WHO/SDE/WSH/05.08/22
English only

Trichloroethene in Drinking-water

Background document for development of
WHO *Guidelines for Drinking-water Quality*

WHO information products on water, sanitation, hygiene and health can be freely downloaded at:
http://www.who.int/water_sanitation_health/

Preface

One of the primary goals of WHO and its member states is that "all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water." A major WHO function to achieve such goals is the responsibility "to propose ... regulations, and to make recommendations with respect to international health matters ...."

The first WHO document dealing specifically with public drinking-water quality was published in 1958 as *International Standards for Drinking-water*. It was subsequently revised in 1963 and in 1971 under the same title. In 1984–1985, the first edition of the WHO *Guidelines for Drinking-water Quality* (GDWQ) was published in three volumes: Volume 1, Recommendations; Volume 2, Health criteria and other supporting information; and Volume 3, Surveillance and control of community supplies. Second editions of these volumes were published in 1993, 1996 and 1997, respectively. Addenda to Volumes 1 and 2 of the second edition were published on selected chemicals in 1998 and on microbial aspects in 2002. The third edition of the GDWQ was published in 2004, and the first addendum to the third edition was published in 2005.

The GDWQ are subject to a rolling revision process. Through this process, microbial, chemical and radiological aspects of drinking-water are subject to periodic review, and documentation related to aspects of protection and control of public drinking-water quality is accordingly prepared and updated.

Since the first edition of the GDWQ, WHO has published information on health criteria and other supporting information to the GDWQ, describing the approaches used in deriving guideline values and presenting critical reviews and evaluations of the effects on human health of the substances or contaminants of potential health concern in drinking-water. In the first and second editions, these constituted Volume 2 of the GDWQ. Since publication of the third edition, they comprise a series of free-standing monographs, including this one.

For each chemical contaminant or substance considered, a lead institution prepared a background document evaluating the risks for human health from exposure to the particular chemical in drinking-water. Institutions from Canada, Denmark, Finland, France, Germany, Italy, Japan, Netherlands, Norway, Poland, Sweden, United Kingdom and United States of America prepared the documents for the third edition and addenda.

Under the oversight of a group of coordinators each of whom was responsible for a group of chemicals considered in the GDWQ, the draft health criteria documents were submitted to a number of scientific institutions and selected experts for peer review. Comments were taken into consideration by the coordinators and authors. The draft documents were also released to the public domain for comment and submitted for final evaluation by expert meetings.

During the preparation of background documents and at expert meetings, careful consideration was given to information available in previous risk assessments carried

out by the International Programme on Chemical Safety, in its Environmental Health Criteria monographs and Concise International Chemical Assessment Documents, the International Agency for Research on Cancer, the Joint FAO/WHO Meetings on Pesticide Residues and the Joint FAO/WHO Expert Committee on Food Additives (which evaluates contaminants such as lead, cadmium, nitrate and nitrite, in addition to food additives).

Further up-to-date information on the GDWQ and the process of their development is available on the WHO Internet site and in the current edition of the GDWQ.

Acknowledgements

The first draft of Trichloroethene in Drinking-water, Background document for development of WHO *Guidelines for Drinking-water Quality*, was prepared by members of the Water Quality and Health Bureau, Health Canada, to whom special thanks are due.

The work of the following working group coordinators was crucial in the development of this document and others contributing to the first addendum to the third edition:

Dr J. Cotruvo, J. Cotruvo Associates, USA (*Materials and chemicals*)
Mr J.K. Fawell, United Kingdom (*Naturally occurring and industrial contaminants*)
Ms M. Giddings, Health Canada (*Disinfectants and disinfection by-products*)
Mr P. Jackson, WRc-NSF, United Kingdom (*Chemicals – practical aspects*)
Prof. Y. Magara, Hokkaido University, Japan (*Analytical achievability*)
Dr E. Ohanian, Environmental Protection Agency, USA (*Disinfectants and disinfection by-products*)

The draft text was discussed at the Working Group Meeting for the first addendum to the third edition of the GDWQ, held on 17–21 May 2004. The final version of the document takes into consideration comments from both peer reviewers and the public. The input of those who provided comments and of participants in the meeting is gratefully acknowledged.

The WHO coordinator was Dr J. Bartram, Coordinator, Water, Sanitation and Health Programme, WHO Headquarters. Ms C. Vickers provided a liaison with the International Programme on Chemical Safety, WHO Headquarters. Mr Robert Bos, Water, Sanitation and Health Programme, WHO Headquarters, provided input on pesticides added to drinking-water for public health purposes.

Ms Penny Ward provided invaluable administrative support at the Working Group Meeting and throughout the review and publication process. Ms Marla Sheffer of Ottawa, Canada, was responsible for the scientific editing of the document.

Many individuals from various countries contributed to the development of the GDWQ. The efforts of all who contributed to the preparation of this document and in particular those who provided peer or public domain review comment are greatly appreciated.

Acronyms and abbreviations used in the text

BMD	benchmark dose
BMDL	lower 95% confidence limit of the benchmark dose
$BMDL_x$	lower 95% confidence limit estimate of dose corresponding to an x% level of risk over background levels
CAS	Chemical Abstracts Service
CH	chloral hydrate
CI	confidence interval
CYP	cytochrome P450
DCA	dichloroacetic acid
DCVC	*S*-dichlorovinyl-L-cysteine
1,1-DCVC	*S*-(1,1-dichlorovinyl)-L-cysteine
1,2-DCVC	*S*-(1,2-dichlorovinyl)-L-cysteine
2,2-DCVC	*S*-(2,2-dichlorovinyl)-L-cysteine
DCVG	*S*-(1,2-dichlorovinyl) glutathione
DCVNac	*N*-acetyl-*S*-dichlorovinyl-L-cysteine
1,2-DCVNac	*N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine
2,2-DCVNac	*N*-acetyl-S-(2,2-dichlorovinyl)-L-cysteine
DNA	deoxyribonucleic acid
EBCT	empty bed contact time
EPA	Environmental Protection Agency (USA)
FAO	Food and Agriculture Organization of the United Nations
GAC	granular activated carbon
GDWQ	*Guidelines for Drinking-water Quality*
GSH	glutathione
GST	glutathione-*S*-transferase
HBV	health-based value
Ieq	ingestion equivalent
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LMS	linearized multistage
LOAEL	lowest-observed-adverse-effect level
LOEL	lowest-observed-effect level
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
OR	odds ratio
PAC	powdered activated carbon
PCE	perchloroethylene (tetrachloroethene)

PPAR	peroxisome proliferator activated receptor
RR	relative risk
SCE	sister chromatid exchange
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SSCP	single-stranded conformation polymorphism
TCA	trichloroacetic acid
TCE	trichloroethene
TCOG	trichloroethanol glucuronide
TCOH	trichloroethanol
TDI	tolerable daily intake
TGF	transforming growth factor
USA	United States of America
US EPA	United States Environmental Protection Agency
UV	ultraviolet
VHL	von Hippel Landau
WHO	World Health Organization

Table of contents

1. GENERAL DESCRIPTION

1.1 Identity

CAS No.:	79-01-6
Molecular formula:	C_2HCl_3

Trichloroethene is also known as trichloroethylene and TCE.

1.2 Physicochemical properties[1]

Property	*Value*	*Reference*
Boiling point	86.7 °C	Windholz et al., 1976
Vapour pressure	8.0–9.9 kPa at 20–25 °C	McNeill, 1979; ATSDR, 1989
Water solubility	1.1–1.4 g/litre	ATSDR, 1997
Log octanol–water partition coefficient	2.29–2.42	Hansch & Leo, 1985; US EPA, 1985b
Henry's law constant	1.1 kPa·m^3/mol at 25 °C	Hine & Mookerjee, 1975

1.3 Organoleptic properties

TCE has a sweet odour. Its odour thresholds are 546–1092 mg/m^3 in air and 0.31 mg/litre in water (Amoore & Hautala, 1983; Ruth, 1986).

1.4 Major uses and sources in drinking-water

TCE is used primarily in metal degreasing operations. It is also used as a solvent for greases, oils, fats and tars, in paint removers, coatings and vinyl resins, and by the textile processing industry to scour cotton, wool and other fabrics. TCE may be used as a chemical intermediate in the production of polyvinyl chloride, pharmaceuticals, flame retardant chemicals and insecticides. It may also be present in household and consumer products, such as typewriter correction fluids (ATSDR, 1997).

Most of the TCE used for degreasing is believed to be emitted to the atmosphere (US EPA, 1985a). TCE may also be introduced into surface water and groundwater in industrial effluents (IPCS, 1985). Poor handling as well as improper disposal of TCE in landfills have been the main causes of groundwater contamination. The biodegradation of another volatile organic pollutant, tetrachloroethene (or perchloroethylene, PCE), in groundwater may also lead to the formation of TCE (Major et al., 1991).

[1] Conversion factor in air: 1 ppm = 5.41 mg/m^3 at 20 °C and 101.3 kPa (Verschueren, 1983).

1.5 Environmental fate

In the atmosphere, TCE is highly reactive and does not persist for any significant length of time (ATSDR, 1993). In surface water, volatilization is the principal route of degradation, while photodegradation and hydrolysis play minor roles. In groundwater, TCE is degraded slowly by microorganisms. Bioconcentration of trichloroethene in aquatic species is low (ATSDR, 1993).

2. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

2.1 Air

TCE has been detected in outdoor and indoor air in Canada. Levels of TCE in air were determined in Toronto and Montreal for 1 year (1984–1985) and in Sarnia and Vancouver for 1 month (autumn 1983). Mean levels for the four cities were 1.9, 0.7, 1.2 and 1.0 μg/m^3, respectively, with maxima of 8.6, 1.7, 3.6 and 3.4 μg/m^3, respectively (Environment Canada, 1986). In another survey, mean concentrations of TCE in ambient air at 11 urban sites and 1 rural site in Canada (1988–1990) ranged from 0.07 to 0.45 μg/m^3 (Vancouver and Calgary, respectively), with an overall mean value of 0.28 μg/m^3 and a maximum single value of 19.98 μg/m^3 reported in Montreal (Dann, 1993).

Recent US data are similar to the levels measured in Canada. In 1998, ambient air measurement data from 115 monitors located in 14 states indicated that TCE levels ranged from 0.01 to 3.9 μg/m^3, with a mean of 0.88 μg/m^3. Mean TCE air concentrations (1985–1998) for rural, suburban, urban, commercial and industrial land uses were 0.42, 1.26, 1.61, 1.84 and 1.54 μg/m^3, respectively (US EPA, 1999a).

The mean air concentration in approximately 750 homes from 10 Canadian provinces surveyed in 1991 was 1.4 μg/m^3, with a maximum value of 165 μg/m^3 (Otson et al., 1992). In two homes tested, it was reported that showering with well water containing extremely high levels of TCE (40 mg/litre) increased levels of TCE in bathroom air from <0.5 to 67–81 mg/m^3 in less than 30 min (Andelman, 1985).

2.2 Water

TCE has been detected frequently in natural water and drinking-water in Canada and other countries. Due to its high volatility, TCE concentrations are normally low in surface water (1 μg/litre). However, in groundwater systems where volatilization and biodegradation are limited, concentrations may be higher if contamination has occurred in the vicinity and leaching has taken place.

Because analytical methods have improved over the years since TCE was first assayed, concentrations that were once considered "non-detectable" are now quantifiable. This confounds the use of historical TCE data, as the values for "non-detectable" have changed over time.

TCE was detected in raw and treated water at 10 potable water supply facilities in Ontario in 1983 at levels ranging from 0.1 to 0.8 µg/litre (Mann Testing Laboratories Ltd, 1983). In 1979, TCE was found in over half of potable water samples taken at 30 treatment facilities across Canada; mean concentrations were 1 µg/litre or less, and the maximum level was 9 µg/litre (Otson et al., 1982).

Monitoring data from eight Canadian provinces for the period 1985–1990 indicated that 95% of 7902 samples from drinking-water supplies (raw, treated or distributed water) had TCE concentrations below 1 µg/litre. The maximum concentration was 23.9 µg/litre (groundwater sample). Most (75%) of the samples in which TCE was detected were from groundwater sources (Department of National Health and Welfare, 1993). More recent data from New Brunswick (1994–2001), Alberta (1998–2001), the Yukon (2002), Ontario (1996–2001) and Quebec (1985–2002) for raw (surface water and groundwater), treated and distributed water indicated that more than 99% of samples contained TCE at concentrations less than or equal to 1.0 µg/litre. The maximum concentration was 81 µg/litre. Of those samples with detectable TCE concentrations, most were from groundwater (Alberta Department of Environmental Protection, New Brunswick Department of Health and Wellness, Ontario Ministry of Environment and Energy, Yukon Department of Health and Social Services and Quebec Ministry of the Environment, personal communications, 2002).

A 2000 survey of 68 First Nations community water supplies (groundwater and surface water) in Manitoba found that TCE concentrations were non-detectable (<0.5 µg/litre) (Yuen & Zimmer, 2001).

