroethylene in the mid-1950s. By 1989, about 85% of the trichloroethylene produced in the United States 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 United States, at 83 and 17%, respectively. In western Europe, 95% was used in vapour degreasing and 5% in other uses (Mertens, 1993). Similar use patterns have been reported for Canada (Moore *et al*., 1991) and Finland (Mroueh, 1993). Tables

3–5 present the uses of trichloroethylene in western Europe, Japan and the United States. Because of environmental and occupational health concerns, industry has attempted to restrict solvent emissions and maximize recovery and recycling. Trichloroethylene is, however, replacing 1,1,1-trichoroethane in some applications (Linak *et al.*, 1992).

Table 3. Use of trichloroethylene in western Europe (thousand tonnes)

Year	Metal cleaning (vapour degreasing)	Metal cleaning (cold cleaning)	Other
1980	164	25	26
1984	137	10	23
1987	124	10	16
1990	120	10	5

From Linak *et al.* (1992), estimates

Table 4. Use of trichloroethylene in Japan (thousand tonnes)

Year	Metal cleaning	Other
1980	49	16
1983	52	11
1987	49	12
1990	30	8

From Linak *et al.* (1992), estimates

Trichloroethylene has also been used, in limited quantities, to control relative molecular mass (by chain transfer) in the manufacture of polyvinyl chloride. An estimated 5500 tonnes are used annually for this application in the United States. It has also been used as a solvent in the rubber industry, some adhesive formulations and in research laboratories. In the textile industry, it is used as a carrier solvent for spotting fluids and as a solvent in dyeing and finishing (Fishbein, 1976; Linak *et al*., 1992; Mertens, 1993). It is also used as a solvent in printing inks, paint, lacquers, varnishes, adhesives and paint strippers. It was used as both an anaesthetic and an analgesic in obstetrics (Smith, 1966). Trichloroethylene has been used in the aerospace industry for flushing liquid oxygen (Sax & Lewis, 1987). In a study of potential sources of indoor air pollution in the United States, 25 of 1159 (2.2%) common household products were found to contain trichloroethylene (Sack *et al*., 1992).

The major use of trichloroethylene is in metal cleaning or 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 is 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 is also used in plastics, appliances, jewellery, automobile, plumbing fixtures, textiles, paper, glass and printing (Papdullo *et al*., 1985; Linak *et al*., 1992).

Table 5. Use of trichloroethylene in the United States (thousand tonnes)

Year	Metal cleaning	Other
1971	200	15
1974	153	4
1977	102	20
1980	84	13
1984	72	14
1987	57	9
1990	46	5

From Linak *et al.* (1992), estimates

Metal cleaning operations are of two types: cold cleaning and vapour cleaning. In cold cleaning, trichloroethylene is applied at room temperature; in vapour degreasing, the solvent vapours are condensed on the part to be cleaned. In cold cleaning, the metal parts are either dipped into the solvent solution or the solution is sprayed and wiped onto the object. The cold process is frequently used in maintenance operations and on small parts. 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).

1.3 Occurrence

1.3.1 Natural occurrence

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

1.3.2 Occupational exposure

The United States National Institute for Occupational Safety and Health (1994b) indicated that about 401 000 employees in 23 225 plants in the United States are potentially exposed to trichloroethylene. This estimate is based on a survey of companies and did not involve actual measurements. Table 6 summarizes the results of studies of occupational exposure.

Table 6. Occupational exposures to trichloroethylene

Country	No. of plants	Job, task or industry	No. of samples[a]	Air concentration (mg/m^3)		Reference
				Mean	Range	
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		[32.2]	
	1	Museum textile restoration	2 (P) 1-h		[3303]	
Netherlands	9	Rubber degreasing, cementing	137	4		Kromhout *et al.* (1994)
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]		Shipman & Whim (1980)
USA	60	Degreasing	433 (P)			Morse & Goldberg (1943)
		Condenser, nonvented	187	[725]	[16–4833]	
		Condenser, vented	149	[515]	[27–2110]	
	NR	Degreasing	146 (A)[b]	86% < [537] 96% < [1074]		Hargarten *et al.* (1961)
	1	Degreasing	11 (P)	[302]	[199–419]	Vandervort & Polakoff (1973)
	1	Degreasing ignition coils	(P)		0–[537]	Bloom *et al.* (1974)
	1	Electronic cleaning	3 (P)	[446]	[408–483]	Gilles & Philbin (1976)
	1	Semi-conductor degreasing	10 (P)	16.1	2–57	Gunter (1977)
	1	Degreasing operator	20 (P)	[736]	[140–2024]	Kominsky (1978)
		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]	
	1	Aircraft degreasing	4 (P)	[21.5]	[5.4–37.6]	Okawa *et al.* (1978)
	1	Tank relining	8 (P)	[1.3]	ND–[5.4]	Burroughs (1980)

Table 6 (contd)

Country	No. of plants	Job, task or industry	No. of samples[a]	Air concentration (mg/m^3)		Reference
				Mean	Range	
USA (contd)	1	Degreasing sheet metal	2 (P) 2 (A)	11 11	10–12 4–18	Johnson (1980)
	1	Degreasing, custom finishing	23 (P) 2 (A)	8.3 6	1–38 4–8	Ruhe & Donohue (1980)
	1	Vapour degreasing	14 (P)	[333]	[26.9–1670]	Burgess (1981)
	1	Degreasing, bus maintenance	3 (A)	3.0	ND–8.9	Love & Kern (1981)
	1	Degreasing	24 (STEL) 9 (TWA)	742 145	56–2000 37–357	Ruhe *et al*. (1981)
	1	Degreasing, plastics	2 (P)	[4.8]	[2.7–7.0]	Burroughs & Moody (1982)
	1	Degreasing, electronics	79 (P)	10.2	ND–209	Lee & Parkinson (1982)
	1	Degreasing, medical	5 (P) 2 (A)	5.4 6.5	1–16 4–9	Ruhe (1982)
	1	Degreasing, energy conservation products	2 (P) 10 (A)	[36.5] [1.1]	[22–51] [0.54–3.2]	Almaguer *et al*. (1984)
	1	Degreasing Silk screening	9 (P) 2 (A) 5 (P)	[716] [184] [23.6]	[39–2288] [0.54–367] [1.6–81.1]	Belanger & Coye (1984)
	1	Degreasing aircraft	29 (TWA, P) 11 (TWA, A) 22 (STEL)	[30.7] [28.5] [320]	[ND–208] [2–121] [ND–1256]	Gorman *et al*. (1984)
	1	Taxidermy	2 (A) 2 (P)	[8.9] [8.9]	[1.1–16.6] [1.7–16]	Kronoveter & Boiano (1984)
	1	Degreasing	(TWA) (STEL)	205 1084	117–357 413–2000	Landrigan *et al*. (1987)

ND, not detected; NR, not reported. Most measurements were taken after observation of operating deficiencies of degreasers between 1952 and 1957.

[a] P, personal air samples (breathing zone); A, area samples; STEL, short-term exposure limit; TWA, time-weighted average

1.3.3 Environmental occurrence

Trichloroethylene has been reported in the air, rainwater, surface waters, drinking-water, seawater, marine sediments, marine invertebrates, marine mammals, foods and human tissues (McConnell *et al*., 1975).

(a) Air

The levels of trichloroethylene in air have been measured throughout the world (Table 7). In a compilation of the results of surveys of ambient air in the United States before 1981 (Brodzinsky & Singh, 1983; United States Agency for Toxic Substances and Disease Registry, 1989), representing 2353 monitoring points, the mean concentrations were 30 ppt [0.2 μg/m^3] in rural areas, 460 ppt [2.5 μg/m^3] in urban and suburban areas and 1200 ppt [64 μg/m^3] in industrialized areas near sources of trichloroethylene emissions. Industrial releases of trichloroethylene to the environment in the United States were 24 430 tonnes in 1988, 22 400 tonnes in 1989, 17 680 tonnes in 1990 and 15 950 tonnes in 1991 (United States Environmental Protection Agency, 1993).

Air emissions in western Europe in 1980 are reported in Table 8. In the Netherlands, emissions of trichloroethylene to the air were 6.5 tonnes in 1970, 5.4 tonnes in 1975, 4.2 tonnes in 1979, 3.7 tonnes in 1980, 2.6 tonnes in 1981 and 2.2 tonnes in 1982 (Besemer *et al*., 1984).

Indoor air concentrations of trichloroethylene can increase when trichloroethylene-contaminated water is used domestically. A community water supply that contained 40 mg/L of trichloroethylene was estimated to contribute about 40 mg/m^3 to the air of a bathroom during showering, and the weekly dose through inhalation was estimated to be 48 mg trichloroethylene (assuming 1-h showering), due to off-gassing of trichloroethylene from the water. About 42 mg of trichloroethylene were ingested from the water per week (Andelman, 1985). Similar conclusions were reached by Bogen *et al*. (1988).

(b) Water

Trichloroethylene occurs at low levels in all water supplies and frequently in groundwater, owing to its widespread use and physical characteristics. Table 9 summarizes the concentrations of trichloroethylene found in surface waters, groundwater and drinking-water worldwide.

Trichloroethylene was detected in an estimated 3% of surface water samples and 19% of groundwater samples analysed, at geometric mean concentrations of 27.3 ppb [μg/L] in groundwater and 40.2 ppb in surface water (United States Environmental Protection Agency, 1989). In a computerized database on water quality, the reported median concentrations of trichloroethylene in 1983–84 were 5.0 μg/L in industrial effluents (19.6% detectable, 1480 samples), 0.1 μg/L (28% detectable, 9295 samples) in ambient water, < 50 μg/kg dry weight (6% detectable, 338 samples) in sediment and < 50 μg/kg (none detectable, 93 samples) in biota (Staples *et al*., 1985).

The concentrations of trichloroethylene in sediment and animal tissue collected near the discharge zone of the Los Angeles County, CA, waste-treatment plant in 1980–81, were 17 μg/L in the effluent, < 0.5 μg/kg dry weight in sediment and 0.3–7 μg/kg wet weight in various marine animal tissues (Gossett *et al*., 1983).

Table 7. Concentrations of trichloroethylene in ambient air

Area	Year	Concentration [μg/m^3]		Reference
		Mean	Range	
Remote				
Pacific Ocean (latitude 37° N)	1977	[0.07]		US Environmental Protection Agency (1985)
Panama Canal Zone (latitude 9° N)	1977	[0.08]		US Environmental Protection Agency (1985)
Northern hemisphere	1985		[0.06–0.09]	US Environmental Protection Agency (1985)
Southern hemisphere	1981		[< 0.02]	Singh *et al*. (1983)
Rural				
Badger Pass, CA, USA	1977	[0.06]	[0.005–0.09]	US Environmental Protection Agency (1985)
Whiteface Mountains, NY, USA	1974	[0.5]	[< 0.3–1.9]	Lillian *et al*. (1975)
Reese River, NV, USA	1977	[0.06]	[0.005–0.09]	US Environmental Protection Agency (1985)
Jetmar, KS, USA	1978	[0.07]	[0.04–0.11]	US Environmental Protection Agency (1985)
Western Ireland	1974	[0.08]		Lovelock (1974)
Urban and suburban				
Phoenix, AZ, USA	1979	[2.6]	[0.06–16.7]	Singh *et al.* (1981)
Los Angeles, CA, USA	1976	[1.7]	[0.14–9.5]	US Environmental Protection Agency (1985)
Lake Charles, LA, USA	1976–78	[8.6]	[0.4–11.3]	US Environmental Protection Agency (1985)
New Jersey, USA	1973–79	[9.1]	[ND–97]	Lillian *et al.* (1975); US Environmental Protection Agency (1985)
New York City, NY, USA	1974	[3.8]	[0.6–5.9]	Lillian *et al.* (1975)
Denver, CO, USA	1980	[1.07]	[0.15–2.2]	US Environmental Protection Agency (1985)
St Louis, MO, US	1980	[0.6]	[0.1–1.3]	US Environmental Protection Agency (1985)
Portland, OR, USA	1984	[1.5]	[0.6–3.9]	Ligocki *et al.* (1985)
Philadelphia, PA, USA	1983–84	[1.9]	[1.6–2.1]	Sullivan *et al.* (1985)
Brussels, Belgium	1974–75	[21.5]	[5.9–31.2]	Su & Goldberg (1976)
Geneva, Switzerland	1974	[31.2]		Su & Goldberg (1976)
Moscow, Russian Federation	1974	[19.3]	[14.0–28.5]	Su & Goldberg (1976)
Paris, France	1975	[4.0]		Su & Goldberg (1976)
Grenoble, France	1975	[19.3]	[6.4–28.5]	Su & Goldberg (1976)
Kyoto, Japan	1975	[5.1]		Su & Goldberg (1976)
Tokyo, Japan	1975	[1.8]		Su & Goldberg (1976)
Yokohama, Nagoya and Kawasaki, Japan	1985–86	[5.4]	[3.4–7.5]	Urano *et al.* (1988)

Table 8. Estimated emissions of trichloroethylene to the air in western Europe, 1981

Country or region	Air emission (tonnes/year)
Netherlands	2.7
Belgium/Grand Duchy of Luxembourg	2.9
Western Germany	46.0
France	45.0
Italy	27.0
Spain	15.0
Austria	5.5
United Kingdom	50.0
Norway	0.9
Sweden	12.0
Finland	2
Portugal	1
Switzerland	7
Denmark	2

From Besemer *et al.* (1984); figures include secondary emissions from water and solid waste

Table 9. Concentrations of trichloroethylene in water

Area	Concentration (μg/L)		Reference
	Mean	Range	
Surface waters			
Seawater			
Eastern Pacific Ocean	0.0003	0.0001–0.0007	Singh *et al.* (1983)
Coastal waters			
Sea coast, industrial area, United Kingdom		0.1–1	Herbert *et al.* (1986)
West coast, Sweden	0.015		Herbert *et al.* (1986)
Northern coast, Greece		0.06–2.8	Fytianos *et al.* (1985)
Rivers			
Tributaries of the Rhine		0.06–7.0	Herbert *et al.* (1986); Bauer (1981a); Hellman (1984)
Elbe, Germany		0.7–52.3	Hellman (1984)
Weser, Germany		0.5–1.5	Herbert *et al.* (1986)
Rhine		0.1–2.4	Herbert *et al.* (1986)
United Kingdom		0.01–1.0	Herbert *et al.* (1986)
Danube, Vienna, Austria	0.6		Herbert *et al.* (1986)
Netherlands		0.1–1.5	Herbert *et al.* (1986)

Table 9 (contd)

Area	Concentration (μg/L)		Reference
	Mean	Range	
Jackfish Bay, Canada		4.1–120	Comba *et al.* (1994)
Canada		< 0.001–42	Moore *et al.* (1991)
Rainwater			
Portland, OR, USA	0.006	0.002–0.02	Ligocki *et al.* (1985)
Groundwater			
Gloucester, Ontario, Canada		< 1–583	Lesage *et al.* (1990)
Zurich, Switzerland		1.1–1.9	Herbert *et al.* (1986)
Dubendorg, Germany	85		Herbert *et al.* (1986)
Northern Switzerland	0.92		Herbert *et al.* (1986)
Frankfurt, Germany		0.4–159	Herbert *et al.* (1986)
Mannheim, Germany		< 0.16–120	Herbert *et al.* (1986)
Italy		0.1–158	Ziglio *et al.* (1984a,b)
United Kingdom		< 0.1–70	Fielding (1981)
Netherlands		< 0.1–1100	Zoeteman *et al.* (1980); Trouwborst (1981)
Minnesota, USA, near landfill		0.7–125	Sabel & Clark (1984)
New Jersey, USA, near landfill		≤ 1530	Burmaster (1982)
Pennsylvania, near landfill		≤ 27 300	Burmaster (1982)
Japan, near electronics factory		≤ 10 000	Hirata *et al.* (1992)
Phoenix, Arizona, USA		8.9–29	Flood *et al.* (1990)
Drinking-water			
Southern Philippines		0.03	Trussell *et al.* (1980)
Northern Philippins		0.01	Trussell *et al.* (1980)
Egypt		1.2	Trussell *et al.* (1980)
United Kingdom		0.4	Trussell *et al.* (1980)
Nicaragua		0.05	Trussell *et al.* (1980)
USA 1976–77		0.2–49	Thomas (1989)
1977–81		Trace–53	
1978		0.5–210	
		Trace–35 000 (with local contamination)	
New Jersey	23.4	Max. 67	Cohn *et al.* (1994)
Woburn, Massachusetts		Max. 267	Lagakos *et al.* (1986)

1.3.4 Food

The concentrations of trichloroethylene in food in the United Kingdom were: 0.3–10 ppb [μg/kg] in dairy products, 12–22 ppb in meat, none detected (ND)–19 ppb in oils and fats, ND–60 ppb in beverages, ND–7 ppb in fruits and vegetables and 7 ppb in cereals. In marine organisms, the concentrations varied from ≤ 1 ppb in invertebrates to 10 ppb in the flesh of fish

to a maximum of 50 ppb in the eggs of sea birds and the blubber of seals (McConnell *et al.*, 1975). Molluscs from Liverpool Bay, United Kingdom, contained a mean of 85 µg/kg on a dry-weight basis (range, 2–250 µg/kg). Various fish had a mean concentration of 106.5 µg/kg (range, 7–479 µg/kg) (Dickson & Riley, 1976).

The average concentrations of trichloroethylene in food in the United States were 0.9 (0–2.7) µg/kg in grain-based foods, 1.8 (0–12) µg/kg in 'table-ready' foods, 73.6 (1.6–980) µg/kg in butter and margarine, 3.8 (0–9.5) µg/kg in cheese products, 0.5 (0–1.7) µg/kg in peanut butter, 3.0 (0–9.2) µg/kg in ready-to-eat cereal products and 1.3 (0–4) µg/kg in highly processed foods (Heikes & Hopper, 1986; Heikes, 1987). In an evaluation of process waters and food commodities collected at 15 food processing plants, trichloroethylene was found at 3–7.8 ppb [µg/L] in three process waters but in none of the food products (Uhler & Diachenko, 1987). It was detected in five of 372 fatty and non-fatty food samples at concentrations of 2–94 µg/kg, with a mean of 49 µg/kg (Daft, 1989).

Trichloroethylene was found at a concentration of 100–500 ppb [µg/kg] in one of 70 samples of margarine taken from shops in the United States in 1980–82 and 1984 but at < 50 ppb in 20 samples. In 1984, the levels were all < 50 ppb (Entz & Diachenko, 1988). The mean daily intake of trichloroethylene from food, water and air in Germany was estimated to be 32–51 µg/day (Bauer, 1981b; von Düszeln *et al.*, 1982).

1.3.5 Biological monitoring

Individual exposure to trichloroethylene in Germany was determined in non-occupational and a number of occupational environments by biological monitoring. Trichloroethylene was detected in 31% of all blood samples from persons not occupationally exposed to volatile halogenated hydrocarbons (median, < 0.1 µg/L; range, < 0.1–1.3 µg/L). The median levels of trichloroacetic acid, a metabolite of trichloroethylene, were 21.4 µg/L (range, 4.8–221 µg/L) in 43 blood samples and 6.0 µg (range, 0.6–261 µg) in 94 samples of 24-h urine from these unexposed persons. The blood levels of trichloroethylene were < 0.1–0.2 µg/L in nine motor vehicle mechanics, < 0.1 µg/L in three painters, 0.1–15.5 µg/L in three precision instrument makers and 0.2–7.1 µg/L in six dry cleaners (Hajimiragha *et al.*, 1986).

In a plant in the United States where trichloroethylene was used in five degreasing operations in the manufacture of steel tubing, the concentrations of trichloroethylene in air were 117–357 mg/m^3, with short-term exposures as high as 2000 mg/m^3. Urine samples collected from exposed workers before the shift contained, on average, 298 mg/L (range, 4–690 mg/L) of total trichloroethylene metabolites, while the mean concentration after the shift was 480 mg/L (range, 63–1050 mg/L) (Ruhe *et al.*, 1981).

The average blood plasma levels of trichloroethylene of 157 employees at two metal-working plants in the United States were 2.5 ppb [µg/L] (range, 0–22 ppb) and undetectable; in the second plant, the major exposure was to a solvent that contained chloroform. A control population living several miles from the first plant also had undetectable levels of trichloroethylene (Pfaffenberger *et al.*, 1984).

The concentration of total trichloro compounds in the urine of workers in a degreasing operation at a United States aircraft factory were 0.5–83.4 mg/g creatinine. These concentrations

correlated well with the air concentrations, which averaged 5.7 ppm [30.6 mg/m^3] (Gorman *et al.*, 1984).

The levels of trichloroacetic acid in the urine of 73 workers in 24 workshops in Switzerland where degreasing was performed were 8–444 mg/L, with a mean of 86.7 mg/L. The levels in the 96 air samples were 1–335 ppm [5.37–1800 mg/m^3] with a mean of 56.7 ppm [304 mg/m^3] (Grandjean *et al.*, 1955).

The relationship between concentrations of trichloroethylene in the air near degreasing operations and urinary excretion of total trichloro compounds was reported in Japan. Eight workers had an average urinary concentration of 243.9 mg/L (range, 95–787 mg/L) total trichloro compounds after exposure to 40.7 ppm [217 mg/m^3] trichloroethylene in air. The calculated estimated air levels corresponding to the urine levels found were 41.7 (range, 22.3–67.4) ppm [224 (120–362) mg/m^3] (Nomiyama, 1971).

A total of 31 employees in 19 vapour degreasing plants in central Sweden were exposed to trichloroethylene at a mean level in ambient air of 27 mg/m^3; 86% of the air samples contained < 50 mg/m^3. A weak correlation was found between the concentrations of *N*-acetyl-β-D-glucosaminidase and trichloroacetic acid in urine ($r = 0.48$; $p < 0.01$), but no correlation was seen with ambient air levels ($r = 0.08$; $p = 0.66$) (Seldén *et al.*, 1993).

In China, the relationship between the time-weighted average exposure to trichloroethylene at the end of a work week and the concentrations of metabolites in urine was investigated in 140 exposed and 114 control workers. In a plant where trichloroethylene was manufactured by chlorination of acetylene followed by dehydrochlorination, 61 men who were exposed to trichloroethylene in air at a concentration of 3–94 ppm [16.1–505 mg/m^3] and 17 women exposed to 2–47 ppm [11–253 mg/m^3] had ≤ 127 mg/L (men) and ≤ 111 mg/L (women) total trichloro compounds in their urine. In a metal-plating plant where trichloroethylene was used for degreasing, 52 men were exposed to concentrations of 1–63 ppm [5.37–338 mg/m^3] and 10 women were exposed to 2–13 ppm [10.7–69.8 mg/m^3]; the urinary levels were ≤ 89 mg/L for the men and ≤ 98 mg/L for the women (Inoue *et al.*, 1989).

The Danish Labour Inspection Service conducted biological monitoring of workers exposed to trichloroethylene in various factories between 1947 and 1987. The concentrations of trichloroacetic acid in 2272 urine samples from workers in 330 factories were similar from the mid-1950s to the mid-1970s and then began to decrease. The average urinary concentrations were 82 mg/L (range, 0–750 mg/L) in 1947–51, 40 mg/L (0–1975 mg/L) in 1950–56, 32 mg/L (0–680 mg/L) in 1957–61, 55 mg/L (0–730 mg/L) in 1962–66, 53 mg/L (0–850 mg/L) in 1967–71, 35 mg/L (0–370 mg/L) in 1972–76, 30 mg/L (0–365 mg/L) in 1977–81 and 18 mg/L (0–130 mg/L) in 1982–86 (Christensen & Rasmussen, 1990).

Blood and urine samples were collected in 1990 from 10 people working in four dry cleaning shops in Croatia, where trichloroethylene was used as the cleaning solvent. The concentration of trichloroethylene in the air was 25–40 ppm [134–215 mg/m^3]. The mean blood levels of trichloroethylene were 0.38 μmol/L [50 μg/L] on Monday morning (range, 0.15–3.58 μmol/L) [20–470 μg/L] and 3.39 μmol/L [445 μg/L] on Wednesday afternoon (range, 0.46–12.71 μmol/L [60–1670 μg/L]). The mean trichloroethanol levels in blood were 3.02 μmol/L (0–10.7 μmol/L) [451 (0–1600 μg/L)] and 7.70 μmol/L (0–26.1 μmol/L) [1150 (0–3894 μg/l)] for the same period, respectively, and the results for trichloroacetic acid were 165 μmol/L (6.12–302

μmol/L) [27 (1–49 mg/L)] and 194 μmol/L (13.5–394 μmol/L) [31 (2–64 mg/L)]. The mean trichloroacetic acid level in urine was 32.5 mmol/mol creatinine (1.3–61.2) [47 (2–89) mg/g] on Monday morning and 37.2 mmol/mol creatinine (1.9–77.4) [54 (3–112) mg/g] on Wednesday afternoon. The mean trichloroethanol levels in urine were 9.7 mmol/mol creatinine (0.4–35.7) [13 (0.5–47 mg/g)] in the Monday morning sample and 54.9 mmol/mol creatinine (5.3–177.7) [73 (7–235) mg/g] in the Wednesday afternoon sample (Skender *et al.*, 1991).

A number of researchers have studied the influence of hourly and daily variations in exposure concentrations on the alveolar concentrations of trichloroethylene and on the urinary excretion of trichloroethanol and trichloroacetic acid (Ogata *et al.*, 1971; Droz & Fernández, 1978). The estimated concentrations of trichloroacetic acid in urine at the end of a workday in which workers were exposed to 270 mg/m^3 trichloroethylene for 8 h per day, five days a week, were 100 mg/g creatinine 0.5 h after exposure, 80 mg/g creatinine after 16 h and 50 mg/g creatinine after 64 h (Monster, 1984).

People exposed to 50 ppm (270 mg/m^3) trichloroethylene for 8 h per day on five days a week were estimated to have alveolar air concentrations of 10–15 ppm [53.7–80.6 mg/m^3] at the end of exposure and 0.1 ppm [0.5 mg/m^3] 64 h after exposure. The blood concentrations were estimated to range from 0.9 to 0.006 mg/L (Monster, 1984).

The airborne concentrations of trichloroethylene at a liquid–vapour degreasing operation in the United States in 1980 were 117–357 mg/m^3, with short-term sampling peaks of 413–2000 mg/m^3. Nine exposed workers had a mean pre-shift urinary concentration of total trichloroethylene metabolites of 298 μg/L; the mean post-shift concentration was 480 μg/L (Landrigan *et al.*, 1987).

Swedish producers of trichloroethylene offered an exposure control programme to customers using trichloroethylene in which free analysis of trichloroacetic acid in urine was conducted annually. On this basis, Axelson *et al.* (1994) categorized the average exposure of 1670 workers as 0–49 mg/L, 50–99 mg/L and ≥ 100 mg/L; 81% were placed in the lowest group. The analytical method used to determine trichloroacetic acid in urine indicated that 50 mg/L was approximately equivalent to an 8-h time-weighted average exposure to 20 ppm [107 mg/m^3] trichloroethylene.

In an ongoing biological monitoring study of workers in various occupations who are exposed to trichloroethylene, tetrachloroethylene or 1,1,1-trichloroethane, conducted by the Finnish Institute of Occupational Health, 11 534 samples representing 3976 workers in 600 workplaces were obtained for the three compounds between 1965 and 1983. Of these workers, 94.4% were monitored for one solvent, 5.2% for two solvents and 0.4% for three solvents. The overall median concentrations of trichloroethylene, reported as trichloroacetic acid in urine, were 63 μmol/L [10.3 mg/L] for women and 48 μmol/L [7.8 mg/L] for men. Before 1970, the mean urinary levels were 80–90 μmol/L [13.1–14.7 mg/L] for men and 60–80 μmol/L [9.8–13.1 mg/L] for women (Anttila *et al.*, 1995).

Trichloroethylene was detected in the blood of 22 of 39 subjects in Zagreb, Croatia, who had no known exposure to solvents, and trichloroacetic acid was found in all plasma and urine samples. The geometric mean concentrations of trichloroethylene were 0.023 μg/L (range, < 0.020–0.090 μg/L) in blood; those of trichloroacetic acid were 45.4 μg/L (13.5–160 μg/L) in

plasma and 24.2 µg/L (1.67–292 µg/L) in urine. The concentration of trichloroethylene in the drinking-water was 4.20 µg/L (0.69–35.9 µg/L) (Skender *et al.*, 1993).

The mean concentration of trichloroacetic acid in sera from 94 subjects who were not exposed to organic solvents in Germany was 23.8 µg/L (range, 4.8–221 µg/L), and the average level of trichloroacetic acid in 24-h urine samples was 7.6 µg (range, 0.6–261.4 µg) (Hajimiragha *et al.*, 1986).

Of the 14 million inhabitants of the Netherlands in the 1980s, 14 000 were estimated to be exposed by all routes to an average trichloroethylene concentration of 10 µg/m^3, resulting in a daily intake of 200 µg; 350 000 were exposed to 4 µg/m^3 with a daily intake of 80 µg; and 13.6 million inhabitants were exposed to 0.8 µg/m^3 for a daily intake of 16 µg (Besemer *et al.*, 1984).

The serum levels of trichloroacetic acid in inhabitants of Milan, Italy, who drank water containing > 2000 µg/L of trichloroethylene was 36.5 µg/L; that in an unexposed group was 8 µg/L (Ziglio *et al.*, 1984c). The ambient air level of trichloroethylene in Milan in 1979 was 7.6 µg/m^3 (Ziglio *et al.*, 1983).

Analysis of human tissue taken *post mortem* showed trichloroethylene concentrations of 2–32 µg/kg wet weight in body fat, 2–5.8 µg/kg in liver, < 1–3 µg/kg in kidney and ≤ 1 µg/kg in brain (McConnell *et al.*, 1975).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for trichloroethylene in a number of countries are presented in Table 10.

WHO (1993) has established a provisional guideline of 70 µg/L trichloroethylene in drinking-water.

The American Conference of Governmental Industrial Hygienists (1994) has recommended several biological exposure indices for trichloroethylene. That for trichloroacetic acid in urine at the end of the work week is 100 mg/g creatinine; that for trichloroacetic acid and trichloroethanol in urine at the end of the shift at the end of the work week is 300 mg/g creatinine; and that for free trichloroethanol in blood at the end of the shift at the end of the work week is 4 mg/L. It is noted that these indices are nonspecific, i.e. other exposures can affect the measurement, and that trichloroethylene in exhaled air and in blood can be used as an indicator of exposure but interpretation of the measurement is only semiquantitative.

Biological indices for exposure to trichloroethylene have been reported. In Finland, the action level for trichloroacetic acid in urine is 360 µmol/L [47.3 mg/L] (Aitio *et al.*, 1995); in Germany, the biological tolerance values are 5 mg/L trichloroethanol in blood and 100 mg/L trichloroacetic acid in urine (Deutsche Forschungsgemeinschaft, 1993); and in Switzerland, the biological tolerance values are 5 mg/L trichloroethanol in blood and 100 mg/g creatinine trichloroacetic acid in urine (Schweizerische Unfallversicherungsanstalt, 1994).

Table 10. Occupational exposure limits and guidelines for trichloroethylene

Country	Year	Concentration (mg/m^3)	Interpretation
Australia	1993	270	TWA
		1080	STEL
Austria	1987	260	TWA
Belgium	1993	269	TWA
		1070	STEL
Brazil	1987	420	TWA
Bulgaria	1993	269	TWA
		537	STEL
Canada	1987	75	TWA
		402	STEL (15 min)
Chile	1987	428	TWA
China	1987	535	TWA
Colombia	1993	269	TWA
		537	STEL
Czech Republic	1993	250	TWA
		1250	STEL
Denmark	1993	160	TWA
Egypt	1987	269	TWA
Finland	1993	160	TWA
		240	STEL
France	1993	405	TWA
		1080	STEL
Germany	1993	270	TWA; suspected carcinogen
Hungary	1987	10	TWA
		40	STEL
India	1987	535	TWA
		800	STEL
Indonesia	1987	535	TWA
Italy	1987	400	TWA
Japan	1993	270	TWA
Jordan	1993	269	TWA
		537	STEL
Mexico	1987	535	TWA
Netherlands	1994	190	TWA
		538	STEL (15 min)
New Zealand	1993	269	TWA
		537	STEL
Norway	1984	105	TWA; carcinogen
Philippines	1993	535	TWA
Republic of Korea	1993	269	TWA
		537	STEL
Poland	1993	50	TWA

Table 10 (contd)

Country	Year	Concentration (mg/m^3)	Interpretation
Romania	1987	200	TWA
		300	STEL
Russian Federation	1993	269	TWA
Singapore	1993	269	TWA
		537	STEL
Sweden	1993	50	TWA
		140	STEL
Switzerland	1994	260	TWA
		1300	STEL
Thailand	1993	537	TWA
		1074	STEL
Turkey	1993	535	TWA
United Kingdom	1993	535	TWA
		805	STEL
USA			
ACGIH	1994	269	TWA
		537	STEL
NIOSH	1994	134	TWA; carcinogen
		11	Ceiling (60 min[a])
OSHA	1994	537	TWA
		1074	Ceiling
		1611	Peak
Venezuela	1987	535	TWA
		800	STEL
Viet Nam	1993	269	TWA
		537	STEL

From Cook (1987); ILO (1991); Deutsche Forschungsgemeinschaft (1993); Työministeriö (1993); American Conference of Governmental Industrial Hygienists (ACGIH) (1994); Arbeidsinspectie (1994); Schweizerische Unfallversicherungsanstalt (1994); United Kingdom Health and Safety Executive (1994); United States National Institute for Occupational Safety and Health (NIOSH) (1994c); United States Occupational Safety and Health Administration (OSHA) (1994)

TWA, time-weighted average; STEL, short-term exposure limit; ceiling, level not to be exceeded during any part of the workday; peak, acceptable maximum peak above acceptable ceiling concentration for an 8-h shift (maximum duration, 5 min in any 2 h)

[a] During use as an anaesthetic

2. Studies of Cancer in Humans

2.1 Case reports

Málek *et al*. (1979) followed-up 57 men who had worked for at least one year in dry cleaning in Prague, Czech Republic, since the 1950s. Nearly 60% of those tested had a urinary trichloroacetic acid concentration in excess of 100 mg/L, with sporadic values in the region of 1000 mg/L. The follow-up period was 5–50 years. Six men were found to have cancer: three had lung cancer, one had cancer of the tongue, one had rectal cancer and one had a bladder cancer and two rectal tumours.

Novotná *et al*. (1979) reviewed the occupational histories of all 63 subjects diagnosed with histologically confirmed carcinoma of the liver in 1972 and 1974 in Prague, Czech Republic. None of them had been employed in workshops where trichloroethylene was used. Paraf *et al*. (1990) reported a case of gall-bladder cancer in a woman aged 64 who had worked as a technician in a laboratory in France where trichloroethylene was used for degreasing metal.

Jalihal and Barlow (1984) reported a case of acute myeloid leukaemia in a 60-year-old dry cleaner in the United Kingdom. He had had heavy exposure for many years first to trichloroethylene and later to tetrachloroethylene.