Groundwater is the sole source of water for an estimated 25–30% of the Canadian population (Statistics Canada, 1994). In 1995, a national review of TCE occurrence data was carried out to determine the extent of groundwater contamination by TCE and the number of people potentially exposed to contaminated drinking-water. The majority of sites were from Ontario and New Brunswick. The review was based on urban groundwater supplies. Of the 481 municipal/communal and 215 private/domestic groundwater supplies (raw water), 8.3% and 3.3%, respectively, contained TCE, at average maximum concentrations of 25 µg/litre and 1680 µg/litre, respectively. This review involved a compilation of data from a variety of sources over different periods of time. Consequently, interpretation of the data is made more difficult by the range of detection limits. A majority of all sites (93%) had non-detectable levels (<0.01–10 µg/litre), 3.6% had a maximum concentration of <1 µg/litre, 1.4% had a maximum of 1–10 µg/litre, 0.43% had a maximum of 10–100 µg/litre and 1.3%[2] had a maximum of >100 µg/litre (Raven and Beck Environmental Ltd, 1995).

[2] Based on the information provided, it was not possible to determine the exact TCE concentration of the seven private/domestic water supply sites (3.3%) with detectable residues; therefore, for the purposes of this calculation, it was assumed that all concentrations were >100 µg/litre.

It was estimated that approximately 1.67 million of the 7.1 million Canadians who relied on groundwater for household use in 1995 were covered by this study. Of the 1.67 million surveyed, the water supplies of 49% had non-detectable levels of TCE (<0.01–10 µg/litre), 48.1% had a maximum of 1–10 µg/litre, 2.1% had a maximum of 10–100 µg/litre and 0.8% had a maximum of >100 µg/litre. Despite the problems associated with the wide range of detection limits reported in this study, the results of the survey suggested that more than 95% of Canadians who rely on groundwater are exposed to less than 10 µg/litre in their drinking-water. In fact, this probably represents a worst-case scenario, since the sampled data were for raw water and may not be representative of water received at households (Raven and Beck Environmental Ltd, 1995).

In the USA, TCE has been the volatile organic contaminant that is most frequently found in groundwater and the one present in the highest concentrations (ATSDR, 1997). TCE was detected (detection limit 0.2 µg/litre) in 91 of 945 (9.6%) samples of finished water using groundwater sources nationwide. The median level in positive samples was 1 µg/litre, and the maximum was 130 µg/litre. In samples taken from tap water in homes near the Love Canal waste site, TCE levels ranged from 10 to 250 ng/litre. In New Jersey, TCE was detected in 388 of 669 (58%) samples taken between 1977 and 1979, with a maximum concentration of 635 µg/litre (ATSDR, 1997). TCE levels ranging from 900 to 27 300 µg/litre were found in contaminated wells in a survey of four states (Pennsylvania, New York, Massachusetts and New Jersey) (ATSDR, 1997). TCE was detected in 28% of 9295 surface water samples taken nationwide between 1980 and 1982 in the USA. A similar percentage was found in two surveys (n = 6322) of the Ohio River system (1978–1979 and 1980–1981), with TCE levels ranging from 0.1 to 1 µg/litre. TCE was detected (maximum level of 32.6 µg/litre) in 261 of 462 (56%) surface water samples collected in New Jersey between 1977 and 1979. In 1981, mean TCE levels of 0.008–0.13 µg/litre were detected in the Niagara River and Lake Ontario (ATSDR, 1997).

2.3 Multiroute exposure through drinking-water

Due to TCE's volatility and lipid solubility, exposure can also occur dermally and through inhalation, especially through bathing and showering. For the purposes of assessing overall TCE exposure, the relative contribution of each exposure route needs to be assessed and is expressed in ingestion equivalents (Ieq) per day. For example, an inhalation exposure of 1.7 Ieq/day means that the daily exposure to TCE via inhalation is equivalent to a person drinking an extra 1.7 litres of water per day.

Bogen et al. (1988) accounted for oral, dermal and inhalation routes of exposure to TCE from household uses of tap water. They proposed lifetime Ieq/day values for 70-kg adults of 2.2 (ingestion), 2.9 (inhalation) and 2 (dermal). The ingestion value was based on the consideration of US age-specific consumption rates, and the dermal number was derived using a generic dermal absorption coefficient value for volatile organic compounds, rather than a TCE-specific value. In addition to the shower scenario, these authors quantified exposure via household air when determining the Ieq/day value for the inhalation route.

Weisel & Jo (1996) concluded that the dermal and inhalation routes contribute internal doses similar to that from ingestion of tap water and that their total contribution is greater than that from ingestion. However, in the absence of data for route-specific doses and the TCE concentration in air, a verification of their conclusions and the determination of Ieq/day values for the various routes are not easily achieved.

Lindstrom & Pleil (1996) outlined simple methodological approaches for the calculation of potential doses received by the ingestion, dermal and inhalation routes. Using a water concentration of 4.4 µg/litre, these authors calculated that the ingested dose was more important than the inhaled dose for a 10-min shower, which, in turn, was greater than the dermal dose.

Krishnan (2003) determined Ieq/day values for dermal and inhalation exposures of adults and children (6-, 10- and 14-year-olds) to TCE (5 µg/litre) in drinking-water for a 10-min shower and a 30-min bath on the basis of the methodological approach of Lindstrom & Pleil (1996), the use of physiologically based pharmacokinetic models and consideration of the fraction absorbed (Laparé et al., 1995; Lindstrom & Pleil, 1996; Poet et al., 2000). The "fraction absorbed" for the dermal and inhalation exposures took into consideration the TCE dose that was absorbed following exposure as well as that portion that was excreted in the following 24 h. It was assumed that 100% of the skin is exposed in both the shower and bath scenarios, and a dermal absorption coefficient specific to TCE was used (Nakai et al., 1999). Complete (100%) absorption of ingested drinking-water was assumed for all subpopulations; this was supported by the extent of hepatic extraction of TCE (Laparé et al., 1995).

Ieq/day values for the inhalation and dermal routes were higher for the 30-min bath scenario than for the 10-min shower for all subpopulations based on the longer exposure time. The highest value was 5.0 Ieq/day (2 litres ingestion, 2.3 litres inhalation, 0.7 litres dermal) for adults. The 5.0 Ieq/day value is considered to be conservative, since most people do not take a 30-min bath on a daily basis. In the event that individuals spend more than 10 min in a shower or are exposed to TCE via other household activities, the calculated 5.0 Ieq/day value (which includes inhalation and dermal exposure from a 30-min bath) should be adequate.

2.4 Food

The US EPA (2001) concluded that exposure to TCE from food was probably low and that there were insufficient food data for reliable estimates of exposure. The daily intakes of TCE in food for Canadian adults (20–70 years old) and children (5–11 years old) were estimated to range from 0.004 to 0.01 µg/kg of body weight per day and from 0.01 to 0.04 µg/kg of body weight per day, respectively (Department of National Health and Welfare, 1993). These numbers were based on TCE concentrations from US food surveys from the mid- to late 1980s as well as Canadian food consumption data. In recent decades, severe restrictions have been placed on the use of TCE in food processing in North America, and the disposal of TCE is more

carefully controlled in other industrial sectors. Therefore, there is no reason to suppose that these values would have increased in the interim.

2.5 Estimated total exposure and relative contribution of drinking-water

In order to assess the approximate contribution of drinking-water (ingestion, inhalation and dermal) to total TCE exposure, scenarios for adults (20–59 years) and children (5–11 years) were calculated[3] using representative TCE concentrations for non-contaminated (1 µg/litre) and contaminated (10 µg/litre) drinking-water. In both scenarios, the average indoor (1.4 µg/m^3) and outdoor (0.28 µg/m^3) air concentrations were used, along with maximum food intake values of 0.01 µg/kg of body weight per day and 0.04 µg/kg of body weight per day for adults and children, respectively (Department of National Health and Welfare, 1993).

In the non-contaminated (1 µg/litre) drinking-water scenario, ≤15% of total exposure was derived from drinking-water for both adults and children. In the contaminated scenario (10 µg/litre), drinking-water comprised up to 65% of total TCE exposure.

3. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

3.1 Absorption

TCE is readily absorbed following both oral and inhalation exposures. Dermal absorption is also possible, but information pertaining to this route of exposure is limited. Significant inter- and intraspecies variability in TCE absorption following all routes of exposure has been well documented.

TCE is rapidly and extensively absorbed from the gastrointestinal tract into the systemic circulation in animals. Mass balance studies using radiolabelled TCE indicated that mice and rats metabolized TCE at 38–100% and 15–100%, respectively, following oral administration in corn oil vehicle. For both species, the lower values were obtained following treatment with large doses in excess of 1000 mg/kg of body weight, implying that the rate of absorption was higher at low doses than at high doses in both species (Daniel, 1963; Parchman & Magee, 1982; Dekant & Henschler, 1983; Dekant et al., 1984; Buben & O'Flaherty, 1985; Mitoma et al., 1985; Prout et al., 1985; Rouisse & Chakrabarti, 1986). Different vehicles affect the rate of absorption, with the rate being almost 15 times greater following dosing in water than following administration in corn oil. Overall, absorption of TCE through the gastrointestinal tract is considerable and, at very low concentration, nearly complete. Although human exposure studies investigating oral absorption of TCE

[3] Adults (60 kg) and children (31 kg) were assumed to consume 2 litres per day and 1 litre per day, respectively (Health Canada, 1998; WHO, 2004). Both groups were assumed to spend 4 h per day outdoors and 20 h per day indoors (IPCS, 1994). Adults and children (5–11 years) had average inhalation volumes of 22 m^3/day and 15 m^3/day, respectively (IPCS, 1994). Ieq/day values of 5.0 litres and 2.95 litres (calculated based on methodology in Krishnan, 2003) were used for adults and children, respectively.

were not identified, numerous case-studies of accidental or intentional ingestion of TCE suggest that absorption of TCE from the gastrointestinal tract in humans is likely to be extensive (Kleinfeld & Tabershaw, 1954; DeFalque, 1961; Bruning et al., 1998).

Pulmonary uptake of TCE into the systemic circulation is rapid in animals, but blood:gas partition coefficients in rodents vary across species, strain and gender (Lash et al., 2000). After inhalation exposure to radiolabelled TCE at 54 or 3200 mg/m^3 over a 6-h period, net pulmonary uptake was 10 times greater at the higher concentration than at the lower concentration in rats, whereas it was similar at both exposure concentrations in mice (Stott et al., 1982). In humans, TCE is rapidly and extensively absorbed by the lungs and into the alveolar capillaries. The blood:air partition coefficient of TCE has been estimated to be approximately 1.5- to 2.5-fold lower in humans than in rodents (Sato et al., 1977; Monster, 1979; Clewell et al., 1995). Under non-steady-state conditions, TCE pulmonary uptake is rapid during the first 30–60 min of exposure, decreasing significantly as TCE concentrations in tissues approach steady state (Fernandez et al., 1977; Monster et al., 1979).

Dermal absorption has been demonstrated in mice (Tsuruta, 1978) and guinea-pigs (Jakobson et al., 1982). Dermal absorption has also been demonstrated in human volunteers (Stewart & Dodd, 1964; Sato & Nakajima, 1978); however, variability between individuals precludes any meaningful interpretation of these data.

3.2 Distribution

Once absorbed, TCE diffuses readily across biological membranes and is widely distributed to tissues and organs via the circulatory system. Studies in animals (e.g., Fernandez et al., 1977; Dallas et al., 1991; Fisher et al., 1991) and humans (De Baere et al., 1997) have found TCE or its metabolites in most major organs and tissues. Primary sites of distribution include the lungs, liver, kidneys and central nervous system. TCE may accumulate in adipose tissue because of its lipid solubility. Consequently, slow release of TCE from adipose stores might act as an internal source of exposure, ultimately resulting in longer mean residence times and bioavailability of TCE (Fernandez et al., 1977; Dallas et al., 1991; Fisher et al., 1991). Age-dependent factors may influence TCE distribution in humans, suggesting greater susceptibility to TCE in children than in adults (Pastino et al., 2000).

3.3 Metabolism

TCE metabolism occurs primarily in the liver, although it may also occur in other tissues, particularly the kidney. There are two main pathways responsible for TCE metabolism: oxidation by cytochrome P450 and conjugation with glutathione (GSH) by glutathione-*S*-transferases (GSTs) (OEHHA, 1999; Lash et al., 2000). In the liver, TCE is metabolized by cytochrome P450 enzymes to an epoxide intermediate, which spontaneously rearranges to chloral. Chloral is further metabolized to trichloroethanol (TCOH), trichloroethanol glucuronide (TCOG) and trichloroacetic acid (TCA) as the principal metabolites. Under certain conditions, TCE-epoxide forms dichloroacetyl chloride, which rearranges to dichloroacetic acid (DCA). Other minor metabolites

include carbon dioxide, *N*-(hydroxyacetyl)aminoethanol and oxalic acid, all believed to be products of hydrolysis of a TCE-epoxide intermediate (Goeptar et al., 1995).

In the conjugation pathway, the reactive electrophilic species produced through the oxidation are deactivated by conjugation to the nucleophilic sulfur atom of GSH. This may be catalysed by various cytosolic and microsomal GSTs or may occur spontaneously via a non-enzymatic addition/elimination reaction. The resulting conjugates undergo further metabolism to yield various metabolites, the most important of which are mercapturic acids, which are rapidly excreted in urine (Goeptar et al., 1995).

The oxidative metabolism of TCE takes place primarily in the liver, although it may occur to some extent in various other tissues, such as the lung (Lash et al., 2000). Four isozymes of cytochrome P450 (primarily CYP2E1) oxidize TCE (OEHHA, 1999; Lash et al., 2000). An intermediate electrophilic epoxide (2,2,3-trichlorooxirane, or TCE-oxide) is suspected to form during oxidative metabolism, although it is not known whether TCE-oxide exists in free form (Lash et al., 2000). TCE-oxide may be metabolized by several pathways, the predominant pathway being spontaneous rearrangement to chloral, which is then hydrated to chloral hydrate (CH) (OEHHA, 1999). CH is metabolized to TCA, which is the main TCE metabolite in the blood, and TCOH. TCA and TCOH may be further metabolized to DCA and TCOG, respectively.

The GSH conjugation also occurs primarily in the liver by GST, although several other tissues (kidney, biliary tract and intestines) are involved (Lash et al., 2000). The GSH conjugation reactions occur more slowly than the cytochrome P450-catalysed oxidation reactions. TCE is converted by GST to *S*-(1,2-dichlorovinyl) glutathione (DCVG), which is excreted into the bile, then reabsorbed through enterohepatic circulation and converted to the cysteine conjugates *S*-(1,1-dichlorovinyl)-L-cysteine (1,1-DCVC) and *S*-(1,2-dichlorovinyl)-L-cysteine (1,2-DCVC) (Lash et al., 2000; Clewell et al., 2001). 1,1-DCVC may undergo *N*-acetylation and be excreted in the urine or metabolized by a lyase enzyme to reactive metabolites, including a thioacetaldehyde, whereas 1,2-DCVC may be metabolized by *N*-acetyltransferase and excreted in the urine or converted by β-lyase to reactive metabolites, including a thioketene (Clewell et al., 2001). Therefore, exposure to TCE clearly results in exposure of tissues to a complex mixture of metabolites (OEHHA, 1999; US EPA, 2001).

Enterohepatic circulation of TCOG is believed to play a very important role in maintaining levels of TCA, which has a major impact on dosimetry and the very high clearance of TCE seen at low doses by first-pass metabolism in the liver (Stenner et al., 1997, 1998; Barton et al., 1999). This appears to control the low-dose behaviour of the metabolites, essentially favouring the oxidative metabolites. It is one of the reasons why the GSH pathway does not seem to contribute much to the clearance of TCE at low doses. Since the oxidative metabolites are clearly responsible for the effects on the liver (both cancer and non-cancer), this implies that the oral route is

most importantly related to liver effects, whereas other routes may preferentially affect other organs (e.g., kidney) (discussed in a later section).

There are several interspecies differences in TCE metabolism. For example, human hepatic microsomes possess less activity towards TCE than rat or mouse hepatic microsomes (Nakajima et al., 1993), and humans are less efficient at metabolizing TCE than rodents. Furthermore, a comparison of renal β-lyase activities in the kidney indicates that rats are more efficient than humans at metabolizing DCVC to reactive metabolites (Clewell et al., 2000). There are also intraspecies differences. In humans, interindividual variations in enzyme expression and activity, such as individual variation in activities of CYP1A2 and CYP2E1, for example, have been observed. As well, males generally have higher GSH conjugation rates than females, and genetic polymorphisms may influence GSH conjugation rates in humans (Lash et al., 2000).

The major metabolism of DCA occurs through GSH transferase (zeta), a family of cytosolic enzymes. DCA's rates of metabolism are very high compared with those of TCA and TCE, explaining why it is difficult to generate sufficient concentrations *in vivo* to measure. However, TCA is unlikely to be responsible for human liver cancer at the levels that are encountered in the environment, based on its mode of action as a peroxisome proliferator and because it produced liver tumours only in mice despite being adequately tested in rats (DeAngelo et al., 1997). One of the issues of most concern with TCE is its conversion to DCA. The relative contributions of DCA and TCA to liver tumours in mice were discussed in Chen (2000). A paper by Bull et al. (2002) strongly suggests that DCA does contribute to the liver cancer response in mice. DCA is clearly carcinogenic in both mice and rats, and its mode of action is clearly different from that of TCA. Therefore, DCA cannot be dismissed as a potential human carcinogen. It is apparent, however, that while DCA may be formed during the metabolism of TCE, it is unlikely to be produced in significant amounts at environmental levels of exposure to TCE.

3.4 Elimination

The database pertaining to the elimination of TCE is large, and TCE clearance is well characterized in both animals and humans. Although the elimination kinetics of TCE and its metabolites vary by route of exposure, elimination pathways appear to be similar for ingestion and inhalation. No data regarding the elimination of TCE and its metabolites following dermal exposures were found.

TCE is eliminated either unchanged in expired air or by metabolic transformation with subsequent excretion, primarily in urine, as TCA, TCOH or TCOG (following oxidative metabolism) or as DCVG or the cysteine conjugate *N*-acetyl-*S*-dichlorovinyl-L-cysteine (DCVNac) (following GSH conjugation). Studies in human volunteers have shown that following TCE exposure, urinary TCOH is first produced more quickly and in larger amounts than urinary TCA. However, over time, TCA production eventually exceeds that of TCOH (Nomiyama & Nomiyama, 1971; Muller et al., 1974; Fernandez et al., 1975; Sato et al., 1977; Monster & Houtkooper, 1979; Monster et al., 1979). Small amounts of metabolized TCE are excreted in the bile or

as TCOH in exhaled air. TCE may also be excreted in breast milk (Pellizzari et al., 1982; Fisher et al., 1987, 1989).

Comparative studies have found that elimination is more rapid in mice than in rats (Lash et al., 2000). However, the formation of the more toxic metabolite TCA is also approximately 10 times faster in mice than in rats. Therefore, differential elimination kinetics help explain interspecies differences in toxicity and toxicokinetics associated with TCE, given that the toxicity of TCE is linked to the formation of its metabolites (Parchman & Magee, 1982; Stott et al., 1982; Dekant et al., 1984; Buben & O'Flaherty, 1985; Mitoma et al., 1985; Prout et al., 1985; Rouisse & Chakrabarti, 1986). In humans, interindividual heterogeneity was seen in the metabolism and elimination of TCE (Nomiyama & Nomiyama, 1971; Fernandez et al., 1975; Monster et al., 1976).

4. EFFECTS ON LABORATORY ANIMALS AND IN VITRO *TEST SYSTEMS*

Many studies of a wide range of toxic end-points using repeated oral exposures to TCE have been reviewed (NTP, 1985, 1986, 1990; Barton et al., 1996; Kaneko et al., 1997). Due to the poor solubility of TCE in water, few studies used water as a vehicle (Tucker et al., 1982), although some drinking-water or water gavage studies have used emulsifying agents. Many of the studies are therefore confounded by the use of corn oil as a vehicle, which has been found to alter the pharmacokinetics of TCE and to affect lipid metabolism and other pharmacodynamic processes.

The best documented systemic effects are neurotoxicity, hepatotoxicity, nephrotoxicity and pulmonary toxicity in adult animals. Reproductive and developmental effects have also been extensively studied.

4.1 Acute exposure

Neurological, lung, kidney and heart effects have been reported in animals acutely exposed to TCE (ATSDR, 1993, 1997). Tests involving acute exposure of rats and mice have shown TCE to have low toxicity from inhalation exposure and moderate toxicity from oral exposure (RTECS, 1993; ATSDR, 1997). The 14-day acute oral LD_{50} values for TCE were determined to be 2400 mg/kg of body weight in mice (Tucker et al., 1982) and 4920 mg/kg of body weight in rats (Smyth et al., 1969; IPCS, 1985; ATSDR, 1993, 1997). The 4-h inhalation LC_{50} was calculated to be 67 600 mg/m^3 in rats (Siegel et al., 1971) and 54 700 mg/m^3 in mice (Fan, 1988). A review of studies of dermal exposure of TCE in rabbits indicates that skin irritation occurs after 24 h at 0.5 ml and degenerative skin changes occur within 15 min at 1 ml in guinea-pigs (Fan, 1988). Instillation of 0.1 ml to rabbit eyes caused conjunctivitis and keratitis, with complete recovery within 2 weeks.

4.2 Short-term exposure

In a 13-week oral study, Fischer 344/N rats and B6C3F1 mice (10 per sex per dose) were administered TCE in corn oil by gavage at doses of up to 1000 mg/kg of body

weight per day in female rats, up to 2000 mg/kg of body weight per day in male rats and up to 6000 mg/kg of body weight per day in mice of both sexes for 5 days per week (NTP, 1990). Body weights were decreased in male rats at 2000 mg/kg of body weight per day. Pulmonary vasculitis involving small veins was reported in female rats at 1000 mg/kg of body weight per day. Mild to moderate cytomegaly and karyomegaly of the renal tubular epithelial cells occurred in rats at 1000 mg/kg of body weight per day (females) or 2000 mg/kg of body weight per day (males). The no-observed-adverse-effect level (NOAEL) in rats was reported as 1000 mg/kg of body weight per day (males) and 500 mg/kg of body weight per day (females). Among the mice, there were decreases in survival in both sexes and body weight gain in males at 750 mg/kg of body weight per day and above. Doses of 3000 mg/kg of body weight per day and above were associated with centrilobular necrosis and multifocal calcification in the liver, as well as mild to moderate cytomegaly and karyomegaly of the renal tubular epithelial cells in both sexes. A NOAEL was set at 375 mg/kg of body weight per day for mice.

In drinking-water studies (Sanders et al., 1982; Tucker et al., 1982), CD-1 and ICR outbred albino mice (140 per sex per dose) were administered TCE in a 1% solution of Emulphor in drinking-water at dose levels of 0, 0.1, 1.0, 2.5 or 5.0 mg/litre (equivalent to 0, 18.4, 216.7, 393 or 660 mg/kg of body weight per day) for 4 or 6 months. Females at 5.0 mg/litre and males at and above 2.5 mg/litre consumed less water than the controls. A decrease in body weight gain in both sexes and an increase ($P < 0.05$) in kidney weight in males occurred at 5.0 mg/litre. In addition, at 5.0 mg/litre, there were elevated urinary protein and ketone levels in both sexes, decreases in leukocyte and red blood cell counts in males, altered coagulation times in both sexes and shortened prothrombin times in females. At 2.5 mg/litre, enlargement of the liver and an increase in urinary protein and ketone levels in males were observed. Inhibition of humoral immunity, cell-mediated immunity and bone marrow stem cell colonization was seen among females at 2.5 mg/litre and greater. The lowest-observed-adverse-effect level (LOAEL) was considered to be 2.5 mg/litre based on decreased water consumption, enlargement of the liver, increases in urinary protein and ketone levels in males (an indication of renal effects) and changes in immunological parameters in females. A NOAEL of 1.0 mg/litre (equivalent to 216.7 mg/kg of body weight per day) was determined as a result of these studies. Several previous oral studies in animals had not documented evidence of renal toxicity in mice or rats exposed to TCE (Stott et al., 1982).

Several studies have evaluated the toxicity of TCE to rodents following short-term inhalation exposure. In a 14-week inhalation study, rats were exposed to 0, 270, 950 or 1800 mg TCE/m^3 for 4 h per day, 5 days per week, for 14 weeks. Another group was exposed to 300 mg TCE/m^3 for 8 h per day, 5 days per week, for 14 weeks. There were significant increases ($P < 0.01$) in the absolute and relative liver weights in treated animals compared with controls, although liver and kidney function tests of treated animals remained within normal limits (Kimmerle & Eben, 1973). In a study in which mice, rats and gerbils (unspecified strains) were exposed to TCE continuously by inhalation at 810 mg/m^3 for 30 days, there was a significant increase ($P < 0.05$) in the liver weights of all three species (Kjellstrand et al., 1981). Renal

effects of inhaled TCE have also been reported (Kjellstrand et al., 1981, 1983a,b). Male and female gerbils exposed to 810 mg/m^3 atmospheres of TCE continuously for 30 days had increased ($P < 0.05$) kidney weight. NMRI mice exposed to TCE at 200, 410, 810 or 1600 mg/m^3 continuously for 30 days had significantly increased ($P < 0.05$) kidney weight at 410 mg/m^3 in males and above 810 mg/m^3 in females. No kidney effects were evident in the remaining strains of mice (Kjellstrand et al., 1983a).

4.3 Long-term exposure

Administration of high doses of TCE by gavage for long durations in rats and mice has been associated with nephropathy, with characteristic degenerative changes in the renal tubular epithelium (NCI, 1976), while toxic nephrosis, characterized by cytomegaly of the renal tubular epithelium, has been reported in cancer bioassays in mice and rats (NTP, 1983, 1988, 1990). The toxicity of TCE was investigated in F344 rats and B6C3F1 mice (50 per sex per dose) given 0, 500 or 1000 mg/kg of body weight per day (rats) and 0 or 1000 mg/kg of body weight per day (mice) in corn oil, 5 days per week for 103 weeks. Survival was reduced in male rats and mice but not in females (NTP, 1983). Toxic nephrosis, characterized as cytomegaly of the renal tubular epithelium, occurred in rats at 500 mg/kg of body weight per day and above and in mice at 1000 mg/kg of body weight per day. LOAELs of 500 mg/kg of body weight per day in rats and 1000 mg/kg of body weight per day in mice were defined for long-term effects. A NOAEL was not determined (NTP, 1990).

4.4 Reproductive and developmental toxicity

In an inhalation reproductive toxicity study, Long-Evans rats were exposed by inhalation to TCE at 9700 mg/m^3 for 6 h per day, 5 days per week, for 12 weeks before mating; for 6 h per day, 7 days per week, only during pregnancy through gestation day 21; or for 6 h per day, 5 days per week, for 2 weeks before mating and for 6 h per day, 7 days per week, during pregnancy through gestation day 21. Incomplete ossification of the sternum, indicative of delay in maturation, occurred in animals exposed during pregnancy, while a significant decrease in postnatal weight gain occurred in offspring of the premating exposed group. No maternal toxicity, teratogenicity or other effects on reproductive parameters were observed (Dorfmueller et al., 1979).