2.2 Descriptive studies

Risks for cancer among workers in industries where there is potential exposure to trichloroethylene have been addressed in a number studies but in which exposure to this compound was not specified (e.g. Krain, 1972; Blair, 1980; Blair & Mason, 1980; Brandt-Rauf *et al.*, 1982, 1986; Brandt-Rauf & Hathaway, 1986; Malker *et al.*, 1986; Dubrow & Gute, 1987). These descriptive studies were not considered relevant in view of the availability of cohort and case–control studies.

Paddle (1983) retrieved records from the Mersey Regional Cancer Registry (United Kingdom) for 1951–77 for all 95 subjects with a diagnosis of primary liver cancer and an address near Runcorn, where there is a plant in which trichloroethylene has been manufactured since 1909. Two members of the personnel department of the company compared the records of tens of thousands of people who had worked at the Runcorn site during 1934–76 with the registry list, and the records of two potential matched persons were subsequently checked at the Department of Health and Social Security. It was concluded that none of the subjects had ever worked at the Runcorn site. [The Working Group noted that the interpretation of this result was hindered by the lack of expected numbers.]

2.3 Cohort studies

The cohort studies available to the Working Group addressed three occupational groups: dry cleaners, workers who had undergone biological monitoring for exposure to trichloroethylene and workers employed in miscellaneous manufacturing industries. The Working Group did not consider that the first group of studies (see the monograph on dry cleaning) was relevant to an evaluation of trichloroethylene *per se*, given the extensive exposure of these people to

other solvents. Workers who were biologically monitored were considered likely to have been exposed to trichloroethylene, but the proportion of workers in the third group of studies who were actually exposed to trichloroethylene varied.

2.3.1 Exposure evaluated by biological monitoring

Axelson *et al.* (1978, 1984 [abstract], 1994) studied a cohort of workers in Sweden who had been exposed to trichloroethylene. Between 1930 and 1986, only one plant in central Sweden produced trichloroethylene for the domestic market, and this producer offered its customers free surveillance of their exposed workers by analysis for trichloroacetic acid in the urine. Files containing data from such monitoring constitute the basis of the study, but some of the files had been destroyed. Axelson *et al.* (1978) originally retrieved records for 518 men, later expanded the cohort to 1424 men (Axelson *et al*., 1984, abstract) and finally included 1727 persons drawn from 115 companies that had used the surveillance service at least once between 1955 and 1975 (Axelson *et al*., 1994). Records were incomplete for 23 persons, four people could not be found in the population register, and 30 had emigrated. The final analysis was thus based on 1670 persons, 1421 men and 249 women, who were followed up for mortality from 1955 through 1986 and for cancer incidence from 1958 through 1987. Swedish national rates were used for the calculation of expected numbers. Exposure was assessed as the mean concentration of trichloroacetic acid in all urinary samples available for a given person: 78% of the person-years for men were accumulated in the category 0–49 mg/L, 14% in the category 50–99 mg/L and 8% in the > 100 mg/L category. A total of 253 deaths were observed [giving an overall standardized mortality ratio (SMR) of 1.0; 95% confidence interval (CI), 0.89–1.1]; and 129 incident cancer cases occurred [giving an overall standardized incidence ratio (SIR) of 1.0; 95% CI, 0.84–1.2]. Among men, a significant excess risk was found for skin cancer (SIR, 2.4; 95% CI, 1.0–4.7; eight observed). There were five cases of non-Hodgkin's lymphoma (1.6; 0.51–3.6) and four cases of liver and biliary tract cancer (1.4; 0.38–3.6). Of the incident cancer cases in men, 77 occurred in men in the lowest exposure category [SIR, 0.92], 18 in the medium category [SIR, 0.93] and 12 [SIR, 1.4] in the highest exposure category.

Anttila *et al*. (1995) studied a cohort of 3974 persons in Finland who were biologically monitored for occupational exposure to three halogenated hydrocarbons (3089 for trichloroethylene, 849 for tetrachloroethylene and 271 for 1,1,1-trichloroethane) during 1965–83. The cohort consisted of those people for whom 10 743 measurements were taken; the persons for whom a further 791 measurements were taken could not be identified. The overall median urinary concentration of trichloroacetic acid was higher for women (63 µmol/L [10.3 mg/L]) than for men (48 µmol/L [7.8 mg/L]). The cohort was followed up for incident cancer cases through 1992, and the expected numbers were calculated on the basis of Finnish national rates. There were 208 cancer cases among people monitored for exposure to trichloroethylene (SIR, 1.1; 95% CI, 0.92–1.2). A significant excess risk was seen for cervical cancer (2.4; 1.1–4.8; eight observed), and the risk was further increased for women with a mean level of exposure ≥ 100 µmol/L [≥ 16.3 mg/L] (4.4; 1.4–10; five observed); no further increase in risk was seen with increasing latency since the time the first measurement was made. The SIR for liver cancer among people with high exposure was 2.7 (0.33–9.9; two observed); a significantly increased SIR was seen with a 20-year latency since first measurement (6.1; 1.3–

18; three observed). The SIR for cancers of the lymphohaematopoietic tissues was increased among people with high exposure (2.1; 0.95–4.0; nine observed) and was further increased with the 20-year latency (3.0; 1.2–6.1; seven observed). The SIRs for stomach cancer were 0.91 (0.25–2.3; four cases) for high exposure and 3.0 (1.2–6.1; seven cases) with a 20-year latency. The SIR for prostatic cancer was 0.68 (0.08–2.4; two cases) with high exposure and 3.6 (1.5–7.0; eight cases) with a 20-year latency.

The population studied by Anttila *et al*. (1995) included most of the workers investigated in a previous study that comprised 2117 Finnish workers in whom urinary trichloroacetic acid was measured or were reported as having been exposed to trichloroethylene during 1963–76 (Tola *et al*., 1980). A total of 11 cancer deaths (14.3 expected) was reported.

2.3.2 Exposure in miscellaneous manufacturing industries

Barret *et al*. (1984) reported in an abstract a study of the death certificates of 235 workers who had been exposed to trichloroethylene and cutting oils; a total of 14 500 had been so employed in 1983. In a comparison of SMRs [method not described] for each site of cancer, the authors found a high risk for cancer of the naso- and oropharynx (SMR, 2.5 [95% CI, 1.4–4.1]; 15 deaths).

Shindell and Ulrich (1985) studied a plant in northern Illinois, United States, where trichloroethylene had been used extensively as a degreasing agent and where the workers drank water containing traces (43 ppb [μg/L]) of trichloroethylene. The plant began operation in 1957. The study included all office employees at this plant and all production employees who had worked for three months or more in this or a nearby facility between 1 January 1957 and 31 July 1983. The cohort consisted of 2646 individuals, of whom 2140 were white men, 76 were non-white men and 430 were women. The cohort was followed up until 31 July 1983; vital status was determined for all but 52 persons. National mortality rates were used to calculate the expected numbers of deaths. A total of 141 persons had died, whereas 181.6 deaths were expected [SMR, 0.78; 95% CI, 0.65–0.92]. There were nine deaths from respiratory cancer [0.74; 0.34–1.4] and 12 deaths from non-respiratory cancer [0.49; 0.25–0.85]. The employees who had the greatest opportunity for occupational exposure to trichloroethylene were assemblers, but their mortality rate generally conformed to the expected value for all types of diseases.

Garabrant *et al*. (1988) followed a cohort of 14 067 persons who had worked for at least four years for a large aircraft manufacturing company in the United States and for at least one day at the company facility in San Diego County between January 1958 and 31 December 1982. The cohort was followed up through 1982. Persons lost to follow-up were included up to the last date at which they were known to be alive. United States national rates and rates from San Diego County were used to calculate the expected numbers of deaths. Data from a relatively small case–control study nested in the cohort indicated that 37% of the jobs held in the plant entailed exposure to trichloroethylene. A total of 1804 deaths was observed (SMR, 0.75; 95% CI, 0.72–0.79), and there were 453 deaths from cancer (0.84; 0.77–0.93). None of the SMRs for individual cancer sites was significantly elevated. There were eight deaths from cancer of the biliary passages and liver (0.94; 0.40–1.9).

Spirtas *et al*. (1991) analysed a cohort of 14 457 civilian employees who had worked for at least one year at an air force base in Utah, United States, between 1 January 1952 and 31 December 1956, where they maintained and overhauled aircraft and missiles, cleaning and repairing small parts. The analysis included 12 538 white workers and 1528 workers of unknown race, who were followed up until 31 December 1982; 97% were successfully traced. At the end of follow-up, 3832 persons had died, and their death certificates were obtained from the State vital statistics office and coded by a nosologist. The expected number of deaths was based on rates for the Utah population. In the early years of operation of the base, 1939–54, cold solvents were used to clean metal parts, and these were primarily Stoddard solvent, carbon tetrachloride, trichloroethylene and alcohols. Of these, Stoddard solvent was used most frequently; however, in 1955, trichloroethylene replaced Stoddard solvent, and in 1968 1,1,1-trichloroethane replaced trichloroethylene. Trichloroethylene was the primary solvent used in vapour degreasing in the base shops from 1939 to 1979, when it was replaced by 1,1,1-trichloroethane. Of the 14 467 cohort members, 10 256 were classified as having been exposed to mixed solvents, 7282 to trichloroethylene, 6977 to Stoddard solvent and 6737 to carbon tetrachloride (Stewart *et al*., 1991). Actual exposure levels could not be quantified, but for each combination of job and organization an index of exposure to trichloroethylene was calculated on the basis of the frequency of exposure, the frequency of peak exposure and duration of use. Cumulative exposure categories were derived by multiplying the exposure index assigned to each combination of job and organization by the time spent in this job and by adding these products. The 3832 deaths in the total cohort resulted in an overall SMR of 0.92 (95% CI, 0.90–0.95). Among white men exposed to trichloroethylene, there were 1508 deaths (0.92; 0.87–0.96), 248 of which were from cancer (0.92; 0.81–1.1). When the data for men and women exposed to trichloroethylene were combined, there were 1694 deaths from all causes [0.90; 0.86–0.95] and 281 deaths from cancer [0.88; 0.78–0.99]; there was an elevated risk for cancer of the biliary passages [2.2; 0.96–4.4]. Nonsignificantly excess risks were also seen for cancer of the bone in men (2.6; 0.54–7.7; three deaths) and for cancer of the cervix (2.2; 0.61–5.7; four deaths) and for non-Hodgkin's lymphoma (2.9; 0.78–7.3; four deaths) in women. There were two deaths from primary liver cancer [1.1; 0.12–4.0]. No evidence of a dose–response relationship was seen when the data were analysed by cumulative exposure to trichloroethylene (scored as categories of < 5, 5–25, > 25) for cancer at any site, including cancer of biliary passages, for which the SMRs were [2.5] (three deaths) for exposure to < 5, [4.3] (three deaths) for exposure to 5–25 and [1.3] (two deaths) for exposure to > 25. Both deaths from liver cancer occurred among men in the lowest category of cumulative exposure.

A retrospective cohort study of renal cancer among workers exposed to trichloroethylene in a cardboard manufacturing factory in Germany was reported by Henschler *et al*. (1995). Measurements of exposure were not available, and workers were classified as exposed or not exposed on the basis of categories of job held in the factory. The exposed group consisted of 169 men who had worked for at least one year during 1956–75; a control group consisting of 190 unexposed workers from the same factory was included for comparison. The average observation period was 34 years. Assessment of cancer occurrence was based on abdominal sonography, records of the medical, personnel and pension departments and interviews with relatives. Causes of death were obtained from hospital records or from the treating physician.

During the period of follow-up, four histologically verified cases of renal-cell carcinoma and one case of urothelial cancer of the renal pelvis were seen in the exposed group, and no case was observed in the controls ($p = 0.03$). The five cancers occurred 18–34 years after first exposure; four of the five men had been exposed for more than 13 years. The excess was confirmed in comparisons with population rates for Denmark (SIR, 8.0; 95% CI, 2.6–19) and for the former German Democratic Republic (9.7; 3.1–23). The incidences of cancers at other sites were not reported. There were 50 deaths from all causes among exposed workers and 52 among controls; 16 cases of cancer of any organ were seen in both exposed and control workers; and two deaths from renal cancer occurred in exposed workers and none in controls. In a comparison with the local population, the SMR for renal cancer in the exposed group was 3.3 (95% CI, 0.40–12). [The Working Group noted that the use of sonography suggested that the study originated from the observation of a cluster of cases of renal cancer.]

The main cohort studies are summarized in Table 11.

2.4 Case–control studies

2.4.1 Primary liver cancer

Hernberg *et al*. (1984) identified 374 cases of primary liver cancer (ICD 155.0) that had been reported to the Finnish Cancer Registry in 1979–80. The notifying hospital could not be identified in nine cases, the hospital refused contact with 38 patients, and the diagnosis was incorrect in 83 cases. For the remaining 244 cases, a questionnaire was sent to either the patient or the next-of-kin. Three deceased patients had no relatives, and in 79 instances no reply was obtained. A further check of the diagnoses revealed that only 126 of the 162 cases for which a reply was obtained were primary liver cancers. For each of the 162 cases, two controls with coronary infarct and without cancer were selected, from the hospital register for living cases and from autopsy records for dead cases. Complete replies were obtained from only 174 controls or their next-of-kin. An industrial hygienist evaluated exposure to solvents on the basis of the reported occupational histories. Eight patients had been exposed to solvents for at least one year (odds ratio, 2.3; 95% CI, 0.8–7.0). Six of the exposed patients were women, one of whom had possibly been exposed to trichloroethylene; none of the female controls had been exposed.

Hernberg *et al*. (1988) subsequently identified 618 persons reported as having primary liver cancer to the Finnish Cancer Registry in 1976–78 and 1981. Five patients alive at the start of the study were excluded, and no relative was found for 87 patients. Questionnaires were sent to relatives of the remaining 526 cases, and a response was obtained from 377. Thirty-three cases were omitted on the basis of an incorrect or unconfirmed diagnosis, leaving 344 cases in the study. Two control groups were selected: one, as in the previous study, comprised 674 patients who had died with a coronary infarct, of whom 116 had no relatives and for 385 of whom the questionnaire was returned; the second control group consisted of 720 deceased stomach cancer patients, 66 of whom had no relatives and for 476 of whom a questionnaire was returned from next-of-kin. Two industrial hygienists coded occupational histories for potential exposure to solvents. In comparison with the two control groups combined, the odds ratios for exposure to solvents were 0.6 [95% CI, 0.3–1.4] for men and 3.4 [1.1–10] for women. None of the exposed women had been a heavy or moderate alcohol drinker. One of the seven solvent-exposed female patients and none of the solvent-exposed controls had been exposed to trichloroethylene.

Hardell *et al*. (1984) studied all cases of liver cancer reported to the Swedish Cancer Registry in 1974–81 in men aged 25–80 living in the Umeå region of Sweden. Six patients who were alive at the start of the study in 1981 were excluded, leaving 166 cases. The diagnosis of 114 cases was confirmed on review. Six patients had been used as controls in a previous study, relatives could not be identified for five patients, and the relatives of one patient refused participation, leaving 102 patients (78 with hepatocellular, 15 with cholangiocellular, five with mixed and four with other types of liver cancer) for whom completed questionnaires were obtained. Two deceased controls matched for age, sex, year of death and municipality were selected from the National Population Register for each case, excluding people who had died from suicide or cancer. Exposure to solvents was assessed on the basis of responses to a questionnaire. The risk ratio for all primary liver cancers (hepatocellular and/or cholangiocellular) was 1.8 (95% CI, 0.99–3.4); that for hepatocellular carcinoma was 2.1 (1.1–4.0). Two of the 22 solvent-exposed patients and one of the 27 solvent-exposed controls had been exposed to trichloroethylene.

2.4.2 Malignant lymphoma

Hardell *et al*. (1981) studied 169 men aged 25–85 with histologically confirmed malignant lymphoma (60 with Hodgkin's disease, 105 with non-Hodgkin's lymphoma and four with unclassified lymphomas) in the Umeå region of Sweden between 1974 and 1978. For each of the 107 living patients, two controls matched for sex, age and residence were selected from the National Population Registry. For each of the 62 deceased patients, two controls matched for sex, age, year of death and municipality were selected from the National Registry for Causes of Death, excluding people who had died from suicide or cancer. Exposure to solvents was assessed on the basis of responses to a questionnaire. Three of the 338 controls did not return the questionnaire but were considered not to have been exposed in matched analyses. The relative risk associated with exposure to styrene, trichloroethylene, tetrachloroethylene or benzene was 4.6 (95% CI, 1.9–11). Seven cases and three controls reported exposure to trichloroethylene.

2.4.3 Hodgkin's disease

Olsson and Brandt (1980) studied 25 men aged 20–65 who were admitted consecutively to the Department of Oncology at the University Hospital of Lund, Sweden, in 1978–79 with Hodgkin's disease. For each case, two male controls, matched for age and residence, were selected from the population register. Twelve of the patients had been exposed to organic solvents, giving a relative risk of 6.6 (95% CI, 1.8–24). Three cases and no control reported exposure to trichloroethylene.

2.4.4 Renal-cell carcinoma

Sharpe *et al*. (1989) identified 403 patients who had been diagnosed with renal-cell carcinoma in nine hospitals in Montréal, Canada, in 1982–87. Of these, 168 were still alive in 1987 and agreed to complete a questionnaire. For each case, one control originally suspected to have renal-cell carcinoma but for whom a non-neoplastic diagnosis was given was matched for sex, age and urologist. Ultimately, 164 patients and 161 controls provided information. Ten

patients and three controls had been exposed to degreasing solvents (odds ratio, 3.4; 95% CI, 0.92–13). Tetrachloroethylene, 1,1,1-trichloroethane, trichloroethylene and dichloromethane were reported to be the agents most widely used.

2.4.5 Cancer of the colon

Fredriksson *et al*. (1989) carried out a case–control study of patients aged 30–75 in whom adenocarcinoma of the large bowel had been diagnosed in 1980–83 in the Umeå region of Sweden. A total of 402 incident cases were identified, but only patients alive in 1984–86 were included, leaving 344 patients, of whom 312 participated. Two population controls, matched by age, sex and county, were included for each case. Data on exposure were collected by a postal questionnaire. The odds ratio for exposure to trichloroethylene was 1.5 (95% CI, 0.4–5.7) and that for exposure to trichloroethylene among dry cleaners was 7.4 (1.1–47).

2.4.6 Brain tumours

Heineman *et al*. (1994) undertook a case–control study of 741 white men who had died from astrocytic brain tumours in two states of the United States between 1978 and 1981. Next-of-kin were identified for 654 patients; 483 of these were interviewed, and a hospital diagnosis of astrocytic brain tumour was confirmed in 300 cases. Of 741 selected deceased controls, 320 were included in the study. Exposure to solvents was assessed on the basis of a job–exposure matrix; 128 case patients had been employed in jobs with potential exposure to trichloroethylene (odds ratio, 1.1; 95% CI, 0.8–1.6). None of the risk estimates for subgroups reached significance.

2.4.7 Childhood leukaemia

Lowengart *et al.* (1987) identified 216 children aged 10 years or less from the Los Angeles County (United States) Cancer Surveillance Program in whom acute leukaemia had been diagnosed in 1980–84. Permission for contact with families was obtained for 202 patients; 159 mothers were interviewed, and information about the fathers was obtained for 154 cases. The mothers of the patients were asked to name a control child from among their child's friends. A total of 136 control mothers were interviewed; information about the fathers was obtained for 130 controls. Data on occupational exposure were obtained by telephone interview. The odds ratios associated with father's exposure to trichloroethylene were 2.0 ($p = 0.16$) for exposure one year before pregnancy, 2.0 ($p = 0.16$) for exposure during pregnancy and 2.7 ($p = 0.07$; 95% CI, 0.64–16) for exposure after delivery. The results of this study were also reported in an abstract (Peters *et al.*, 1984).

2.4.8 Childhood brain tumours

Peters *et al.* (1981) studied the occupations of the parents of 92 children under the age of 10 with brain tumours and of 92 matched controls in Los Angeles County, United States. Interviews with the fathers showed that those of 12 children with brain tumours and those of two controls had worked in the aircraft industry; the fathers of only two children with brain tumours reported exposure to trichloroethylene. The results of this study were also reported in an abstract (Peters *et al.*, 1984).

2.4.9 Multiple sites

Siemiatycki (1991) studied men aged 35–70 in Montréal, Canada, during 1979–85. A total of 3730 people with cancers at 21 sites and 533 population controls were interviewed about their occupations in detail, and their exposure to 293 agents or mixtures was then estimated by a group of chemists. The estimated prevalence of exposure to trichloroethylene was 2%. Both case–case and case–control comparisons were conducted. After control for confounding, increased odds ratios were found in the case–case comparison for cancer of the rectum (1.9 [95% CI, 0.9–3.9] and for skin melanoma (2.6 [1.2–5.8]) in relation to presumed exposure to trichloroethylene; for 'substantial' exposure (at least five years of exposure at a presumably medium or high concentration and frequency), elevated odds ratios were reported for prostatic cancer (1.8 [0.7–4.7]) and for skin melanoma (2.3 [0.8–7.0]), while the risk for rectal cancer was no longer elevated (0.8 [0.2–2.8]). The increased risk for skin melanoma was restricted to French Canadians; in the latter group, the risk for lung adenocarcinoma was also elevated (odds ratio for any exposure, 2.6 [0.8–8.4]; odds ratio for substantial exposure, 4.5 [1.1–18]). The risk was not increased for cancers of the bladder (0.6 [0.3–1.4]) or kidney (0.8 [0.3–2.1]) or for non-Hodgkin's lymphoma (1.1 [0.5–2.4]).

2.5 Studies of drinking-water

Cancer occurrence in populations exposed to drinking-water contaminated with various concentrations of trichloroethylene has been compared in a number of studies. The interpretation of some of these studies is complicated by several methodological problems:

(i) information on the concentration of trichloroethylene in water was obtained subsequently to or contemporaneously with the period over which cancer occurrence was measured, although cancer rates should be correlated with exposure before occurrence of the disease;

(ii) exposure was generally measured at the community level and does not necessarily reflect the exposure of individuals;

(iii) the problem of migration in and out of the populations under study was not addressed; and

(iv) the possible confounding effects of other characteristics of the populations being compared (socioeconomic, industrial and cultural factors) were not taken into account.

Isacson *et al*. (1985) tabulated the average annual age-adjusted incidence rates of cancers of the bladder, breast, colon, lung, prostate or rectum per 100 000 population in towns in Iowa, United States, in 1969–81 by the level of detectable volatile organic compounds in finished groundwater supplies. The levels of trichloroethylene were < 0.15 µg/L in one group of areas and ≥ 0.15 µg/L in another. There were virtually no differences in the incidences between these two groups.

Lagakos *et al*. (1986) studied childhood leukaemia in a community in Massachusetts, United States, where water from two wells was contaminated with trichloroethylene. Measurements made in 1979 showed a concentration of 267 ppb [µg/L] trichloroethylene in the well water. Twenty cases of childhood leukaemia were diagnosed in the community in 1964–83, and these were associated with 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; $p = 0.03$).

A study conducted in New Jersey, United States, during 1979–87 included 75 towns (Cohn *et al.*, 1994), of which 27 were included in a study reported by Fagliano *et al.* (1990). Trichloroethylene concentrations were measured during 1984–85, and an average level was assigned to each town. The highest level assigned was 67 µg/L. The water supply of six towns contained > 5 µg/L trichloroethylene (average, 23.4 µg/L). Women in these towns had a significantly higher total incidence of leukaemia than the inhabitants of towns where the concentration of trichloroethylene in drinking-water was < 0.1 µg/L (relative risk, 1.4; 95% CI, 1.1–1.9); no such effect was seen for men (1.1, 0.84–1.4). The risk among women was particularly elevated for acute lymphocytic leukaemia, chronic lymphocytic leukaemia and chronic myelogenous leukaemia. The risk for acute lymphocytic leukaemia in childhood was also significantly increased, in girls but not in boys. Increased risks for non-Hodgkin's lymphoma were apparent in towns in the highest category of trichloroethylene contamination (0.2; 0.94–1.5 for men and 1.4; 1.1–1.7 for women) and was particularly elevated for high-grade lymphomas.

Studies were conducted in two counties in Arizona, United States, 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 neoplasms and testicular cancer (Pima County, Arizona Department of Health Services, 1990). In Maricopa County, two wells that were occasionally used to supplement the water supply were found to contain 8.9 and 29. 0 ppb [µg/L] trichloroethylene in 1982; they were then taken out of service. The concentrations of trichloroethylene in contaminated wells in Pima County were 1–239 µg/L, with levels as high as 4600 µg/L 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 both Maricopa and Pima counties were comparable to those in other areas included in the United States SEER programme.

Vartiainen *et al*. (1993) collected 24-h 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's lymphoma, which occurred in a 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; 0.3–1.1; 14 cases), neither overall cancer incidence nor the incidence of liver cancer or lymphohaematopoietic cancers was increased in the two villages.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 Mouse

Groups of 50 male and 50 female B6C3F1 mice, five weeks of age, were administered trichloroethylene (purity, > 99%; containing 0.19% epoxybutane and 0.09% epichlorohydrin [see IARC, 1987c] as stabilizers) in corn oil by gavage on five days a week for 78 weeks. The time-weighted average doses of trichloroethylene were 1169 and 2339 mg/kg bw per day for males and 869 and 1739 mg/kg bw per day for females. All surviving animals were killed 90 weeks after the start of treatment and submitted to complete necropsy and histopathological evaluation. Groups of 20 male and 20 female vehicle controls were included. The numbers of survivors at the end of the study were 8/20 male vehicle controls, 36/50 males at the low dose and 22/48 males at the high dose; and 20/20 female vehicle controls, 42/50 females at the low dose and 39/47 females at the high dose. The survival-adjusted (Cox and Tarone test) incidences of hepatocellular carcinomas were increased in animals of each sex in relation to dose; males: 1/20 in vehicle controls, 26/50 ($p = 0.004$) at the low dose, 31/48 ($p < 0.001$) at the high dose; females: 0/20 in vehicle controls, 4/50 at the low dose, 11/47 ($p = 0.008$) at the high dose. One male at the high dose developed a forestomach papilloma (United States National Cancer Institute, 1976).

In a subsequent study, groups of 50 male and 50 female B6C3F1 mice, eight weeks of age, were administered 1000 mg/kg bw trichloroethylene (purity, > 99.9%; containing no epichlorohydrin) in corn oil by gavage on five days a week for up to 103 weeks. Groups of 50 mice of each sex served as vehicle controls. Survival of treated males was significantly reduced ($p = 0.004$) in comparison with controls; at the end of the experiment, 33 control and 16 treated males and 32 control and 23 treated females were still alive. Histopathological evaluation revealed increased incidences (incidental tumour test) of hepatocellular tumours in treated animals. In males, hepatocellular adenomas occurred in 7/48 controls and 14/50 ($p = 0.048$) treated animals; hepatocellular carcinomas were found in 8/48 controls and 31/50 ($p < 0.001$) treated animals; and the combined numbers of animals bearing hepatocellular adenomas and/or carcinomas were 14/48 controls and 39/50 ($p < 0.001$) treated animals. In females, hepatocellular adenomas were seen in 4/48 control and 16/49 ($p = 0.001$) treated animals; hepatocellular carcinomas occurred in 2/48 control and 13/49 ($p = 0.002$) treated animals; and the combined numbers of animals bearing hepatocellular adenomas and/or carcinomas were 6/48 controls and 22/49 ($p < 0.001$) treated animals. There was no significant treatment-related increase in the incidence of tumours at other sites. Toxic nephrosis (cytomegaly) was seen in 90% of treated males and in 98% of treated females (United States National Toxicology Program, 1990).

Two groups of 30 male and 30 female ICR:Ha Swiss mice, six to eight weeks of age, were each administered 0 or 0.5 mg trichloroethylene [purity unspecified] by gavage in 0.1 ml trioctanoin once a week for at least 74 weeks. Only sections of lung, liver and stomach were taken for histopathological examination. The incidence of forestomach tumours was reported not to be increased; findings were not given for other sites (Van Duuren *et al.*, 1979). [The Working Group noted the low dose used and the inadequate conduct and reporting of the study.]

3.1.2 *Rat*

Groups of 50 male and 50 female Osborne-Mendel rats, six weeks of age, were administered trichloroethylene (purity, > 99%; containing 0.19% epoxybutane and 0.09% epichlorohydrin as stabilizers) in corn oil by gavage on five days a week for 78 weeks. The time-weighted average doses of trichloroethylene were 549 (low dose) and 1097 mg/kg bw per day (high dose) for animals of each sex. All surviving animals were killed 110 weeks after the start of treatment and were submitted to complete necropsy. Groups of 20 male and 20 female vehicle controls were included. Large proportions of treated and control rats died during the experiment; the numbers of animals alive at the end of the study were 3/20 male vehicle controls, 8/50 males at the low dose and 3/50 males at the high dose; of the females, there were 8/20 vehicle controls, 13/48 at the low dose and 13/50 at the high dose. There was no significant difference in tumour incidence at any site between treated and control rats (United States National Cancer Institute, 1976). [The Working Group noted the high rates of early mortality in both control and treated rats and the limited duration of treatment.]

In a subsequent study, groups of 50 male and 50 female Fischer 344/N rats, eight weeks of age, were administered 0, 500 or 1000 mg/kg bw trichloroethylene (purity, > 99.9%; containing no epichlorohydrin) in corn oil by gavage on five days a week for up to 103 weeks. A group of 50 male and 50 female rats were used as untreated controls. Survival of low-dose and high-dose males was significantly reduced ($p < 0.005$) in comparison with vehicle controls; the numbers of survivors at the end of the experiment were 35 male vehicle controls, 20 at the low dose and 16 at the high dose; and 37 female vehicle controls, 33 at the low dose and 26 at the high dose. An increased incidence of renal tubular-cell adenocarcinomas was seen in males: 0/49 untreated controls, 0/48 vehicle controls, 0/49 at the low dose and 3/49 at the high dose ($p = 0.028$; incidental tumour test). Two males at the low dose had renal tubular-cell adenomas. The incidence of tumours in female rats was not increased at any site. Toxic nephrosis of the kidney occurred in 96/98 treated males and in all of the treated females but not in vehicle control rats of either sex (United States National Toxicology Program, 1990). [The Working Group noted the uncommon occurrence of renal tubular-cell tumours in untreated Fischer 344/N rats.]

Groups of 50 males and 50 females of four strains (ACI, August, Marshall and Osborne-Mendel), 6.5–8 weeks of age, were administered 0, 500 or 1000 mg/kg bw trichloroethylene (purity, > 99.9%) in corn oil by gavage on five days a week for 103 weeks. Additional groups of 50 rats of each sex and strain served as untreated controls. Survival was reduced significantly in low-dose and high-dose males and high-dose females of the ACI strain, in both treated groups of males and females of the Marshall strain, and in high-dose female Osborne-Mendel rats. The numbers of survivors at the end of the study were: ACI males – 36 untreated controls, 37 vehicle controls, 19 at the low dose, 11 at the high dose; ACI females – 36 untreated controls, 33 vehicle controls, 20 at the low dose, 17 at the high dose; August males – 24 untreated controls, 21 vehicle controls, 13 at the low dose, 15 at the high dose; August females – 26 untreated controls, 23 vehicle controls, 26 at the low dose, 24 at the high dose; Marshall males – 32 untreated controls, 26 vehicle controls, 12 at the low dose, 6 at the high dose; Marshall females – 31 untreated controls, 30 vehicle controls, 12 at the low dose, 10 at the high dose; Osborne-Mendel males – 18 untreated controls, 22 vehicle controls, 17 at the low dose, 14 at the high dose; Osborne-Mendel females – 19 untreated controls, 18 vehicle controls, 10 at the low dose, 7 at

the high dose. Many early deaths occurred accidentally. The incidence of renal cytomegaly was > 80% in all treated males and females, and toxic nephropathy (described as dilated tubules lined by elongated and flattened epithelial cells) occurred at rates of 17–80% in the treated groups; however, there was no difference in kidney toxicity between males and females of any strain. Neither of these two renal lesions was seen in untreated or vehicle controls. The incidences of renal tubular-cell hyperplasia and tubular-cell adenoma were increased in male Osborne-Mendel rats at the low dose: hyperplasia – 0/50 untreated controls, 0/50 vehicle controls, 5/50 at the low dose, 3/50 at the high dose; adenoma – 0/50 untreated controls, 0/50 vehicle controls, 6/50 (p = 0.007; survival-adjusted incidental tumour test) at the low dose, 1/50 at the high dose. One renal tubular-cell adenocarcinoma occurred in a male at the high dose. The incidences of interstitial-cell tumours of the testis were increased in Marshall rats exposed to trichloroethylene: 16/46 untreated controls, 17/46 vehicle controls, 21/48 ($p < 0.001$; survival-adjusted incidental tumour test) at the low dose, 32/48 ($p < 0.001$) at the high dose. No significant increase in tumour incidence was reported for ACI or August rats (United States National Toxicology Program, 1988). [The Working Group noted the poor survival among all strains and the fact that five of the six renal adenomas in male Osborne-Mendel rats at the low dose occurred among the 17 rats alive at the end of the study.]

Groups of 30 male and 30 female Sprague-Dawley rats, 12–13 weeks of age, were administered 0, 50 or 250 mg/kg bw trichloroethylene (purity, 99.9%; containing no epoxide) in olive oil by gavage on four to five days per week for 52 weeks and observed for life. Data on survival were not provided, but the authors reported a nonsignificant increase in mortality among treated females. Renal tubular-cell cytokaryomegaly was observed only in male rats at the high dose (46.7% [14/30]; $p < 0.01$). A nonsignificant increase in the incidence of leukaemias was observed in males: none in controls, 6.7% [2/30] at the low dose and 10.0% [3/30] at the high dose (Maltoni *et al.*, 1986). [The Working Group noted the short period of exposure.]

3.2 Inhalation

3.2.1 Mouse

Groups of 30 male and 30 female NMRI mice [age unspecified] were exposed to air containing trichloroethylene (purity, > 99.9%; stabilized with 0.0015% triethanolamine) at a concentration of 0, 100 or 500 ppm (0, 540 or 2700 mg/m^3) for 6 h per day on five days per week for 18 months. The experiment was terminated after 30 months. At the end of exposure (75 weeks), there was no difference in the probability of survival among the females; in males, the probability of survival was reduced from 83% in controls to 63% in low-dose and 56% in high-dose groups. Histopathological examination of spleen, liver, kidney, lung, heart, stomach, central nervous system and all tumours indicated increased age-adjusted incidences of lymphomas in treated female mice: 9/29 controls, 17/30 at the low dose ($p < 0.001$) and 18/28 ($p = 0.01$) at the high dose (Henschler *et al.*, 1980).