In a two-generation reproductive toxicity study, male and female Fischer 344 rats were fed diets containing microencapsulated TCE at doses of approximately 0, 75, 150 or 300 mg/kg of body weight per day from 7 days before mating right through to the birth of the F_2 generation. Although left testicular and epididymal weights decreased in the F_0 and F_1 generation, no associated histopathological changes were observed. The weight changes were attributed to general toxicity, rather than reproductive toxicity (NTP, 1986). In a similar two-generation reproductive toxicity study in CD-1 mice given TCE up to 750 mg/kg of body weight daily, sperm motility was reduced by 45% in F_0 males and 18% in F_1 males, but there were no

treatment-related effects on mating, fertility or reproductive performance in the F_0 or F_1 animals (NTP, 1985).

A number of teratogenicity studies have been conducted using TCE by both oral and inhalation routes. Swiss Webster mice exposed to TCE by inhalation at 1600 mg/m^3 for 7 h per day on gestation days 6–15 did not have any observable treatment-related maternal toxicity or terata (Leong et al., 1975). When Swiss Webster mice and Sprague-Dawley rats were exposed to TCE by inhalation at a concentration of 1600 mg/m^3, 7 h per day on gestation days 6–15, a significant decrease ($P < 0.05$) in maternal weight gain and some evidence of haemorrhages in the cerebral ventricles were observed, but no teratogenic or reproductive effects were seen (Schwetz et al., 1975). In contrast, a significant decrease in fetal weight and some increase in fetal resorptions were reported in rats (strain not specified) exposed to TCE at 540 mg/m^3 for 4 h per day during gestation days 8–21 (Healy et al., 1982).

In a study of the effect of exposure to TCE on developmental/reproductive function, female Sprague-Dawley rats were exposed to TCE in drinking-water at 0, 1.5 or 1100 mg/litre (equal to 0, 0.18 or 132 mg/kg of body weight per day) in one of three dose regimens: for 3 months before pregnancy; for 2 months before and 21 days during pregnancy; or for 21 days during pregnancy only (Dawson et al., 1993). No maternal toxicity was observed at any dose level or regimen. An increase in incidence of fetal heart defects (3% controls, 8.2% and 9.2%) was observed in treated animals at both dose levels (0.18 or 132 mg/kg of body weight per day) in dams exposed before and during pregnancy and only at the high (132 mg/kg of body weight per day) dose (10.4% versus 3% in controls) in animals exposed only during pregnancy. The LOAEL was set at 0.18 mg/kg of body weight per day, based on the increased incidence of heart defects in fetuses born to dams that were exposed prior to and during gestation. However, the study was limited in that it expressed the incidence of malformation only as a proportion of the total number of fetuses in the dose group and did not attempt to establish the incidence of heart defects on a per litter basis. Notwithstanding that shortcoming, the study lends support to similar findings of increased congenital defects in epidemiological studies (Goldberg et al., 1990; Bove et al., 1995), despite lack of a clear dose–response relationship.

A subsequent study (Fisher et al., 2001) conducted with Sprague-Dawley rats treated with TCE, TCA and DCA at dose levels as high as 400 mg/kg of body weight per day failed to reproduce the heart malformations reported in Dawson et al. (1993). However, there were differences in design between the two studies, which may partially account for the incongruence of the results. First, the Fisher et al. (2001) study used soybean oil vehicle, while the Dawson et al. (1993) study used water as a vehicle. Second, the Fisher et al. (2001) study administered a very large dose of TCE (400 mg/kg of body weight per day) in soybean oil in boluses from gestation days 5 to 16 only, whereas the Dawson et al. (1993) study administered TCE in drinking-water at relatively lower doses (maximum 1100 mg/litre, or 129 mg/kg of body weight per day) *ad libitum* either during the entire gestation period (gestation days 1–21) or prior to and throughout pregnancy; both the form of test agent and the timing of the dosage may partially account for the variations between the two studies. Third, the Fisher et

al. (2001) study had a very high background incidence of heart malformations (on a per litter basis) among the soybean oil control fetuses (52%), a rate much higher than the incidence of heart malformations in the parallel water controls (37%), whereas the Dawson et al. (1993) study reported a much lower incidence of heart malformations (25% on a per fetus basis) in the water control fetuses; the high background incidence of heart malformations associated with the TCE vehicle controls in the Fisher et al. (2001) study might have masked the effects in the TCE treatment groups. Finally, it is also possible that slight strain differences in the Sprague-Dawley rats and differences in the purity of the test agents used may account for the incongruent findings in the two studies. Curiously, the Fisher et al. (2001) study failed to reproduce heart malformations in animals treated with high doses of TCA or DCA, which had been previously shown to cause heart malformations in Sprague-Dawley rats (Johnson et al., 1998a,b) and Long-Evans rats (Smith et al., 1989, 1992; Epstein et al., 1992).

A recent developmental toxicity study by Johnson et al. (2003) used a study design and experimental protocol similar to those in the Dawson et al. (1993) study and was able to corroborate the treatment-related heart malformations reported in Dawson et al. (1993). In that study (Johnson et al., 2003), pregnant Sprague-Dawley rats were exposed to TCE throughout pregnancy. There was a significant increase in the percentage of abnormal hearts in the treated groups. The percentage of litters with abnormal hearts ranged from 0 to 66.7%, while 16.4% of control litters had abnormal hearts. Although this study appears to suggest the presence of a dose–response, with the effects beginning to manifest at a dose of 250 µg/litre (0.048 mg/kg of body weight per day) and a NOAEL at 2.5 µg/litre (0.00045 mg/kg of body weight per day), the dose–response is not as clear as might first appear on closer examination of the data.

While the study authors' conclusion that their data support the cardiac teratogenicity of TCE seems quite reasonable, their assertion that the threshold is below 250 µg/litre seems less sure when the dose–response is closely scrutinized. While the authors do point out that the doses, even the no-effect dose, are well in excess of those in epidemiological studies, there is still a need for more data, perhaps with larger dose groups and a wider range of dose levels; however, this end-point, which results from very short term (acute) exposure, deserves close scrutiny and is chosen as the critical end-point on the basis of the currently available data.

4.5 Mutagenicity and related end-points

A range of assays, covering a wide spectrum of genetic end-points, has been performed to assess possible genotoxic effects produced by TCE or its metabolites. DNA- or chromosome-damaging effects have been evaluated in bacteria, fungi, yeast, plants, insects, rodents and humans. The genetic end-points measured by these assays include forward and reverse mutation, sister chromatid exchange (SCE), unscheduled DNA synthesis, gene conversion, chromosomal aberrations, micronuclei formation and mitotic recombination. Induction of DNA repair and covalent binding to DNA have also been examined.

The evidence of TCE genotoxicity is often conflicting, in part because of the presence of impurities or mutagenic stabilizers in the test material. In fact, the information from many of the early studies may not be adequate for complete evaluation of the genotoxic potential of TCE, as few of the studies identified the grade and purity of the test TCE. In addition, some TCE samples used contained a mutagenic stabilizer, and other assays used pure samples without stabilizers, which may have decomposed to chemicals with mutagenic activity, further confounding the interpretation of the significance of the findings.

Genotoxicity studies conducted until the mid-1990s often reported conflicting results, so the evidence to support TCE or its metabolites being potent mutagens is quite limited. TCE is weakly active both *in vitro* and *in vivo*, inducing recombination responses, including SCE, and aneuploidies, including micronuclei; however, it appears to be unable to induce gene mutations or structural chromosomal aberrations (Crebelli & Carere, 1989; Fahrig et al., 1995). TCE was also observed to induce increased DNA synthesis and mitosis in mouse liver *in vivo* (Dees & Travis, 1993). Despite the apparent lack of "typical" genetic toxicity, TCE could be involved in the expression of carcinogen-induced mutations due to its potential to induce recombination and aneuploidy (Fahrig et al., 1995). In general, TCE , TCA and DCA have all been shown to cause DNA strand breaks in rodent liver cells *in vivo* and in culture, at high concentrations, as either the parent molecule or its metabolites (Bull, 2000). However, the results of some studies appear to contradict these findings (Styles et al., 1991; Chang et al., 1992), and it is still unclear whether DNA strand breaks are produced by TCE itself or by its metabolites.

Many genotoxicity studies have been conducted for the major metabolites of TCE. In a recent review, Moore & Harrington-Brock (2000) concluded that TCE and its metabolites CH, DCA and TCA require very high doses to be genotoxic, but that there was not enough information to draw any conclusions for TCOH and the conjugates DCVC and DCVG. Definitive conclusions as to whether TCE will induce tumours in humans via a mutagenic mode of action cannot, therefore, be drawn from the available information.

Overall, while the genotoxicity data are not fully conclusive, there appears to be evidence to show that TCE has a weak, likely indirect, genotoxic effect at high doses. Therefore, the mutagenic potential for this compound cannot be disregarded.

4.6 Carcinogenicity

Carcinogenicity studies of TCE by the oral route in rodents have demonstrated treatment-related liver tumours in mice in both sexes and kidney tumours in rats of both sexes (NCI, 1976; NTP, 1983, 1988, 1990). Oral exposure to TCE has also been shown to increase malignant lymphomas in female mice (US EPA, 2001). An increase in the incidence of testicular interstitial cell tumours was also reported in male rats. However, due to inadequacies of the study, a conclusive interpretation of the interstitial cell tumour incidence data could not be reached (NTP, 1988). Carcinogenicity studies of TCE by the inhalation route have shown treatment-related

tumours in the lungs of female and male mice (Fukuda et al., 1983; Maltoni et al., 1986), testes of rats (Maltoni et al., 1986), the lymphoid system (lymphomas) in female mice (Henschler et al., 1980), the kidney in male rats and the liver in mice of both sexes (Maltoni et al., 1986). However, the early oral studies were confounded by the use of impure test material (TCE), which was stabilized with other compounds, such as epichlorohydrin, that are themselves known to be carcinogenic.

In a carcinogenicity assay exposing rodents to TCE by gavage (NTP, 1983), there was a significant increase in the incidences of hepatocellular carcinomas ($P < 0.05$) at 1000 mg/kg of body weight per day in male mice (13/49 relative to 8/48 in controls) and hepatocellular adenomas ($P < 0.05$) in female mice (8/49 compared with 2/48 in controls). There were no treatment-related liver tumours in rats. The male rats at 1000 mg/kg of body weight per day that survived until the end of the study exhibited a higher ($P = 0.028$) incidence of renal tubular cell adenocarcinomas (3/16 compared with 0/33 among controls). These kidney tumours were considered biologically significant, given the rarity of kidney tumours in that rat strain.

In another carcinogenicity study (NTP, 1988) exposing four different rat strains (ACI, August, Marshall and Osborne-Mendel) to TCE by gavage, male Osborne-Mendel rats exhibited a statistically significant ($P < 0.05$) increase in the incidence of renal cell adenomas and adenocarcinomas (6/44 at 500 mg/kg of body weight per day and 2/33 at 1000 mg/kg of body weight per day, compared with none in controls). The incidence of testicular interstitial cell tumours was also increased in the male Marshall rats (21/33 at 500 mg/kg of body weight per day and 32/39 at 1000 mg/kg of body weight per day, compared with 16/46 for untreated control and 17/46 for vehicle control). However, closer audits of this study indicated that the documentation of many aspects of the study was inadequate to support proper interpretation of the reported tumour incidence data, although, given the rarity of kidney tumours in rats, this finding was still considered significant. No other treatment-related tumours were reported in these rat strains.

In a more recent carcinogenicity study (NTP, 1990) exposing B6C3F1 mice and F344/N rats to TCE by gavage, there was a significant ($P < 0.05$) increase in the incidences of combined hepatocellular carcinomas and adenomas ($P < 0.05$) in female mice (22/49 at 1000 mg/kg of body weight per day compared with 6/48 in untreated control). No treatment-related kidney tumours were observed in mice. Although the study authors considered the results equivocal due to reduced survival in the treated groups, the kidney tumour incidences in rats were statistically significant ($P < 0.05$) when adjusted for reduced survival (2/46 at 500 mg/kg of body weight per day and 3/33 at 1000 mg/kg of body weight per day, compared with none in controls) and were considered toxicologically significant due to the rarity of kidney tumours in the rats.

In a long-term carcinogenicity study by the inhalation route (Maltoni et al., 1986), the increased incidence of renal tubular adenocarcinomas in male rats (4/122 at 675 mg/m^3 compared with none at 0, 112.5 and 337.5 mg/m^3) was statistically significant ($P < 0.05$) when adjusted for survival (US EPA, 2001). The authors indicated that the

findings were biologically significant due to the rarity of renal tubular adenocarcinomas in control animals and the rarity of kidney tumours in historical controls (0/460) (Maltoni et al., 1986).

Overall, animal carcinogenicity studies conducted using pure TCE showed that chronic exposure to this compound by the oral route resulted in malignant liver tumours in mice of both sexes and kidney tumours in male rats, while inhalation exposure led to lymphomas in female mice, malignant liver and lung tumours in mice of both sexes and malignant kidney tumours in male rats.

4.7 Modes of action of TCE

The similarity between carcinogenic effects induced by the parent compound and metabolites supports the conclusion that TCE metabolites are mostly responsible for the liver and kidney tumours observed in TCE bioassays. This is particularly true for renal cell carcinoma, with additional supporting evidence of human GST isozyme dependence and DNA adducts formed from genotoxic DCVC metabolites. TCE-induced human renal carcinomas potentially have a mode of action of von Hippel Landau (VHL) tumour suppressor gene mutation followed by induction of neoplasia (Bruning et al., 1997a). Indeed, multiple mutations of the VHL tumour suppressor genes, primarily C to T changes, including nucleotide 454, were found in renal carcinoma patients with high prolonged TCE exposure (Bruning et al., 1997b; Brauch et al., 1999). These findings augment the characterization of exposure to TCE at high levels as highly likely to produce kidney cancer in humans.

The complexity of TCE metabolism and clearance complicates the identification of a metabolite that could be identified as responsible for TCE-induced effects. More than one mode of action may explain TCE-induced carcinogenicity, and several hypotheses have been put forward. In all likelihood, a number of events would be significant to tumour development in the rodent under bioassay conditions. Uncertainty exists, however, as to which events may be more relevant to human exposure to TCE at environmental levels.

It has been considered that mouse liver carcinogenesis arises in parallel with peroxisome proliferation in the liver by TCE metabolites. Although peroxisome proliferation has been correlated with carcinogenesis, the actual mechanism of carcinogenesis as it relates to peroxisome proliferation is unknown (Bull, 2000). Peroxisome proliferation is more substantial in mice than in rats (Bogen & Gold, 1997). The prevailing view of TCE-induced mouse liver carcinogenesis has been that these tumours arise in parallel with peroxisome proliferation in the liver by TCE metabolites (Elcombe, 1985; Elcombe et al., 1985; Goldsworthy & Popp, 1987; Melnick et al., 1987; DeAngelo et al., 1989; Cattley et al., 1998). However, the role of peroxisome proliferation has been questioned as a mechanism of action for human liver carcinogenesis. As peroxisome proliferation has not been observed in humans, agents that produced this result in the rodent would be unlikely to present a liver carcinogenic hazard to humans.

Modification of cell signal pathways by TCA and DCA, resulting in alterations in cell replication, selection and apoptosis (programmed cell death), is likely an important contributor to the hepatocarcinogenicity of TCE and its metabolites (Bull, 2000). The ability of TCA to activate the peroxisome proliferator activated receptor (PPAR) and the subsequent cascade of responses, including effects on gene transcription, are an example of cell signalling. DCA exposure has additionally been shown to influence other cell signalling pathways, and observed perturbations provide insight on mode-of-action hypotheses regarding induction of DCA tumours.

The potential for peroxisome proliferation to play a role in TCE-induced kidney toxicity has been assessed and is considered unlikely (Lash et al., 2000). While TCE has been reported to cause peroxisome proliferation in rat and mouse kidney, with mice showing a greater response, TCE has not been shown to induce kidney cancer in mice. In addition, studies indicate that renal peroxisomes are generally less responsive to peroxisome proliferators than hepatic peroxisomes (Lash et al., 2000).

Alpha-2u globulin is a major component of urinary protein unique to male rats, and its accumulation was previously considered to contribute to TCE-induced kidney tumours. More recent information indicates that TCE does not cause α_{2u} globulin accumulation (Goldsworthy et al., 1988). In addition, TCE has been identified as causing kidney damage in both male and female rats (Barton & Clewell, 2000). As such, α_{2u} globulin accumulation does not appear to be a mode of action of TCE-induced kidney toxicity, as was previously thought.

The cysteine and GSH intermediates formed during the metabolism of TCE, DCVC and DCVG, have been shown to be capable of inducing point mutations in *Salmonella* genotoxicity assays. Furthermore, DCVC induces the expression of proto-oncogenes, including *c-jun*, *c-fos* and *c-myc*, in mouse liver tumours (Tao et al., 2000a,b). The proto-oncogene *c-myc* is believed to be involved in the control of cell proliferation and apoptosis, which also points towards epigenetic mechanisms for the induction of liver tumours in mice. The cysteine intermediate DCVC has also been shown to induce DNA double-strand breaks and unscheduled DNA synthesis in LLC-PK_1 cells (Lash et al., 2000). There is also evidence that DCVC and DCVG can induce primary DNA damage in mammalian cells (OEHHA, 1999). Other evidence supports the cytotoxic mode of action. Most rats chronically exposed to TCE in the National Cancer Institute and National Toxicology Program bioassays developed toxic nephrosis, and more than 90% of rats (and mice) developed cytomegaly, which was most evident in male rats. Associated with these findings, kidney tumours were increased only in male rats. The TCE conjugates 1,2-DCVC and *S*-(2,2-dichlorovinyl)-L-cysteine (2,2-DCVC) and the corresponding mercapturic acids — *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine (1,2-DCVNac) and *N*-acetyl-*S*-(2,2-dichlorovinyl)-L-cysteine (2,2-DCVNac) — are rodent, and possibly human, nephrotoxicants. These compounds can produce proximal tubular necrosis and other lesions in rat kidney after conversion to reactive mutagenic intermediates by cytosolic cysteine conjugate β-lyase (Goeptar et al., 1995).

It is thought that TCE-induced kidney tumours may occur as a result of cellular necrosis and activation of repair processes that lead to cellular proliferation. Study into this mode of action has also focused on DCVG and DCVC. These metabolites, through the β-lyase enzyme or other enzymatic processes, lead to the production of reactive species, which may be responsible for nephrotoxicity (Lash et al., 2000; Vaidya et al., 2003). The reactive species can lead to mitochondrial dysfunction, protein or DNA alkylation and oxidative stress. These effects lead to additional cytotoxic effects as well as repair and proliferative responses along a continuum that may ultimately result in tumorigenesis (Lash et al., 2000; Vaidya et al., 2003). The *in vivo* formation of DCVG and DCVC in animals and humans indicates that this mode of action may be relevant to assessing the mode of action in humans. While cytotoxicity may play an important role in TCE-induced kidney cancer in rodents, it is uncertain what role it might play in human cancers induced by TCE at exposure levels below those expected to cause frank kidney toxicity.

It has also been hypothesized that formic acid plays a role in kidney toxicity (Green et al., 1998). Increased excretion of formic acid occurs with exposure to TCE and may be related to folate deficiency. Kidney toxicity has been reported in humans and rabbits with exposure to formic acid. However, data indicating that formic acid induces kidney tumours are lacking (Bogen & Gold, 1997).

The accumulation of the TCE metabolite CH is thought to be the cause of TCE lung carcinogenicity, as CH exposure results in lung lesions identical to TCE-induced tumours (Green et al., 1997; Green, 2000). The accumulation of CH in the Clara cells of the lung is thought to lead to lung tumours by causing cell damage and compensatory cell replication, which, in turn, leads to tumour formation (Green et al., 1997; Green, 2000). It is thought that the mechanism by which CH results in tumour formation in animals may not be pertinent to humans, as there is little CYP2E1 activity in human lungs (Green et al., 1997; Green, 2000). Lung tumours were induced in female mice following exposure to TCE (Odum et al., 1992). A specific lesion, characterized by vacuolization of Clara cells, was seen only in mice, and mice exposed to chloral at 600 mg/m^3 had similar lesions. Only mild effects were seen with inhaled TCOH, and none with intraperitoneally administered TCA. These results suggest that acute lung toxicity of TCE may be due to accumulation of chloral in Clara cells in mice. Since chloral is also genotoxic, the toxicity observed with intermittent exposures is likely to exacerbate any genotoxic effect through compensatory cell proliferation in rodents.

In conclusion, the mode of action for tumour induction by TCE may be attributed to non-genotoxic processes related to cytotoxicity, peroxisome proliferation and altered cell signalling; genotoxic processes, such as the production of genotoxic metabolites (e.g., chloral and DCVC); or the production of reactive oxygen species related to peroxisomal induction in the liver. The potential role of several mutagenic or carcinogenic metabolites of TCE cannot be ignored, particularly given the supporting evidence of human DNA adducts formed from genotoxic DCVC metabolites and the evidence of VHL tumour suppressor gene mutation in TCE-exposed kidney cancer patients (Bruning et al., 1997a).

Information on the mode of action for non-cancer effects of TCE is more limited, and support for hypotheses is largely based on observations of common activities with other agents. The major endocrine system effects associated with TCE exposure include the development of testicular (Leydig cell) tumours in rats (Maltoni et al., 1988; NTP, 1988). TCE and its metabolites TCA and TCOH have been found to partition in the male reproductive organs of rats following inhalation exposure (Zenick et al., 1984). The same compounds have been identified in seminal fluids of humans occupationally exposed to TCE (Forkert et al., 2003).

Generally, agents that affect steroid hormone levels, such as testosterone, estradiol and luteinizing hormone, will also induce Leydig cell tumours in the rat (Cook et al., 1999). Peroxisome proliferating chemicals have been shown to induce Leydig cell tumours via a modulation of growth factor expression by estradiol (Cook et al., 1999). Peroxisome proliferating chemicals induce hepatic aromatase activity, which can increase serum and testis estradiol levels. The increased interstitial fluid estradiol levels can modulate growth factors, including transforming growth factor-α (TGFα), and stimulate Leydig cell proliferation (Cook et al., 1999). Since steroid hormones are regulated through the hypothalamic–pituitary–testis axis in both rats and humans, agents that induce Leydig cell tumours in rats by disruption of this axis may pose a hazard to humans (Cook et al., 1999). The occurrence of Leydig cell tumours in rats exposed to TCE may therefore act as a signal for disturbance of the endocrine system and be indicative of potential endocrine disturbances in humans. The effect of endocrine disruption in human populations exposed to TCE is an area requiring further research.

Studies of the mode of action hypotheses for observed developmental effects seen with TCE, TCA and DCA exposure and data specific to TCE exposure are also scant. Developmental effects that have been associated with TCE or TCE metabolite exposure include eye defects (microphthalmia and anophthalmia) in rats and cardiac defects in rats and humans. Microphthalmia has been reported in human offspring with maternal alcohol and retinoic acid exposures. Both retinoic acid and ethanol have, in common with TCE, peroxisome receptor activity. It is possible that PPARα activation may be important to the development of eye anomalies following TCE exposure, although no data currently support this hypothesis (Narotsky & Kavlock, 1995; Narotsky et al., 1995).

The mode of action for TCE-induced cardiac teratogenicity is being evaluated as to whether the gene expression critical for normal heart development is affected during cardiogenesis. Treatment with TCE (equivalent to 110 mg/litre) produced a dose-dependent inhibition of mesenchymal cell transformation (a critical event in development of the heart) in progenitors of the valves and septa in the heart *in vitro* (Boyer et al., 2000). Although debate continues regarding the experimental evidence linking observed cardiac anomalies in the developmental assays, TCE appears to affect events important to the development of the heart, events that are consistent with an induction of cardiac anomalies (Boyer et al., 2000).

The TCE metabolites TCA and DCA both produce cardiac anomalies in rats (Smith et al., 1989, 1992; Epstein et al., 1993; Johnson et al., 1998a,b). DCA also concentrates in rat myocardial mitochondria (Kerbey et al., 1976), freely crosses the placenta (Smith et al., 1992) and has known toxicity to tissues dependent on glycolysis as an energy source (Stacpoole et al., 1979; Katz et al., 1981; Yount et al., 1982; Cicmanec et al., 1991). More research into TCE and its metabolites is needed to more fully elucidate possible modes of action for the effects observed in standard developmental protocols.

5. EFFECTS ON HUMANS

Central nervous system effects were the primary effects noted from acute inhalation exposure to TCE in humans, with symptoms including sleepiness, fatigue, headache, confusion and feelings of euphoria (ATSDR, 1997). Simultaneous exposure to TCE and ethanol results in a marked inhibition of the metabolism of TCE, which leads to an accumulation of TCE in blood and increases the extent of central nervous system depression (Muller et al., 1975). Effects on the liver, kidneys, gastrointestinal system and skin have also been noted (ATSDR, 1997). In its wide use as an inhalant anaesthetic drug in humans, concentrated solutions of TCE have proved quite irritating to the gastrointestinal tract and have caused nausea and vomiting (DeFalque, 1961).

Information from medium- to long-term TCE exposures via inhalation and dermal routes has been reviewed (ATSDR, 1997). These studies indicated that the central nervous system is the most sensitive organ for toxicity, with the liver and kidneys the next most sensitive sites for the chronic toxicity of TCE exposure. Case reports of intermediate and chronic occupational exposures included effects such as dizziness, headache, sleepiness, nausea, confusion, blurred vision, facial numbness and weakness. The liver effects noted included liver enlargement and increases of serum levels of liver enzymes, and the kidney effects included increased *N*-acetyl-β-D-glucosaminidase. Cardiovascular, immunological, reproductive and carcinogenic effects were also observed (ATSDR, 1997).

The demonstration of TCE-induced genetic toxicity in humans has been largely inconclusive. Four studies of SCE tests in peripheral lymphocyte cultures from exposed workers showed no or only minor effects on SCE frequencies (Gu et al., 1981a,b; Nagaya et al., 1989; Brandom et al., 1990; Seiji et al., 1990). Although the studies by Gu et al. (1981a,b) suggested that TCE or a metabolite may have caused chromosomal aberrations or SCE in chronically exposed humans, exposure to additional compounds, including TCE contaminants, cannot be ruled out. Konietzko et al. (1978) found a higher incidence of hypodiploid cells and a greater frequency of chromosome breaks in exposed workers compared with an unmatched control group; the authors did not consider this increase to be biologically significant, and no statistical evaluation of the data was provided. Rasmussen et al. (1988) found a highly significant increase in the frequency of structural aberrations and hyperdiploid cells in cultured lymphocytes from TCE degreasers. However, even though the control group used in that study consisted of physicians and was therefore not equivalent to the

exposed group, the study did not account for the different lifestyles of the two groups and confounding factors such as smoking, as well as possible simultaneous exposure to a number of other substances, possibly including genotoxic polycyclic aromatic hydrocarbons.