Groups of 49–50 female ICR mice, seven weeks of age, were exposed to air containing trichloroethylene (purity, 99.8%; containing 0.13% carbon tetrachloride and > 0.02% benzene and epichlorohydrin) at concentrations of 0, 50, 150 or 450 ppm (0, 270, 810 or 2430 mg/m^3) for 7 h per day on five days per week for up to 104 weeks. There were no significant differences in

survival between the control and exposed groups. Complete necropsy was carried out on all animals. Histopathological evaluation revealed a significant increase (Fisher's exact test) in the incidence of lung adenocarcinomas: 1/49 controls, 3/50 at the low dose, 8/50 ($p < 0.05$) at the middle dose and 7/46 ($p < 0.05$) at the high dose. [The Working Group found a significant dose–response trend: $p = 0.034$, Cochran-Mantel-Haenszel test.] The incidences of adenomas and adenocarcinomas of the lung combined in the groups at the middle (13/50) and high doses (11/46) were not significantly increased in comparison with controls (6/49). The average number of lung tumours was, however, increased in mice at the middle and high doses in comparison with controls: 0.12 in controls, 0.10 at the low dose, 0.46 at the middle dose and 0.39 at the high dose (Fukuda *et al*., 1983).

Groups of 90 male and 90 female Swiss mice, 11 weeks of age, and groups of 90 male and 90 female B6C3F1 mice, 12 weeks of age, were exposed to air containing trichloroethylene (purity, 99.9%; containing no epoxide) at concentrations of 0, 100, 300 or 600 ppm (0, 540, 1620 or 3240 mg/m^3) for 7 h per day on five days a week for 78 weeks and were then observed for life. Data on survival were not provided, but the authors reported that mortality was higher ($p < 0.05$) in treated male B6C3F1 mice than in controls. Dose-related increases in the incidences of lung and liver tumours were observed in male Swiss mice [Fisher's exact test or Cochran-Armitage linear trend test]. The percentages of male Swiss mice bearing a malignant pulmonary tumour were: control, 11.1% [10/90]; low-dose, 12.2% [11/90]; mid-dose, 25.5% [23/90] ($p < 0.05$); and high-dose, 30.0% [27/90] ($p < 0.01$); the percentages of male mice bearing a hepatoma were: control, 4.4% [4/90]; low-dose, 2.2% [2/90]; mid-dose, 8.9% [8/90]; and high-dose, 14.4% [13/90] ($p < 0.05$). In B6C3F1 mice, a dose-related increase in the incidence of lung tumours was observed in females: control, 4.4% [4/90]; low-dose, 6.7% [6/90]; mid-dose, 7.8% [7/90]; and high-dose, 16.7% [15/90] ($p < 0.05$) (Maltoni *et al*., 1986, 1988).

3.2.2 Rat

Groups of 30 male and 30 female Wistar rats [age unspecified] were exposed to air containing trichloroethylene (purity, > 99.9%; stabilized with 0.0015% triethanolamine) at concentrations of 0, 100 or 500 ppm (0, 540 or 2700 mg/m^3) for 6 h per day on five days per week for 18 months. The experiment was terminated after 36 months. No differences in survival were reported; the probability of survival in each group at the end of the experiment was: 46.7% of male controls, 23.3% of males at the low dose, 36.7% of males at the high dose, 16.7% of female controls, 13.3% of females at the low dose and 16.7% of females at the high dose. Histopathological and gross examination of spleen, liver, kidney, lung, heart, stomach, central nervous system and all tumours revealed no increase in tumour incidence (Henschler *et al*., 1980).

Groups of 49–51 female Sprague-Dawley rats, seven weeks of age, were exposed to air containing trichloroethylene (purity, 99.8%) at concentrations of 0, 50, 150 or 450 ppm (0, 270, 810 or 2430 mg/m^3) for 7 h per day on five days per week for 104 weeks. Survival was significantly higher in the exposed groups than in controls: about 75% of the rats in the three treated groups and 50% of controls were alive at 100 weeks. Gross and histopathological examination revealed no difference in the incidence of tumours between the control and exposed groups (Fukuda *et al*., 1983).

Groups of 130–145 male and female Sprague-Dawley rats, 12 weeks of age, were exposed to air containing trichloroethylene (purity, 99.9%; containing no epoxide) at a concentration of 0, 100, 300 or 600 ppm (0, 540, 1620 or 3240 mg/m^3) for 7 h per day on five days per week for 104 weeks. All animals were observed for their lifetime. Data on survival were not provided, but the authors reported no excess mortality in any of the exposed groups. A significant, dose-related increase in the incidence of Leydig cell (interstitial) tumours of the testis was observed [$p < 0.001$; Cochran-Mantel-Haenszel test]; the percentages of male rats bearing these tumours were 4.4% [6/135] of controls, 12.3% [16/130] at the low dose [$p < 0.05$; Fisher's exact test], 23.1% [30/130] at the middle dose [$p < 0.01$; Fisher's exact test] and 23.8% [31/130] at the high dose [$p < 0.01$; Fisher's exact test]. Four renal tubular adenocarcinomas (3.1%) were observed in the high-dose male rats; no such tumours were observed in the lower dose groups, in controls or in the historical control database for Sprague-Dawley rats at the study laboratory. Cytokaryomegaly of renal tubular cells was also observed: in none of the control or low-dose rats, in 16.9% at the middle dose and in 77.7% at the high dose (Maltoni *et al*., 1986, 1988).

3.2.3 Hamster

Groups of 30 male and 30 female Syrian hamsters [age unspecified] were exposed to air containing trichloroethylene (purity, > 99.9%; stabilized with 0.0015% triethanolamine) at concentrations of 0, 100 or 500 ppm (0, 540 or 2700 mg/m^3) for 6 h per day on five days per week for 18 months. The experiment was terminated after 30 months. The probability of survival was similar in exposed and control groups. Histopathological examination of spleen, liver, kidney, lung, heart, stomach, central nervous system and all tumours revealed no significant increase in tumour incidence (Henschler *et al*., 1980).

3.3 Topical application

Mouse: In a study of two-stage carcinogenesis on mouse skin, single doses of 1.0 mg trichloroethylene [purity unspecified] in 0.1 ml of acetone were applied to the shaven dorsal skin of 30 female ICR:Ha Swiss mice aged six to eight weeks; 14 days later, topical applications of 12-*O*-tetradecanoylphorbol 13-acetate (TPA; 2.5 μg in 0.1 ml of acetone, three times per week) were begun, for at least 49 weeks. Nine skin papillomas were found in 4/30 treated mice, and 10 papillomas were found in 9/120 TPA-treated controls. Trichloroethylene was also administered by repeated topical application (three times per week) to groups of 30 female ICR:Ha Swiss mice, six to eight weeks of age, for 83 weeks at a dose of 1.0 mg per mouse. No tumours were observed at the site of application (Van Duuren *et al*., 1979).

3.4 Subcutaneous injection

Mouse: Groups of 30 female ICR:Ha Swiss mice, six to eight weeks of age, were given subcutaneous injections of 0.5 mg trichloroethylene [purity unspecified] in 0.05 ml trioctanoin once a week for at least 74 weeks, or received the vehicle alone. No tumours were observed at the injection site in either group (Van Duuren *et al*., 1979).

3.5 Administration with known carcinogens

Mouse: Five groups of 50 male and 50 female ICR:Ha Swiss mice, five weeks of age, were administered either industrial-grade trichloroethylene (purity, 99.4%; containing 0.11% epichlorohydrin and 0.20% 1,2-epoxybutane) in corn oil by gavage, purified trichloroethylene (purity, > 99.9%) in corn oil by gavage, purified trichloroethylene with added epichlorohydrin (0.8%), purified trichloroethylene with added 1,2-epoxybutane (0.8 %) or purified trichloroethylene with 0.25% epichlorohydrin plus 0.25% 1,2-epoxybutane, on five days per week for 18 months. The doses of trichloroethylene that were administered were 2.4 g/kg bw for males and 1.8 g/kg bw for females. Groups of 50 mice of each sex given corn oil served as vehicle controls. The treatment period was followed by a six-month observation period. The probabilities of survival were significantly reduced ($p < 0.001$) in all groups of treated males in comparison with controls; in females, the probabilities of survival were reduced ($p < 0.05$) in the group receiving purified trichloroethylene and in that receiving purified trichloroethylene plus epichlorohydrin ($p < 0.001$). At the end of the study, there were no more than two survivors in any treatment group. Complete necropsies were performed on all animals. The incidence of squamous-cell carcinomas of the forestomach was increased in several of the treatment groups over that in controls (0/50 for males and females); males: purified trichloroethylene, 0/50; industrial-grade trichloroethylene, 0/49; purified trichloroethylene plus epichlorohydrin, 5/49 ($p < 0.001$); purified trichloroethylene plus 1,2-epoxybutane, 3/49 ($p = 0.029$); and purified trichloroethylene plus epichlorohydrin and 1,2-epoxybutane, 2/49 ($p = 0.036$); females: controls, 0/50; purified trichloroethylene, 0/50; industrial-grade trichloroethylene, 3/50; purified trichloroethylene plus epichlorohydrin, 9/50 ($p < 0.001$); purified trichloroethylene plus 1,2-epoxybutane, 1/48; and purified trichloroethylene plus epichlorohydrin and 1,2-epoxybutane, 9/50 ($p < 0.001$). No significant increase in the incidences of tumours at other sites was reported. The authors attributed the increased incidence of forestomach cancers to the direct alkylating effects of epichlorohydrin and 1,2-epoxybutane (Henschler *et al.*, 1984). [The Working Group noted that the incidences of hepatocellular tumours (adenomas and carcinomas combined) in male mice were: controls, 3/50; purified trichloroethylene, 6/50; and industrial-grade trichloroethylene, 9/50; and that no survival-adjusted analysis of tumour incidence was performed.]

Groups of 23–33 male B6C3F1 mice, 15 days of age, were given a single intraperitoneal injection of *N*-ethylnitrosourea in 0.1 mol/L sodium acetate at doses of 0, 2.5 or 10 mg/kg bw. When the mice were four weeks of age, a 61-week treatment period was begun with 0, 3 or 40 mg/L trichloroethylene (purity, > 99%) in the drinking-water. The highest concentration of trichloroethylene was equivalent to a daily dose of 6 mg/kg bw. The incidences of hepatocellular adenomas and carcinomas were not increased in mice that received trichloroethylene alone in comparison with vehicle controls, and trichloroethylene did not promote liver tumours in mice initiated with *N*-ethylnitrosourea (Herren-Freund *et al.*, 1987). [The Working Group noted the low dose of trichloroethylene used.]

3.6 Carcinogenicity of metabolites

Studies of the carcinogenicity of the known metabolites of trichloroethylene, dichloroacetic acid, trichloroacetic acid and chloral hydrate, are summarized in separate monographs in this volume.

3.6.1 Mouse

A single dose of 1.0 mg of trichloroethylene oxide, a putative metabolite [purity unspecified], in 0.1 ml of acetone was applied to the dorsal skin of 30 female ICR:Ha Swiss mice, six to eight weeks of age; 14 days later, topical applications of TPA (2.5 μg in 0.1 ml of acetone, three times per week) were begun and continued for more than 61 weeks. The incidence of tumours at the site of application was not increased in the group treated with trichloroethylene plus TPA (three mice each had a single papilloma) in comparison with mice receiving TPA alone (10 papillomas in 9/120 mice) (Van Duuren *et al*., 1979).

Trichloroethylene oxide was administered to a group of 30 female ICR:Ha Swiss mice, six to eight weeks of age, by repeated skin application for 82 weeks (2.5 mg/mouse in 0.1 ml acetone three times weekly); 30 mice served as vehicle controls. No tumour was observed at the site of application in either group. Further groups of 30 female ICR:Ha Swiss mice, six to eight weeks of age, were given 0 or 500 μg/mouse trichloroethylene oxide in 0.05 ml tricaprylin once a week for up to 80 weeks. One fibrosarcoma occurred at the injection site in treated animals (Van Duuren *et al*., 1983).

1,2-Dichlorovinyl cysteine, a minor metabolite [purity unspecified], was administered at a concentration of 0, 10 or 50 mg/L in drinking-water to three groups of 30 Swiss-Webster mice [age and sex unspecified] for 14 weeks, beginning one day after administration of *N*-nitrosodimethylamine (NDMA) (six intraperitoneal injections of 5.0 mg/kg bw administered every other day). The average daily doses of 1,2-dichlorovinyl cysteine were 2.4 and 12.6 mg/kg bw, respectively. Renal tumours occurred after 50 weeks in 2/16 mice receiving NDMA alone, 2/15 receiving NDMA plus the low dose of 1,2-dichlorovinyl cysteine and 3/16 receiving NDMA plus the high dose of 1,2-dichlorovinyl cysteine [not significant]. Multiple renal tumours were found in 7/40 mice treated with NDMA plus 1,2-dichlorovinyl cysteine, whereas none were found in 21 mice treated with NDMA alone [$p = 0.043$; Fisher's exact test] (Meadows *et al*., 1988).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

The biotransformation and the kinetics of trichloroethylene have been described in many studies of workers and of volunteers. Pulmonary uptake of trichloroethylene is rapid, the rate of

uptake being dependent on the rate of respiration, and uptake increases about twofold with exercise (Monster *et al.*, 1976). Distribution to the tissues has not been described, but the concentrations of trichloroethylene should be proportional to the duration and concentration of exposure, and the distribution is probably similar to that in animals. The blood:air partition coefficient for trichloroethylene in human volunteers was about 15 (Monster *et al.*, 1979), and the fat:air partition coefficient was about 700 (Sherwood, 1976; Steward *et al.*, 1973); there is therefore a tendency for deposition in fat from blood, the fat:blood partition coefficient being about 50 (700/15).

After inhalation, 40–70% of an administered dose of trichloroethylene is metabolized, the unmetabolized fraction being cleared by exhalation. Metabolism was proportional to the concentration of trichloroethylene in air up to 315 mg/m^3 for 3 h (Ikeda & Imamura, 1973; Monster *et al.*, 1976; Ikeda, 1977; Nomiyama & Nomiyama, 1977). No saturation of biotransformation has been detected with concentrations up to 380 ppm [1976 mg/m^3].

Trichloroethanol, its glucuronide and trichloroacetic acid are major metabolites in urine, and chloral hydrate is a transient metabolite in blood (Cole *et al.*, 1975). After controlled exposure of males to 200 ppm (1040 mg/m^3) trichloroethylene for 6 h, oxalic acid and *N*-(hydroxyacetyl)aminoethanol were detected as minor metabolites (Dekant *et al.*, 1984). Traces of *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-L-cysteine were present in the urine of workers exposed to unknown concentrations of trichloroethylene in air (Birner *et al.*, 1993). Trichloroethanol and its glucuronide are rapidly eliminated in urine, with half-lives of about 10 h, and trichloroacetic acid is eliminated slowly, with a half-life of about 52 h (range, 35–70 h) (Müller *et al.*, 1972, 1974). Repeated exposure of volunteers to 50 ppm (260 mg/m^3) trichloroethylene for 4 h per day on five consecutive days resulted in slightly higher concentrations of trichloroethylene and trichloroethanol in blood than after a single exposure to 40 ppm (208 mg/m^3) for 4 h (Ertle *et al.*, 1972). Urinary excretion of trichloroethanol by five male volunteers exposed to 70 ppm [364 mg/m^3] for 4 h per day for five days stabilized rapidly and remained constant until the end of the exposure, whereas urinary excretion of trichloroacetic acid continued to rise (Monster *et al.*, 1979).

4.1.2 Experimental systems

The biotransformation of trichloroethylene has been reviewed (Bonse & Henschler, 1976; Kimbrough *et al.*, 1985; Dekant, 1986; Bruckner *et al.*, 1989; Davidson & Beliles, 1991).

The absorption, distribution, metabolism and excretion of trichloroethylene at doses outside the range of those tested experimentally have been predicted from a number of physiologically based pharmacokinetic models constructed from the existing experimental data (Dallas *et al.*, 1991; Fisher *et al.*, 1991; Allen & Fischer, 1993).

The absorption and excretion of trichloroethylene have been studied in rats and mice. The compound is rapidly absorbed from the gastrointestinal tract and through the lungs; skin absorption after exposure to the vapour is negligible. In male Sprague-Dawley rats exposed to 50 ppm [260 mg/m^3] or 500 ppm [2600 mg/m^3] trichloroethylene for 2 h through a miniaturized one-way breathing valve (Dallas *et al.*, 1991), the uptake decreased from > 95% at the beginning of exposure to a relatively constant, almost steady-state level of 70%. The concentrations of trichloroethylene in exhaled breath towards the end of the exposure period were 34.6 ± 1.1 ppm

[185 ± 6 mg/m^3] after exposure to 50 ppm and 340.8 ± 10.6 ppm [1830 ± 60 mg/m^3] after exposure to 500 ppm. This direct proportionality was not reflected in the arterial blood concentrations, where the 10-fold increase in dose resulted in a 25- to 30-fold increase in blood levels and only an 8.7-fold increase in total absorbed dose.

The blood:air partition coefficient is about 14 in mice (Fisher *et al*., 1991) and about 18 in rats (Andersen *et al*., 1987; Fisher *et al*., 1989). The corresponding fat:blood values are about 36 and 27, and the liver:blood partition coefficients are about 1.8 and 1.3. At the end of 4-h exposures of Fischer 344 rats to 529 ppm [2751 mg/m^3] (males) and 600 ppm [3120 mg/m^3] (females), the concentrations of trichloroethylene in blood were about 35.5 μg/ml (males) and 25.8 μg/ml (females). The concentrations of trichloroethylene in the blood of B6C3F1 mice were much lower: the highest mean blood concentrations seen during exposure of males to 110–748 ppm [572–3890 mg/m^3] and females to 42–889 ppm [218–4623 mg/m^3] were 7.3 μg/ml after exposure to 748 ppm [3890 mg/m^3] (males) and 6.3 μg/ml after exposure to 368 ppm [1914 mg/m^3] (females) (Fisher *et al*., 1991).

The distribution of trichloroethylene in mice after a 10-min inhalation (approximate dose, 280 mg/kg bw) was studied by whole-body autoradiography of animals killed at intervals over 8 h. Trichloroethylene was distributed throughout the body into well-perfused organs; after 30 min, redistribution to adipose tissues had occurred (Bergman, 1983a).

The urinary excretion of trichloroacetic acid by rats exposed to 55 ppm [286 mg/m^3] trichloroethylene for 8 h per day for 14 weeks reached a maximum after two days and remained constant until the end of the exposure, whereas urinary excretion of trichloroethanol increased steadily over the first 10 weeks of the study (Kimmerle & Eben, 1973).

Mice have consistently higher rates of biotransformation than rats (Fisher *et al.*, 1991). The metabolism of trichloroethylene in rats can be described by Michaelis-Menten kinetics and is saturated after exposure by inhalation to more than 500–600 ppm (2600–3120 mg/m^3). Saturation of metabolism in rats at 500 ppm was also seen in the experiments of Dallas *et al*. (1991), described above. The atmospheric concentration at which elimination shifts from first-order to zero-order kinetics was found to be 65 ppm [338 mg/m^3] in rats in a closed exposure system (Filser & Bolt, 1979). Metabolic saturation occurs after oral administration of > 200–500 mg/kg bw trichloroethylene to rats; in mice, the rate of biotransformation is linear up to a dose of 2000 ppm (10 400 mg/m^3) by inhalation and up to 2000 mg/kg bw by oral administration (Stott *et al.*, 1982; Buben & O'Flaherty, 1985; Green & Prout, 1985; Prout *et al.*, 1985).

Mice have been shown to biotransform 2.6 times more trichloroethylene on a body weight basis than rats after exposure by inhalation to 600 ppm (3120 mg/m^3) (Dekant *et al*., 1986a). Trichloroacetic acid concentrations in blood reached significantly higher values in B6C3F1 mice than in Fischer 344 rats at the end of a 4-h exposure by inhalation. The peak concentrations were 23.3 μg/ml in male rats and 39.6 μg/ml in female rats exposed to 505 ppm [2626 mg/m^3] and 600 ppm [3120 mg/m^3], respectively, while the values for mice were 129.6 μg/ml in males exposed to 748 ppm [3890 mg/m^3] and 94.3 μg/ml in females exposed to 889 ppm [4623 mg/m^3] (Fisher *et al.*, 1991). After exposure to low doses, the rate of metabolism in mice and rats is similar, and about 90% of an oral dose of 2 or 10 mg/kg bw trichloroethylene was eliminated as metabolites within 72 h by female Wistar and NMRI mice (Dekant *et al.*, 1986b).

After an oral dose of 2000 mg/kg bw, 78% of the dose was exhaled as unchanged trichloroethylene by rats but only 14% by mice (Prout *et al.*, 1985).

As a result of the higher biotransformation rate in mice, their blood levels of trichloroethanol and trichloroacetic acid were four- and sixfold higher than those in rats, and peak concentrations were reached within 2 h in mice and up to 10 h in rats. In mice, the high levels of trichloroacetate in blood persisted for over 30 h (Prout *et al.*, 1985). After dosing by gavage with 1.5 mmol/kg bw (200 mg/kg bw) trichloroethylene, the peak blood concentrations of trichloroacetic acid and the area under the integrated time–concentration curve were higher in mice (216 nmol/ml [35 μg/ml] and 2.5 μmol-h/ml) [408 μg-h/ml] than in rats (81 nmol/ml [13 μg/ml] and 1.5 μmol-h/ml [245 μg-h/ml]) (Larson & Bull, 1992a). The highest concentration of trichloroacetic acid that was found in the blood of rats after oral administration of trichloroethylene in corn oil was equivalent to about 50 mg/kg bw of trichloroacetic acid (Elcombe, 1985). Blood concentrations of the chloroacetic acids resulting from their administration to mice and rats are described in the relevant monographs in this volume.

Several excretory metabolites have been identified in mice and rats (see Figure 1). Most of the metabolites in urine can be accounted for by cytochrome P450-catalysed oxidation reactions of trichloroethylene to chloral hydrate. Trichlorethanol and its glucuronide are formed by reduction of chloral hydrate; trichloroacetic acid is formed by oxidation of this intermediate (Butler, 1949; Daniel, 1963; Kimmerle & Eben, 1973). The glucuronide of trichloroacetic acid has been identified in the urine of non-human primates treated by intramuscular injection with trichloroethylene (Müller *et al.*, 1982). The mechanism of formation of dichloroacetic acid has been postulated as a rearrangement of 1,1,2-trichlorooxirane and subsequent hydrolysis (Hathway, 1980), but it may also be formed by biotransformation of chloral hydrate or trichloroacetic acid (Larson & Bull, 1992b). Oxalic acid may be formed as a urinary metabolite of trichloroethylene as an end-product of 1,1,2-trichlorooxirane, by enzymatic or non-enzymatic cleavage of the epoxide followed by spontaneous elimination of two equivalents of hydrochloric acid, reaction with water and oxidation (Dekant *et al.*, 1984). Oxalic acid may also be formed by oxidation of dichloroacetic acid (Larson & Bull, 1992a,b). The formation of *N*-(hydroxyacetyl)-aminoethanol is proposed to proceed by the reaction of trichloroethylene-derived oxidative intermediates with ethanol amine or with phosphatidylethanol amine and enzymic breakdown of the acylated lipids (Dekant *et al.*, 1984).

Traces of metabolites indicative of conjugation of trichloroethylene with glutathione are also excreted in urine after high oral doses of trichloroethylene. The presence of *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-L-cysteine indicates trichloroethylene conjugation with glutathione followed by catabolism and acetylation by the enzymes of the mercapturic acid pathway (Dekant *et al.*, 1986a; Commandeur & Vermeulen, 1990; Dekant *et al.*, 1990). Chloroacetic acid is another trace metabolite of trichloroethylene in rats (Green & Prout, 1985); it may be formed by hydrolysis of the intermediate electrophile, chlorothioketene, which is a cysteine conjugate β-lyase-catalysed cleavage product of *S*-(1,2-dichlorovinyl)-L-cysteine (Dekant *et al.*, 1986c, 1988). Monochloroacetate may be formed by reduction of dichloroacetic acid (Larson & Bull, 1992b).

Species and strain differences in the biotransformation of trichloroethylene have been reported. Higher peak blood levels of dichloroacetic acid were reported in B6C3F1 mice

(35 nmol/ml [4.5 μg/ml]) dosed with 1.5 mmol/kg bw (200 mg/kg bw) trichloroethylene orally than in rats (< 4 nmol/ml [< 0.5 μg/ml]) receiving 23 mmol/kg bw (3000 mg/kg bw) (Larson & Bull, 1992a). These differences are not, however, reflected in the urinary excretion of dichloroacetic acid (Green & Prout, 1985; Dekant *et al.*, 1986b): In both mice and rats, the blood levels of dichloroacetic acid are at least one order of magnitude lower than those of trichloroacetic acid (Larson & Bull, 1992a). Strain differences among mice in the metabolism of trichloroethylene to trichloroacetic acid are also apparent: In Swiss and B6C3F1 mice, trichloroacetic acid in urine accounts for 7–12% of an oral dose of trichloroethylene; in NMRI mice, trichloroacetic acid is only a trace metabolite of trichloroethylene (Dekant *et al.*, 1986b).

The elimination rates of the major trichloroethylene metabolites differ markedly. Trichloroethanol and chloral hydrate are cleared from the blood with a half-life of 1–2 h, whereas high concentrations of trichloroacetic acid are present for up to 30 h and are cleared only slowly (Kimmerle & Eben, 1973). The amounts of trichloroethylene that are cleared by exhalation depend on the administered dose.

No changes in metabolite profiles were observed after exposure of rats to 55 ppm (286 mg/m^3) trichloroethylene by inhalation for 14 weeks (Kimmerle & Eben, 1973). Daily administration by gavage of 1000 mg/kg bw trichloroethylene to male B6C3F1 mice for 180 days did not induce the overall metabolism of trichloroethylene (Green & Prout, 1985).

Cytochrome P450 activity in mouse lung Clara cells was reduced following exposure to 100 ppm [537 mg/m^3] trichloroethylene for 6 h; the activities of glutathione *S*-transferases were unaffected. Studies with isolated mouse lung Clara cells showed oxidative metabolism of trichloroethylene, leading to accumulation of chloral in the cells, which were presumably unable to metabolize chloral further to trichloroethanol, as occurs in the liver (Odum *et al.*, 1992).

The metabolism of trichloroethylene in liver microsomes from mice and rats has been studied by determining changes in trichloroethylene concentrations in the headspace of incubation vials containing liver subfractions. The apparent Michaelis-Menten constant (K_m) and the maximal metabolic velocity (V_{max}) in microsomal fractions were 4.2 μmol/L and 8.0 mmol/mg protein per 10 min, respectively, for substrate concentrations of 0.3–34 μmol/L (Kim *et al.*, 1994). Chloral hydrate was found consistently as an end-product of trichloroethylene biotransformation. The formation of chloral hydrate and the cofactor requirements suggest that a cytochrome P450 (probably 2E1) catalyses the formation of chloral hydrate from trichloroethylene (Byington & Leibman, 1965; Leibman & McAllister, 1967; Leibman, 1968; Costa *et al.*, 1980; Guengerich *et al.*, 1991). Other cytochrome P450 enzymes may also catalyse the oxidation of trichloroethylene but have a lower affinity (Nakajima *et al.*, 1990, 1992).

An epoxide (1,2,2-trichlorooxirane) was postulated as an intermediate during the oxidation of trichloroethylene to chloral hydrate (Bonse *et al.*, 1975; Greim *et al.*, 1975; Bonse & Henschler, 1976; Henschler, 1977; Henschler & Bonse, 1977; Hathway, 1980); however, later studies on the biotransformation of trichloroethylene and other chlorinated olefins and knowledge of the mechanisms of oxidation by cytochrome P450 enzymes suggest a stepwise oxidation of trichloroethylene to chloral hydrate, in which the epoxide is not an obligatory intermediate (Miller & Guengerich, 1982; Liebler & Guengerich, 1983; Miller & Guengerich, 1983). Mouse liver microsomes had a threefold higher capacity for the oxidative biotransformation of trichloroethylene than rat liver microsomes (Miller & Guengerich, 1982).

Incubation of trichloroethylene with liver microsomes and liver cytosol from rats in the absence of cofactors for oxidative biotransformation by cytochrome P450 and in the presence of glutathione resulted in the formation of *S*-(1,2-dichlorovinyl)glutathione at low rates (Dekant *et al.*, 1990).

4.1.3 Comparison of humans and animals

A quantitative comparison of the metabolism of trichloroethylene in humans and rats and mice by application of physiologically based pharmacokinetic models suggests that humans have a lower rate of metabolism (14.9 mg/kg bw per h) than B6C3F1 mice (23.2 mg/kg bw per h in females and 32.7 mg/kg bw per h in males) but a slightly higher rate than Fischer 344 rats (11 mg/kg bw per h) (Allen & Fisher, 1993). In the absence of comparative studies, the role of saturable metabolism in humans cannot be assessed; however, in the occupationally and environmentally relevant range of exposures, the metabolism of trichloroethylene after exposure by inhalation seems to be similar in humans and rats. Qualitatively, the pathways of biotransformation in humans and animals are identical, and most metabolites identified in experimental animals have also been found in humans; however, whereas the urinary excretion of trichloroacetic acid remains constant in rats exposed repeatedly to trichloroethylene, the quantity increases steadily in humans over five days. The opposite trend is observed for trichloroethanol, the urinary excretion increasing in rats and remaining constant in humans. The kinetics of the biotransformation of trichloroethylene to trichloroacetic acid in isolated hepatocytes was markedly species dependent: The V_{max}/K_m values ('intrinsic clearance') in mouse, rat and human hepatocytes were 3.8×10^{-6}, 1.2×10^{-7} and 3.25×10^{-8} L/min per 10^6 cells, respectively (Elcombe, 1985).

4.2 Toxic effects

4.2.1 Humans

The acute toxicity of trichloroethylene in humans is characterized mainly by depression of the central nervous system: In 288 cases of acute intoxication with trichloroethylene, effects on the central nervous system were the major toxic manifestations. Liver toxicity was seen in only five individuals, and there was no renal damage (McCarthy & Jones, 1983).

Chronic exposure to trichloroethylene has been reported to be hepatotoxic, and trichloroethylene has also been implicated in the so-called 'psycho-organic syndrome' (McCarthy & Jones, 1983). There was no direct evidence for renal toxicity in humans exposed chronically to low levels of trichloroethylene (50 mg/m^3) (Seldén *et al.*, 1993).

4.2.2 Experimental systems

The oral LD_{50} values for trichloroethylene are 7183 mg/kg bw in rats (Smyth *et al.*, 1969) and 2400–2850 mg/kg bw in mice (Aviado *et al.*, 1976; Tucker *et al.*, 1982). The LC_{50} in rats was 26 300 ppm [136 760 mg/m^3] for a 1-h exposure (Vernot *et al.*, 1977) and 12 500 ppm [65 000 mg/m^3] for a 4 h-exposure (Siegel *et al.*, 1971).

The major toxic effects in animals are depression of central nervous function and sensitization of cardiac function to adrenalin. After acute exposure of Fischer 344 rats to high doses

of trichloroethylene, liver damage was observed, characterized by increased activities of serum glutamic–oxaloacetic acid and glutamic–pyruvic transaminases. Administration of high doses of trichloroethylene after pretreatment with phenobarbital also induced renal damage (Chakrabarti & Tuchweber, 1988). High oral doses of trichloroethylene (> 2000 mg/kg bw) damaged Clara cells in mouse lung (Scott *et al.*, 1988; Forkert & Birch, 1989), and dose-dependent damage to mouse Clara cells was observed after single exposures to 200–1000 ppm [1040–5200 mg/m^3] by inhalation for 6 h; no effect was seen at 20 ppm [104 mg/m^3]. The effect seems to be species-specific, since inhalation of 1000 ppm [5200 mg/m^3] trichloroethylene for 6 h had no toxic effects on the rat lung (Odum *et al*., 1992).

In male Sprague-Dawley rats injected once intraperitoneally with trichloroethylene at 1 mmol/kg bw [131 mg/kg bw], the activities of serum bile acids, particularly cholic and taurocholic acids, were increased 4 and 8 h after dosing. These times reflect those at which high levels of trichloroethylene and trichloroethanol appear in serum and liver. The selected dose did not induce hepatotoxic effects, and it was suggested that the changes in bile acid activity were due to perturbation of a physiological process (Bai & Stacey, 1993; Hamdan & Stacey, 1993).

Studies on the longer-term toxicity of trichloroethylene in rats and mice exposed orally and by inhalation showed consistent increases in relative liver weight and associated histopathological and biochemical changes. The effects described in kidney included increased relative weights in mice exposed continuously to > 75 ppm (> 390 mg/m^3) trichloroethylene for 30 days and renal dysfunction in the absence of marked histopathological changes in rats exposed to > 50 ppm [> 260 mg/m^3] for 12 weeks (Kjellstrand *et al.*, 1981a,b; Stott *et al.*, 1982; Tucker *et al.*, 1982; Kjellstrand *et al.*, 1983a,b; Elcombe *et al.*, 1985; Nomiyama *et al.*, 1986).

Oral administration of 500–1500 mg/kg bw trichloroethylene for 10 consecutive days increased the weight of the liver and the synthesis of DNA and decreased hepatic DNA concentrations in B6C3F1 and Alderley Park mice (Elcombe *et al.*, 1985). Increased hepatic DNA synthesis and mitosis, but no unscheduled DNA synthesis (see section 4.4.2), have been reported in mice dosed with trichloroethylene by gavage or inhalation (Stott *et al.*, 1982; Dees & Travis, 1993).

Trichloroethylene has been shown to induce hepatic peroxisome proliferation in mice, causing substantial increases in cyanide-insensitive palmitoyl coenzyme-A oxidase activity and peroxisomal volume density. The minimal daily dose of trichloroethylene reported to induce this effect in mice is 100 mg/kg bw over 10 days (Elcombe, 1985). Increased hepatic cyanide-insensitive palmitoyl coenzyme A oxidase activity has been reported in Fischer 344 rats treated by gavage with much higher doses of trichloroethylene (1200 mg/kg bw for 14 days, 130%; 1000 mg/kg bw for 10 days, 180% increase) (Goldsworthy & Popp, 1987; Melnick *et al*., 1987). Increases of 786% and 625% in the activity of this enzyme were reported in B6C3F1 mice treated with 1000 mg/kg bw per day for 10 days (Elcombe *et al.*, 1985; Goldsworthy & Popp, 1987).

Trichloroethylene has been shown to induce a small increase in cyanide-insensitive palmitoyl coenzyme A oxidation activity in the kidneys of both mice and rats after oral dosing with 1000 mg/kg bw per day for 10 days. Greater effects were observed in mice than in rats (Goldsworthy & Popp, 1987).

Two metabolites of trichloroethylene, dichloroacetic acid and trichloroacetic acid (see monographs, this volume), have also been shown to induce peroxisome proliferation in mice and rats (Elcombe, 1985; Goldsworthy & Popp, 1987; DeAngelo *et al.*, 1989). Trichloroacetic acid induced peroxisome proliferation in the kidney of mice, but not rats (Goldsworthy & Popp, 1987).