Most epidemiological studies have found no association between adverse reproductive effects in humans and exposure to TCE in contaminated drinking-water (IPCS, 1985; ATSDR, 1997). Although an epidemiological study of 2000 male and female workers exposed to TCE via inhalation found no increase in malformations in babies born following exposure (IPCS, 1985), an association was found between the occurrence of congenital heart disease in children and a drinking-water supply contaminated with TCE and other similar chemicals (IPCS, 1985). These earlier studies were confounded by, among other factors, potential exposure to many other contaminants or compounds that produce similar metabolites, the lack of characterization of the exposure levels and the exposed populations, and failure to characterize the nature of the "congenital heart disease," which may not necessarily be equivalent to cardiac anomalies. Therefore, their use in inferring a causal association between TCE and congenital cardiac anomalies remains very limited. More recent epidemiological studies of women exposed to degreasing solvents, including TCE, have reported elevated risks for cardiac anomalies in their offspring (Goldberg et al., 1990; Ferencz et al., 1997; Wilson et al., 1998). Large, statistically significant excesses were observed for specific cardiac defects: left-sided obstructive defects (odds ratio [OR] = 6.0, 95% confidence interval [CI] = 1.7–21.3) and hypoplastic left heart (OR = 3.4, 95% CI = 1.6–6.9), with an attributable risk[4] of 4.6% (Wilson et al., 1998). Neural tube defects have also been noted with either occupational or drinking-water exposure to solvents, including TCE (Holmberg & Nurminen, 1980; Holmberg et al., 1982; Bove et al., 1995). Overall, these epidemiological studies are plagued by lack of clarity on the background co-exposure. For example, in the Wilson et al. (1998) study, the investigators asked subjects about their exposure to "solvents/de-greasing compounds" but not specifically to TCE. Generally, however, it is acknowledged that subjects at air force bases are exposed to jet fuels as well as other solvents on a daily basis (Stewart et al., 1991), yet it is unlikely that the individuals know the exact compounds contained in the degreasing compounds or solvents. This suggests that, based on currently available human studies, TCE cannot be specifically implicated; however, these studies can be used as supporting evidence, complementary to developmental-reproductive effects reported in animal studies. In a study in which semen parameters of workers exposed to TCE were evaluated (Chia et al., 1996), sperm density showed a significant difference between low- and high-exposure subjects. In a recent study involving a small number of subjects, TCE and its metabolites were identified in seminal fluids of workers exposed to TCE (Forkert et al., 2003), suggesting that TCE may play a role in the observed effects on sperm parameters.

[4] Attributable risk is the risk or rate difference that may be attributable to the exposure (Rothman, 1986).

The carcinogenicity of TCE has been investigated in several epidemiological studies in exposed populations. An association between any specific type of cancer and exposure to TCE has not been consistently observed in these studies. Cancer occurrence in populations exposed to drinking-water contaminated with various concentrations of TCE has been compared in several studies, but the interpretation of these studies is complicated by methodological problems.

The evidence for TCE-induced cancers in humans has been reviewed in depth by IARC (1995). Three cohort studies were considered to be relevant to TCE evaluation. Two of these studies, in Sweden and Finland (Axelson et al., 1994; Anttila et al., 1995), involved people who had been monitored for exposure to TCE by measurement of TCA in urine. The third study, in the USA (Spirtas et al., 1991), covered workers exposed to TCE during maintenance of military aircraft and missiles, some of whom were also exposed to other solvents. In none of the available cohort studies was it possible to control for potential confounding factors, such as smoking (IARC, 1995). Most importantly, an elevated risk for liver and biliary tract cancer was observed, in addition to a modestly elevated risk for non-Hodgkin lymphoma seen in cohort studies. A marginally increased risk for non-Hodgkin lymphoma was suggested to exist in areas where groundwater is contaminated with TCE (IARC, 1995). The occurrence of renal cancer was not elevated in the cohort studies, although a study of German workers exposed to TCE yielded five cases of renal cancer compared with none in a control comparison group (IARC, 1995).

After meta-analysis of the four occupational studies (Garabrant et al., 1988; Spirtas et al., 1991; Axelson et al., 1994; Anttila et al., 1995), the following standardized mortality ratios (SMRs) resulted: liver cancer, 1.32; prostate cancer, 1.09; kidney cancer, 1.09; bladder cancer, 1.15; and non-Hodgkin lymphoma, 1.25. However, the small number of cases (except for prostate cancer), even though they were aggregated across four studies, limits the interpretation of these findings. Other limitations include narrowly defined exposure groups, lack of data on potential confounders, such as smoking, diet and exposure to other solvents, and no direct measure of personal exposure.

The authors of a retrospective cohort study conducted on 169 workers in a cardboard factory in Germany who were exposed to TCE for at least 1 year between 1956 and 1975 claim a causal link between cancer and TCE exposure (Henschler et al., 1995a,b). By the close of the study in 1992, 50 members of the study group had died, 16 from malignant neoplasms. In 2/16 cases, kidney cancer was the cause of death (SMR = 3.28, versus local population). Five workers were diagnosed with kidney cancer: four with renal cell cancer and one with a urothelial cancer of the renal pelvis (standardized incidence ratio [SIR] = 7.77, 95% CI = 2.50–18.59). After the close of the observation period, two additional kidney tumours (one renal and one urothelial) were diagnosed in the study group. By the end of the study, 52 members of the control group, which consisted of 190 unexposed workers from the same plant, had died — 16 from malignant neoplasms, but none from kidney cancer. No case of kidney cancer was diagnosed in the control group. For the seven cases of kidney cancer, the average exposure duration was 15.2 years (range 3–19.4 years).

The GST gene family encodes multifunctional enzymes that catalyse several reactions between GST and electrophilic as well as hydrophobic compounds (Raunio et al., 1995). Certain defective GST genes are known to be associated with an increased risk of different kinds of cancer. A recent case–control study (Bruning et al., 1997b) investigated the role of GST polymorphisms on the incidence of renal cell cancer in two occupational groups exposed to high levels of TCE. The data indicate a higher risk for development of renal cell cancer if TCE-exposed persons carry either the GSTT1 or GSTM1 gene. The authors concluded that this genetic polymorphism may indicate predisposition for TCE-induced renal cell cancer. These results tend to support the view of the mode of action of TCE-induced kidney cancer as involving metabolites derived from the GSH-dependent pathway, at least in humans, and are supported by the study of Henschler et al. (1995a), which reaffirms the relevance of increased incidences of renal cell tumours in a cohort of cardboard workers exposed to TCE.

The epidemiological studies of TCE and PCE as they relate to risk of renal cell cancer were critically reviewed by McLaughlin & Blot (1997). The authors state that there was little evidence of an increased risk of renal cell cancer with exposure to TCE or PCE. The few studies with elevations in risk suffered from important methodological shortcomings. Although it was virtually impossible, using epidemiological data, to conclusively rule out a small increase in risk of renal cell cancer, the totality of the epidemiological evidence clearly did not support a causal association with TCE or PCE (McLaughlin & Blot, 1997). Although McLaughlin & Blot (1997) criticized the Henschler et al. (1995a) study, it is impossible to ignore the findings of Henschler et al. (1995a), particularly in light of the authors' response to the published critique (Henschler et al., 1995b).

Over 80 published papers and letters on the cancer epidemiology of people exposed to TCE were reviewed by Wartenberg et al. (2000). Evidence of excess cancer incidence among occupational cohorts with the most rigorous exposure assessment is found for kidney cancer (relative risk [RR] = 1.7, 95% CI = 1.1–2.7), liver cancer (RR = 1.9, 95% CI = 1.0–3.4) and non-Hodgkin lymphoma (RR = 1.5, 95% CI = 0.9–2.3), as well as for cervical cancer, Hodgkin disease and multiple myeloma. However, since few studies isolate TCE exposure, results are likely confounded by exposure to other solvents and risk factors. More recently, a positive association between renal cancer and prolonged occupational exposure to high levels of TCE has been reaffirmed (Bruning et al., 2003) in a case–control study in Germany involving 134 renal cell cancer patients and 410 controls, comprising workers from industries with and without TCE exposure. When the results were adjusted for age, gender and smoking, a significant excess risk was determined for the longest-held job in industries with TCE exposure (OR = 1.80, 95% CI = 1.01–13.32). Any exposure to degreasing agents was found to be a risk factor for renal cell cancer (OR = 5.57, 95% CI = 2.33–13.32), while self-reported narcotic symptoms, an indication of peak exposures, were associated with an excess risk for renal cell cancer (OR = 3.71, 95% CI = 1.80–7.54). However, the levels of occupational exposure in that study were very high and unlikely to be reached from environmental exposure. The prolonged exposure to high

levels likely affects the metabolism of TCE, with the net production of active metabolites underlying the development of renal cell cancer in occupationally exposed industrial workers.

A recent novel feature of the cancer database for TCE has been the molecular information on the VHL tumour suppressor gene. Mutations in the VHL tumour suppressor gene have been associated with increased risk of renal cell carcinoma. Recent studies provide evidence that TCE exposure may be associated with VHL mutations among renal cell carcinoma patients (Bruning et al., 1997a; Brauch et al., 1999). Bruning et al. (1997a) examined VHL mutation by single-stranded conformation polymorphism (SSCP) in 23 renal cell carcinoma patients with documented high occupational TCE exposure. All (100%) TCE-exposed renal cell carcinoma patients had VHL mutations, which was higher than the background frequency (33–55%) among unexposed renal cell carcinoma patients. Brauch et al. (1999), in a follow-up study that determined VHL mutations by SSCP and direct sequencing of mutations in renal tissue from 44 TCE-exposed renal cell carcinoma patients, found that 75% of TCE-exposed patients had VHL mutations and 39% had a C to T mutation at nucleotide 454. All the C to T transitions in the control renal cell carcinoma patients were relatively rare (6% of the total incidence). In the Brauch et al. (1999) study, the VHL mutations were detected in patients with medium and high, but not low, TCE exposure, although only three patients were classified as having low exposure. These data indicate a highly significant association (P = 0.0006) between TCE exposure and multiplicity of VHL mutations.

In summary, although several studies have indicated a positive association between exposure to solvents, including TCE, and human cancer, further study is still necessary to better specify the specific agents that confer this risk and to estimate the magnitude of that risk (Wartenberg et al., 2000).

6. PRACTICAL ASPECTS

6.1 Analytical methods and analytical achievability

For the determination of TCE in water, the practical quantification limit considered to be achievable by most good laboratories is 5 µg/litre.

Four methods for measuring TCE in drinking-water have been approved by the US Environmental Protection Agency (EPA). EPA Method 502.2, which employs purge and trap capillary gas chromatography with photoionization detectors and electrolytic conductivity detectors in series, has a detection limit in the range 0.01–3.0 µg/litre (US EPA, 1999b). EPA Method 524.2, which uses purge and trap capillary gas chromatography with mass spectrometric detectors in series, has a detection limit of 0.5 µg/litre (US EPA, 1999b). EPA Method 503.1 employs purge and trap capillary gas chromatography with photoionization conductivity detectors and has a detection limit of 0.01–3.0 µg/litre (US EPA, 1999b). EPA Method 551.1 uses liquid–liquid extraction and gas chromatography with electron capture detectors; this method has a method detection limit of 0.01 µg/litre (US EPA, 1999b).

6.2 Treatment and control methods and technical achievability

A TCE concentration below 2 µg/litre should be achievable by air stripping, possibly in combination with granular activated carbon (GAC) adsorption.

Aeration has been used to treat contaminated well water (27 µg/litre) at pilot scale. For an air to water ratio of 10, a rate of 25 m/h and a 3.75-m contact height, the process achieved a 67% reduction in TCE (Simon & Mitchell, 1992).

Pilot-scale tests using air stripping achieved TCE removals from water with an influent concentration of 204 µg/litre of between 82% and 87% for air to water ratios of 75:1 and 125:1, respectively (McKinnon & Dykesen, 1984). Other pilot-scale studies using diffused aeration have achieved removals between 70% and 92% using an air to water ratio of 4:1 and a 10-min contact time (Kruithof et al., 1985). One study investigated the effect of media depth on the removal rate. A packed tower with a media depth of 4.5 m, an air to water ratio of 30:1 and a liquid loading rate of 13.8 litre/m^2·s achieved a removal of 98.2%, whereas a packed tower with a media depth of 1.2 m achieved a removal of 45% under the same conditions (Amy et al., 1987).

It has been reported that full-scale spray aeration of well water containing TCE achieved 90% removal (Kruithof & Koppers, 1989). The experiments found spray aeration to be efficient at removing TCE to below 1 µg/litre, with an influent concentration up to 10 µg/litre.