Trichloroethylene has been reported to inhibit the activity of the natural immune system (natural killer, natural cytotoxic and natural P815 killer cells) in Sprague-Dawley rats and B6C3F1 mice (Wright *et al.*, 1991). The inhibition was particularly evident in the liver after administration *in vivo* and in both liver and spleen after exposure *in vitro*. The background activities of natural immune activities had previously been reported to be higher in species and strains with lower background incidences of liver tumours (Wright & Stacey, 1991). More recently, trichloroethylene has been shown to inhibit aspects of the natural immune system in cells isolated from human liver (Wright *et al.*, 1994). Inhibition of natural immunity may therefore enhance the likelihood of tumour development.

Nuclear magnetic resonance was used to show that trichloroethylene interacts non-specifically with lipid molecules and that, in phosphatidylcholine bilayers, interaction occurs predominantly with the interfacial region rather than the hydrocarbon interior (Bhakuni & Roy, 1994).

4.3 Reproductive and prenatal effects

4.3.1 Humans

(a) Endocrine and gonadal effects

Out of a group of 99 metal workers in Aarhus (Denmark), 15 men who degreased parts with trichloroethylene for more than 20 h per week were asked to deliver a semen specimen (Rasmussen *et al.*, 1988). Twelve were included in the analysis and compared with 14 un-exposed physicians. There was no difference between the two groups in terms of sperm count or morphology, but the exposed group had a small, non-significant increase in the prevalence of mature spermatozoa containing two fluorescent Y bodies, which may indicate Y-chromosomal nondisjunction.

(b) Fertility

Taskinen *et al*. (1989) conducted a nested case–control study of 120 cases of spontaneous abortion and 251 controls on the basis of a file of 6000 Finnish workers who had been biologically monitored for exposure to solvents. Information about their marriages and their wives' pregnancies and spontaneous abortions were obtained from national registries; data on paternal occupational exposure to solvents were collected by means of a questionnaire sent to workers and covered the period of spermatogenesis. The likelihood of exposure was defined in three categories: unexposed, potentially exposed (i.e. use of solvents was possible but no exposure was reported or measured) and probably exposed (i.e. exposure was measured or reported). No association was found between paternal occupational exposure to trichloroethylene and spontaneous abortion (crude odds ratio, 1.0; 95% CI, 0.6–2.0).

(c) Pregnancy

Pregnancies occurring among 3265 women biologically monitored for exposure to solvents in 1965–83 were identified from a Finnish database (Lindbohm *et al*., 1990). Only one pregnancy per woman was included, resulting in a total of 120 cases of spontaneous abortion; 336 age-matched controls were randomly selected among women who had only normal births during the study period. Data on workplace, occupational exposure, medical history, alcohol and smoking habits were obtained from a postal questionnaire, to which 85.5% of subjects responded. For each potential exposure, women were classified, without knowledge of their case or control status, into one of three categories: unexposed, potentially exposed (i.e. work tasks might have involved use of solvents, but exposure was not reported or measured) or exposed (i.e. exposure was measured or reported). The analysis addressed 73 women who had had a spontaneous abortion and 167 controls who reported a pregnancy of interest and detailed information on occupational exposures during pregnancy. The odds ratio for spontaneous abortion, adjusted for previous spontaneous abortions, parity, smoking, use of alcohol and exposure to other solvents, was 0.6 (95% CI, 0.2–2.3) for exposure to trichloroethylene.

The 852 women for whom a spontaneous abortion was certified in one of the 11 hospital laboratories in Santa Clara County, CA (United States) were compared with 1618 controls randomly selected among County residents who had had a live birth and frequency matched by date of last menstrual period and hospital (Windham *et al*., 1991). All participants were contacted by telephone and asked about occupational use of 18 solvents or products during the first 20 weeks of pregnancy. An excess risk for spontaneous abortion was observed for those women who reported exposure to trichloroethylene (crude odds ratio, 3.1; 95% CI, 0.92–10.4) [adjusted odds ratio not calculated]; four of the seven women who reported exposure to trichloroethylene had also used tetrachloroethylene. The odds ratio increased for women who reported more ‘intense’ exposure, primarily on the basis of detection of odour (odds ratio, 3.9; $p = 0.04$). Odds ratios adjusted for maternal age, race, education, prior fetal loss, smoking, average number of hours worked and quality of response were nonsignificant when the whole group of halogenated solvents was considered (odds ratio for any use, 1.0; 95% CI, 0.65–1.6; odds ratio for use > 10 h per week, 1.5, 95%CI, 0.73–3.0).

Information on 7316 pregnancies was obtained from the hospital discharge register for 9186 women identified as working in Finnish laboratories (Taskinen *et al*., 1994).The pregnancies resulted in 5663 births, 687 spontaneous abortions and 966 induced abortions, and a case–referent study was conducted within the cohort. Questionnaires were posted requesting confirmation of the study pregnancy and data on exposures; the response rate was 78%. The 206 women with only one registered spontaneous abortion and 329 controls randomly selected among women who had given birth to a normal infant were included in the analysis of spontaneous abortion. The analysis of congenital malformations involved 36 cases and 105 referents. Seven women who had had a spontaneous abortion and nine controls reported exposure to trichloroethylene, giving an odds ratio of 1.6 (95% CI, 0.5–4.8), adjusted for employment, smoking, alcohol consumption, parity, previous miscarriages, failed birth control and febrile disease during pregnancy. The odds ratios associated with exposure to halogenated solvents as a group were 0.6 (0.4–1.1) for exposure on one to two days per week and 1.8 (0.9–3.7) for exposure on three to five days per week. The odds ratio for congenital malformations

associated with exposure to halogenated solvents was 0.8 (0.2–2.5), adjusted for alcohol consumption, parity, previous miscarriages and failed birth control.

In 1981, the groundwater in a small area in the southwestern part of the city of Tucson, Arizona (United States), was found to be contaminated with trichloroethylene and, to a lesser extent, with dichloroethylene and chromium (Goldberg *et al.*, 1990). The parents of 707 children with congenital heart disease who had conceived their child and spent the beginning of the pregnancy (one month before and the first trimester) in the Tucson valley between 1969 and 1987 were interviewed. The prevalence of congenital heart disease among children born to mothers who had been exposed (0.68%) was higher than that of mothers who lived outside the area (0.26%; $p < 0.001$). The ratio decreased to near unity for new arrivals in the contaminated area after closure of the well.

4.3.2 Experimental systems

Trichloroethylene and its metabolites appear to cross the placenta readily in many species (Helliwell & Hutton, 1949, 1950; Lanham, 1970; Withey & Karpinski, 1985; Ghantous *et al.*, 1986). In mice, inhalation of trichloroethylene resulted in accumulation of its metabolite, trichloroacetic acid (see also Land *et al.*, 1981), in amniotic fluid (Ghantous *et al.*, 1986).

A significant increase in the percentage of abnormal spermatozoa was observed in mice exposed to 0.2% trichloroethylene for 4 h per day for five days over that in controls and in mice exposed to 0.02% trichloroethylene (Land *et al.*, 1981). No sperm toxicity was induced in male Long-Evans rats exposed by gavage to up to 1000 mg/kg bw, trichloroethylene on five days per week for six weeks (Zenick *et al.*, 1984). Mating of untreated female NMRI mice with male mice that had been exposed to up to 450 ppm [2417 mg/m^3] trichloroethylene by inhalation for 24 h did not influence fertilization or pre- or post-implantation rates and did not induce dominant lethal mutation (Slacik-Erben *et al.*, 1980). No modification of mating performance or female fertility was observed in groups of female Long-Evans rats exposed to trichloroethylene by gavage for two weeks before mating at doses up to 1000 mg/kg bw, which was a toxic dose (Manson *et al.*, 1984). Administration of trichloroethylene in the diet of mice and rats at concentrations equivalent to doses of up to 300 mg/kg bw per day for two generations resulted in marginal effects on testicular weight and on survival of pups of both the F_1 and F_2 generations at the highest dose. No other signs of reproductive toxicity were observed (United States National Toxicology Program, 1985, 1986).

Female Long-Evans rats were exposed by inhalation to 1800 ± 200 ppm [9666 ± 1074 mg/m^3] trichloroethylene for two weeks before and/or during gestation. Post-natal body weight was decreased in the offspring of mothers that had been exposed before gestation. Significant increases in the incidence of skeletal and soft-tissue anomalies, indicative of developmental delay in maturation rather than teratogenesis, were observed in the group exposed during pregnancy alone (Dorfmueller *et al.*, 1979). A significant increase in the incidence of cardiac malformations was reported in newborn Sprague-Dawley rats after maternal exposure to trichloroethylene in drinking-water (1.5 or 1100 ppm [mg/L]) for seven days before and throughout gestation. [The actual dose could not be calculated from the available data.] No signs of maternal toxicity or other signs of fetal toxicity were observed (Dawson *et al.*, 1993). No increase in the frequency of birth defects has been reported in most other studies of rat or mouse

dams exposed by various routes to various concentrations of trichloroethylene, except for a predictable impairment of fetal growth associated with maternally toxic doses (Schwetz *et al.*, 1975; Leong *et al.*, 1975; Healy & Wilcox, 1978; Hardin *et al.*, 1981; Cosby & Dukelow, 1992).

The male offspring of female rats exposed to trichloroethylene in the drinking-water at up to 1250 mg/L before and during gestation and postpartum up to day 21 had enhanced locomotor activity and exploratory behaviour (Taylor *et al.*, 1985). Impairment of myelinization of the central nervous system and decreased glucose uptake by whole brain and cerebellum were observed in the offspring of rats exposed to 312 or 625 mg/L trichloroethylene in the drinking-water before and during gestation and postpartum (Noland-Gerbee *et al.*, 1986; Isaacson & Taylor, 1989). The specific gravity of brain tissue was reduced in the offspring of mice exposed to 150 ppm [806 mg/m^3] trichloroethylene by inhalation four weeks before and during gestation (Westergren *et al.*, 1984).

4.4 Genetic and related effects

4.4.1 Humans

Cytogenetic damage in lymphocytes: In a study of 28 male degreasers exposed to trichloroethylene, nine were reported to have > 13% hypodiploid cells in cultured peripheral lymphocytes (Konietzko *et al.*, 1978). These men had been exposed to a higher mean maximal concentration of trichloroethylene (206 ppm [1106 mg/m^3]) than those considered to have normal rates of hypodiploidy (116 ppm [623 mg/m^3]). A correlation ($r = 0.46$; $p < 0.05$) was also seen between the hypodiploidy rate and the average daily or average maximal exposure to trichloroethylene. The mean rate of hypodiploid cells was 10.9% (SD, 4.5; $n = 27$, excluding one man with karyotype 47, XY, +mar), in comparison with 6.5% (SD, 3.2) among 10 male controls. The exposed workers also had a fivefold higher mean rate of chromosomal breaks per 100 mitoses (3.1; SD, 3.7; $n = 27$) than the controls (0.6; SD, 0.7; $n = 10$), but these data were not commented upon. The effects of age and cigarette smoking could not be judged from the report. [The Working Group noted that the hypodiploidy rate among controls was very high.]

In a study of 22 workers who had constantly used trichloroethylene in their [unspecified] jobs for an average of 9.7 years (range, 0.7–34) and 22 controls matched for age, sex and smoking habits, no increase in the frequency of sister chromatid exchange was seen in peripheral lymphocyte (Nagaya *et al.*, 1989). Spot urine samples collected at the same time as the blood samples from the exposed workers showed a concentration of 19.1–1066.4 mg/L (mean, 183.6 mg/L) total trichloro compounds. Smoking increased the frequency of sister chromatid exchange.

A group of 15 workers involved in metal degreasing with trichloroethylene for more than 20 h per week in a half-open vapour plant had a significantly greater frequency of chromosomal aberrations, excluding gaps and hyperdiploid cells, in cultured lymphocytes than 669 controls; seven of the degreasers were also painters. The mean urinary concentration of trichloroacetate was fairly low: 3.7 mg/L (range, 0.02–26.9), and the mean number of cumulative working years was 4.6 (range, 0.8–22.0) (Rasmussen *et al.*, 1988). The effects of smoking and age could not be judged from the paper. The authors considered the reference group ‘not ideal’ but reported that the distribution of confounding factors was no different from that in the average population.

Sperm counts and the frequencies of abnormal sperm heads and of sperm with two fluorescent Y bodies were not significantly different in the 12 workers and 14 controls from whom semen samples containing sperm were taken.

Sister chromatid exchange was analysed in 22 male and 16 female workers in trichloroethylene synthesis and degreasing and in 26 control male and 25 female subjects who worked filling tanks with hydrogen, nitrogen and oxygen or as lathe operators (Seiji *et al.*, 1990). No effect of the occupational exposure was seen among nonsmokers, but the eight exposed smokers (all males) had a significantly higher mean frequency of sister chromatid exchange per cell (7.06) than seven male smoking controls (5.10). Sister chromatid exchange was also studied in nine male and 10 female tetrachloroethylene synthesis workers who had been exposed to an 8-h time-weighted geometric mean concentration of 8 ppm [43.0 mg/m^3] trichloroethylene (75th percentile, 49 ppm [263 mg/m^3]; maximum, 521 ppm [2798 mg/m^3]) and 17 ppm [115 mg/m^3] tetrachloroethylene (75th percentile, 28 ppm [190 mg/m^3]; maximum, 567 ppm [3844 mg/m^3]). They were compared with a control group of nine men and nine women and an extended control group consisting of 21 men and 23 women. Occupational exposure was reported to have affected the frequency of sister chromatid exchange in exposed male smokers, on the basis of a comparison of the frequency in these five men (7.33) with that in six nonsmoking male controls in the small (5.72; $p < 0.05$) and nine controls in the extended (5.48; $p < 0.01$) groups; the mean frequency of sister chromatid exchange in exposed male smokers was also higher than that in the 12 male smokers in the extended control group (5.7). No significant differences were reported between exposed and unexposed smokers. [Comparison of exposed smokers and unexposed nonsmokers may not be justified, especially as smoking usually induces sister chromatid exchange, although in this study such an effect could not be shown.]

4.4.2 Experimental systems (see also Tables 12 and 13 and Appendices 1 and 2)

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; European Centre for Ecotoxicology and Toxicology of Chemicals, 1994). The mechanisms of the possible genotoxicity of trichloroethylene were discussed by Henschler (1987).

(a) DNA binding

Trichloroethylene was reported to bind to DNA *in vitro* after metabolic activation; the binding was enhanced by the addition of glutathione and reduced by addition of SKF-525-A, an inhibitor of mixed-function oxidases. High-performance liquid chromatography indicated a possible DNA adduct, which could not be identified (Mazzullo *et al.*, 1992). DNA binding could not be demonstrated *in vivo* in several tissues of mice in one study (Bergman, 1983b) or in the liver of rats in another study (Parchman & Magee, 1982); however, the latter authors noted incorporation of label into normal nucleosides. A low level of covalent interaction was reported with the DNA of rat and mouse liver, kidney, lungs and stomach (estimated at 0.15 adducts per 10^6 nucleotides; Mazzullo *et al.*, 1992) and of mouse liver (maximum, 0.62 alkylations per 10^6 nucleotides; Stott *et al.*, 1982).

(b) Mutation and allied effects

The stabilizers often used in commercial preparations of trichloroethylene, such as epichlorohydrin and 1,2-epoxybutane, are mutagenic, rendering problematic the interpretation of positive results in assays for the mutagenicity of trichloroethylene *per se* (McGregor *et al*., 1989). Humans are exposed mostly, if not exclusively, to preparations containing stabilizers.

Apart from two reports in which trichloroethylene weakly induced mutation in *Salmonella typhimurium* TA1535, purified trichloroethylene did not induce gene mutation in various strains of *Salmonella* in the absence of metabolic activation; however, trichloroethylene containing directly mutagenic epoxide stabilizers did. Purified trichloroethylene also did not usually induce mutation in *Salmonella* in the presence of exogenous metabolic activation systems, except in two tests with *S. typhimurium* TA100.

Trichloroethylene (pure or of unspecified purity) gave negative results in the SOS chromotest in *Escherichia coli* with and 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*.

Trichloroethylene (pure or of unspecified purity) induced gene conversion in *Saccharomyces cerevisiae* in two of three studies and induced reverse mutation in all four studies available in the presence of a metabolic activation system. In a single study, pure trichloroethylene or trichloroethylene containing stabilizers did not induce forward mutation in *Schizosaccharomyces pombe*. Pure trichloroethylene induced forward mutation in one study of growing cultures of *Aspergillus nidulans*, which are capable of some metabolic activation reactions, whereas no such effect was 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*. Pure trichloroethylene did not cause recessive lethal mutations in *Drosophila melanogaster* after injection, and equivocal results were obtained after feeding.

Unscheduled DNA synthesis *in vitro* was reported in four studies, one with mouse and three with rat hepatocytes. Positive results were obtained with trichloroethylene (of unspecified purity) in mouse cells and in one study of rat cells, while negative results were obtained in the other two studies of rat primary hepatocytes, in one of which trichloroethylene of high and of unspecified purity were compared. Pure trichloroethylene induced gene mutation in mouse lymphoma L5178Y cells in the presence of exogenous metabolic activation. In a single study, pure trichloroethylene weakly induced sister chromatid exchange in Chinese hamster cells *in vitro* with and without metabolic activation. Pure trichloroethylene did not increase the frequency of chromosomal aberrations in Chinese hamster cells *in vitro*. In three different assays, trichloroethylene (of unspecified purity) weakly induced cell transformation in mouse, Syrian hamster and (pure trichloroethylene) rat cells *in vitro*, without exogenous metabolic activation. Pure trichloroethylene inhibited intercellular communication in mouse hepatocytes but not in rat hepatocytes *in vitro*.

A 95% pure formulation weakly induced sister chromatid exchange in the absence of metabolic activation in one study. No induction of gene mutation was seen in human lymphoblastoid cells exposed to pure trichloroethylene.

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.

Pure trichloroethylene induced DNA single-strand breaks/alkaline-labile sites *in vivo* in mouse liver and kidney and in rat liver. Unscheduled DNA synthesis was not augmented in mouse or rat hepatocytes after treatment with trichloroethylene (pure or of unspecified purity) *in vivo*, and pure trichloroethylene did not induce a significant response in a mouse spot test. Trichloroethylene did not induce chromosomal aberrations in mouse bone marrow *in vivo*. Micronuclei were reported to be induced by trichloroethylene (pure or of unknown purity) in mouse bone-marrow polychromatic erythrocytes in two studies (one was reported in an abstract), while two other studies showed no such effect. A significant increase ($p = 0.028$) observed in one of the latter studies was considered to be due to an exceptionally low control value. Micronuclei were not induced in mouse spermatocytes. In a study in which mice and rats were exposed by inhalation to reagent-grade trichloroethylene (purity, > 99%), micronuclei were induced in the bone-marrow cells of rats but not of mice; neither micronuclei, chromosomal aberrations nor sister chromatid exchange were induced in the peripheral lymphocytes of rats or the splenocytes of mice (Kligerman *et al*., 1994). In a single study, pure trichloroethylene did not induce dominant lethal mutation in mice. Trichloroethylene increased the frequency of S-phase in mouse hepatocytes *in vivo* but did not produce enzyme-altered foci in rat liver.

(c) Genetic effects of trichloroethylene metabolites

The genetic toxicology of dichloroacetic and trichloroacetic acids is reviewed in the relevant monographs in this volume. Dichloroacetyl chloride, a presumed metabolite of trichloroethylene, did not induce prophage in *E. coli*, but was weakly mutagenic in *S. typhimurium* TA100 in the absence of metabolic activation in one study.

The minor urinary metabolite, *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine, was mutagenic in *S. typhimurium* TA2638 in the presence of kidney cytosol, which allows deacetylation to the corresponding cysteine conjugate (Vamvakas *et al*., 1987). The presumed intermediate metabolite, *S*-(1,2-dichlorovinyl)-L-cysteine, was mutagenic to *S. typhimurium* TA100 and TA2638 in the presence and absence of metabolic activation (Green & Odum, 1985; Dekant *et al*., 1986c). *S*-(1,2-Dichlorovinyl)glutathione, the precursor of the cysteine conjugate, was also mutagenic to *S. typhimurium* TA2638 in the presence of rat kidney microsomes, which allow degradation to the cysteine conjugate (Vamvakas *et al*., 1988). Both the cysteine and the glutathione conjugate induced a low rate of unscheduled DNA synthesis in a cultured pig kidney cell line (Vamvakas *et al*., 1989). In the same cell line, *S*-(1,2-dichlorovinyl)-L-cysteine induced

DNA double-strand breaks and expression of the proto-oncogenes c-*fos* and c-*myc* (Vamvakas *et al.*, 1992; Vamvakas & Köster, 1993; Vamvakas *et al*., 1993). DNA single-strand breaks were observed in mouse kidney and double-strand breaks in rat kidney after intraperitoneal injection of *S*-(1,2-dichlorovinyl)-L-cysteine (Jaffe *et al*., 1985; McLaren *et al*., 1994).

^{35}*S*-(1,2-Dichlorovinyl)-L-cysteine metabolites bound to isolated DNA *in vitro* (Bhattacharya & Schulze, 1972).

(d) Mutations in proto-oncogenes in tumours from trichloroethylene-treated animals

A group of 110 male B6C3F1 mice, eight weeks of age, were given trichloroethylene in corn oil orally by gavage at a dose of 1700 mg/kg bw per day on five days per week for up to 76 weeks. There were two concurrent control groups, each consisting of 50 male mice: one was untreated and the other received corn oil at a dose of 10 ml/kg bw. Ten control mice in each group were killed at 76 weeks, and the remainder were killed at 96, 103 and 134 weeks [numbers not stated]. At death, liver tumours 0.5 cm in diameter were taken for histological examination and for analysis of oncogenes. At the time of the terminal kill, there were 24 untreated controls, 32 vehicle controls and 75 animals treated with trichloroethylene. The numbers of hepatocellular adenomas per mouse in animals in these three groups were 0.9 ± 0.06 (8%), 0.13 ± 0.06 (13%) and 1.27 ± 0.14 (67%); the corresponding numbers of hepatocellular carcinomas were 0.09 ± 0.06 (8%), 0.12 ± 0.06 (12%) and 0.57 ± 0.10 (39%), respectively. The authors noted numerous foci of cellular alteration (presumed preneoplastic lesions) in the livers of treated mice but only rare foci in the livers of controls. No neoplasms related to treatment were found at other sites. The frequency of mutations in codon 61 of H-*ras* was not significantly different in 76 hepatocellular tumours from trichloroethylene-treated mice and in those from the 74 combined historical and concurrent controls (51% *versus* 69%). The spectra of these mutations, however, showed a significant decrease in AAA and an increase in CTA in the tumours from treated mice in comparison with those from controls. Other H-*ras* and K-*ras* mutations each contributed 4% to the total in the treated mice, whereas their frequency appeared to be very low in the concurrent controls and none were seen in the historical controls. The authors interpreted these findings as suggesting that exposure to trichloroethylene provides the environment for a selective growth advantage for spontaneous CTA mutations in codon 61 of H-*ras* (Anna *et al*., 1994).

5. Summary and Evaluation

5.1 Exposure data

Trichloroethylene, a chlorinated solvent, has been produced commercially since the 1920s in many countries by chlorination of ethylene or acetylene. Its use in vapour degreasing began in the 1920s. In the 1930s, it was introduced for use in dry cleaning, but it has had limited use in that way since the 1950s. Currently, 80–90% of trichloroethylene worldwide is used for degreasing metals. Use for all applications in western Europe, Japan and the United States in 1990 was about 225 thousand tonnes.

Trichloroethylene has been detected in air, water, soil, food and animal tissues. The most heavily exposed people are those working in the degreasing of metals, who are exposed by inhalation.

5.2 Human carcinogenicity data

Three cohort studies were considered to be particularly relevant for the evaluation of trichloroethylene. Two of these studies, conducted in Sweden and Finland, involved people who had been monitored for exposure to trichloroethylene by measurement of trichloroacetic acid in urine. The levels in samples from most of the people in the two cohorts indicated relatively low levels of exposure. The third study, from the United States, covered workers exposed to trichloroethylene during maintenance of military aircraft and missiles, some of whom were also exposed to other solvents.

A fourth cohort study included all workers in an aircraft manufacturing company in the United States. This study was considered less relevant, as only one-third of the jobs in the plant entailed exposure to trichloroethylene and the exposures of the workers could not be classified.

In none of the available cohort studies was it possible to control for potential confounding factors, such as those associated with social class with regard to cervical cancer and smoking in respect of urinary bladder cancer.

Case–control studies have been conducted to investigate a number of cancer sites, including a multisite study from Montréal, Canada, in which other cancer cases were used as controls. Most of these studies do not provide risk estimates for exposure to trichloroethylene separately but only for groups of chemicals.

The results of the three most informative cohort studies consistently indicate an excess relative risk for cancer of the liver and biliary tract, with a total of 23 observed cases, whereas 12.87 were expected. The risk for these cancers was not elevated in the fourth, less informative cohort study. Results for liver cancer were given separately in the study from Finland and for the maintenance workers in the study in the United States. A total of seven cases were observed, whereas 4.00 were expected. Three case–control studies of primary liver cancer indicated elevated relative risks for people exposed to solvents, but only a few of the subjects in each study reported exposure to trichloroethylene.

With regard to non-Hodgkin's lymphoma, the results of the three most informative cohort studies were consistent; the data indicated a modest excess relative risk, with 27 cases observed and 18.9 expected. The risk for non-Hodgkin's lymphoma was not increased in the fourth, less informative study. In a case–control study covering all malignant lymphomas, an elevated odds ratio for exposure to trichloroethylene was indicated on the basis of seven exposed cases. The risk for non-Hodgkin's lymphoma was not increased among people assumed to have been exposed to trichloroethylene in the study in Montréal.

A twofold risk for cervical cancer was observed in two cohort studies.

The occurrence of cancer of the kidney was not elevated in the cohort studies; however, a study of German workers exposed to trichloroethylene revealed five cases of renal cancer whereas no case was found in an unexposed comparison group. The study may, however, have been initiated after the observation of a cluster. A case–control study and the multisite cancer

study, both from Montréal, Canada, provided discordant results with regard to cancer of the kidney.

The incidence of urinary bladder cancer was not increased in the two cohort studies from Sweden and Finland, whereas slightly increased numbers of deaths were seen in the two United States cohorts. The incidence of urinary bladder cancer was not increased in people assumed to be exposed to trichloroethylene in the Montréal study.

Data on cancer incidence or mortality have been reported from five areas in which groundwater was contaminated with trichloroethylene. A weak association between contamination and the incidence of leukaemia was indicated in two of these studies, from Massachusetts and New Jersey, United States. The cohort studies of trichloroethylene-exposed workers did not indicate an association with the occurrence of leukaemia. Two studies, from Finland and New Jersey, suggested a marginal increase in the occurrence of non-Hodgkin's lymphoma in areas with contaminated groundwater.

Overall, the most important observations are the elevated risk for cancer of the liver and biliary tract and the modestly elevated risk for non-Hodgkin's lymphoma in all three of the most informative cohort studies. Two of these studies reported data for primary liver cancer separately. Finally, the suggested marginally increased risk for non-Hodgkin's lymphoma in areas with trichloroethylene-contaminated groundwater is noted.

5.3 Animal carcinogenicity data

Trichloroethylene, with and without stabilizers, was tested for carcinogenicity by oral administration in two adequate experiments in mice. The studies showed significant increases in the incidences of benign and malignant liver tumours. Of seven studies in which trichloroethylene was given orally to rats, most were inconclusive because of reduced survival or a too short treatment. In two of the studies, the incidence of uncommonly occurring renal-cell tumours was significantly increased in male rats, and in one study an increased incidence of interstitial-cell testicular tumours was seen.

Trichloroethylene was tested for carcinogenicity by inhalation in four experiments in mice. One study showed an increased incidence of lymphomas, one study showed increased incidences of liver tumours, and three studies showed increased incidences of lung tumours. One of three experiments in which rats were exposed by inhalation showed an increased incidence of interstitial testicular tumours and a marginal increase in that of renal-cell tumours in males. No increase in tumour incidence was observed in one study in hamsters exposed by inhalation.

In limited studies, trichloroethylene and its proposed metabolite trichloroethylene oxide did not increase the incidence of skin tumours or local sarcomas in mice when administered by topical application or subcutaneous injection.

5.4 Other relevant data

In rodents, trichloroethylene is rapidly absorbed from the gastrointestinal tract and through the lungs, whereas absorption of the vapour through the skin is negligible. The major pathway is oxidative metabolism leading to the formation of chloroacetic acids. Mice showed consistently

higher rates of oxidative biotransformation than rats. A minor pathway in rodents and humans involves the formation of mercapturic acids.

The acute toxicity of trichloroethylene in rodents and humans is low. After high doses of trichloroethylene are administered repeatedly to rodents, damage is seen in liver and kidney (in mice and rats) and in lung (in mice only). Repeated exposure of humans in the workplace appears to have no marked toxic effects on the kidney or liver. Trichloroethylene is a more potent peroxisome proliferator in the livers of mice than of rats.

The available studies show no consistent effect of trichloroethylene on the human reproductive system. Trichloroethylene is metabolized to trichloroacetic acid in the placenta or fetus of many species. There is little evidence of toxic effects in developing rats or mice.

Studies of structural chromosomal aberrations, aneuploidy and sister chromatid exchange in peripheral lymphocytes of workers exposed to trichloroethylene were inconclusive.

Pure trichloroethylene did not induce chromosomal aberrations, dominant lethal mutations, sister chromatid exchange or unscheduled DNA synthesis in rodents, whereas an increased induction of micronuclei and DNA single-strand breaks/alkaline labile sites was observed.

In single studies with human cells *in vitro*, trichloroethylene of low purity slightly increased the frequencies of sister chromatid exchange and unscheduled DNA synthesis. Pure trichloroethylene did not induce gene mutation in human cells. In mammalian cells *in vitro*, pure trichloroethylene induced cell transformation, sister chromatid exchange and gene mutation, but not chromosomal aberrations. In fungi, trichloroethylene (pure or of unspecified purity) induced aneuploidy, gene mutation and mitotic recombination and induced gene conversion in the presence of metabolic activation.

Gene mutation or DNA damage was usually not induced in prokaryotes by pure trichloroethylene, while preparations containing epoxide stabilizers were mutagenic. Sulfur-containing metabolites formed by a minor trichloroethylene biotransformation pathway were genotoxic in bacteria and cultured renal cells.

5.5 Evaluation[1]

There is *limited evidence* in humans for the carcinogenicity of trichloroethylene.

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

Overall evaluation[2]

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

In making the overall evaluation, the Working Group considered the following evidence:

(i) Although the hypothesis linking the formation of mouse liver tumours with peroxisome proliferation is plausible, trichloroethylene also induced tumours at other sites in mice and rats.

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[1] For definition of the italicized terms, see Preamble.

[2] Dr N.H. Stacey disassociated himself from the overall evaluation.

(ii) Several epidemiological studies showed elevated risks for cancer of the liver and biliary tract and for non-Hodgkin's lymphoma.

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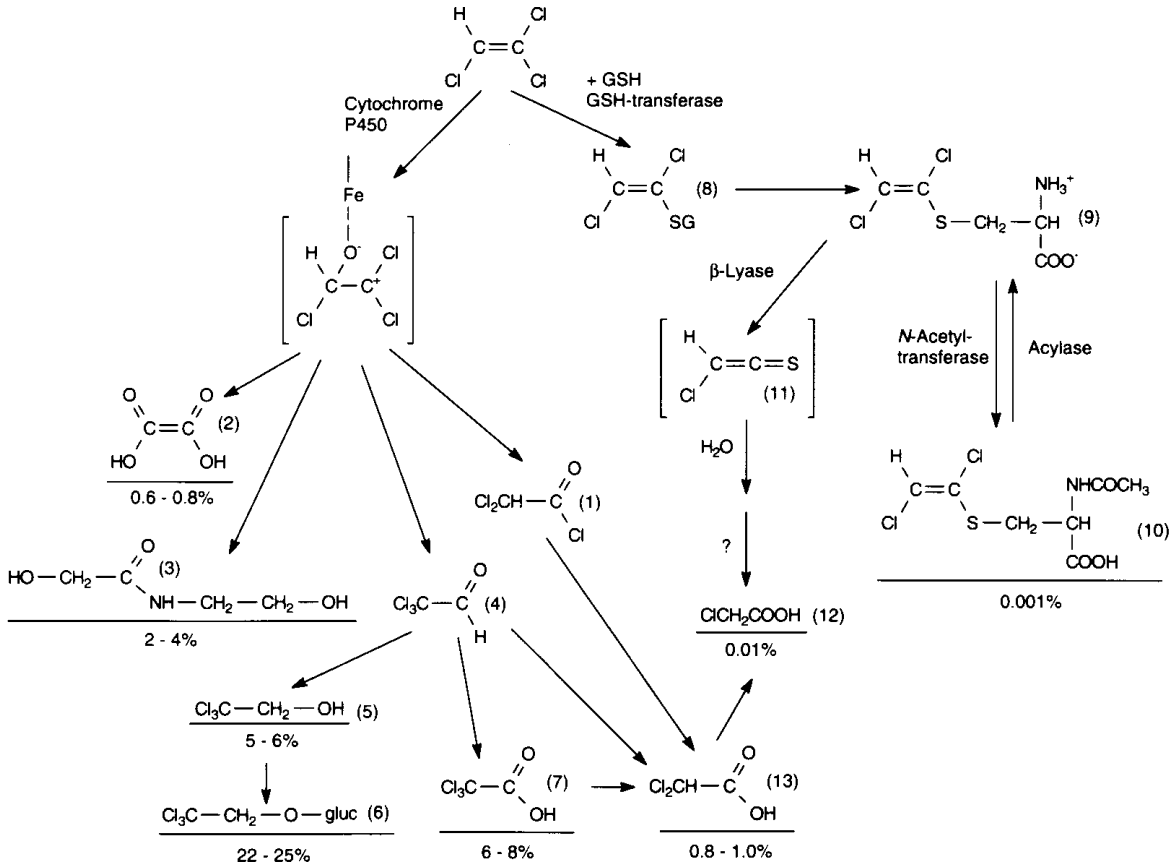
Figure 1. Proposed biotransformation of trichloroethylene to urinary metabolites in rats



Modified from Dekant *et al.* (1984); Dekant (1986)
Identified urinary metabolites are underlined; percentages are those of an oral dose of 200 mg/kg bw excreted as individual metabolites
1, Dichloroacetyl chloride; 2, oxalic acid; 3, *N*-(hydroxyacetyl)aminoethanol; 4, chloral; 5, trichloroethanol; 6, trichloroethanol glucuronide; 7, trichloroacetic acid; 8, dichlorovinylglutathione; 9, *S*-1,2-dichlorovinylcysteine; 10, *S*-1,2-dichlorovinyl-*N*-acetylcysteine; 12, monochloroacetic acid; 13, dichloroacetic acid

Table 11. Summary of data from four cohort studies of trichloroethylene

Cancer site	Axelson *et al*. (1994) 1421 men using trichloroethylene and monitored for exposure (Sweden, 1958–87)			Anttila *et al*. (1995) 3089 men and women using trichloroethylene and monitored for exposure (Finland, 1967–92)			Spirtas *et al*. (1991) 7282 men and women employed in aircraft maintenance and exposed to trichloroethylene (USA, 1953–82)			Garabrant *et al*. (1988) 14 067 men and women employed in aircraft manufacture (USA, 1958–82)		
	SIR	95% CI	Obs	SIR	95% CI	Obs	SMR	95% CI	Obs	SMR	95% CI	Obs
All cancers	0.96	0.80–1.2	107	1.1	0.92–1.2	208	[0.88]	[0.78–0.99]	281	0.84	0.77–0.93	453
Oesophagus	NR			NR			[1.0]	[0.37–2.2]	6	1.1	0.62–1.9	14
Stomach	0.70	0.23–1.6	5	1.3	0.75–2.0	17	[0.78]	[0.43–1.3]	14	0.40	0.18–0.76	9
Colon	1.0	0.44–2.0	8	0.84	0.36–1.7	8	[1.0]	[0.67–1.4]	29	0.96	0.71–1.3	47
Liver and biliary tract	1.4	0.38–3.6	4	[1.9]	[0.86–3.6]	9	[1.9]	[0.91–3.5]	10	0.94	0.40–1.9	8
Primary liver cancer				2.3	0.74–5.3	5	[1.1]	[0.14–4.0]	2			
Biliary tract				1.6	0.43–4.0	4	[2.2]	[0.96–4.4]	8			
Cervix	NR			2.4	1.1–4.8	8	2.2	0.61–5.7	4	0.61[a]	0.25–1.3	7
Prostate	1.3	0.84–1.8	26	1.4	0.73–2.4	13	0.80	0.50–1.2	22	0.93	0.60–1.4	25
Kidney	1.2	0.42–2.5	6	0.87	0.32–1.9	6	[1.1]	[0.46–2.1]	8	0.93	0.48–1.6	12
Urinary bladder	1.0	0.44–2.0	8	0.82	0.27–1.9	5	[1.4]	[0.70–2.5]	11	1.3	0.74–2.0	17
Skin	2.4	1.0–4.7	8	NR			[1.0][b]	[0.38–2.3]	6	0.7[c]	0.29–1.5	7
Brain and nervous system	NR			1.1	0.50–2.1	9	[0.78]	[0.36–1.5]	9	0.78	0.42–1.3	13
Lymphohaemato-poietic system				1.5	0.92–2.3	20	[0.94]	[0.66–1.3]	37	0.78	0.56–1.1	38
Non-Hodgkin's lymphoma	[1.5][d]	0.5–3.6	5	1.8[d]	0.78–3.6	8	[1.3][d]	[0.68–2.1]	14	0.82	0.44–1.4	13
Hodgkin's disease	1.1	0.03–6.0	1	1.7	0.35–5.0	3	[0.87]	[0.24–2.2]	4	0.73	0.20–1.9	4
Leukaemia	NR			1.1	0.35–2.5	5	[0.73][e]	[0.37–1.3]	11	0.82[d]	0.47–1.3	16

SIR, standardized incidence ratio; CI, confidence interval; Obs, observed; SMR, standardized mortality ratio; NR, not reported

[a] Female genital organs

[b] Malignant melanoma

[c] Includes five cases of malignant melanoma

[d] Including ICD 202

[e] Including aleukaemia

Table 12. Genetic and related effects of trichloroethylene without mutagenic stabilizers

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, SOS chromotest, *Escherichia coli* PQ37	–	–	7325[c]	Mersch-Sundermann *et al*. (1989)
SAF, *Salmonella typhimurium* BAL13, forward mutation (*ara* test)	–	–	190	Roldán-Arjona *et al.* (1991)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	(+)	160 vapour[c]	Simmon *et al.* (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	160 vapour[c]	Baden *et al*. (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	420 (8% vapour) 16h	Bartsch *et al.* (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	18 vapour	Crebelli *et al*. (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	260 vapour[d]	Shimada *et al.* (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	167[c]	Mortelmans *et al.* (1986)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	–	1050 vapour	McGregor *et al.* (1989)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	526 vapour[c]	Baden *et al.* (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	(+)	0	50[c]	Kringstad *et al*. (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	(+)	–	50 vapour[d]	Shimada *et al.* (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	167[c]	Mortelmans *et al.* (1986)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	167[c]	Mortelmans *et al.* (1986)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	167[c]	Mortelmans *et al.* (1986)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	–	1050 vapour	McGregor *et al.* (1989)
SCG, *Saccharomyces cerevisiae* D7, gene conversion	–	+	2600	Bronzetti *et al*. (1978)
SCR, *Saccharomyces cerevisiae* D7, reverse mutation	–	+	1300	Bronzetti *et al*. (1978)
ANG, *Aspergillus nidulans*, diploid *yA2*/+ strain 35x17, quiescent conidia, mitotic crossing-over	–	0	3660	Crebelli *et al.* (1985)
ANG, *Aspergillus nidulans*, diploid *yA2*/+ strain 35x17, growth-mediated assay, mitotic crossing-over	–	0	90 vapour	Crebelli *et al.* (1985)
SZF, *Schizosaccharomyces pombe* P1, stationary phase, forward mutation	–	–	3280	Rossi *et al.* (1983)
SZF, *Schizosaccharomyces pombe* P1, growing cells, forward mutation	–	–	13 140	Rossi *et al.* (1983)
ANF, *Aspergillus nidulans*, haploid strain 35, quiescent conidia, forward mutation (methionine suppressor)	–	0	100 vapour	Crebelli *et al*. (1985)
ANF, *Aspergillus nidulans*, haploid strain 35, 'growth-mediated assay', forward mutation (methionine suppressor)	+	0	13 vapour	Crebelli *et al*. (1985)

Table 12 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
ANN, *Aspergillus nidulans*, diploid *yA2*/+ strain 35×17, quiescent conidia, nondisjunctional diploids	–	0	3660	Crebelli *et al*. (1985)
ANN, *Aspergillus nidulans*, diploid *yA2*/+ strain 35×17, quiescent conidia, haploids	–	0	3660	Crebelli *et al*. (1985)
ANN, *Aspergillus nidulans*, diploid *yA2*/+ strain 35×17, 'growth-mediated assay', nondisjunctional diploids	+	0	40 vapour	Crebelli *et al*. (1985)
ANN, *Aspergillus nidulans*, diploid *yA2*/+ strain 35×17, 'growth-mediated assay', haploids	+	0	90 vapour	Crebelli *et al*. (1985)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	–		2500[c] injection	Foureman *et al.* (1994)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	?		5000 feeding[c]	Foureman *et al.* (1994)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	0	130 vapour[d]	Shimada *et al.* (1985)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	+	146[c]	Caspary *et al*. (1988)
SIC, Sister chromatid exchange, Chinese hamster ovary (CHO) cells *in vitro*	(+)	(+)	401[c]	Galloway *et al.* (1987)
CIC, Chromosomal aberrations, Chinese hamster ovary (CHO) cells *in vitro*	–	–	14 900[c]	Galloway *et al.* (1987)
TRR, Cell transformation, RLV/Fischer rat F1706 embryo cells *in vitro*	+	0	144	Price *et al.* (1978)
GIH, Gene mutation, human lymphoblastoid TK6 cells *in vitro*	–	–	600	Caspary *et al.* (1988)
ICR, Inhibition of intercellular communication, B6C3F1 mouse hepatocytes *in vitro*	+	0	1.3	Klaunig *et al.* (1989)
ICR, Inhibition of intercellular communication, F344 rat hepatocytes *in vitro*	–	0	13	Klaunig *et al.* (1989)
HMM, Host-mediated assay, gene conversion in *Saccharomyces cerevisiae* D4 recovered from CD-1 mouse liver, lungs and kidneys	+		400 po × 1[e]	Bronzetti *et al.* (1978)
HMM, Host-mediated assay, gene conversion in *Saccharomyces cerevisiae* D7 recovered from CD-1 mouse liver and kidneys	+		400 po × 1	Bronzetti *et al.* (1978)
HMM, 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)
HMM, Host-mediated assay, forward mutation in *Schizosaccharomyces pombe* P1, CD-1 × C57Bl hybrid mouse	–		2000 iv or ip × 1	Rossi *et al.* (1983)
DVA, DNA single-strand breaks, mouse liver *in vivo*	–		2000 ip × 1	Parchman & Magee (1982)
DVA, DNA single-strand breaks (alkaline unwinding) in liver and kidney of male NMRI mice *in vivo*	+[f]		790 ip × 1	Walles (1986)

Table 12 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DVA, DNA single-strand breaks (alkaline unwinding), mouse liver *in vivo*	+		1500 po × 1[c]	Nelson & Bull (1988)
DVA, DNA single-strand breaks (alkaline unwinding), rat liver *in vivo*	+		3000 po × 1[c]	Nelson & Bull (1988)
MST, Mouse spot test *in vivo*	–		350 ip × 1	Fahrig (1977)
UVM, Unscheduled DNA synthesis, CD-1 mouse primary hepatocytes *in vivo*	–		1000 po × 1	Doolittle *et al*. (1987)
MVM, Micronucleus induction, mouse bone-marrow erythrocytes *in vivo*	+		750 po × 2	Duprat & Gradiski (1980)
MVM, Micronucleus induction, B6C3F1 mouse bone-marrow erythrocytes *in vivo*	–		2500 ip × 3[c]	Shelby *et al.* (1993)
MVM, Micronucleus induction, mouse spermatocytes *in vivo* (spermatids examined) *in vivo*	–		565 inh 6 h/d × 5	Allen *et al.* (1994)
MVM, Micronucleus induction, mouse splenocytes *in vivo*	–		9800 inh 6 h	Kligerman *et al*. (1994)
MVR, Micronucleus induction, rat bone-marrow erythrocytes *in vivo*	+		5 inh 6 h	Kligerman *et al*. (1994)
MVR, Micronucleus induction, rat bone-marrow erythrocytes *in vivo*	–		960 inh 6 h × 4	Kligerman *et al*. (1994)
MVR, Micronucleus induction, rat peripheral lymphocytes *in vivo*	–		8800 inh 6 h	Kligerman *et al*. (1994)
MVR, Micronucleus induction, rat peripheral lymphocytes *in vivo*	–		960 inh 6 h × 4	Kligerman *et al*. (1994)
SVA, Sister chromatid exchange, rat peripheral lymphocytes *in vivo*	–		8800 inh 6 h	Kligerman *et al*. (1994)
SVA, Sister chromatid exchange, rat peripheral lymphocytes *in vivo*	–		960 inh 6 h × 4	Kligerman *et al*. (1994)
SVA, Sister chromatid exchange, mouse splenocytes *in vivo*	–		9800 inh 6 h	Kligerman *et al*. (1994)
CLA, Chromosomal aberrations, rat peripheral lymphcytes *in vivo*	–		8800 inh 6 h	Kligerman *et al*. (1994)
CLA, Chromosomal aberrations, rat peripheral lymphcytes *in vivo*	–		960 inh 6 h × 4	Kligerman *et al*. (1994)
CVA, Chromosomal aberrations, mouse splenocytes *in vivo*	–		9800 inh 6 h	Kligerman *et al*. (1994)
DLM, Dominant lethal mutation, male NMRI-Han/BGA mice *in vivo*	–		3400 inh 24 h[c]	Slacik-Erben *et al.* (1980)
BID, Binding (covalent) to salmon sperm DNA *in vitro*	–	+	270	Banerjee & Van Duuren (1978)
BID, Binding (covalent) to calf thymus DNA *in vitro*	–	+	340[c]	Bergman (1983b)
BID, Binding (covalent) to calf thymus DNA *in vitro*	0	+	13	Miller & Guengerich (1983)
BID, Binding (covalent) to DNA of isolated rat hepatocytes *in vitro*	+	0	13	Miller & Guengerich (1983)
BID, Binding (covalent) to DNA of isolated mouse hepatocytes *in vitro*	+	0	13	Miller & Guengerich (1983)
BID, Binding (covalent) to calf thymus DNA *in vitro*	0	+	131	DiRenzo *et al*. (1982)
BVP, Binding (covalent) to RNA of NMRI mouse spleen, lung, liver, kidney, pancreas, testis and brain *in vivo*	–[g]		67 ip × 5[c]	Bergman (1983b)

Table 12 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BVD, Binding (covalent) to DNA of NMRI mouse spleen, pancreas, lung, testis, kidney and brain *in vivo*	–[g]		67 ip × 5	Bergman (1983b)
BVD, Binding (covalent) to DNA of NMRI mouse liver *in vivo*	?		67 ip × 5	Bergman (1983b)
BVD, Binding (covalent) to DNA of B6C3F1 mouse liver *in vivo*	?		1200 po × 1	Stott *et al*. (1982)
BVD, Binding (covalent) to DNA of B6C3F1 mouse liver *in vivo*	?		250 ip × 1	Parchman & Magee (1982)
BVD, Binding (covalent) to DNA of rat liver *in vivo*	?		1000 ip × 1	Parchman & Magee (1982)
Dichloroacetyl chloride				
PRB, λ Prophage induction, *Escherichia coli* WP2	–	–	10 000	DeMarini *et al*. (1994)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	(+)	3	DeMarini *et al*. (1994)

[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] 99% purity or greater
[d] 0.001% stabilizers
[e] Also positive by gavage at 150 mg/kg for 5 days a week, 22 times with 400 mg/kg on the last day
[f] No DNA strand breaks in lungs of mice treated with 1300 mg/kg ip × 1
[g] Metabolic incoporation of ^{14}C into nucleotides was observed.

Table 13. Genetic and related effects of trichloroethylene containing mutagenic stabilizers or for which information on purity was not sufficiently clear

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, SOS chromotest, *Escherichia coli* PQ37	–	–	0.00	von der Hude *et al*. (1988)
***, Mutatox assay, derepression of luminescence operon, *Photobacterium phosphorium*	–	0	0.00	Elmore & Fitzgerald (1990)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	14 650	Henschler *et al.* (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	525 vapour	Waskell (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	260 vapour[c]	Shimada *et al.* (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	0.00	Milman *et al*. (1988)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	(+)	130 vapour	McGregor *et al*. (1989)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	50 vapour[c]	Shimada *et al.* (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	0.00	Milman *et al.* (1988)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	33 vapour	McGregor *et al.* (1989)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	0.00	Milman *et al*. (1988)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	525 vapour	Waskell (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	0.00	Milman *et al*. (1988)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	65 vapour	McGregor *et al*. (1989)
ECK, *Escherichia coli* K12, forward mutation	–	–	434	Greim *et al.* (1975)
ECK, *Escherichia coli* K12, reverse mutation (*arg*$^+$)	–	+	434	Greim *et al*. (1975)
ECK, *Escherichia coli* K12, reverse mutation (*gal*$^+$)	–	–	434	Greim *et al.* (1975)
ECK, *Escherichia coli* K12, reverse mutation (*nad*$^+$)	–	–	434	Greim *et al.* (1975)
SCG, *Saccharomyces cerevisiae* D7, log-phase cultures, gene conversion	0	+	1970	Callen *et al.* (1980)
SCG, *Saccharomyces cerevisiae* D7, log-phase and stationary cultures, gene conversion	–	–	2900	Koch *et al.* (1988)
SCG, *Saccharomyces cerevisiae* XV185-14C, reverse mutation (*lys*1-1, *his*1-7, *hom*3-10)	0	+	1460	Shahin & Von Borstel (1977)
SCR, *Saccharomyces cerevisiae* D7, log-phase cultures, reverse mutation	0	+	1970	Callen *et al.* (1980)
SCH, *Saccharomyces cerevisiae* D7, log-phase cultures, mitotic recombinants or otherwise genetically altered colonies (*ade*2)	0	+	1970	Callen *et al.* (1980)

Table 13 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SCR, *Saccharomyces cerevisiae* D7, log-phase and stationary cultures, reverse mutation	–	(+)	2900	Koch *et al.* (1988)
SZF, *Schizosaccharomyces pombe* P1, stationary phase, forward mutation	–	–	3280	Rossi *et al.* (1983)
SZF, *Schizosaccharomyces pombe* P1, growing cells, forward mutation	–	–	13 140	Rossi *et al.* (1983)
SCN, *Saccharomyces cerevisiae* D61.M, growing cells, aneuploidy	0	+	725	Koch *et al.* (1988)
TSM, *Tradescantia* species, mutation	+	0	0.0003	Schairer & Sautkulis (1982)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	0	130 vapour	Shimada *et al.* (1985)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	0	0.00	Milman *et al.* (1988)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	0	1445	Williams *et al.* (1989)
UIA, Unscheduled DNA synthesis, B6C3F1 mouse primary hepatocytes *in vitro*	+	0	0.00	Milman *et al.* (1988)
SIC, Sister chromatid exchange, Chinese hamster ovary (CHO) cells *in vitro*	0	–	9	White *et al.* (1979)
CIC, Chromosomal aberrations, Chinese hamster lung (CHL) cells *in vitro*	–	–	1000	Sofuni *et al.* (1985)
TBM, BALB/c–3T3 mouse cells, cell transformation *in vitro*	(+)	0	250	Tu *et al.* (1985)
TFS, Syrian hamster embryo cells, morphological transformation *in vitro*	(+)	0	25	Amacher & Zelljadt (1983)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	(+)	0	178	Gu *et al.* (1981)
HMM, Host-mediated assay, forward mutation in *Schizosaccharomyces pombe* P1 recovered from CD-1 mouse kidneys and lungs	–	0	2000 po × 1	Loprieno & Abbondandolo (1980)
HMM, Host-mediated assay, forward mutation in *Schizosaccharomyces pombe* P1 recovered from CD-1 mouse liver	(+)	0	2000 po × 1	Loprieno & Abbondandolo (1980)
HMM, Host-mediated assay, *Schizosaccharomyces pombe* P1, forward mutation, in CD-1 mouse peritoneum	(+)	0	1000 po × 1	Loprieno & Abbondandolo (1980)
HMM, Host-mediated assay, *Schizosaccharomyces pombe* P1, forward mutation, in Spraque-Dawley rat peritoneum	–	0	1000 po × 1	Loprieno & Abbondandolo (1980)
HMM, Host-mediated assay, forward mutation in *Schizosaccharomyces pombe* P1, CD-1 × 7BL hybrid mouse	–	0	2000 iv or ip × 1	Rossi *et al.* (1983)
UPR, Unscheduled DNA synthesis, Fischer-344 male rat hepatocytes *in vivo*	–		1000 po × 1	Mirsalis *et al.* (1989)
UVM, Unscheduled DNA synthesis, male and female B6C3F1 mouse hepatocytes *in vivo*	–		1000 po × 1	Mirsalis *et al.* (1989)

Table 13 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
CBA, Chromosomal aberrations, CD-1 mouse bone marrow cells *in vivo*	–		1000 po × 1	Loprieno & Abbondandolo (1980)
CBA, Chromosomal aberrations, mouse bone marrow cells *in vivo*	–		1200 po × 1	Sbrana *et al.* (1985) (abstract)
CBA, Chromosomal aberrations, mouse bone marrow cells *in vivo*	–		795 inh 7 h × 50[c]	Sbrana *et al.* (1985) (abstract)
MVM, Micronucleus induction, mouse bone marrow erythrocytes *in vivo*	+		1200 po × 1	Sbrana *et al.* (1985) (abstract)
MVM, Micronucleus induction, mouse bone marrow erythrocytes *in vivo*	+		460 ip × 1	Hrelia *et al.* (1995)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	(+)		0.00	Gu *et al*. (1981)
BID, Binding (covalent) to calf thymus DNA *in vitro*	0	+	3.2	Mazzullo *et al*. (1992)
BVD, Binding (covalent to DNA of BALB/c mouse liver, kidney, lung and stomach *in vivo*	(+)		0.76 ip × 1	Mazzullo *et al*. (1992)
BVD, 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, 5 d/week, 7 weeks	Milman *et al*. (1988)
***, Enzyme-altered foci in male Osborne-Mendel rat liver *in vivo*, initiation protocol, phenobarbital as promoter	–		1300 mg/kg	Milman *et al*. (1988)
***, S-Phase induction, male and female B6C3F1 mouse hepatocytes *in vivo*	+		200 mg/kg	Mirsalis *et al.* (1989)

NDEA, *N*-nitrosodiethylamine

[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, mg/ml; in-vivo tests, mg/kg bw; 0.00, dose not reported; ip, intraperitoneally; po, orally

[c]5 days/week, 10 weeks

***, Not included on profile

Appendix B: Excerpts from the 1990 National Toxicology Program (NTP) Technical Report Toxicology and Carcinogenesis Studies of Trichloroethylene (Without Epichlorohydrin) [CAS No. 79-01-6] in F344/N Rats and B6C3F1 Mice (Gavage Studies) (TR-243) pp. 16-60

NTP TECHNICAL REPORT

ON THE

CARCINOGENESIS STUDIES OF

TRICHLOROETHYLENE

(WITHOUT EPICHLOROHYDRIN)

(CAS NO. 79-01-6)

IN F344/N RATS AND B6C3F$_1$ MICE

(GAVAGE STUDIES)

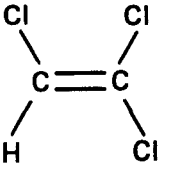
NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

May 1990

NTP TR 243

NIH Publication No. 90-1779

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health



TRICHLOROETHYLENE

CAS NO. 79-01-6
C_2HCl_3 Mol. Wt. 131.40

Trichloroethylene (TCE) is an industrial solvent used for vapor degreasing and cold cleaning of fabricated metal parts. TCE has also been used as a carrier solvent for the active ingredients of insecticides and fungicides, as a solvent for waxes, fats, resins, and oils, as an anesthetic for medical and dental use, and as an extractant for spice oleoresins and for caffeine from coffee. Trichloroethylene may be found in printing inks, varnishes, adhesives, paints, lacquers, spot removers, rug cleaners, disinfectants, and cosmetic cleansing fluids. TCE may also be used as a chain terminator in polyvinyl chloride production and as an intermediate in the production of pentachloroethane (Kirk-Othmer, 1963 and 1979; IARC, 1979; Defalque, 1961; Wetterhahn, 1972; U.S. CFR, 1976; Valle-Riestra, 1974; Waters et al., 1977). Trichloroethylene is no longer used with food, drugs, or cosmetics (IARC, 1979; Food Chemical News, 1978). Before 1976, tolerances for TCE in decaffeinated ground coffee were set at 25 ppm (U.S. CFR, 1976).

An estimated 3.5 million workers are exposed to TCE (Page, 1979). The threshhold limit value for TCE is 100 ppm (Federal Register, 1975). In 1979 production of TCE was 319,432,000 pounds (USITC, 1980).

Trichloroethylene has been found in various foodstuffs in England at the following concentrations: packet tea, 60 ppm; pig's liver, 22 ppm; butter, 10 ppm; and fresh bread, 7 ppm (McConnell et al., 1975). Trichloroethylene has also been found in commercial deionized charcoal-filtered water (Dowty et al., 1975) and in drinking water in various cities (Kavlock et al., 1979).

The oral LD_{50} is reported to be 5,200 mg/kg in rats (National Clearinghouse for Poison Control Centers, 1967). The intraperitoneal LD_{50} for mice is 3,200 mg/kg (Klaassen and Plaa, 1966). Trichloroethylene is a central nervous system depressant (Clayton and Clayton, 1981).

The results of mutagenicity testing of TCE are difficult to interpret because few authors provide analytical data regarding the purity of their test materials. The sensitivities of most of these tests are such that the presence of trace levels of potent genotoxic contaminants, such as epichlorohydrin, could affect the results generated. Differences in purity could explain the diversity of results reported from various laboratories. For example, "pure" TCE has been reported to be weakly mutagenic, equivocally mutagenic, or nonmutagenic for *S. typhimurium* TA100 (Baden et al., 1979; Bartsch et al., 1979; Simmon et al., 1977; Waskell, 1978). TCE did not cause mutations in *Salmonella typhimurium* TA98, TA100, TA1535, or TA1537, with or without metabolic activation; using Chinese hamster ovary (CHO) cells, TCE did not induce chromosome aberrations and the results for sister chromatid exchanges were considered equivocal (NTP unpublished results).

The results and conclusions of other workers employing a variety of test methods are similarly inconsistent. Cerna and Kypenova (1977) reported that TCE was mutagenic (without metabolic activation) in *in vitro* tests with *S. typhimurium* TA1535 and 1538 and in host-mediated assays with TA1950, 1951, and 1952. Greim et al. (1975) reported that microsomally activated TCE was slightly mutagenic for *Escherichia coli* K 12, but Loprieno et al. (1979) found no mutagenic activity in a series of short-term tests. Slacik-Erben (1980) studied TCE (99.5% pure) in a dominant lethal test in male Han/BGA NMRI mice and found no mutagenic activity.

Evidence for a carcinogenic effect of TCE was presented by the National Cancer Institute (NCI, 1976) after the completion of a 78-week bioassay of industrial grade (>99% pure) TCE in $B6C3F_1$ mice and Osborne-Mendel rats; without additional TCE administration, rats were observed for another 32 weeks and mice for 12 more weeks. In mice, time-weighted-average gavage doses of 1,169 and 2,339 mg/kg in males and 869 and 1,739 mg/kg in females were associated with significant increases in the incidence of hepatocellular carcinoma. In Osborne-Mendel rats, time-weighted-average gavage doses of 549 and 1,097 mg/kg (both sexes) did not increase the incidence of primary tumors. However, as in several earlier bioassays of chlorinated ethanes and ethylenes (hexachloroethane, NCI, 1978a; 1,1,2,2-tetrachloroethane, NCI, 1978b; 1,1,2-trichloroethane, NCI, 1978c; tetrachloroethylene, NCI, 1977; and pentachloroethane, NTP, 1982), the survival of the rats was compromised by the dosage regimen. The results of most of these earlier carcinogenicity studies have been summarized (Weisburger, 1977) and reviewed (IARC, 1979). The International Agency for Research on Cancer considered the TCE bioassay in Osborne-Mendel rats to be inadequate for evaluation and the bioassay in $B6C3F_1$ mice to provide limited evidence of carcinogenicity; that is, carcinogenic in one species (IARC, 1979). IARC evaluated TCE as being "carcinogenic to mice after its oral administration, producing liver and lung neoplasms" (IARC, 1982).

The interpretation of the earlier TCE study (NCI, 1976) was complicated by the presence of certain contaminants, particularly epichlorohydrin (0.09%) in the test material. Epichlorohydrin had been previously shown to induce local sarcomas in mice following subcutaneous injection (Van Duuren et al., 1974) and has subsequently been reported to cause nasal carcinomas in rats after inhalation exposure (Laskin et al., 1980). Further, epichlorohydrin is a potent mutagen for *S. typhimurium* TA100 (Simmon, 1977). Therefore, although the carcinogenicity of industrial-grade TCE in $B6C3F_1$ mice was firmly established, unequivocal statements regarding its carcinogenicity in rats and the carcinogenicity of pure TCE in mice could not be made.

Results of long-term inhalation studies with purified TCE (less than 0.000025% of each of 5 chlorinated hydrocarbon impurities by gc/ms; stabilized with 0.0015% triethanolamine) have been reported (Henschler et al., 1980). In these studies, male and female Wistar rats, NMRI mice, and Syrian hamsters were exposed to air containing up to 500 ppm of TCE for 18 months (6 hours per day, 5 days per week). This regimen failed to produce compound-related increases in primary tumors in these species. The investigators did report an increase in the incidence of malignant lymphomas in female mice, but the relationship of this lesion to TCE exposure was considered questionable because of the high incidence of lymphomas in control mice.

The evidence for the carcinogenicity of TCE, like that of the chlorinated ethanes and ethylenes tested earlier (hexachloroethane, tetrachloroethane, trichloroethane, tetrachloroethylene, and pentachloroethane) comes mainly from data obtained in experiments conducted in mice. For the most part, the carcinogenic classification of these materials is based upon dose-related increases in the incidences of hepatocellular carcinoma in $B6C3F_1$ mice. Because this is a relatively common tumor in males of this strain of mouse (seen in approximately 18% of control males and 3% of control females), the significance of the lesion is frequently questioned. Also, the reason for the apparent insensitivity of Osborne-Mendel rats to members of this chemical class remains unknown. It may be related to the reduced survival times of dosed animals. Therefore, the failure of the doses to increase tumor incidences in rats could have been due to the animals not surviving long enough to develop the lesions. However, in most of the earlier studies the survival times of both rats and mice were shortened by compound administration. In light of this observation, it is possible that Osborne-Mendel rats are not susceptible to the carcinogenicity of these chemicals. Inter- or intra-species differences in susceptibility to TCE could be mediated through inherited pharmacokinetic factors. Stott et al. (1982) reported that $B6C3F_1$ mice can metabolize more TCE (on a mg/kg basis) than can Osborne-Mendel rats. A similar quantitative difference in TCE metabolism between $B6C3F_1$ mice and Sprague-Dawley rats has recently been reported (Parchman and Magee, 1982). If a carcinogenic effect of TCE requires biotransformation of the parent molecule to a reactive metabolite, the $B6C3F_1$ mouse might be expected to be more sensitive than the Osborne-Mendel rat. Trichloroethylene epoxide has been suggested as an electrophilic metabolite of TCE (Van Duuren,

1975), but Miller and Guengerich (1982) have reported the results of *in vitro* experiments which suggest that the epoxide may not be an intermediate of TCE metabolism.

The possibility of a strain difference in susceptibility to TCE prompted the National Cancer Institute to initiate a series of chronic carcinogenicity studies of a variety of chlorohydrocarbons, including TCE, in several strains of rats. Most of these studies are still in progress; the NTP is preparing draft reports from completed carcinogenesis studies on four strains of rats (Marshall, ACI, Osborne-Mendel, and August) using the oral route, and Dr. C. Maltoni (Institute of Oncology, Bologna, Italy) is currently (August 1983) examining the histopathology portion of TCE inhalation experiments using Sprague-Dawley rats and Swiss and $B6C3F_1$ mice. The comparative testing of epichlorohydrin-free trichloroethylene administered by gavage to $B6C3F_1$ mice and F344/N rats has been completed. This report describes the results of that study.

II. MATERIALS AND METHODS

CHEMICAL ANALYSES

DOSE PREPARATION

THIRTEEN-WEEK STUDY

TWO-YEAR STUDIES

Study Design

Source and Specifications of Test Animals

Animal Maintenance

Clinical Examinations and Pathology

Data Recording and Statistical Methods

CHEMICAL ANALYSES

High purity "Hi-Tri" trichloroethylene was obtained in two lots. Lot No. TB 05-206AA was obtained from Dow Chemical Co. (Richmond, VA) and was used for the 13-week study and for the first 19 months of the 2-year study. Lot No. TB 08-039AA was obtained from Missouri Solvents (Kansas City, MO) and was used for the final 5 months of the 2-year study.

Purity and identity analyses were conducted at Midwest Research Institute. The results of elemental analyses for both lots were consistent with the theoretical values. Twelve impurities with areas totalling less than 0.04% of the area of the major peak were detected in Lot No. TB 05-206AA by gas-liquid chromatography in one system (Appendix G). Eight impurities having areas less than 0.02% that of the major peak were detected in a second system. One impurity with an area of 0.02% that of the major peak was detected in Lot No. TB 08-039AA. The area of all other impurities in this lot totaled less than 0.01% that of the major peak. These impurities were not identified. The infrared and nuclear magnetic resonance spectra of both lots were consistent with the literature spectra. "Hi-Tri" trichloroethylene contains 8 ppm of an amine stabilizer (diisopropylamine) and, if present, no more than 0.001% epichlorohydrin stabilizers as determined by gas chromatography/mass spectrometry.

Throughout the course of this study, the trichloroethylene was stored at 4°C. Papanicolaou periodically analyzed the chemical versus a standard, maintained at −20°C, by gas-liquid chromatography using a 10% OV-101 glass column at 70°C. The chemical showed no decrease in purity over the course of the study, even though a white flocculent material was noticed in the July 1979 reanalysis. A 5-gallon can of this material was returned to Midwest Research Institute for attempted purification. The flocculent material was present at a level of 25-30 ppm (Appendix H). Results of infrared and mass spectroscopy indicated that the precipitate was a mixture of long chain alkene or alkanes and inorganic carbonate. Midwest Research Institute shipped the filtered trichloroethylene back to Papanicolaou in October 1979.

The new lot (TB 08-039AA) was received at Papanicolaou in December 1979 and was used immediately. Both lots were considered to be greater than 99.9% pure.

DOSE PREPARATION

Doses were administered at a constant volume. Mice received 0.5 ml per dose and rats received 1.0 ml per dose. A stock solution of trichloroethylene in corn oil was prepared and appropriately diluted (Table 1). Trichloroethylene in corn oil (1% w/v) was found to be stable for 7 days at room temperature (Appendix I). Later, stock solutions of trichloroethylene in corn oil were found to be stable at 4°C for 4 weeks (Appendix J). Stock solutions were prepared once per week for the first 16 weeks of the study and once per month for the remainder of the study. Stock solutions were analyzed by gas chromatography and found to be within 10% of the target concentrations (Appendix K).

THIRTEEN-WEEK STUDIES

Neither single-dose nor 14-day studies were conducted. Dosage levels for the 13-week study were based on earlier experiences with TCE in rats and mice (NCI, 1976).

Five-week-old male and female F344/N rats and 3-week-old B6C3F$_1$ mice were obtained from Frederick Cancer Research Center. Rats were observed for 2 weeks and mice for 4 weeks. Animals were assigned to cages according to a table of random numbers. Cages were then assigned to dosed and control groups according to another table of random numbers.

Rats and mice were housed five per cage in polycarbonate cages (Table 1). Cages and bedding were changed twice per week. Purina® Lab Chow and water (via an automatic watering system) were available *ad libitum*.

Groups of 10 male rats were administered 0, 125, 250, 500, 1,000, or 2,000 mg/kg trichloroethylene in corn oil by gavage, 5 days per week for 13 weeks. Groups of 10 female rats were administered 0, 62.5, 125, 250, 500, or 1,000 mg/kg, and groups of 10 mice of each sex received 0, 375, 750, 1,500, 3,000, or 6,000 mg/kg on the same schedule. Doses of trichloroethylene were calculated on the basis of mean body weights from the previous weighing period.

Animals were checked for mortality and signs of morbidity twice daily. Each animal was given a clinical examination weekly.