GAC has been used to remove high concentrations of TCE at pilot scale. The carbon removed effectively 100% of the influent concentration (approximately 2500 µg/litre), for 30 bed volumes at an empty bed contact time (EBCT) of 2.5 min and 40 bed volumes at an EBCT of 10 min (Hand et al., 1994). The presence of humic substances (33 mg of total organic carbon per litre) decreased GAC adsorption of TCE by 10–20% (Urano, 1991). GAC adsorption capacity for TCE at saturation, for an influent concentration of 20.8 ± 5.2 µg/litre and an EBCT of 2.5 min, was between 1.33 and 2.12 mg/g, depending upon the specific type of GAC used (Qi et al., 1992).

It has been reported that the combination of aeration and GAC adsorption has been used effectively to remove TCE from groundwater (McKinnon & Dykesen, 1984). As the TCE levels in the groundwater abated, it was possible to use aeration alone to effectively remove the contaminant.

It has been reported that ozone doses of 2, 6 and 20 mg/litre achieved TCE removals of 39%, 76% and 95%, respectively (Fronk, 1987). Pilot plant studies have shown that ozonation can virtually completely remove trace concentrations of TCE from groundwater (Slagle, 1990).

Removal of TCE by ozone in combination with ultraviolet (UV) irradiation has been studied: the log ozone dose versus log (TCE concentration / initial concentration) was linear. The initial concentration of TCE was 100–600 µg/litre. UV enhanced the

destruction of TCE by more than 10 times compared with ozone alone (Kusakabe et al., 1991). In another study, ozone alone (2–5 mg/litre, 5 min contact time) removed approximately 25% of influent TCE (initial concentration 65–85 µg/litre), compared with 30–80% removal when the same ozone dose range was combined with hydrogen peroxide (0.4 w/w) (Duguet, 1990).

A combination of hydrogen peroxide and UV radiation has been used to treat groundwater contaminated with volatile organic compounds, including TCE (0.89–1.30 mg/litre). Operated at 38 litres/min, with a reactor volume of 57 litres and with hydrogen peroxide dosed at 65 mg/litre, the effluent concentration was generally below detection limits (maximum removal efficiency was >99.9%) (Topudurti et al., 1994). Other research has confirmed that TCE is readily removed from water by ozone and that UV irradiation gave only a slight improvement; 75 mg/litre was removed by an ozonation rate of 6 mg/litre and UV radiation flux of 100 $mW \cdot s/cm^2$ (Pailard et al., 1987).

Cross-flow microfiltration, combined with application of powdered activated carbon (PAC) to the influent stream, has been used to remove TCE. The tests used a bench-scale, continuous-flow system (5-h hydraulic retention time), a ceramic membrane and a PAC dose of 50 mg/litre. The recycled fraction resulted in accumulation of PAC to 2000–3000 mg/litre (3–5 days' retention) in the influent stream. The influent TCE concentration (mean 200 µg/litre) was reduced to <0.5 µg/litre (99.8% removal) at steady state (Pirbazari, 1992).

Laboratory studies have shown that TCE is readily extracted from water by air stripping across a hollow-fibre membrane (Semmens et al., 1989).

7. PROVISIONAL GUIDELINE VALUE

7.1 Cancer risk assessment

There are now several epidemiological studies that suggest that TCE is carcinogenic and that show consistency in terms of target tissues and tumour types. However, some fail to reach a level of statistical significance or are confounded by simultaneous exposure to other substances in drinking-water or in industrial settings and therefore may be inadequate to infer a causal relationship between TCE and cancer in humans. Nevertheless, there is adequate evidence of TCE carcinogenicity in two species of rodents, although the sites and types of tumour vary with gender and species. Confidence in the relevance to humans of these findings is enhanced by concordance in target tissues between animals and humans for non-cancer and cancer end-points and by consideration of mechanistic information in the context of species differences in metabolism. Carcinogenicity has been observed in animals exposed to TCE by both inhalation and ingestion, and responses tend to increase with dose.

Several metabolites of TCE are genotoxic, and some are established as known or likely human carcinogens. Some metabolites of TCE are suspected to be carcinogenic and likely involve non-genotoxic mechanisms of effect, such as cytotoxicity and

altered cell signalling, both of which may be relevant to humans. Furthermore, animals and humans with cancer or tumours related to TCE exposure have been shown to excrete similar TCE metabolites (Birner et al., 1993; Lash et al., 2000). There is a substantial body of evidence that several different mechanisms are responsible for the observed carcinogenicity of TCE in animals, and these appear to be related to the effect mechanisms of the TCE metabolites. It is feasible that the different tumour responses to TCE are attributable to the pharmacokinetic differences between genders and species.

The results considered most pertinent in assessing the weight of evidence of carcinogenicity of TCE in humans are principally the significant increases in kidney tumours in rats (NTP, 1983, 1990), pulmonary tumours in mice (Fukuda et al., 1983; Maltoni et al., 1986, 1988; NTP, 1988) and testicular tumours in rats (Maltoni et al., 1986, 1988; NTP, 1988). Although there is some doubt about the human relevance of pulmonary tumours in mice, it cannot be concluded that the potential tumour induction mechanism in this species does not also occur in humans exposed to TCE. In addition, TCE appears to be weakly genotoxic in *in vitro* and *in vivo* assays (IPCS, 1985). In view of the sufficient weight of evidence of carcinogenicity in two species of experimental animals with supporting human data, IARC (1995) classified TCE as Group 2A, probably carcinogenic to humans.

The cancer risk assessment for TCE was based on kidney tumours, which were observed in rats of both sexes and in humans. The evidence surrounding kidney tumours is reasonable on several levels. Although the tumours were few, the finding was repeatable. Such tumours are historically rare in rats, so their appearance among dosed animals was considered biologically significant. Such tumours were also observed in Sprague-Dawley rats exposed to TCE by the inhalation route (Maltoni et al., 1986). There are similarities between sites and histopathological characteristics of the tumours observed in human patients and in rat bioassays (Vamvakas et al., 1993, 1998). The metabolites derived from the likely intermediates of bioactivation of TCE are identical in humans and in experimental animals (Dekant et al., 1986; Birner et al., 1993). Small increases in renal tumours in male rats at doses inducing renal damage cannot be dismissed as irrelevant to humans; epidemiological evidence supports the conclusion that TCE may cause kidney tumours in humans. The new evidence associating human TCE exposure with transformation (VHL gene mutations) at nucleotide 454 is important evidence specific to TCE exposure, which provides a genetic fingerprint associating kidney tumours with TCE exposure (Bruning et al., 1997a,b).

The linearized multistage (LMS) model was used (Health Canada, 2003a) to calculate unit risks for the kidney tumour types observed in rats. Use of a linear (LMS) approach is supported by the possible genotoxicity associated with some TCE metabolites, particularly DCVC and DCVG, although a non-linear approach could be argued due to a possible mixed mode of action (mutagenicity and cytogenicity) of TCE and enhanced susceptibility of the rat to nephropathy. The unit risks were calculated for the data on kidney tumours (NTP, 1988, 1990). An animal-to-human

kinetic adjustment factor, expressed as $(0.35/60)^{1/4}$, was applied to the final unit risks, assuming a rat weighs 0.35 kg and a human weighs 60 kg.

The unit risks calculated (Health Canada, 2003a) for pooled combined tubular cell adenomas and adenocarcinomas of the kidneys in rats (ACI, Augusta, Marshall and Osborne-Mendel strains) following oral exposure to TCE for 103 weeks (NTP, 1988, 1990) were 7.80×10^{-4} (mg/kg of body weight per day)$^{-1}$ in males and 4.63×10^{-4} (mg/kg of body weight per day)$^{-1}$ in females, while the unit risks for renal tubular adenocarcinomas in rats following inhalation exposure for 104 weeks (Maltoni et al., 1986) were 1.16×10^{-4} (mg/m^3)$^{-1}$ in males and 7.84×10^{-5} (mg/m^3)$^{-1}$ in females. The unit risk value of 7.80×10^{-4} (mg/kg of body weight per day)$^{-1}$ for pooled combined tubular cell adenomas and adenocarcinomas of the kidneys in male rats (oral study) was chosen among the above values. This corresponds to the highest unit risk and therefore the most conservative value.

For the cancer risk assessment, a health-based value (HBV) for TCE in drinking-water associated with an upper-bound excess lifetime cancer risk of 10^{-5} can be calculated as follows:

$$\text{HBV} = \frac{60 \text{ kg} \times 10^{-5}}{7.80 \times 10^{-4} \text{ (mg/kg of body weight per day)}^{-1} \times 2.0 \text{ litres/day}}$$

$$\approx 0.4 \text{ mg/litre (400 µg/litre)}$$

where:

- 60 kg is the average body weight of an adult
- 10^{-5} is the upper-bound risk of one additional cancer case per 100 000 of the population ingesting drinking-water containing TCE at the HBV for 70 years
- 7.80×10^{-4} (mg/kg of body weight per day)$^{-1}$ is the unit risk calculated using the LMS model[5]
- 2.0 litres/day is the daily volume of water consumed by an adult.

Unit risk values were similarly calculated using the LMS method for the various pertinent tumour types (including liver, testis and lymphomas) observed in the rodent carcinogenicity studies with TCE. These unit risk values were used to estimate health-based values, which were then compared with the value obtained using the reproductive-developmental end-point below. Overall, even with the use of the probably more conservative LMS method, the health-based values based on carcinogenicity were higher than that determined for the reproductive-developmental end-point.

[5] The potency estimates were converted to human equivalence (in (mg/kg of body weight per day)$^{-1}$) using an allometric scaling factor of $(0.35/60)^{1/4}$ for scaling from rat to adult 60-kg human.

7.2 Non-cancer risk assessment

For effects other than cancer, a tolerable daily intake (TDI) can be derived by considering all studies and selecting the critical effect that occurs at the lowest dose, selecting a dose (or point of departure) at which the critical effect either is not observed or would occur at a relatively low incidence (e.g., 10%) and reducing this dose by an uncertainty factor to reflect the differences between study conditions and conditions of human environmental exposure.

Choice of the developmental toxicity study (Dawson et al., 1993) for non-cancer risk assessment was based on the appropriateness of the vehicle used (drinking-water), the low dose at which the effects were observed, which coincides with the lowest adverse effect level in all animal studies reviewed, the severity of the end-point (heart malformations) and the presence of evidence for similar effects (e.g., cardiac anomalies) from epidemiological studies (Lagakos et al., 1986; Goldberg et al., 1990; MDPH, 1994; Bove et al., 1995), as well as the observation of similar malformations in studies of TCE metabolites (Smith et al., 1989, 1992; Epstein et al., 1992, 1993; Johnson et al., 1998a,b). Although it is recognized that the Dawson et al. (1993) study is not the ideal key study to use in a risk assessment because of its inherent methodological limitations, it was chosen for the guideline derivation because it was considered the best available study that used a drinking-water vehicle and studied the most sensitive (i.e., reproductive) end-point. Furthermore, the same cardiac anomalies reported in Dawson et al. (1993) were corroborated by Johnson et al. (2003). Although the Johnson et al. (2003) study could be used in the risk assessment, the Dawson et al. (1993) study was deemed more appropriate as the key study, because it showed a clearer dose–response relationship. Finally, the choice of a key study investigating reproductive effects was made in recognition of advancing research into the developmental health effects of TCE and to exercise the precautionary principle — in other words, to protect against the potential for reproductive effects even if the cause-and-effect relationship has not been fully established scientifically.

As only a LOAEL was identified in the critical study, the benchmark dose (BMD) approach was used to estimate the NOAEL. This approach has recently gained acceptance for the risk assessment of non-cancer effects (Haag-Gronlund et al., 1995; US EPA, 1995) due to its many advantages over the NOAEL/LOAEL/uncertainty factor methodology. For example, the BMD is derived on the basis of data from the entire dose–response curve for the critical effect rather than from the single dose group at the NOAEL, and it can be calculated from data sets in which a NOAEL was not determined (as in this case), thus eliminating the need to apply an additional uncertainty factor to the LOAEL (IPCS, 1994; Barton & Das, 1996; Clewell, 2000). A lower confidence limit of the benchmark dose (BMDL) has been suggested as an appropriate replacement of the NOAEL (Crump, 1984; Barton & Das, 1996). More specifically, a suitable BMDL is defined as a lower 95% confidence limit estimate of dose corresponding to a 1–10% level of risk over background levels (Barton & Das, 1996). Definition of the BMD as a lower confidence limit accounts for the statistical power and quality of the data (IPCS, 1994).

The BMD method was therefore used (Health Canada, 2003b) to estimate a dose at which the critical effect either would not be observed or would occur at a relatively low incidence, based on the teratogenicity data of the critical study by Dawson et al. (1993). Although these are developmental toxicology data, standard bioassay techniques were used, since individual pup-by-dam data were not available. Typically, developmental toxicology data contain extra-binomial variation due to the "litter effect"; that is, pups from the same dam are more similar than pups from other dams. Due to a lack of data, this variability could not be accounted for in this analysis. The key dosing scenario was the one in which dams were exposed both prior to and during pregnancy, since this most closely mimics what would be expected in the human population. Specifically, the incidence of heart abnormalities among pups was 7/238 (2.9%), 23/257 (8.2%) and 40/346 (9.2%) at doses of 0, 1.5 and 1100 mg/litre (0, 0.18 and 132 mg/kg of body weight per day).

Using the data from this dosing regimen, the BMD and its lower 95% confidence limit (BMDL) corresponding to a 1%, 5% and 10% increase in extra risk of fetal heart malformations over background were calculated using the THRESH (Howe, 1995) software. A chi-square lack of fit test was performed for the model fit, yielding a significant *P*-value of <0.0001. The fitted model provided $BMDL_{01}$, $BMDL_{05}$ and $BMDL_{10}$ values of 0.014, 0.071 and 0.146 mg/kg of body weight per day, respectively (Health Canada, 2003b).