At the end of the 13-week study, survivors were killed with carbon dioxide. Necropsies were performed on animals, unless precluded in whole or in part by autolysis or cannibalization. Thus the number of animals from which organs or tissues were examined microscopically varies and does not necessarily represent the number of animals that were placed on study in each group. Liver weights from animals of all dosed mouse groups were measured. The following were examined for the control and high dose rats and for the control and the two highest dosed groups of mice: gross lesions, tissue masses, abnormal lymph nodes, skin, mandibular lymph nodes, mammary gland, salivary gland, thigh muscle, sciatic nerve, bone marrow, costochondral junction (rib), thymus, larynx, trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, duodenum, jejunum, ileum, colon, mesenteric lymph nodes, liver, gallbladder (mice), pancreas, spleen, kidneys, adrenals, urinary bladder, seminal vesicles/prostate/testes or ovaries/uterus, nasal cavity, brain, pituitary, and spinal cord. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

TWO-YEAR STUDIES

Study Design

Groups of 50 rats of each sex were administered 500 or 1,000 mg/kg trichloroethylene in corn oil for up to 103 weeks. Groups of 50 mice of each sex were administered 1,000 mg/kg for up to 103 weeks. Doses of trichloroethylene administered were calculated on the basis of mean body weights from the previous weighing period. Groups of 50 rats and mice of each sex received corn oil only and served as vehicle controls (Table 1). In addition, groups of 50 rats of each sex were used as untreated controls. Tumor incidence data from these groups were not used for routine statistical analyses, but the incidences are shown in Appendix A.

Source and Specifications of Test Animals

Five and one-half-week-old male and female F344/N rats and B6C3F$_1$ mice were obtained from Harlan Industries (Indianapolis, IN), observed for 2.5 weeks, and assigned to individual cages according to a table of random numbers. The cages were then assigned to control and dosed groups according to another table of random numbers. Animals that died during the first 1.5 weeks of the study as a result of gavage error were replaced.

Animal Maintenance

Rats were housed five per cage in polycarbonate cages. Mice were housed 10 per cage for the first 8 months and then transferred to smaller cages housing five animals each. Cages and bedding were changed twice per week. Tap water, via an automatic watering system, and diet were available *ad libitum* (Table 1).

The temperature in the animal rooms was 22°-24°C and the humidity was 40%-60%. Ten to fifteen changes of room air per hour were provided. Fluorescent lighting provided illumination 12 hours per day.

Clinical Examinations and Pathology

All animals were observed twice daily for signs of morbidity or mortality. Clinical signs were recorded monthly. Body weights, by cage, were recorded weekly for the first 12 weeks and then every fourth week thereafter. The mean body weight of each group was calculated by dividing the total weight of animals in the group by the number of animals in the group. Moribund animals and animals that survived to the end of the bioassay were killed with carbon dioxide and necropsied.

Examinations for grossly visible lesions were performed on major tissues or organs. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following were examined microscopically: tissue masses, abnormal lymph nodes, skin, mammary gland, salivary gland, thymus, larynx, trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, duodenum, ileum, colon, mesenteric lymph nodes, liver, gallbladder (mice), pancreas, spleen, kidneys, adrenals, urinary bladder, seminal vesicles/prostate/testes or ovaries/uterus, brain, pituitary, and spinal cord.

Necropsies were performed on all animals not excessively autolyzed or cannibalized. The number of animals from which particular organs or tissues were examined microscopically varies and is not necessarily equal to the number of animals that were placed on study in each group.

Neoplastic nodules were classified according to the recommendations of Squire and Levitt (1975) and the National Academy of Sciences (1980). When the pathology examination was completed, the slides, individual animal data records, and summary tables were sent to an independent quality assurance laboratory. Individual animal records and tables were compared for accuracy, slides and tissue counts were verified, and histotechniques were evaluated. All tumor diagnoses, all target tissues, and all tissues from a randomly selected 10 percent of the animals were evaluated by an experienced rodent pathologist. Slides of all target tissues and those on which the original and quality assurance pathologists disagreed were submitted to the Chairperson of the Pathology Working Group (PWG) for the evaluation. Representative slides selected by the PWG Chairperson were reviewed blindly by the PWG's experienced rodent pathologists, who reached a consensus and compared their findings with the original diagnoses. When conflicts were found, the PWG sent the appropriate slides and their comments to the original pathologist for review. (This procedure is described, in part, by Maronpot and Boorman, in press.) The final diagnosis represents a consensus of contractor pathologists and the NTP Pathology Working Group.

Data Recording and Statistical Methods

Data on this experiment were recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969).

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing. Animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals in which that site was examined. In most instances, the denominators included only those animals for which that site was examined histologically.

However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling, or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the number of animals necropsied.

For the statistical analysis of tumor incidence data, two different methods of adjusting for intercurrent mortality were employed. Each used the classical methods for combining contingency tables developed by Mantel and Haenszel (1959). Tests of significance included pairwise comparisons of high and low dose groups with controls and tests for overall dose-response trends.

The first method of analysis assumed that all tumors of a given type observed in animals dying before the end of the study were "fatal;" i.e., they either directly or indirectly caused the death of the animal. According to this approach, the proportions of tumor-bearing animals in the dosed and control groups were compared at each point in time at which an animal died with a tumor of interest. The denominators of these proportions were the total number of animals at risk in each group. These results, including the data from animals killed at the end of the study, were then combined by the Mantel-Haenszel method to obtain an overall P value. This method of adjusting for intercurrent mortality is the life table method of Cox (1972) and of Tarone (1975).

The second method of analysis assumed that all tumors of a given type observed in animals dying before the end of the study were "incidental"; i.e., they were merely observed at autopsy in animals dying of an unrelated cause. According to this approach, the proportions of animals found to have tumors in dosed and control groups were compared in each of five time intervals: 0-52 weeks, 53-78 weeks, 79-92 weeks, week 93 to the week before the terminal kill period, and the terminal kill period. The denominators of these proportions were the number of animals actually autopsied during the time interval. The individual time interval comparisons were then combined by the previously described methods to obtain a single overall result. (See Peto et al., 1980, for the computational details of both methods.)

In addition to these tests, one other set of statistical analyses was carried out and reported in the tables analyzing primary tumors: the Fisher's exact test for pairwise comparisons and the Cochran-Armitage linear trend test for dose-response trends (Armitage, 1971; Gart et al., 1979). These tests were based on the overall proportion of tumor-bearing animals. All reported P values are one-sided.

For studies in which there is little effect of compound administration on survival, the results of the three alternative analyses will generally be similar. When differing results are obtained by the three methods, the final interpretation of the data will depend on the extent to which the tumor under consideration is regarded as being the cause of death.

TABLE 1. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS

	13-Week Studies
Experimental Design	
Size of Test Groups	10 males and 10 females of each species
Doses	Male rats: 0, 125, 250, 500, 1,000, or 2,000 mg/kg body weight in corn oil by gavage
	Female rats: 0, 62.5, 125, 250, 500, or 1,000 mg/kg body weight in corn oil by gavage
	Mice: 0, 375, 750, 1,500, 3,000, or 6,000 mg/kg body weight in corn oil by gavage
Duration of Dosing	Five days per week for 13 weeks
Type and Frequency of Observation	Observed twice daily for morbidity and mortality
Necropsy and Histologic Examination	All animals necropsied. All control and high dose rats and the control and the two highest dosed groups of mice were examined histologically
Animals and Animal Maintenance	
Species	F344/N rats; $B6C3F_1$ mice
Animal Source	Frederick Cancer Research Center (Frederick, MD)
Time Held before Start of Test	Rats: 2 weeks Mice: 4 weeks
Age When Placed on Study	Rats: 7 weeks Mice: 7 weeks
Age When Killed	Rats: 20 weeks Mice: 20 weeks
Method of Animal Distribution	Assigned to cages according to a table of random numbers and then to groups according to another table of random numbers
Feed	Purina® Rodent Chow 5001 *ad libitum* (Distributed by O.K. Feed Store, Miami, FL)
Bedding	Sani Chip® hardwood, Pinewood Products Co. (Distributed by O.K. Feed Store, Miami, FL)
Water	Tap water via Edstrom Automatic Watering System (Waterford, WI)
Cages	Polycarbonate, Lab Products (Rochelle Park, NJ); cages changed and sanitized twice weekly; racks sanitized every 2 weeks
Animals per Cage	5
Cage Filters	Cerex spun nylon, Florida Filters (Miami, FL); filters changed every 2 weeks
Animal Room Environment	22°-24°C; 40%-60% relative humidity; 12 hours fluorescent light per day; room air changed 18-20 times per hour
Other Chemicals on Test in Same Room	None
Chemical/Vehicle Mixture	
Preparation	Trichloroethylene was dissolved in Mazola® corn oil so as to allow the proper dose to be contained in 1 ml (rats) or 0.5 ml (mice)
Maximum Storage Time	One week
Storage Conditions	2°-5°C

TABLE 1. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS (Continued)

2-Year Studies	
Experimental Design	
Size of Test Groups	50 males and 50 females of each species
Doses	Rats: 0, 500, or 1,000 mg/kg body weight in corn oil by gavage; 1.0 ml per dose Mice: 0 or 1,000 mg/kg body weight in corn oil by gavage; 0.5 ml per dose
Duration of Dosing	Five days per week for 103 weeks
Type and Frequency of Observation	Observed twice daily for morbidity and mortality
Necropsy and Histologic Examination	All animals necropsied and examined histologically
Animals and Animal Maintenance	
Species	F344/N rats; $B6C3F_1$ mice
Animal Source	Harlan Industries (Indianapolis, IN)
Time Held before Start of Test	2.5 weeks
Age When Placed on Study	Rats: 8 weeks Mice: 8 weeks
Age When Killed	Rats: 111-115 weeks Mice: 112-115 weeks
Method of Animal Distribution	Assigned to cages according to a table of random numbers and then to groups according to another table of random numbers
Feed	Purina® Rodent Chow 5001 *ad libitum* (Distributed by O.K. Feed Store, Miami, FL)
Bedding	Sani Chip® hardwood, Pinewood Products Co. (Distributed by O.K. Feed Store, Miami, FL)
Water	Tap water via Edstrom Automatic Watering System (Waterford, WI)
Cages	Polycarbonate, Lab Products (Rochelle Park, NJ); cages changed and sanitized twice weekly; racks sanitized every 2 weeks
Animals per Cage	5 rats per cage; 10 mice per cage for the first 8 months and then 5 per cage
Cage Filters	Cerex spun nylon, Florida Filters (Miami, FL); filters changed every 2 weeks
Animal Room Environment	22°-24°C; 40%-60% relative humidity; 12 hours fluorescent light per day; room air changed 10-15 per hour
Other Chemicals on Test in Same Room	None
Chemical/Vehicle Mixture	
Preparation	Trichloroethylene was dissolved in Mazola® corn oil so as to allow the proper dose to be contained in 1 ml (rats) or 0.5 ml (mice)
Maximum Storage Time	One week for the first 16 weeks and one month thereafter
Storage Conditions	Refrigerated at 2°-5°C in glass bottles sealed with Teflon® septa and aluminum caps

III. RESULTS

RATS

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

MICE

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

THIRTEEN-WEEK STUDIES

All animals survived to the end of the study and only male rats dosed with 2,000 mg/kg/day of TCE exhibited >10% decrements in body weight (Table 2). The body final weight gains for both males and females dosed with 1,000 mg/kg/day or less were considered to be normal.

Histopathological examination of the tissues from animals receiving the highest doses of TCE revealed pulmonary vasculitis, usually involving small veins, in 6/10 males and 6/10 females. This change was also seen in 1/10 male and 1/10 female control animals. Most of these animals also had mild interstitial pneumonitis. Minimal or mild cytomegaly and karyomegaly of the renal tubular epithelial cells in the inner cortex was seen in 8/9 males receiving 2,000 mg/kg/day and the same effect, graded as equivocal or minimal, was seen in 5/10 females that had received the 1,000 mg/kg/day dose. These renal effects were so minimal that they were diagnosed only during a reevaluation of the tissues. The reevaluation was prompted by the production of definite renal toxicity in the 2-year study.

The results of this 13-week study in F344/N rats are essentially similar to those of an earlier 8-week study conducted in Osborne-Mendel rats (NCI, 1976). In that earlier study, only doses in excess of 5,000 mg/kg/day were lethal to rats. Doses of 1,000 mg/kg/day had no effect on body weight gains in males, but depressed weight gains in females by approximately 15%.

In view of the survival of all rats, the minimal effects on weight gain, and the relatively minor nature of the histological changes, dosage levels for the 2-year study were set at 500 and 1,000 mg/kg/day for both sexes.

TABLE 2. SURVIVAL AND MEAN BODY WEIGHTS OF F344/N RATS ADMINISTERED TRICHLOROETHYLENE IN CORN OIL BY GAVAGE FOR 13 WEEKS

Dose (mg/kg)	Survival *(a)*	Mean Body Weights (grams)			Final Body Weight Relative to Controls *(b)* (Percent)
		Initial	Final	Change	
MALES					
0	10/10	87	312	+225	—
125	10/10	88	292	+204	- 6
250	10/10	92	301	+209	- 4
500	10/10	95	313	+218	0
1,000	10/10	101	303	+202	- 3
2,000	10/10	83	238	+155	-24
FEMALES					
0	10/10	81	181	+100	—
62.5	10/10	72	168	+ 96	- 7
125	10/10	74	179	+105	- 1
250	10/10	75	177	+102	- 2
500	10/10	73	176	+103	- 3
1,000	10/10	76	177	+101	- 2

(a) Number surviving/number per group

(b) Weight Relative to Controls ▫

$$\frac{\text{Weight (Dosed Group)} - \text{Weight (Control Group)}}{\text{Weight (Control Group)}} \times 100$$

TWO-YEAR STUDIES

Body Weights and Clinical Signs

The growth curves for rats administered 500 or 1,000 mg/kg doses of TCE for 103 weeks are shown in Figure 1, and body weights are summarized in Table 3. The 1,000 mg/kg dose reduced weight gain in male rats, with an 11% decrement relative to controls being observed after 20 weeks. This effect was maintained throughout the experimental period, with high dose males exhibiting a 13% decrement after 99 weeks on study. Although mean body weight for the male rats administered the 500 mg/kg dose appears to be lower than that of the controls (Figure 1), the initial weights were lower than those of the controls (141 g versus 161 g) and therefore the differences in body weight in the 500 mg/kg male rats was not considered compound related. Both dosage levels of TCE reduced body weight gains in female rats, and after these animals were on study for approximately 60 weeks, the decreases in weight gain were dose related.

Gross observations of the appearance and behavior of the rats did not reveal any compound-related clinical signs.

Antibody Titers

Viral antibody titers are shown in Appendix F. At the end of 6 months, positive titers were identified for pneumonia (PVM) and Sendai viruses. Titer values for these viruses diminished over the course of the study and were negative at the 24-month test. Rat corona virus had a significant titer at 24 months.

Survival

Two female rats (one high dose and one vehicle control) were replaced, due to gavage error, during the initial 1.5 weeks of the study. Estimates of the probabilities of survival of male and female rats administered trichloroethylene and those of the vehicle controls are shown by the Kaplan and Meier curves in Figure 2. (The following animals died as a result of gavage error: 1 male vehicle control, 3 low dose males, 10 high dose males, 2 female vehicle controls, 5 low dose females, and 5 high dose females. These animals were censored from the Kaplan and Meier curves at the date of death.) The survival of the dosed male rats was significantly reduced when compared with that of the vehicle controls (low dose, P=0.005; high dose, P=0.001). No significant differences were observed between the dosed males or between any groups of females.

Among male rats, 35/50 (70%) of the controls, 20/50 (40%) of the low dose group, and 16/50 (32%) of the high dose group lived to the end of the study. Among female rats, 37/50 (74%) of the controls, 33/50 (66%) of the low dose group, and 26/50 (52%) of the high dose group lived to the end of the study. The survival data indicated above include one high dose male, two control males, and three control females that died during the termination period of the study (weeks 103-107). For the statistical evaluations of tumor incidences, these animals are considered to have been killed at termination.

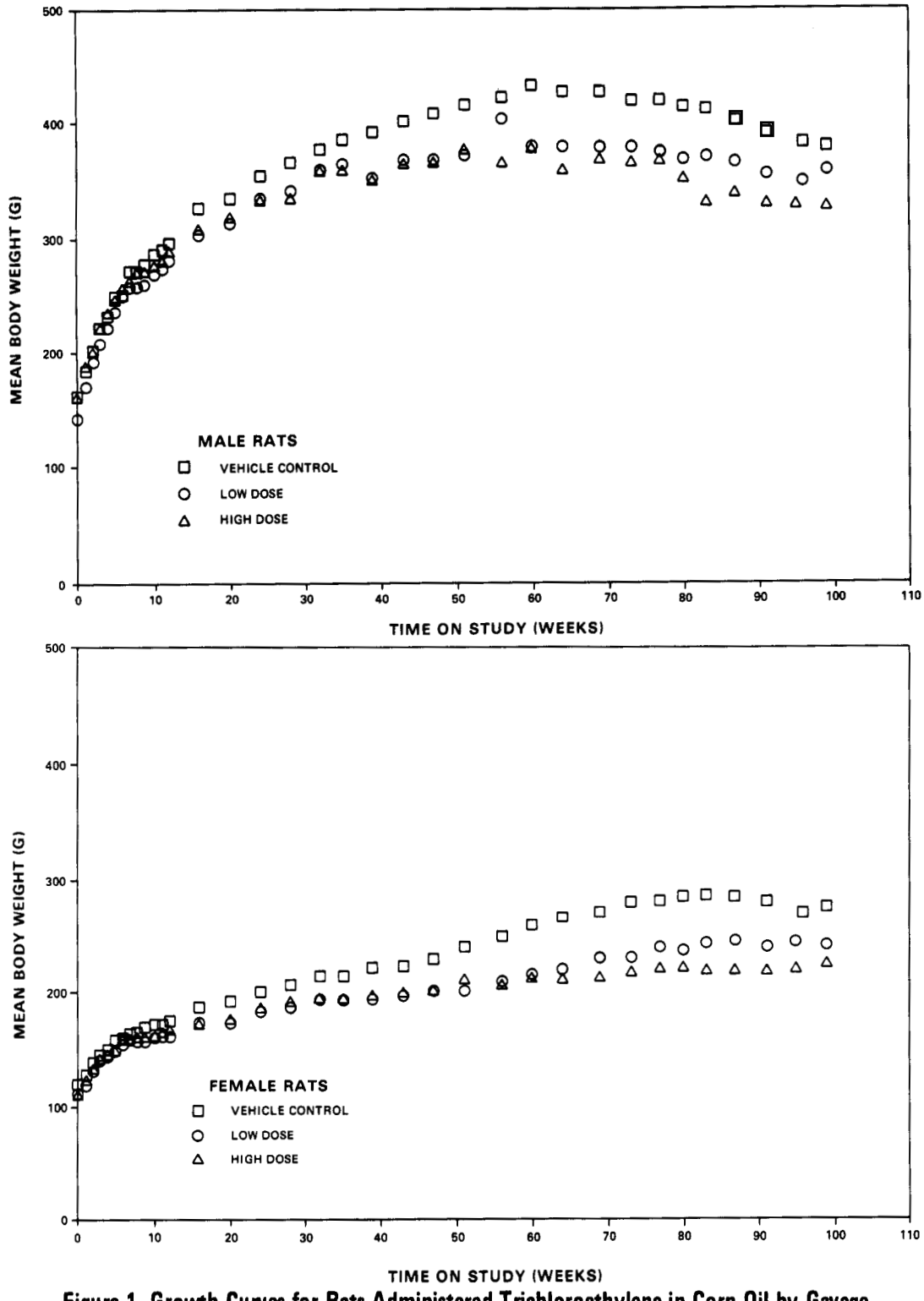


Figure 1. Growth Curves for Rats Administered Trichloroethylene in Corn Oil by Gavage

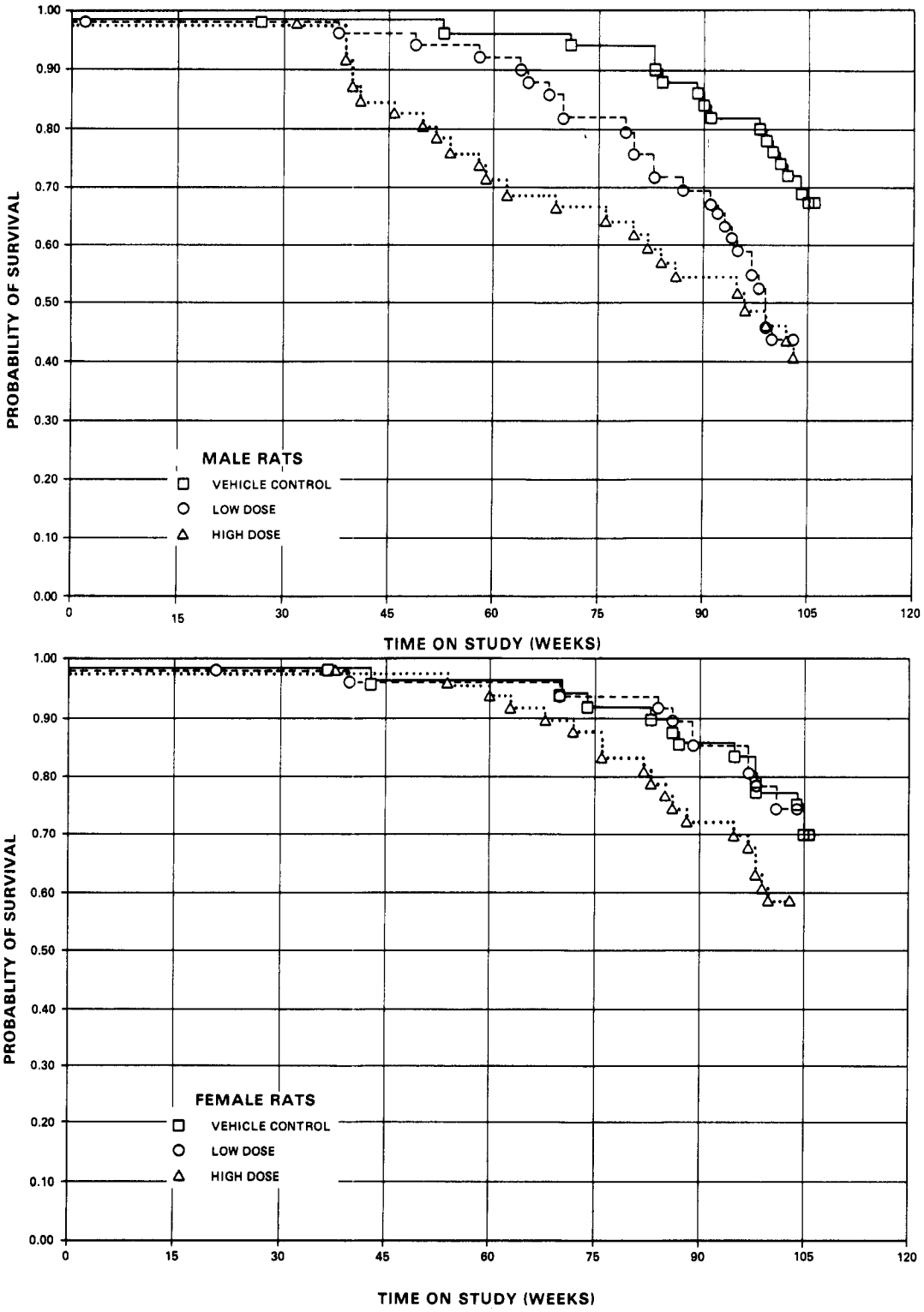


Figure 2. Survival Curves for Rats Administered Trichloroethylene in Corn Oil by Gavage

TABLE 3. MEAN BODY WEIGHTS (RELATIVE TO CONTROLS) OF RATS ADMINISTERED TRICHLOROETHYLENE IN CORN OIL BY GAVAGE FOR TWO YEARS

		Mean Body Weight (grams)			Body Weight Relative to Controls *(a)* (Percent)	
	Week No.	Control	Low Dose	High Dose	Low Dose	High Dose
Males						
	0	161	141	160	-12	- 1
	1	184	169	187	- 8	+ 2
	20	335	314	318	- 6	- 5
	39	392	353	350	-10	-11
	60	433	379	377	-12	-13
	80	414	367	352	-11	-15
	99	378	358	327	- 0	-13
Females						
	0	120	112	111	- 7	- 8
	1	128	119	123	- 7	- 4
	20	191	173	176	- 9	- 8
	39	221	193	197	-13	-11
	60	259	215	212	-17	-18
	80	285	236	221	-17	-22
	99	276	242	225	-12	-18

(a) Weight Relative to Controls = $\frac{\text{Weight (Dosed Group)} - \text{Weight (Control Group)}}{\text{Weight (Control Group)}} \times 100$

Pathology and Statistical Analyses of Results

Histopathologic findings on neoplasms in rats are summarized in Appendix A, Tables A1 and A2. Tables A3 and A4 give the survival and tumor status for each individual animal in the male and female rat studies, respectively. Findings on nonneoplastic lesions are summarized in Appendix C, Tables C1 and C2. Tables 4 and 5 contain the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. Because of the reduced survival in dosed male rats, the statistical procedures that adjust for intercurrent mortality (life table and incidental tumor tests) were regarded as more meaningful than the "unadjusted" analysis in the evaluation of tumor incidence data in these groups. Many of the lymph nodes, thymuses, tracheae, pituitaries, thyroids, parathyroids, and ovaries were not examined microscopically. These omissions appear to be the result of faulty histological technique.

Kidney: Renal tubular adenocarcinoma occurred with a significant positive trend in male rats that survived to the end of the study (P=0.009), and the pairwise comparison between the control and high dose groups was significant (terminal incidences: control, 0/33; low dose, 0/20; high dose, 3/16; P=0.028). These tumors were not found in animals that died before the termination of the study. Additional tumors found in the kidneys of male rats included a transitional cell papilloma of the renal pelvis in an untreated control, a transitional-cell carcinoma of the renal pelvis and two tubular cell adenomas in low dose animals and one carcinoma (NOS) of the renal pelvis in a high dose animal. Among female rats, one high dose animal had a renal tubular adenocarcinoma.

Small renal tubular cell adenomas consisted of solid collections of renal tubular epithelial cells filling several contiguous tubules. The tumor cells were enlarged and had abundant cytoplasm, large nuclei, and prominent nucleoli. In larger tumors, there was an increasing degree

of cellular atypia. There was no sharp demarcation between the larger adenomas and the tubular cell adenocarcinomas. The adenocarcinomas contained solid sheets of cells and focal areas of necrosis, and the cells were often quite atypical, with large irregular nuclei and prominent nucleoli. Local invasion was a common feature of these tumors.

Toxic nephrosis, designated "cytomegaly," was present in 98% of the dosed male rats and in 100% of the dosed females, but was not found in any of the vehicle control rats of either sex. The lesion was first noticed in dosed rats that died early in the test and was shown microscopically as frank enlargement of the nucleus and cytoplasm of scattered individual tubular cells that had brush borders and that were located near the cortico-medullary junction.

Progression of the lesion became evident in kidneys taken from rats that died early or were killed during the test. As exposure time increased, affected tubular cells continued to enlarge and additional tubules and tubular cells were affected. Occasionally, some tubules were enlarged or dilated to the extent that they were difficult to identify as tubules. In animals that survived longer, there was a decrease in the numbers of the enlarged cells, the corresponding tubules were dilated, and portions of the basement membrane had a stripped appearance. Special stains (Periodic Acid Schiff) were not useful in attempts to determine if the apparently stripped basement membrane was in fact naked or covered by a thin cytoplasmic membrane extending from the one or more remaining cytomegalic tubular cells. In the most advanced stage, the lesion extended to the subcapsular cortex, where enlarged tubular cells were readily found. Development of cytomegaly did not completely overshadow development of the spontaneous rat nephropathy which also was present but was recognized in a lower percentage of dosed animals than controls.

The cytomegaly was graded in each instance by subjective microscopic evaluation; the designations used as measures of the degree of severity were: slight (1), moderate (2), well marked (3), and severe (4). In this context, slight (1) indicates a subtle change that is often detected only at high microscopic magnifications, involves a limited part of the organ, and probably would not affect the function of the organ. Severe (4) indicates an obvious lesion that is readily visible at low microscopic magnifications, involves a substantial part of the organ, would significantly affect the function of the organ, and might be life threatening. Moderate (2) and well marked (3) are intermediate grades between the two extremes.

The average numerical grade of the lesion in each group of rats was: 0.0 for male and female controls; 2.8 for low dose males; 1.9 for low dose females; 3.1 for high dose males; and 2.7 for high dose females. Cytomegaly was more severe in males than in females, and the high dose males were most severely affected.

Peritoneum: Malignant mesotheliomas were observed in increased incidence in the low dose males compared with the controls ($P=0.042$, life table test; control, 1/50, 2%; low dose, 5/50, 10%; high dose, 0/49, 0%). This tumor was not observed among female rats.

Hematopoietic System: Leukemia occurred in reduced ($P<0.05$) incidence in the low dose female group compared with the controls. Both leukemia and leukemia or lymphoma (combined) occurred in reduced but not statistically significant proportions in the high dose male and high dose female groups. The following incidences of leukemia were observed: males, control, 5/50 (10%); low dose, 5/50 (10%); high dose, 1/49 (2%); females, control, 14/50 (28%); low dose, 4/50 (8%); high dose, 9/50 (18%). A malignant lymphoma was found in one control male rat.

Pituitary: Chromophobe adenomas were observed in decreased incidence in both male and female dosed rats (males: control, 7/42, 17%; low dose, 2/35, 6%; high dose, 1/26; 4%; females: control, 13/37, 35%; low dose, 6/34, 18%; high dose, 6/41, 15%). The pairwise comparison between the high dose and control groups was statistically significant ($P=0.040$, incidental tumor test) for female rats.

Uterus: Endometrial stromal polyps were observed among female rats in a statistically significant negative trend ($P=0.035$, incidental tumor test; control, 15/48, 31%; low dose, 8/48, 17%; high dose, 6/46, 13%).

Testis: Interstitial cell tumors occurred in male rats with a decreased incidence in the high dose group relative to controls (control, 47/49, 96%; low dose, 47/49, 96%; high dose, 32/46, 70%). However, this effect was not statistically significant when survival differences were taken into account (incidental tumor tests).

TABLE 4. ANALYSIS OF PRIMARY TUMORS IN MALE RATS *(a)*

	Untreated Control	Vehicle Control	Low Dose	High Dose
Subcutaneous Tissue: Fibroma				
Tumor Rates				
Overall *(b)*	3/50 (6%)	4/50 (8%)	1/50 (2%)	0/49 (0%)
Adjusted *(c)*		10.2%	5.0%	0.0%
Terminal *(d)*		2/35 (6%)	1/20 (5%)	0/16 (0%)
Statistical Tests *(e)*				
Life Table		P=0.108N	P=0.356N	P=0.208N
Incidental Tumor Test		P=0.064N	P=0.216N	P=0.141N
Cochran-Armitage Trend Test		P=0.027N		
Fisher Exact Test			P=0.181N	P=0.061N
Skin or Subcutaneous Tissue: Fibroma				
Tumor Rates				
Overall *(b)*	3/50 (6%)	4/50 (8%)	1/50 (2%)	1/49 (2%)
Adjusted *(c)*		10.2%	5.0%	6.3%
Terminal *(d)*		2/35 (6%)	1/20 (5%)	1/16 (6%)
Statistical Tests *(e)*				
Life Table		P=0.318N	P=0.356N	P=0.475N
Incidental Tumor Test		P=0.238N	P=0.216N	P=0.373N
Cochran-Armitage Trend Test		P=0.104N		
Fisher Exact Test			P=0.181N	P=0.187N
Lung: Alveolar/Bronchiolar Carcinoma				
Tumor Rates				
Overall *(b)*	1/49 (2%)	3/50 (6%)	2/50 (4%)	2/49 (4%)
Adjusted *(c)*		7.6%	9.5%	10.9%
Terminal *(d)*		2/35 (6%)	1/20 (5%)	1/16 (6%)
Statistical Tests *(e)*				
Life Table		P=0.461	P=0.656	P=0.568
Incidental Tumor Test		P=0.583N	P=0.494N	P=0.639N
Cochran-Armitage Trend Test		P=0.415N		
Fisher Exact Test			P=0.500N	P=0.510N
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma				
Tumor Rates				
Overall *(b)*	1/49 (2%)	4/50 (8%)	2/50 (4%)	2/49 (4%)
Adjusted *(c)*		10.4%	9.5%	10.9%
Terminal *(d)*		3/35 (9%)	1/20 (5%)	1/16 (6%)
Statistical Tests *(e)*				
Life Table		P=0.584N	P=0.570N	P=0.669
Incidental Tumor Test		P=0.439N	P=0.368N	P=0.516N
Cochran-Armitage Trend Test		P=0.259N		
Fisher Exact Test			P=0.339N	P=0.349
Hematopoietic System: Leukemia				
Tumor Rates				
Overall *(b)*	11/50 (22%)	5/50 (10%)	5/50 (10%)	1/49 (2%)
Adjusted *(c)*		13.6%	17.9%	5.9%
Terminal *(d)*		4/35 (11%)	2/20 (10%)	0/16 (0%)
Statistical Tests *(e)*				
Life Table		P=0.394N	P=0.336	P=0.368N
Incidental Tumor Test		P=0.255N	P=0.548	P=0.309N
Cochran-Armitage Trend Test		P=0.094N		
Fisher Exact Test			P=0.630	P=0.107N

TABLE 4. ANALYSIS OF PRIMARY TUMORS IN MALE RATS *(a)* (Continued)

	Untreated Control	Vehicle Control	Low Dose	High Dose
Hematopoietic System: Leukemia or Lymphoma				
Tumor Rates				
Overall *(b)*	12/50 (24%)	6/50 (12%)	5/50 (10%)	1/49 (2%)
Adjusted *(c)*		15.4%	17.9%	5.9%
Terminal *(d)*		4/35 (11%)	2/20 (10%)	0/16 (0%)
Statistical Tests *(e)*				
Life Table		P=0.287N	P=0.455	P=0.270N
Incidental Tumor Test		P=0.121N	P=0.512N	P=0.126N
Cochran-Armitage Trend Test		P=0.051N		
Fisher Exact Test			P=0.500N	P=0.059N
Kidney: Tubular-Cell Adenocarcinoma				
Tumor Rates				
Overall *(b)*	0/49 (0%)	0/48 (0%)	0/49 (0%)	3/49 (6%)
Adjusted *(c)*		0.0%	0.0%	18.8%
Terminal *(d)*		0/33 (0%)	0/20 (0%)	3/16 (19%)
Statistical Tests *(e)*				
Life Table		P=0.009	*(f)*	P=0.028
Incidental Tumor Test		P=0.009	*(f)*	P=0.028
Cochran-Armitage Trend Test		P=0.038		
Fisher Exact Test			*(f)*	P=0.125
Kidney: Tubular-Cell Adenoma or Adenocarcinoma				
Tumor Rates				
Overall *(b)*	0/49 (0%)	0/48 (0%)	2/49 (4%)	3/49 (6%)
Adjusted *(c)*		0.0%	5.6%	18.8%
Terminal *(d)*		0/33 (0%)	0/20 (0%)	3/16 (19%)
Statistical Tests *(e)*				
Life Table		P=0.019	P=0.194	P=0.028
Incidental Tumor Test		P=0.030	P=0.327	P=0.028
Cochran-Armitage Trend Test		P=0.084		
Fisher Exact Test			P=0.253	P=0.125
Pituitary: Chromophobe Adenoma				
Tumor Rates				
Overall *(b)*	4/39 (10%) *(g)*	7/42 (17%) *(h)*	2/35 (6%) *(i)*	1/26 (4%)
Adjusted *(c)*		21.2%	8.2%	7.7%
Terminal *(d)*		7/33 (21%)	1/18 (6%)	1/13 (8%)
Statistical Tests *(e)*				
Life Table		P=0.147N	P=0.285N	P=0.258N
Incidental Tumor Test		P=0.125N	P=0.250N	P=0.258N
Cochran-Armitage Trend Test		P=0.150N		
Fisher Exact Test			P=0.128N	P=0.111N
Adrenal: All Pheochromocytomas				
Tumor Rates				
Overall *(b)*	8/45 (18%)	4/45 (9%)	3/42 (7%)	1/44 (2%)
Adjusted *(c)*		13.3%	15.8%	3.2%
Terminal *(d)*		4/30 (13%)	3/19 (16%)	0/16 (0%)
Statistical Tests *(e)*				
Life Table		P=0.348N	P=0.571	P=0.393N
Incidental Tumor Test		P=0.270N	P=0.571	P=0.254N
Cochran-Armitage Trend Test		P=0.140N		
Fisher Exact Test			P=0.539N	P=0.187N

TABLE 4. ANALYSIS OF PRIMARY TUMORS IN MALE RATS *(a)* (Continued)

	Untreated Control	Vehicle Control	Low Dose	High Dose
Thyroid: C-Cell Carcinoma				
Tumor Rates				
Overall *(b)*	0/44 (0%)	2/44 (5%)	3/43 (7%)	0/39 (0%)
Adjusted *(c)*		6.5%	15.9%	0.0%
Terminal *(d)*		2/31 (6%)	2/15 (13%)	0/14 (0%)
Statistical Tests *(e)*				
Life Table		P=0.470N	P=0.231	P=0.425N
Incidental Tumor Test		P=0.429N	P=0.281	P=0.425N
Cochran-Armitage Trend Test		P=0.232N		
Fisher Exact Test			P=0.489	P=0.278N
Thyroid: C-Cell Adenoma or Carcinoma				
Tumor Rates				
Overall *(b)*	5/44 (11%)	4/44 (9%)	3/43 (7%)	0/39 (0%)
Adjusted *(c)*		12.9%	15.9%	0.0%
Terminal *(d)*		4/31 (13%)	2/15 (13%)	0/14 (0%)
Statistical Tests *(e)*				
Life Table		P=0.224N	P=0.464	P=0.202N
Incidental Tumor Test		P=0.197N	P=0.519	P=0.202N
Cochran-Armitage Trend Test		P=0.061N		
Fisher Exact Test			P=0.513N	P=0.074N
Preputial Gland: Adenoma				
Tumor Rates				
Overall *(b)*	4/50 (8%)	4/50 (8%)	0/50 (0%)	1/49 (2%)
Adjusted *(c)*		11.4%	0.0%	6.2%
Terminal *(d)*		4/35 (11%)	0/20 (0%)	1/16 (6%)
Statistical Tests *(e)*				
Life Table		P=0.253N	P=0.154N	P=0.473N
Incidental Tumor Test		P=0.253N	P=0.154N	P=0.473N
Cochran-Armitage Trend Test		P=0.084N		
Fisher Exact Test			P=0.059N	P=0.187N
Preputial Gland: Adenoma or Adenocarcinoma				
Tumor Rates				
Overall *(b)*	4/50 (8%)	5/50 (10%)	1/50 (2%)	3/49 (6%)
Adjusted *(c)*		13.5%	5.0%	13.4%
Terminal *(d)*		4/35 (11%)	1/20 (5%)	1/16 (6%)
Statistical Tests *(e)*				
Life Table		P=0.553	P=0.254	P=0.536
Incidental Tumor Test		P=0.457N	P=0.211N	P=0.541N
Cochran-Armitage Trend Test		P=0.272N		
Fisher Exact Test			P=0.102N	P=0.369N
Testis: Interstitial-Cell Tumor				
Tumor Rates				
Overall *(b)*	44/47 (94%)	47/49 (96%)	47/49 (96%)	32/46 (70%)
Adjusted *(c)*		100.0%	100.0%	96.8%
Terminal *(d)*		35/35 (100%)	20/20 (100%)	14/15 (93%)
Statistical Tests *(e)*				
Life Table		P=0.004	P<0.001	P=0.008
Incidental Tumor Test		P=0.252N	P=0.190	P=0.594N
Cochran-Armitage Trend Test	P<0.001N			
Fisher Exact Test			P=0.691	P=0.001N

TABLE 4. ANALYSIS OF PRIMARY TUMORS IN MALE RATS *(a)* (Continued)

	Untreated Control	Vehicle Control	Low Dose	High Dose
Peritoneum: Malignant Mesothelioma				
Tumor Rates				
Overall *(b)*	1/50 (2%)	1/50 (2%)	5/50 (10%)	0/49 (0%)
Adjusted *(c)*		2.9%	16.1%	0.0%
Terminal *(d)*		1/35 (3%)	0/20 (0%)	0/16 (0%)
Statistical Tests *(e)*				
Life Table		P=0.518	P=0.042	P=0.656N
Incidental Tumor Test		P=0.348N	P=0.274	P=0.656N
Cochran-Armitage Trend Test		P=0.407N		
Fisher Exact Test			P=0.102	P=0.505N
Peritoneum: All Mesotheliomas				
Tumor Rates				
Overall *(b)*	1/50 (2%)	1/50 (2%)	5/50 (10%)	1/49 (2%)
Adjusted *(c)*		2.9%	15.6%	6.3%
Terminal *(d)*		1/35 (3%)	0/20 (0%)	1/16 (6%)
Statistical Tests *(e)*				
Life Table		P=0.286	P=0.042	P=0.578
Incidental Tumor Test		P=0.583	P=0.274	P=0.578
Cochran-Armitage Trend Test		P=0.585		
Fisher Exact Test			P=0.102	P=0.747

(a) Dosed groups received doses of 500 or 1,000 mg/kg of trichloroethylene by gavage.
(b) Number of tumor bearing animals/number of animals examined at the site.
(c) Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.
(d) Observed tumor incidence at terminal kill.
(e) Beneath the control incidence are the P values associated with the trend test. Beneath each dosed group incidence is the P value corresponding to the pairwise comparison between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as non-fatal. The Cochran-Armitage and Fisher's exact test compare directly the overall incidence rates. A negative trend or lower incidence is indicated by (N).
(f) Not significant; no tumors were observed in dosed or control groups.
(g) Two chromophobe carcinomas, one adenoma, NOS, and one carcinoma, NOS, were also observed.
(h) One chromophobe carcinoma was also observed.
(i) One adenoma, NOS, was also observed.

TABLE 5. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS *(a)*

	Untreated Control	Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma				
Tumor Rates				
Overall *(b)*	0/49 (0%)	1/50 (2%)	1/49 (2%)	3/50 (6%)
Adjusted *(c)*		2.7%	3.0%	7.5%
Terminal *(d)*		1/37 (3%)	1/33 (3%)	0/26 (0%)
Statistical Tests *(e)*				
Life Table		P=0.151	P=0.736	P=0.246
Incidental Tumor Test		P=0.326	P=0.736	P=0.541
Cochran-Armitage Trend Test		P=0.202		
Fisher Exact Test			P=0.747	P=0.309
Hematopoietic System: Leukemia				
Tumor Rates				
Overall *(b)*	10/49 (20%) *(f)*	14/50 (28%)	4/50 (8%)	9/50 (18%)
Adjusted *(c)*		33.8%	10.7%	29.1%
Terminal *(d)*		10/37 (27%)	2/33 (6%)	6/26 (23%)
Statistical Tests *(e)*				
Life Table		P=0.316N	P=0.019N	P=0.446N
Incidental Tumor Test		P=0.110N	P=0.004N	P=0.182N
Cochran-Armitage Trend Test		P=0.121N		
Fisher Exact Test			P=0.009N	P=0.171N
Pituitary: Chromophobe Adenoma				
Tumor Rates				
Overall *(b)*	18/43 (42%)	13/37 (35%)	6/34 (18%)	6/41 (15%)
Adjusted *(c)*		37.5%	24.0%	22.0%
Terminal *(d)*		9/29 (31%)	5/23 (22%)	3/22 (14%)
Statistical Tests *(e)*				
Life Table		P=0.131N	P=0.135N	P=0.191N
Incidental Tumor Test		P=0.036N	P=0.075N	P=0.040N
Cochran-Armitage Trend Test		P=0.022N		
Fisher Exact Test			P=0.081N	P=0.032N
Pituitary: All Adenomas or Carcinomas				
Tumor Rates				
Overall *(b)*	19/43 (44%)	13/37 (35%)	8/34 (24%)	6/41 (15%)
Adjusted *(c)*		37.5%	30.3%	22.0%
Terminal *(d)*		9/29 (31%)	6/23 (26%)	3/22 (14%)
Statistical Tests *(e)*				
Life Table		P=0.151N	P=0.297N	P=0.191N
Incidental Tumor Test		P=0.038N	P=0.188N	P=0.040N
Cochran-Armitage Trend Test		P=0.024N		
Fisher Exact Test			P=0.209N	P=0.032N
Adrenal: Cortical Adenoma				
Tumor Rates				
Overall *(b)*	2/45 (4%)	1/46 (2%)	2/48 (4%)	3/47 (6%)
Adjusted *(c)*		3.0%	6.1%	12.0%
Terminal *(d)*		1/33 (3%)	2/33 (6%)	3/25 (12%)
Statistical Tests *(e)*				
Life Table		P=0.142	P=0.500	P=0.210
Incidental Tumor Test		P=0.142	P=0.500	P=0.210
Cochran-Armitage Trend Test	P=0.227			
Fisher Exact Test		P=0.516	P=0.317	

TABLE 5. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS *(a)* (Continued)

	Untreated Control	Vehicle Control	Low Dose	High Dose
Thyroid: C-Cell Adenoma				
Tumor Rates				
Overall *(b)*	2/36 (6%) *(g)*	4/41 (10%)	1/45 (2%)	1/44 (2%)
Adjusted *(c)*		10.9%	3.3%	4.2%
Terminal *(d)*		3/34 (9%)	1/30 (3%)	1/24 (4%)
Statistical Tests *(e)*				
Life Table		P=0.168N	P=0.214N	P=0.290N
Incidental Tumor Test		P=0.127N	P=0.168N	P=0.210N
Cochran-Armitage Trend Test		P=0.086N		
Fisher Exact Test			P=0.152N	P=0.159N
Mammary Gland: Fibroadenoma				
Tumor Rates				
Overall *(b)*	10/49 (20%)	9/50 (18%)	12/50 (24%)	4/50 (8%)
Adjusted *(c)*		22.9%	32.6%	14.5%
Terminal *(d)*		7/37 (19%)	9/33 (27%)	3/26 (12%)
Statistical Tests *(e)*				
Life Table		P=0.307N	P=0.240	P=0.285N
Incidental Tumor Test		P=0.169N	P=0.353	P=0.177N
Cochran-Armitage Trend Test		P=0.114N		
Fisher Exact Test			P=0.312	P=0.117N
Uterus: Endometrial Stromal Polyp				
Tumor Rates				
Overall *(b)*	10/45 (22%)	15/48(31%)	8/48 (17%)	6/46 (13%)
Adjusted *(c)*		40.1%	23.1%	21.0%
Terminal *(d)*		13/35(37%)	7/33 (21%)	4/25 (16%)
Statistical Tests *(e)*				
Life Table		P=0.074N	P=0.098N	P=0.120N
Incidental Tumor Test		P=0.035N	P=0.069N	P=0.056N
Cochran-Armitage Trend Test		P=0.019N		
Fisher Exact Test			P=0.075N	P=0.030N
Uterus: Endometrial Stromal Polyp or Sarcoma				
Tumor Rates				
Overall *(b)*	10/45 (22%)	15/48 (31%)	8/48 (17%)	7/46(15%)
Adjusted *(c)*		40.1%	23.1%	23.5%
Terminal *(d)*		13/35 (37%)	7/33 (21%)	4/25(16%)
Statistical Tests *(e)*				
Life Table		P=0.127N	P=0.098N	P=0.193N
Incidental Tumor Test		P=0.060N	P=0.069N	P=0.086N
Cochran-Armitage Trend Test		P=0.037N		
Fisher Exact Test			P=0.075N	P=0.055N

(a) Dosed groups received doses of 500 or 1,000 mg/kg of trichloroethylene by gavage.
(b) Number of tumor bearing animals/number of animals examined at the site.
(c) Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.
(d) Observed tumor incidence at terminal kill.
(e) Beneath the control incidence are the P values associated with the trend test. Beneath each dosed group incidence is the P value corresponding to the pairwise comparison between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as non-fatal. The Cochran-Armitage and Fisher's exact test compare directly the overall incidence rates. A negative trend or lower incidence is indicated by (N).
(f) One malignant lymphoma, histiocytic type, was also observed.
(g) One C-cell carcinoma was also observed.

THIRTEEN-WEEK STUDIES

The survival, body weight gains, and relative liver weights for mice receiving TCE for 13 weeks are summarized in Table 6. Deaths occurred in all males and 9/10 females receiving 6,000 mg/kg, 7/10 males and 1/10 females receiving 3,000 mg/kg, and 2/10 males and 1/10 females receiving 1,500 mg/kg. Mean body weights of male mice receiving 750, 1,500, or 3,000 mg/kg doses were depressed 11%, 19%, and 17%, respectively, relative to controls. Mean body weights of control and dosed groups of female mice were similar.

Liver weights (both absolute and as a percent of body weight) increased with dose. Liver weights were increased by more than 10% relative to controls for males receiving 750 mg/kg or more and for females receiving 1,500 mg/kg or more.

The most prominent hepatic lesion detected in the mice was centrilobular necrosis, observed in 6/10 males and 1/10 females administered 6,000 mg/kg. Although centrilobular necrosis was not seen in either males or females administered 3,000 mg/kg, 2/10 males had multifocal areas of calcification scattered throughout their livers. These areas of calcification are considered to be evidence of earlier hepatocellular necrosis. Multifocal calcification was also seen in the liver of a single female mouse that survived the 6,000 mg/kg dosage regimen. One female mouse administered 3,000 mg/kg also had an hepatocellular adenoma, an extremely rare lesion in female mice of this age (20 weeks).

As in the rat study, reevaluation of the kidney tissues revealed the presence of mild to moderate cytomegaly and karyomegaly of the renal tubular epithelial cells of the inner cortex. These lesions appear to be a response to repeated doses of TCE, since they were found in only 1 of the 13 males and 10 females that died after receiving doses of 3,000 or 6,000 mg/kg for up to 6 weeks. All four of the male mice that died after receiving the 3,000 mg/kg dose for 7-13 weeks had the lesions, as did all animals that survived the 6,000 mg/kg (1/10 females) and the 3,000 mg/kg doses (3/10 males and 9/10 females). Tissues from mice receiving lower doses of TCE were not examined.

The single dose chosen for the 2-year study in B6C3F$_1$ mice (1,000 mg/kg) was selected on the basis of results of the present 13-week study and the earlier 2-year study (NCI, 1976). While the relatively low incidence of major histopathological lesions among animals administered 3,000 mg/kg and the lack of clinical signs among animals administered 1,500 mg/kg suggested that 1,500 mg/kg might be an acceptable dose level for the 2-year study, the two deaths in males and one death in females at this level indicated toxicity. (The single death in females at 750 mg/kg occurred during week 2 and may not have been due to a toxic effect of trichloroethylene.) Mean liver weights and liver weight/body weight ratios also were increased at 1,500 mg/kg in both males and females, compared to controls. Doses in the range of 1,500 mg/kg were associated with significantly shorter survival times among female mice in the earlier 2-year study. Consequently, the 1,000 mg/kg dose was selected in an attempt to achieve improved survival in the new study.

TABLE 6. SURVIVAL, MEAN BODY WEIGHTS, AND MEAN LIVER WEIGHTS OF MICE ADMINISTERED TRICHLOROETHYLENE IN CORN OIL BY GAVAGE FOR 13 WEEKS

Dose (ppm)	Survival *(a)*	Mean Body Weights (grams)			Final Body Weights Relative to Controls *(b)* (Percent)	Mean Liver Weights (Grams)	Relative Liver Weight (Percent of Body Weight)
		Initial	Final	Change			
MALES							
0	10/10	21	36	+15	—	2.10	5.8
375	10/10	20	35	+15	- 3	1.74	5.0
750	10/10	21	32	+11	-11	2.14	6.8
1,500	8/10 *(c)*	19	29	+10	-19	2.27	7.6
3,000	3/10 *(d)*	20	30	+10	-17	2.78	8.5
6,000	0/10 *(e)*	22	—	—	—	—	—
FEMALES							
0	10/10	18	26	+ 8	—	1.40	5.5
375	10/10	17	26	+ 9	0	1.31	5.0
750	9/10 *(f)*	17	26	+ 9	0	1.55	5.8
1,500	9/10 *(g)*	17	26	+ 9	0	1.80	6.5
3,000	9/10 *(g)*	15	26	+11	0	2.06	7.8
6,000	1/10 *(h)*	15	27	+12	+ 4	2.67	9.5

(a) Number surviving/number per group

(b) Weight Relative to Controls =

$$\frac{\text{Weight (Dosed Group)} - \text{Weight (Control Group)}}{\text{Weight (Control Group)}} \times 100$$

(c) Week of death: 2, 7

(d) Week of death: 1, 6, 6, 7, 9, 10, 13

(e) All mice died during the first week

(f) One mouse died during the second week

(g) One mouse died during the fifth week

(h) Eight mice died during the first week; one mouse died during the third week

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Mean body weights of dosed male mice were lower than those of controls throughout the study (Table 7 and Figure 3). Mean body weights of dosed and control female mice were comparable. No compound-related clinical signs were reported.

TABLE 7. MEAN BODY WEIGHTS (RELATIVE TO CONTROLS) OF MICE ADMINISTERED TRICHLOROETHYLENE IN CORN OIL BY GAVAGE FOR TWO YEARS

		Mean Body Weight (grams)		Body Weight Relative to Controls *(a)* (Percent)
	Week No.	Control	Dosed	Dosed
Males				
	0	27	27	0
	1	28	29	+ 4
	20	36	34	- 6
	39	46	39	-15
	60	46	41	-11
	80	46	40	-13
	99	41	37	-10
Females				
	0	21	22	+ 5
	1	22	23	+ 5
	20	28	28	0
	39	35	32	- 9
	60	39	36	- 8
	80	38	39	+ 3
	99	35	33	- 6

(a) Weight of the dosed group relative to that of the controls = $\frac{\text{Weight (Dosed Group)} - \text{Weight (Control Group)}}{\text{Weight (Control Group)}} \times 100$

Antibody Titers

Viral antibody titers are shown in Appendix F. Sendai virus titers were positive at the 6-, 12-, and 18-month tests. At the 24-month test, all titers were negative.

Survival

Ten males (4 vehicle control and 6 dosed) and 11 females (6 vehicle control and 5 dosed) were replaced, due to gavage error, during the initial 1.5 weeks of the study. Estimates of the probabilities of survival of male and female mice administered trichloroethylene at the concentrations used in this bioassay and the estimates for the control groups are shown by the Kaplan and Meier curves in Figure 4. Two male vehicle controls, three dosed males, one female vehicle control, and three dosed females died as a result of gavage error. These animals were censored from the Kaplan and Meier curves at the date of death. The survival of the male dosed group was significantly reduced ($P=0.004$) when compared with that of the controls. No significant difference was observed between the female control and dosed groups.

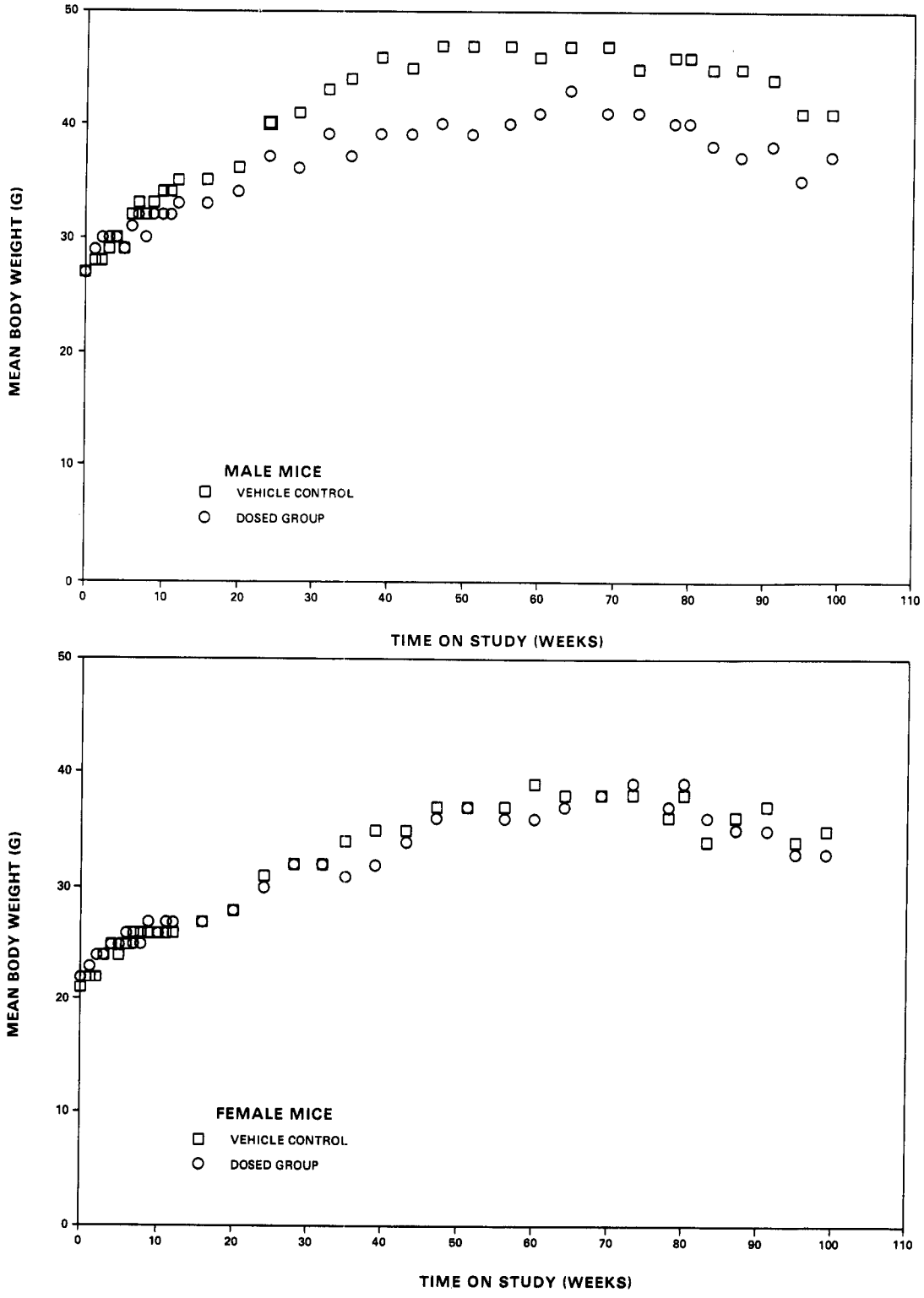


Figure 3. Growth Curves for Mice Administered Trichloroethylene in Corn Oil by Gavage

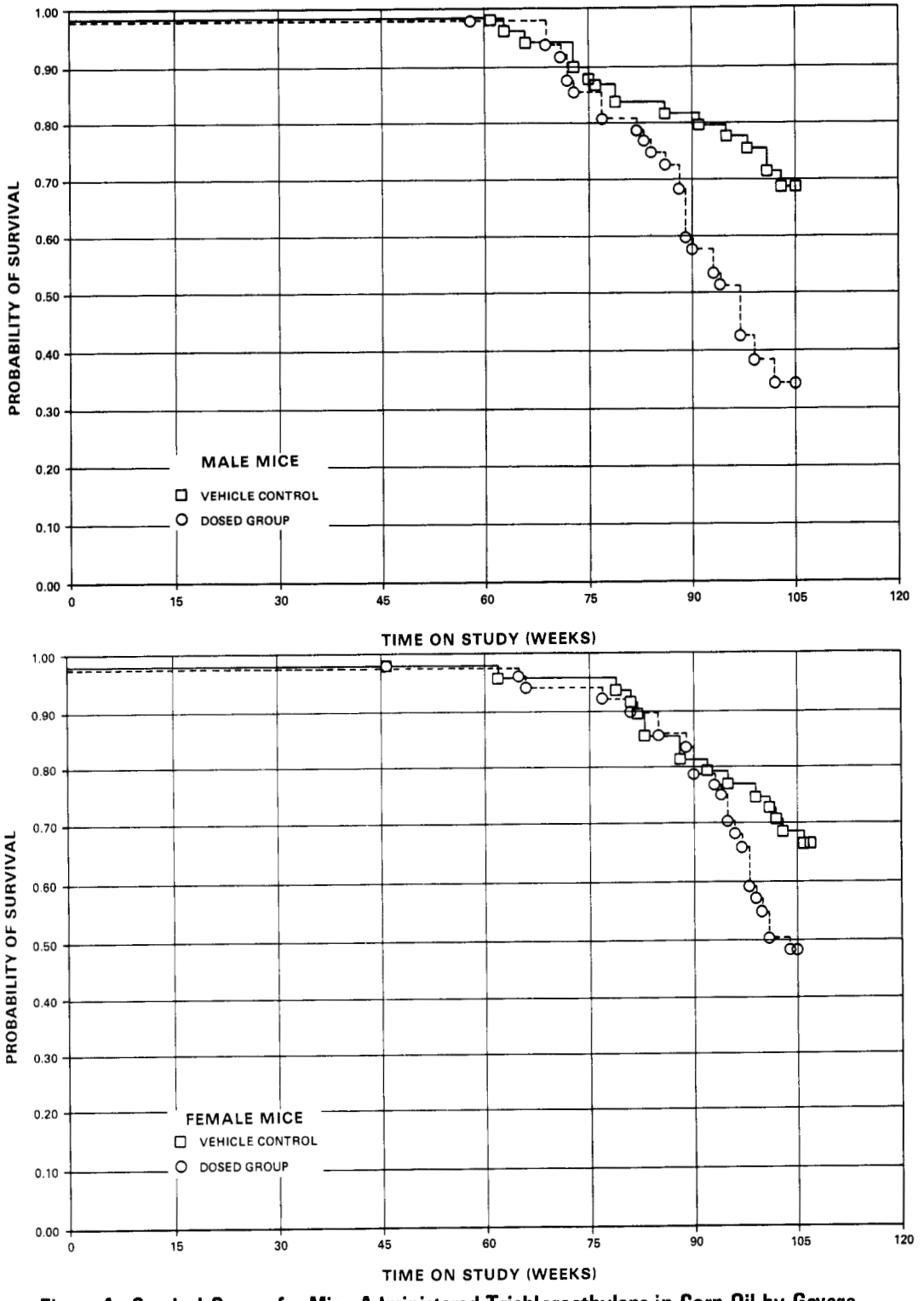


Figure 4. Survival Curves for Mice Administered Trichloroethylene in Corn Oil by Gavage

In male mice, 33/50 (66%) of the controls and 16/50 (32%) of the dosed group lived to the end of the study. In female mice, 32/50 (64%) of the controls and 23/50 (46%) of the dosed group lived to the end of the study. The survival data include four control males, one control female, and one dosed female that died during the termination period of the study (weeks 104-107). For the statistical evaluation of tumor incidences, these animals are considered to have been killed at termination.

Pathology and Statistical Analyses of Results

Histopathologic findings on neoplasms occurring in mice are summarized in Appendix B, Tables B1 and B2. Tables B3 and B4 give the survival and tumor status for each individual animal in the male and female mouse studies, respectively. Findings on nonneoplastic lesions are summarized in Appendix D, Tables D1 and D2. Tables 8 and 9 contain the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in either group. Many of the lymph nodes, thymuses, tracheae, pituitaries, thyroids, parathyroids, and ovaries were not examined microscopically. These omissions appear to be the result of faulty histological technique.

Liver: The incidence of hepatocellular carcinoma was significantly increased in dosed male and female mice (males: control, 8/48; dosed, 31/50; $P<0.001$; females: control, 2/48; dosed, 13/49; $P\leq0.002$). In five dosed males and one control male, these tumors metastasized to the lung. The incidence of hepatocellular adenomas was increased in dosed mice (males: control, 7/48; dosed, 14/50; females: control, 4/48; dosed, 16/49; $P<0.05$).

Microscopically, the hepatocellular adenomas were circumscribed areas of distinctive hepatic parenchymal cells with a perimeter of normal appearing parenchyma in which there were areas that appeared to be undergoing compression from expansion of the tumor. Mitotic figures were sparse or absent, but the tumors lacked typical lobular organization.

The hepatocellular carcinomas had markedly abnormal cytology and architecture. Abnormalities in cytology included increased cell size, decreased cell size, cytoplasmic eosinophilia, cytoplasmic basophilia, cytoplasmic vacuolization, cytoplasmic hyaline bodies and variations in nuclear appearance. In many instances, several or all of the abnormalities were present in different areas of the tumor. There also were variations in architecture. Some of the hepatocellular carcinomas had areas of trabecular organization. The general microscopic appearance was that of a diffusely infiltrating hepatocellular tumor that was replacing the normal architectural pattern of the liver. Often the boundary between recognizable tumor and nonneoplastic liver was vague. In some areas, subtle infiltration of tumor cells among persistent remnants of portal areas created a confusion of microscopic details that were difficult to interpret. Mitosis was variable in amount and location.

Lung: Alveolar/bronchiolar adenomas occurred at an increased incidence in dosed female mice (life table, $P=0.040$; control, 0/48; dosed, 4/48). However, the incidence of alveolar/bronchiolar adenoma or carcinoma (combined) was not significantly elevated in dosed female mice.

Hematopoietic System: Lymphoma (all malignant) and lymphoma or leukemia occurred at increased incidences in dosed female mice ($P<0.05$, life table test; lymphoma, control, 7/48, 15%; dosed, 13/49, 27%; lymphoma or leukemia, control, 7/48, 15%; dosed 14/49, 29%). In male mice, these tumors did not occur in statistically significant proportions.

Stomach: Squamous-cell papillomas were found in two dosed female mice, and a squamous cell carcinoma was found in a third dosed female mouse. A squamous cell carcinoma was also found in a male vehicle control.

Kidney: A compound related toxic nephrosis, designated as "cytomegaly," was present in 90% of the dosed male and 98% of the dosed female mice but not in the controls. The pathologic development was basically similar to the comparable cytomegaly found in dosed rats, but it was relatively less severe and did not develop to a stage in which there was extensive loss of cytomegalic epithelial cells and tubular dilation. The cytomegaly in mice was generally graded as slight (1) or moderate (2); in only one instance was a grade of well marked (3) assigned (average numerical grade, 0.0 for control males and females, 1.5 for dosed males, and 1.8 for dosed females). One control male had a renal tubular cell adenoma, and one dosed male had a renal tubular cell adenocarcinoma.

Harderian Gland: Harderian gland adenomas were detected in 4 dosed male and 3 dosed female mice. None were observed in male or female vehicle control mice. Only Harderian glands considered abnormal at necropsy were examined microscopically.

TABLE 8. ANALYSIS OF PRIMARY TUMORS IN MALE MICE *(a)*

	Vehicle Control	Dosed
Lung: Alveolar/Bronchiolar Adenoma		
Tumor Rates		
Overall *(b)*	4/49 (8%)	5/50 (10%)
Adjusted *(c)*	10.5%	27.1%
Terminal *(d)*	2/33 (6%)	4/16 (25%)
Statistical Tests *(e)*		
Life Table		P=0.197
Incidental Tumor Test		P=0.375
Fisher Exact Test		P=0.513
Lung: Alveolar/Bronchiolar Carcinoma		
Tumor Rates		
Overall *(b)*	3/49 (6%)	1/50 (2%)
Adjusted *(c)*	9.1%	4.0%
Terminal *(d)*	3/33 (9%)	0/16 (0%)
Statistical Tests *(e)*		
Life Table		P=0.553N
Incidental Tumor Test		P=0.407N
Fisher Exact Test		P=0.301N
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma		
Tumor Rates		
Overall *(b)*	7/49 (14%)	6/50 (12%)
Adjusted *(c)*	19.2%	30.0%
Terminal *(d)*	5/33 (15%)	4/16 (25%)
Statistical Tests *(e)*		
Life Table		P=0.310
Incidental Tumor Test		P=0.575
Fisher Exact Test		P=0.484N
Hematopoietic System: Malignant Lymphoma, Undifferentiated Type		
Tumor Rates		
Overall *(b)*	3/50 (6%)	5/50 (10%)
Adjusted *(c)*	6.7%	17.4%
Terminal *(d)*	0/33 (0%)	1/16 (6%)
Statistical Tests *(e)*		
Life Table		P=0.258
Incidental Tumor Test		P=0.590
Fisher Exact Test		P=0.357
Hematopoietic System: Malignant Lymphoma, Mixed Type		
Tumor Rates		
Overall *(b)*	3/50 (6%)	3/50 (6%)
Adjusted *(c)*	8.8%	14.6%
Terminal *(d)*	2/33 (6%)	1/16 (6%)
Statistical Tests *(e)*		
Life Table		P=0.348
Incidental Tumor Test		P=0.663N
Fisher Exact Test		P=0.661

TABLE 8. ANALYSIS OF PRIMARY TUMORS IN MALE MICE (a) (Continued)

	Vehicle Control	Dosed
Hematopoietic System: Lymphoma, All Malignant		
Tumor Rates		
Overall(b)	11/50 (22%)	13/50 (26%)
Adjusted (c)	25.2%	45.7%
Terminal (d)	3/33 (9%)	3/16 (19%)
Statistical Tests (e)		
Life Table		P=0.116
Incidental Tumor Test		P=0.398N
Fisher Exact Test		P=0.408
Liver: Adenoma		
Tumor Rates		
Overall(b)	7/48 (15%)	14/50 (28%)
Adjusted (c)	20.6%	53.1%
Terminal (d)	6/33 (18%)	6/16 (37%)
Statistical Tests (e)		
Life Table		P=0.002
Incidental Tumor Test		P=0.048
Fisher Exact Test		P=0.084
Liver: Carcinoma		
Tumor Rates		
Overall(b)	8/48 (17%)	31/50 (62%)
Adjusted (c)	22.1%	92.9%
Terminal (d)	6/33 (18%)	14/16 (88%)
Statistical Tests (e)		
Life Table		P<0.001
Incidental Tumor Test		P<0.001
Fisher Exact Test		P<0.001
Liver: Adenoma or Carcinoma		
Tumor Rates		
Overall(b)	14/48 (29%)	39/50 (78%)
Adjusted (c)	38.4%	100%
Terminal (d)	11/33 (33%)	16/16 (100%)
Statistical Tests (e)		
Life Table		P<0.001
Incidental Tumor Test		P<0.001
Fisher Exact Test		P<0.001
Harderian Gland: All Adenomas		
Tumor Rates		
Overall(b)	0/50 (0%)	4/50 (8%)
Adjusted (c)	0.0%	12.0%
Terminal (d)	0/33 (0%)	0/16 (0%)
Statistical Tests (e)		
Life Table		P=0.044
Incidental Tumor Test		P=0.216
Fisher Exact Test		P=0.059

TABLE 8. ANALYSIS OF PRIMARY TUMORS IN MALE MICE *(a)* (Continued)

(a) The dosed group received doses of 1,000 mg/kg of trichloroethylene by gavage.
(b) Number of tumor bearing animals/number of animals examined at the site.
(c) Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.
(d) Observed tumor incidence at terminal kill.
(e) Beneath the dosed group incidence is the P value corresponding to the pairwise comparison between the dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as non-fatal. The Fisher exact test compares directly the overall incidence rates. A negative trend or lower incidence is indicated by N.

TABLE 9. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE *(a)*

	Vehicle Control	Dosed
Lung: Alveolar/Bronchiolar Adenoma		
Tumor Rates		
Overall *(b)*	0/48 (0%)	4/48 (8%)
Adjusted *(c)*	0.0%	14.3%
Terminal *(d)*	0/32 (0%)	2/22 (9%)
Statistical Tests *(e)*		
Life Table		P=0.040
Incidental Tumor Test		P=0.064
Fisher Exact Test		P=0.059
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma		
Tumor Rates		
Overall *(b)*	1/48 (2%)	4/48 (8%)
Adjusted *(c)*	2.5%	14.3%
Terminal *(d)*	0/32 (0%)	2/22 (9%)
Statistical Tests *(e)*		
Life Table		P=0.132
Incidental Tumor Test		P=0.184
Fisher Exact Test		P=0.181
Hematopoietic System: Malignant Lymphoma, Undifferentiated Type		
Tumor Rates		
Overall *(b)*	1/48 (2%)	3/49 (6%)
Adjusted *(c)*	2.2%	10.1%
Terminal *(d)*	0/32 (0%)	0/23 (0%)
Statistical Tests *(e)*		
Life Table		P=0.252
Incidental Tumor Test		P=0.476
Fisher Exact Test		P=0.316
Hematopoietic System: Malignant Lymphoma, Lymphocytic Type		
Tumor Rates		
Overall *(b)*	0/48 (0%)	3/49 (6%)
Adjusted *(c)*	0.0%	9.6%
Terminal *(d)*	0/32 (0%)	0/23 (0%)
Statistical Tests *(e)*		
Life Table		P=0.096
Incidental Tumor Test		P=0.324
Fisher Exact Test		P=0.125
Hematopoietic System: Malignant Lymphoma, Histiocytic Type		
Tumor Rates		
Overall *(b)*	1/48 (2%)	3/49 (6%)
Adjusted *(c)*	2.8%	10.9%
Terminal *(d)*	0/32 (0%)	2/23 (9%)
Statistical Tests *(e)*		
Life Table		P=0.228
Incidental Tumor Test		P=0.323
Fisher Exact Test		P=0.316

TABLE 9. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE (a) (Continued)

	Vehicle Control	Dosed
Hematopoietic System: Malignant Lymphoma, Mixed Type		
Tumor Rates		
Overall(b)	3/48 (6%)	2/49 (4%)
Adjusted (c)	9.1%	7.5%
Terminal (d)	2/32 (6%)	1/23 (4%)
Statistical Tests (e)		
Life Table		P=0.636N
Incidental Tumor Test		P=0.424N
Fisher Exact Test		P=0.490N
Hematopoietic System: Lymphoma, All Malignant		
Tumor Rates		
Overall(b)	7/48 (15%)	13/49 (27%)
Adjusted (c)	18.8%	38.0%
Terminal (d)	3/32 (9%)	3/23 (13%)
Statistical Tests (e)		
Life Table		P=0.047
Incidental Tumor Test		P=0.331
Fisher Exact Test		P=0.114
Hematopoietic System: Lymphoma or Leukemia		
Tumor Rates		
Overall(b)	7/48 (15%)	14/49 (29%)
Adjusted (c)	18.8%	39.3%
Terminal (d)	3/32 (9%)	3/23 (13%)
Statistical Tests (e)		
Life Table		P=0.032
Incidental Tumor Test		P=0.287
Fisher Exact Test		P=0.076
Liver: Adenoma		
Tumor Rates		
Overall(b)	4/48 (8%)	16/49 (33%)
Adjusted (c)	12.5%	55.6%
Terminal (d)	4/32 (13%)	11/23 (48%)
Statistical Tests (e)		
Life Table		P<0.001
Incidental Tumor Test		P=0.001
Fisher Exact Test		P=0.003
Liver: Carcinoma		
Tumor Rates		
Overall(b)	2/48 (4%)	13/49 (27%)
Adjusted (c)	6.2%	43.9%
Terminal (d)	2/32 (6%)	8/23 (35%)
Statistical Tests (e)		
Life Table		P<0.001
Incidental Tumor Test		P=0.002
Fisher Exact Test		P=0.002

TABLE 9. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE (a) (Continued)

	Vehicle Control	Dosed
Liver: Adenoma or Carcinoma		
Tumor Rates		
Overall(b)	6/48 (13%)	22/49 (45%)
Adjusted (c)	18.7%	69.7%
Terminal (d)	6/32 (19%)	4/23 (61%)
Statistical Tests (e)		
Life Table		P<0.001
Incidental Tumor Test		P<0.001
Fisher Exact Test		P<0.001
Stomach: Squamous Cell Papilloma or Carcinoma		
Tumor Rates		
Overall(b)	0/47 (0%)	3/47 (6%)
Adjusted (c)	0.0%	8.1%
Terminal (d)	0/32 (0%)	0/22 (0%)
Statistical Tests (e)		
Life Table		P=0.112
Incidental Tumor Test		P=0.261
Fisher Exact Test		P=0.121
Pituitary: Chromophobe Adenoma		
Tumor Rates		
Overall(b)	3/27 (11%)	0/28 (0%)
Adjusted (c)	13.1%	0.0%
Terminal (d)	2/19 (11%)	0/14 (0%)
Statistical Tests (e)		
Life Table		P=0.183N
Incidental Tumor Test		P=0.081N
Fisher Exact Test		P=0.111N
Harderian Gland: Adenoma		
Tumor Rates		
Overall(b)	0/48 (0%)	3/49 (6%)
Adjusted (c)	0.0%	8.3%
Terminal (d)	0/32 (0%)	0/23 (0%)
Statistical Tests (e)		
Life Table		P=0.114
Incidental Tumor Test		P=0.171
Fisher Exact Test		P=0.125

(a) The dosed groups received doses of 1,000 mg/kg of trichloroethylene by gavage.
(b) Number of tumor-bearing animals/number of animals examined at the site.
(c) Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.
(d) Observed tumor incidence at terminal kill.
(e) Beneath the control incidence is the P value associated with the trend test. Beneath the dosed group incidence is the P value corresponding to the pairwise comparison between the dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as non-fatal. The Fisher exact test compares directly the overall incidence rates. A negative trend or lower incidence is indicated by N.

IV. DISCUSSION AND CONCLUSIONS

IV. DISCUSSION AND CONCLUSIONS

Carcinogenesis studies of epichlorohydrin-free trichloroethylene were conducted by administering the test chemical in corn oil by gavage to groups of 50 male and 50 female F344/N rats and $B6C3F_1$ mice. Trichloroethylene was administered five times per week for 103 weeks, and surviving animals were killed between weeks 103 and 107. Groups of 50 rats and 50 mice of each sex received corn oil by gavage on the same schedule and served as vehicle controls. Groups of 50 male and 50 female rats were used as untreated controls.

Groups of 10 male and 10 female rats received TCE by gavage at doses of 125 to 2,000 mg/kg (males) and 62.5 to 1,000 mg/kg (females) for 13 weeks. Groups of 10 male and 10 female mice received gavage doses of 375 to 6,000 mg/kg of TCE for 13 weeks. Survival, body weight gains, and previous experience with TCE were used to select doses for the 2-year studies. All rats survived the 13-week studies; males receiving 2,000 mg/kg exhibited a 24% difference in final body weight compared to controls. The doses selected for the 2-year study in rats were 500 and 1,000 mg/kg for both sexes. These dose levels were lower than the initial doses used in the earlier bioassay in Osborne-Mendel rats (650 and 1,300 mg/kg for both sexes). Two dose levels were used in the rat portion of the study because TCE had not been tested in a bioassay with F344/N rats, and in the earlier bioassay in Osborne-Mendel rats the chemical was not shown to be carcinogenic.

All male mice receiving 750 mg/kg, 8/10 males given 1,500 mg/kg, and 9/10 female mice administered 750 or 1,500 mg/kg survived the 13-week experimental period. The single dosage level selected for the 2-year study in mice was 1,000 mg/kg for both sexes. This dose was less than the high doses used in the earlier bioassay in $B6C3F_1$ mice (2,339 mg/kg for males and 1,739 for females). A single dose level was used in the mouse study because TCE containing epichlorohydrin had been shown to be carcinogenic in $B6C3F_1$ mice in the earlier bioassay (NCI, 1976). The mice in the present study served as "positive" controls.

The earlier bioassay of TCE (NCI, 1976) established the kidneys of both Osborne-Mendel rats and $B6C3F_1$ mice as target organs for non-neoplastic lesions (toxic nephrosis) induced by long-term administration of TCE. The results of the present study confirmed the effect in $B6C3F_1$ mice, and the data indicate that the kidney is a target organ in F344/N rats as well.

Toxic nephropathy was present in 98% of the dosed male rats, in 100% of the dosed female rats, in 90% of the dosed male mice, and in 98% of the dosed female mice; and was not present in any of the vehicle control rats or mice of either sex. First noticed in rats that died early, the lesions were diagnosed as frank enlargement of the nucleus and cytoplasm of scattered individual tubular cells with brush borders and located near the cortico-medullary junction. Progression of the lesion was evident. As exposure time increased, affected tubular cells continued to enlarge and additional tubules and tubular cells were affected. Occasionally, some tubules were enlarged or dilated to the extent that they were difficult to identify as tubules. Eventually, there was loss of some enlarged cells. Corresponding tubules became dilated and portions of the basement membrane had a stripped appearance. In the most advanced stage, the lesion had progressed to the subcapsular cortex, with enlarged tubular cells. Development of toxic nephropathy did not completely overshadow development of the "spontaneous" rat nephropathy which was recognized in a smaller number of dosed animals than of controls. The toxic nephropathy was graded as slight (1), moderate (2), well marked (3), and severe (4). The numerical value calculated for each group of rats was 0.0 for male and female controls, 2.8 for low dose males, 1.9 for low dose females, 3.1 for high dose males, and 2.7 for high dose females. In mice, the pathologic development was basically similar to the comparable cytomegaly found in dosed rats, but it was relatively less severe and did not develop to a stage in which there was extensive loss of cytomegalic epithelial cells and tubular dilation. The cytomegaly in mice was generally graded as slight (1) or moderate (2); in only one instance was a grade of well marked (3) assigned (average numerical grade, 0.0 for control males and females, 1.5 for dosed males, and 1.8 for dosed females).

In addition to the nonneoplastic renal lesions, the present study has established the kidney of the male F344/N rat as a target organ for neoplastic changes induced by long term administration of TCE. In male rats, the high dose of TCE was associated with a significant ($P<0.05$) increase in the incidence of renal tubular cell adenocarcinoma in rats killed at the end of the experiment (control, 0/33; low dose, 0/20; high dose, 3/16). In addition, two low dose male rats had tubular cell adenoma. Other types of neoplasms found in the kidneys of male rats

included a transitional cell carcinoma of the kidney pelvis in a low dose animal and a carcinoma (NOS) of the renal pelvis in a high dose animal. One high dose female also had a renal adenocarcinoma. No renal neoplasms were found in vehicle control animals, but one untreated control male had a transitional cell papilloma of the renal pelvis.

The primary criterion used to differentiate small tubular cell adenocarcinomas from tubular cell adenomas in this study was evidence of basement membrane invasion. Marked cellular atypia, aggressive growth, and increased mitotic activity characterized the adenocarcinoma. Because of their morphologic similarities, there is little doubt that tubular cell adenomas have the potential to progress to renal adenocarcinomas. For this reason, there is validity in combining renal tubular cell adenomas and adenocarcinomas for determining the carcinogenicity of TCE. The number of renal tubular cell neoplasms in male rats is control, 0/48; low dose, 2/49; and high dose, 3/49.

Renal tumors are rare among F344/N male rats, with 3/748 (0.4%) corn oil control rats in the Bioassay Program having had adenocarcinoma, adenoma, or carcinoma of the renal pelvis (Appendix E, Table E1). All three of these tumors were adenocarcinomas, and all three occurred in animals killed at the end of their respective studies. The incidence of renal tumors among the dosed male rats in this study exceeds both historical and concurrent control rates.

The renal tumors observed in dosed male rats were probably produced by TCE administration; these tumors may have been secondary to the nephrotoxicity. However, TCE produced the same nephrotoxicity in female rats and in both sexes of mice without inducing these tumors; thus, nephrotoxicity did not lead to carcinogenicity in three of four experiments.

Both dose levels of TCE used in this study were toxic for male F344/N rats; the mechanism or causes of the early deaths among rats in this study have not been established. This decreased survival might have reduced the sensitivity of the bioassay to detect a higher incidence of adenocarcinomas or other neoplasms. Ten (20%) high dose male rats were killed accidentally by gavage error (these deaths were not considered to have been due to chemical toxicity and even with these excluded, the decrease in survival of high dose males was significant, P=0.001). There appears to be a dose-response relationship in the incidence of animals accidentally killed by gavage error (males: 1 vehicle control, 3 low dose, 10 high dose; females: 2 vehicle controls, 5 low dose, 5 high dose).

The incidence of mesotheliomas of the peritoneum among low dose male rats was increased (control, 1/50; low dose, 5/50; high dose, 1/49; $P<0.05$). The first mesothelioma to be diagnosed was in a low dose animal during week 64 (the others were at weeks 70, 97, 99, and 100), and 29/50 of the high dose males survived longer than 64 weeks. The mesothelioma found among high dose males was detected at 103 weeks, whereas the one in controls was observed at week 106. The incidence of this tumor in the low dose males exceeds the historical incidence (16/752, 2.1%, Appendix E, Table E2). This increase in mesotheliomas may have been related to the administration of TCE.

The results of the present study with epichlorohydrin-free TCE in mice are similar to those with epichlorohydrin-containing TCE (NCI, 1976). In both studies, TCE administration produced significant increases in the incidence of male and female $B6C3F_1$ mice with hepatocellular carcinoma. In the present study, a higher percentage of dosed male mice dying before the end of the study (16/34, 47%) had hepatocellular carcinoma compared with control males dying early (2/15, 13%). The earliest hepatocellular carcinoma found among dosed males was at week 57, and the earliest diagnosis among control males was at week 75. The incidence of dosed male mice having this tumor detected at the end of the study was 14/16 (88%) versus 6/33 (18%) in the control groups. The incidence of dosed male mice with hepatocellular carcinoma exceeded the rates in either concurrent or historical control groups (31/50, 62%, versus 8/48, 17%, or 120/656, 18%; Appendix E, Table E7). Pulmonary metastasis of the hepatocellular carcinomas was found in 5/31 (16%) dosed males and 1/8 (13%) control males.

The incidence of dosed female mice with hepatocellular carcinoma (13/49, 27%) was seven to nine times greater than that in either concurrent (2/48, 4%) or historical (22/751, 2.9%) control groups. Five of the 13 (31%) dosed females with this tumor died before the end of the study, while both of the control females with hepatocellular carcinoma survived to the scheduled termination. No metastases were found among female mice. Likewise, hepatocellular adenomas were increased in both sexes (males:

control, 3/48; dosed, 8/50; females: control, 2/48; dosed, 8/49).

Harderian gland adenomas were detected in 4/50 (8%) dosed male and 3/49 (6%) dosed female mice, all of which died prior to the end of the study. No Harderian gland tumors were found in control mice. Among males, the incidence was significant by life table analysis ($P<0.05$), but not by the incidental tumor test. The incidental tumor test is the most appropriate method of analysis because this type of tumor is not life threatening. The incidental tumor test also adjusts for the survival differences between dosed and control groups. Historically, Harderian gland tumors occur in 2.4% of male and 0.8% of female B6C3F$_1$ mice (Appendix E, Table E10). Harderian glands were examined microscopically only when found to be grossly abnormal at necropsy; those animals not having visible lesions were not examined similarly.

The increased incidence of dosed female mice with malignant lymphoma ($P<0.05$) and lymphoma or leukemia ($P<0.05$) are not considered to be related to the administration of TCE. The incidence in the concurrent control group was somewhat lower than that in the historical controls, and the incidences in dosed females were within the ranges of historical incidences (Appendix E, Table E9).

Although the incidence of alveolar/bronchiolar adenomas was significantly increased in dosed female mice ($P<0.05$, life table analysis), the significance was lost when adenomas were combined with carcinomas. While this increase in adenomas cannot be ignored, to distinguish between some alveolar/bronchiolar adenomas and carcinomas is sometimes difficult or arbitrary. Therefore, the most meaningful measure of these tumors is considered to be their combined incidence. Because of this, the increased incidence of alveolar/bronchiolar adenomas in dosed female mice is not considered to be due to TCE administration. Using the data available from the previous study (NCI, 1976), IARC considered the increased incidences of lung tumors as being caused by TCE (IARC, 1979; 1982).

Three tumors of the nonglandular stomach were found among dosed female mice, but their incidence was not significantly increased when compared with that of the controls. One animal (dead at week 65) had a squamous cell carcinoma, and two others (dead at week 89 and week 99) had squamous cell papillomas. Squamous cell papillomas have been detected previously in 3/656 (0.5%) gavage control females, while squamous cell carcinomas have never been reported in gavage control females in the Bioassay Program. One vehicle control male in the present study (dead at week 63) had a squamous cell carcinoma. While these stomach lesions in dosed female mice may have been due to the 2-year gavage administration of TCE, their appearance is not considered to be an indication of carcinogenicity per se. The earlier bioassay of TCE (NCI, 1976) did not reveal evidence of chemically-related stomach neoplasms.

In both the rat and mouse portions of this study, many of the lymph nodes, thymuses, tracheae, pituitaries, thyroids, parathyroids, and ovaries were not examined microscopically. These omissions appear to be the result of faulty histological techniques. Because none of these tissues are likely target organs for TCE, these losses are of lesser significance with respect to this study. The results in male F344/N rats were considered equivocal for detecting a carcinogenic response because both groups receiving TCE showed significantly reduced survival compared to vehicle controls (35/50, 70%; 20/50, 40%; 16/50, 32%) and because 20% of the animals in the high dose group were killed accidently by gavage error.

As of August 1983, Dr. C. Maltoni (Institute of Oncology, Bologna, Italy) was examining the histopathological portion of several long term carcinogenesis studies of TCE administered by inhalation at 0, 10, 100, 300, and 600 ppm and for different periods of time up to two years to male and female Sprague-Dawley rats and to male and female Swiss and B6C3F$_1$ mice, and by gavage (0, 50, and 250 mg/kg body weight in olive oil) to male and female Sprague-Dawley rats. Animals were kept until natural death. The studies included 3,500 to 4,000 animals, most of which were treated by inhalation.

Conclusions: Under the conditions of these studies, epichlorohydrin-free trichloroethylene caused renal tubular-cell neoplasms in male F344/N rats, produced toxic nephrosis in both sexes, and shortened the survival time of males. This experiment in male F344/N rats was considered to be inadequate to evaluate the presence or absence of a carcinogenic response to trichloroethylene. For female F344/N rats receiving trichloroethylene, containing no epichlorohydrin, there was no evidence of carcinogenicity. Trichloroethylene (without epichlorohydrin) was carcinogenic for B6C3F$_1$ mice, causing increased incidences of hepatocellular carcinomas in males and females and of hepatocellular adenomas in females.

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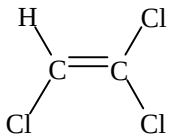
Appendix C: Report on Carcinogens (RoC), 9th Edition Profile for Trichloroethylene. C-1 – C-5

TRICHLOROETHYLENE
CAS No. 79-01-6
First listed in the *Ninth Report on Carcinogens*



CARCINOGENICITY

Trichloroethylene (TCE) is *reasonably anticipated to be a human carcinogen* based on limited evidence of carcinogenicity from studies in humans, sufficient evidence of malignant tumor formation in experimental animals, and convincing relevant information that trichloroethylene acts through mechanisms indicating it would likely cause cancer in humans.

Epidemiological data are limited for evaluating the carcinogenicity of trichloroethylene in humans. Studies have suggested that occupational exposure to TCE causes cancer of the liver and biliary tract, and also non-Hodgkin's lymphoma (IARC V.63, 1995). Another study has indicated that occupational exposure to TCE has been associated with cancer of the kidneys (Henschler et al., 1995a,b; Brüning et al., 1997). Results of three cohort studies consistently indicate an excess relative risk for cancer of the liver and biliary tract, with a total of 23 observed cases, whereas 12.87 were expected (RR = 1.8), and a moderately elevated risk for non-Hodgkin's lymphoma (IARC V.63, 1995). Further, the suggested marginally increased risk for non-Hodgkin's lymphoma in areas with trichloroethylene-contaminated ground water deserves mention (IARC V.63, 1995). For a cohort of cardboard workers exposed almost exclusively to high levels of TCE, the standardized incidence ratio for kidney cancer was 7.97 (95% CI = 2.59-18.59) (Henschler et al., 1995a).

The findings in humans are predated and supported by evidence in experimental animals. Target site concordance for TCE-induced tumors is consistent between humans and rodents. In mice, TCE causes increases in benign and malignant tumors of the liver (NCI 2, 1976; Maltoni et al., 1988; cited by IARC V.63, 1995; NTP 243, 1990), increases in tumors of the lung (Maltoni et al., 1988; cited by IARC V.63, 1995), and lymphomas (Henschler et al., 1980). In rats, TCE induces cancers of the kidney (Maltoni et al., 1988; cited by IARC V.63, 1995; NTP 243, 1990; NTP 273, 1988), interstitial cell tumors of the testis (Maltoni et al., 1988; cited by IARC V.63, 1995; NTP 273, 1988), and possibly leukemias (Maltoni et al., 1988; cited by IARC V.63, 1995).

ADDITIONAL INFORMATION RELEVANT TO CARCINOGENESIS OR POSSIBLE MECHANISMS OF CARCINOGENESIS

Mechanistically, renal cell carcinomas (RCCs) from workers occupationally exposed to high levels of TCE exhibited somatic mutations of the von Hippel-Landau (VHL) tumor suppressor gene, a gene that has been associated with renal cell carcinomas (Brüning et al., 1997). RCC tissues from all 23 TCE-exposed persons [mainly from Henschler et al. (1995a) cohort] analyzed thus far showed aberrations of the VHL gene, with 30% having aberrations in exon 1, 44% in exon 2, and 26% in exon 3. By comparison to TCE-unexposed RCC patients, VHL mutation frequencies of 33-55% were found in different cohorts, with about 24% affecting exon 2.

There is biological plausibility of the kidney tumors observed and TCE exposures because (1) site and histopathological characteristics of the tumors observed in patients and in experimental animals are identical (Vamvakas et al., 1993); (2) the molecular mechanism of this type of nephrocarcinogenicity has been elucidated (Dekant et al., 1986; cited by IARC V.63, 1995 and Bernauer et al., 1996); (3) the metabolites derived from the likely ultimate electrophilic intermediates of the bioactivation of TCE are identical in humans and in experimental animals (Birner et al., 1993; cited by IARC V.63, 1995 and Clewell et al., 1995); and (4) taking the key urinary metabolites (mercapturic acids) as an indicator of the bioactivation of TCE (Birner et al., 1993; cited by IARC V.63, 1995 and Clewell et al., 1995), humans seem to be more sensitive than rats in developing the primary biochemical lesion leading to the induction of renal cancer.

Rodents exposed to TCE typically exhibit dose-related cytomegaly of the kidneys, the lesion often being more severe in males, with none or few being found in male or female vehicle-control mice or rats. Toxic nephropathy commonly occurs in the solvent-exposed rodents, likewise being more frequent and more severe than seen in controls. In humans, substantially more cases of tubule cell damage were found among renal cell carcinoma patients who had been exposed to high levels of TCE over many years than among RCC patients who had not been exposed to TCE (Henschler et al., 1995a).

Studies of chromosomal aberrations, aneuploidy, and sister chromatid exchanges in peripheral lymphocytes of workers exposed to TCE were considered inconclusive. In rodents, TCE did not induce chromosomal aberrations, dominant lethal mutations, sister chromatid exchange, or unscheduled DNA synthesis, whereas an increase in micronuclei and DNA single-strand breaks/alkaline labile sites was observed. TCE did not induce gene mutations in human cells. In mammalian cells *in vitro*, TCE induced cell transformation, sister chromatid exchange, and gene mutations, but not chromosome aberrations (IARC V.63, 1995).

PROPERTIES

TCE is a colorless liquid with a sweet, chloroform-like odor. Upon combustion TCE produces irritants and toxic gases, which may include hydrogen chloride. In the presence of moisture and light, it decomposes by forming hydrochloric acid (HSDB, 1997).

USE

TCE is used mainly as a degreaser for metal parts. Five main industrial groups use TCE in vapor or cold degreasing operations: furniture and fixtures, fabricated metal products, electrical and electronic equipment, transport equipment, and miscellaneous manufacturing industries (IARC V.63, 1995). TCE can be used as an extraction solvent and a chemical intermediate and as a component in adhesives, lubricants, paints, varnishes, paint strippers, pesticides, and cold metal cleaners (ATSDR, 1995-H008).

PRODUCTION

IARC (V.63, 1995) reported that two companies in the United States produced TCE ca. 1992 with a combined annual capacity of 160,000 tons (145,000 metric tons or Mg). The SRI *Directory of Chemical Producers in the United States* listed only one producer (SRIa, 1996).

EXPOSURE

Air is the primary route of potential environmental exposure to TCE. Most of the TCE emissions into the atmosphere are from vapor degreasing operations. Mean TCE background levels in air range from 0.03 parts per billion (ppb) (0.16 $\mu g/m^3$) in rural areas to 0.46 ppb (2.5 $\mu g/m^3$) for urban and suburban areas. Areas near emission sources have up to 1.2 ppb (6.4 $\mu g/m^3$) TCE in the air (ASTDR, 1995-H008).

The Toxic Chemical Release Inventory for 1995 (TRI95, 1997) contains reports on environmental releases of TCE from 717 U.S. facilities. Of these, 591 reported releases to the atmosphere of more than 2000 lb (0.9072 Mg), with releases ranging from 2000 to > 200,000 lb. The total amount of TCE released in 1995 by the 717 facilities was 25,484,235 lb (11,559 Mg), while the 17 greatest emitters together released 6.1 million lb (2770.4 Mg). The greatest releases were generally from metalworking facilities, with 3 sites each reporting under Standard Industrial Classification (SIC) codes 3317 (steel pipe and tubes) and 3714 (motor vehicle parts and accessories). Other facilities (1 each) reported under SICs 3089 (plastics and plastic products, not elsewhere classified), 3671 (electron tubes), and 3721 (aircraft).

TCE is one of the volatile organic compounds (VOCs) measured in the U.S. EPA's large-scale Total Exposure Assessment Methodology (TEAM) studies (Wallace et al., 1996). In studies in the United States (Maryland, New Jersey, and California) in the 1980s (1981-1987), determination of TCE exposure via personal air monitors carried by 750 persons for two consecutive 12-hour periods revealed TCE median personal air concentrations of 0.3 to 3.0 $\mu g/m^3$. Breath samples taken in the evenings after several hours at home from 50 to 350 persons in two New Jersey cities in 1981-1983 and 75 persons in two California towns in 1984 had 0.1 to 0.9 μg TCE/m^3 (median personal air concentrations of 1.7-3.0 $\mu g/m^3$). However, in 1984 and 1987, TCE was not detected in the breath of 140 persons in Los Angeles, CA (personal air levels were 0.3-1.2 $\mu g/m^3$), nor in 1987 in 75 persons in Baltimore, MD (personal air levels were 1.1 μg TCE/m^3).

Industrial discharges of wastewater streams are the primary release of TCE into aquatic systems. TRI95 (1997) includes data from 28 facilities that had each released more than 10 lb (4.5 kg) TCE to water in 1995. Five facilities each released 250 to 280 lb (114 to 127 kg). The total release of TCE to water was 1477 lb (0.670 Mg). Four of the five facilities were metalworking plants; one was a plant that produced TCE as a by-product and for onsite use and processing. TCE background levels in large bodies of water range from 0.001 to 0.007 ppb ($\mu g/L$), while values reported for rainwater and snow are 0.0008 to 0.039 ppb ($\mu g/L$) TCE (Gist and Burg, 1995). In the U.S. EPA's Contract Laboratory Program Statistical Database, TCE was found in approximately 3% of surface water samples and 19% of groundwater samples at geometric mean surface water concentration of 40.2 ppb (individual sample values ranged from 0.0001 to 120 ppb) and geometric mean ground water concentration of 27.3 ppb (individual sample values ranged from <0.1 to ≤27300 ppb) (USEPA, 1989; cited by IARC V.63, 1995). The total releases of TCE to land and underground injection wells in 1995 were 3577 lb (1.622 Mg) and 550 lb (0.249 Mg), respectively (TRI95, 1997).

TCE is present in typewriter correction fluids, paint removers, strippers, adhesives, spot removers, and rug-cleaning fluids (Gist and Burg, 1995). Former uses of TCE as an extraction solvent for cosmetic and drug products and as a dry cleaning agent have been discontinued (IARC V.63, 1995).

TCE has been found in a variety of foods with the highest levels being found in meats, 12-16 ppb (0.09-0.12 $\mu mol/kg$), and U.S. margarine, 440-3,600 ppb (3.35-27.4 $\mu mol/kg$)

(ATSDR, 1995-H008). TCE had been used as an extraction solvent for natural fats and oils, spices, hops, and caffeine (from coffee), but FDA banned these uses in 1977 (IARC V.63, 1995).

According to the National Institute for Occupational Safety and Health (NIOSH, 1990), 401,373 employees in 23,225 plants in the United States National Occupational Exposure Survey (1981-1983) were potentially exposed to TCE.

REGULATIONS

EPA regulates TCE as a Hazardous Air Pollutant under the Clean Air Act (CAA) 1990 Amendments and as a Volatile Organic Compound (VOC) subject to emission standards under the CAA Section 111 (40 CFR Part 60, 1995) (CHEMLIST, 1997).

Under the Safe Drinking Water Act, the Maximum Contaminant Level (MCL) for community and nontransient, noncommunity water systems is set at 0.005 mg/L (40 CFR Part 141, 1996) (CHEMLIST, 1997). The World Health Organization (WHO, 1993) recommended a provisional guideline value for TCE in drinking water of 0.070 mg/L. Based on a 1985 study by Buben and O'Flaherty, WHO (1993) calculated a total daily intake (TDI) of 0.0238 mg/kg bw by applying an uncertainty factor of 3000 to the study's LOAEL (lowest observable adverse effect level) of 100 mg/kg bw/day when mice were exposed for 5 days/week for 6 weeks. The observed adverse effects were minor effects in relative liver weight. Ten percent of the TDI was allocated to drinking water to derive the provisional guideline value of 70 µg/L.

TCE is regulated under RCRA as a Halogenated Organic Compound (HOC) and under the Land Disposal Restrictions. Under the latter, hazardous wastes that contain total concentrations of HOCs of at least 1000 mg/L (liquids) or 1000 mg/kg (nonliquids) are prohibited from land disposal. Under 40 CFR 268.40 and 268.48, treatment standards are given for wastewater and nonwastewater extract concentrations, or the applicable Technology Code (40 CFR 268.42) is given (CHEMLIST, 1997).

TCE is regulated under Sections 110 and 313 of the Superfund Amendment Reauthorization Act (SARA). Priority data needs established under Section 110 include exposure levels in humans living near hazardous waste sites and other populations and epidemiological studies on health effects, including carcinogenicity. Under EPCRA Section 313 (Community Right-to-Know and the Toxic Chemical Release Inventory [TRI], 40 CFR Part 372 Subpart D, 1992), TCE is one of the 19 substances for which the de minimus for reporting changes from 1.0 percent to 0.1 percent. Under TRI, since 1989, manufacturers of at least 25,000 lb/yr (11,350 kg/yr) and other handlers of at least 10,000 lb/yr (4,540 kg/lb) must report releases of TCE to any environmental medium. Under 40 CFR Part 302 Table 302.4, TCE is on the CERCLA List of Hazardous Substances with an RQ for reporting releases of 100 lb (45.4 kg) or more (CHEMLIST, 1997).

TCE is regulated under the Clean Water Act (CWA) Sections 301, 307, and 311 (40 CFR Part 423, 1996; 40 CFR Parts 116 and 117, 1996). TCE is a priority pollutant in final discharges resulting from steam electric power generation. It is designated a hazardous substance if discharged to navigable waters. The Reportable Quantity (RQ) for notification is 100 lb (45.4 kg) (CHEMLIST, 1997).

FDA regulations govern the presence of TCE in color additives, bottled water, food as extraction solvent residues, and as indirect additives as migrants from adhesives, etc., used in food packaging.

The OSHA Permissible Exposure Limit (PEL) for time-weighted average (TWA) exposure in a 40-hour work week to TCE in workroom air is 100 ppm (537 mg/m^3) with a ceiling value of 200 ppm (1070 mg/m^3) (29 CFR 1910.1000, 1996 [CHEMLIST, 1997]). NIOSH considers TCE to be a potential occupational carcinogen, recommending that exposure be limited to the lowest feasible concentration. NIOSH recommends a REL (Recommended Exposure Level) of 2 ppm (11 mg/m^3) during use of TCE as an anesthetic and a 10-hour TWA of 25 ppm (130 mg/m^3) during all other exposures (Ludwig, 1994). The Threshold Limit Value (TLV®) recommended by ACGIH is 50 ppm (269 mg/m^3); the Short-Term Exposure Limit or Ceiling recommended is 100 ppm (537 mg/m^3). ACGIH (1996) classified TCE as A5 (*Not Suspected as a Human Carcinogen*). Regulations are summarized in Volume II, Table B-117.