The $BMDL_{10}$ was chosen as a default value, as has been proposed and used elsewhere (Haag-Gronlund et al., 1995; Barton & Das, 1996). This value remains an uncertain estimate of the NOAEL due to the following: (1) the data do not elucidate the shape of the dose–response curve in the range of the $BMDL_{10}$; (2) only two dose groups were used to estimate the $BMDL_{10}$, since the top group was removed to eliminate lack of fit; and (3) it is not known with certainty which BMDL level best represents the NOAEL. However, Haag-Grondlund et al. (1995), applying the same method for non-cancer risk assessment for TCE, found all no-observed-effect levels (NOELs) to be higher than the BMD corresponding to 1% extra risk and 42% of the NOELs and 93% of the lowest-observed-effect levels (LOELs) to be higher than the BMD corresponding to 10% extra risk. Therefore, the $BMDL_{10}$ of 0.146 mg/kg of body weight per day was chosen to best represent the NOAEL.

The TDI for TCE can be calculated as follows:

$$\text{TDI} = \frac{\text{0.146 mg/kg of body weight per day}}{100}$$

$$= \text{0.00146 mg/kg of body weight per day (1.46 µg/kg of body weight per day)}$$

where:

- 0.146 mg/kg of body weight per day is the $BMDL_{10}$, derived as described above
- 100 is the uncertainty factor (×10 for interspecies variation, ×10 for intraspecies variation).

Using the TDI derived with the BMD method, a health-based value (HBV) can be calculated as follows:

$$\text{HBV} = \frac{0.00146 \text{ mg/kg of body weight per day} \times 60 \text{ kg} \times 0.5}{2 \text{ litres/day}}$$

$$\approx 0.02 \text{ mg/litre (20 µg/litre)}$$

where:

- 0.00146 mg/kg of body weight per day is the TDI, as derived above
- 60 kg is the average body weight of an adult
- 0.5 is the proportion of total daily intake that is allocated to drinking-water
- 2 litres/day is the daily volume of water consumed by an adult.

7.3 Selection of provisional guideline value

Both cancer and non-cancer end-points were considered in the derivation of the guideline value for TCE in drinking-water. The health-based value of 0.02 mg/litre derived for reproductive effects was selected as the guideline value, as it is protective for both cancer and non-cancer end-points. It should be noted that the allocation factor of 50% of the TDI for drinking-water was used rather than the 20% that was used previously, since the discontinuation of TCE in many medical applications and some consumer products has decreased exposure to this contaminant in these situations. The guideline remains provisional on the basis of uncertainties in the toxicological database.

Exposure data (see section 2.5) suggest that contributions of TCE to total exposure come from four areas: ingestion of drinking-water; inhalation of indoor air largely due to volatilization from drinking-water; inhalation and dermal exposure during showering or bathing; and ingestion of food. All but food exposure arise primarily from drinking-water (5.0 Ieq/day). It should be noted that for non-contaminated (<1 µg/litre) drinking-water sources, ≤15% of the total exposure to TCE is derived from drinking-water, whereas in a contaminated scenario (10 µg/litre), drinking-water comprises up to 65% of the total exposure to TCE for both adults and children. This is particularly important in countries with low rates of ventilation in houses and high rates of showering and bathing. In these countries, consideration should be given to taking this additional exposure into account in developing national standards from the provisional guideline value.

The provisional guideline value of 20 µg/litre is both analytically and technically achievable (see section 6).

8. REFERENCES

Amoore JE, Hautala E (1983) Odor as an aid to chemical safety: odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution. *Journal of Applied Toxicology*, 3:272–290.

Amy GL, Narbaitz RM, Cooper WJ (1987) Removing VOCs from groundwater containing humic substances by means of coupled air stripping and adsorption. *Journal of the American Water Works Association*, 79(8):49–54.

Andelman JB (1985) Inhalation exposure in the home to volatile organic contaminants of drinking water. *The Science of the Total Environment*, 47:443–460.

Anttila A et al. (1995) Cancer incidence among Finnish workers exposed to halogenated hydrocarbons. *Journal of Occupational and Environmental Medicine*, 37:797–806.

ATSDR (1989) *Toxicological profile for trichloroethylene*. Atlanta, GA, US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, 139 pp. (ATSDR/TP-88/24).

ATSDR (1993) *Toxicological profile for trichloroethylene*. Atlanta, GA, US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

ATSDR (1997) *Toxicological profile for trichloroethylene (TCE)*. Atlanta, GA, US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

Axelson O et al. (1994) Updated and expanded Swedish cohort study on trichloroethylene and cancer risk. *Journal of Occupational Medicine*, 36:556–562.

Barton HA, Clewell HJ, III (2000) Evaluating non cancer effects of trichloroethylene: Dosimetry, mode of action, and risk assessment. *Environmental Health Perspectives*, 108(Suppl. 2):323–334.

Barton HA, Das S (1996) Alternatives for a risk assessment on chronic noncancer effects from oral exposure to trichloroethylene. *Regulatory Toxicology and Pharmacology*, 24:269–285.

Barton HA, Flemming CD, Lipscomb JC (1996) Evaluating human variability in chemical risk assessment: hazard identification and dose response assessment for non-cancer oral toxicity of trichloroethylene. *Toxicology*, 11:271–287.

Barton HA et al. (1999) Dichloroacetate (DCA) dosimetry: Interpreting DCA-induced liver cancer dose response and the potential for DCA to contribute to trichloroethylene-induced liver cancer. *Toxicology Letters*, 106:9–21.

Birner G et al. (1993) Nephrotoxic and genotoxic *N*-acetyl-*S*-dichlorovinyl-L-cysteine is a urinary metabolite after occupational 1,1,2-trichloroethylene exposure in humans: implications for risk of trichloroethylene exposure. *Environmental Health Perspectives*, 99:281–284.

Bogen KT, Gold LS (1997) Trichloroethylene cancer risk: simplified calculation of PBPK-based MCLs for cytotoxic end points. *Regulatory Toxicology and Pharmacology*, 25:26–42.

Bogen KT et al. (1988) *Health risk assessment of trichloroethylene (TCE) in California drinking water*. Livermore, CA, University of California, Lawrence Livermore National Laboratory, Environmental Sciences Division.

Bove FL, Fulcomer MC, Klotz JB (1995) Public drinking water contamination and birth outcomes. *American Journal of Epidemiology*, 141:850–862.

Boyer AS, Finch WT, Runyan RB (2000) Trichloroethylene inhibits development of embryonic heart valve precursors *in vitro*. *Toxicological Sciences*, 53:109–117.

Brandom WF et al. (1990) Sister chromatid exchanges and chromosome aberration frequencies in plutonium workers. *International Journal of Radiation Biology*, 58:195–207.

Brauch H et al. (1999) Trichloroethylene exposure and specific somatic mutations in patients with renal cell carcinoma. *Journal of the National Cancer Institute*, 91:854–861.

Bruning T et al. (1997a) Renal cell carcinomas in trichloroethylene (TRI) exposed persons are associated with somatic mutations in the Von Hippel-Lindau (VHL) tumour suppressor gene. *Archives of Toxicology*, 71:332–335.

Bruning T et al. (1997b) Influence of polymorphisms of GSTM1 and GSTT1 for risk of renal cell cancer in workers with long-term high occupational exposure to trichloroethylene. *Archives of Toxicology*, 71:596–599.

Bruning T et al. (1998) Acute intoxication with trichloroethylene: clinical symptoms, toxicokinetics, metabolism, and development of biochemical parameters for renal damage. *Toxicological Sciences*, 41:157–165.

Bruning T et al. (2003) Renal cell cancer risk and occupational exposure to trichloroethylene: Results of a consecutive case–control study in Arnsberg, Germany. *American Journal of Industrial Medicine*, 43(3):274–285.

Buben JA, O'Flaherty EJ (1985) Delineation of the role of metabolism in the hepatotoxicity of trichloroethylene and perchloroethylene: A dose–effect study. *Toxicology and Applied Pharmacology*, 78:105–122.

Bull RJ (2000) Mode of action for liver tumor induction by trichloroethylene and its metabolites, trichloroacetate and dichloroacetate. *Environmental Health Perspectives*, 108(Suppl. 2):241–259.

Bull RJ et al. (2002) The contribution of dichloroacetate and trichloroacetate to liver tumor induction in mice by trichloroethylene. *Toxicology and Applied Pharmacology*, 182:55–65.

Cattley RC et al. (1998) Do peroxisome proliferating compounds pose a hepatocarcinogenic hazard to humans? *Regulatory Toxicology and Pharmacology*, 27:47–60.

Chang LW, Daniel FB, DeAngelo AB (1992) Analysis of DNA strand breaks induced in rodent liver *in vivo*, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes. *Environmental and Molecular Mutagenesis*, 20:277–288.

Chen CW (2000) Biologically based dose–response model for liver tumors induced by trichloroethylene. *Environmental Health Perspectives*, 108(Suppl. 2):335–342.

Chia SE et al. (1996) Semen parameters in workers exposed to trichloroethylene. *Reproductive Toxicology*, 10:295–299.

Cicmanec JL, Condie LW, Olson GR (1991) 90-day toxicity study of dichloroacetate in dogs. *Fundamental and Applied Toxicology*, 17:376–389.

Clewell HJ et al. (1995) Considering pharmacokinetic and mechanistic information in cancer risk assessments for environmental contaminants: examples with vinyl chloride and trichloroethylene. *Chemosphere*, 31:2561–2578.

Clewell HJ et al. (2000) Development of a physiologically based pharmacokinetic model of trichloroethylene and its metabolites for use in risk assessment. *Environmental Health Perspectives*, 108(Suppl. 2):283–305.

Clewell H et al. (2001) Under what conditions is trichloroethylene likely to be a carcinogen in humans. *Human and Ecological Risk Assessment*, 7(4):687–716.

Cook JC et al. (1999) Rodent Leydig cell tumorigenesis: a review of the physiology, pathology, mechanisms and relevance to humans. *Critical Reviews in Toxicology*, 29:169–261.

Crebelli R, Carere A (1989) Genetic toxicology of 1,1,2-trichloroethylene. *Mutation Research*, 221:11–37.

Crump KS (1984) A new method for determining allowable daily intakes. *Fundamental and Applied Toxicology*, 4:854–871.

Dallas CE et al. (1991) Physiological pharmacokinetic modeling of inhaled trichloroethylene in rats. *Toxicology and Applied Pharmacology*, 110:303–314.

Daniel JW (1963) The metabolism of ^{36}Cl-labelled trichloroethylene and tetrachloroethylene in the rat. *Biochemical Pharmacology*, 12:795–802.

Dann T (1993) Unpublished data on concentrations of trichloroethylene at Canadian sites. Environment Canada, Ottawa, Ontario [cited in Department of National Health and Welfare, 1993].

Dawson BV et al. (1993) Cardiac teratogenesis of halogenated hydrocarbon-contaminated drinking water. *Journal of the American College of Cardiology*, 21:1466–1472.

DeAngelo AB et al. (1989) Species and strain sensitivity to the induction of peroxisome proliferation by chloroacetic acids. *Toxicology and Applied Pharmacology*, 101:285–298.

DeAngelo AB et al. (1997) Failure of monochloroacetic acid and trichloroacetic acid administered in the drinking water to produce liver cancer in male F344/N rats. *Journal of Toxicology and Environmental Health*, 52:425–445.

De Baere S et al. (1997) Tissue distribution of trichloroethylene and its metabolites in a forensic case. *Analytical Toxicology*, 21:223–227.

Dees C, Travis C (1993) The mitogenic potential of trichloroethylene in B6C3F1 mice. *Toxicology Letters*, 69:129–137.

DeFalque RJ (1961) Pharmacology and toxicology of trichloroethylene. A critical review of world literature. *Clinical Pharmacology and Therapeutics*, 2:665–688.

Dekant W, Henschler D (1983) New pathways of trichloroethylene metabolism. *Developments in Toxicology and Environmental Science*, 11:399–402.

Dekant W, Metzler M, Henschler D (1984) Novel metabolites of trichloroethylene through dechlorination reactions in rats, mice, and humans. *Biochemical Pharmacology*, 33:2021–2037.

Dekant W, Metzler M, Henschler D (1986) Identification of *S*-1,2-dichlorovinyl-*N*-acetylcysteine as a urinary metabolite of trichloroethylene: A possible explanation of its nephrocarcinogenicity in male rats. *Biochemical Pharmacology*, 3:2455–2458.

Department of National Health and Welfare (1993) *Trichloroethylene. Supporting documentation, health related sections for the Canadian Environmental Protection Act (CEPA) Priority Substances List assessment report.* Ottawa, Ontario, Health Canada.

Dorfmueller MA et al. (1979) Evaluation of teratogenicity and behavioral toxicity with inhalation exposure of maternal rats to trichloroethylene. *Toxicology*, 14:153–166.

Duguet JP (1990) New advances in oxidation processes: Some examples of application of ozone/hydrogen peroxide combination for the removal of micropollutants from drinking water. *Water Supply*, 8(3/4):465–474.

Elcombe CR (1985) Species differences in carcinogenicity and peroxisome proliferation due to trichloroethylene: a biochemical human hazard assessment. *Archives of Toxicology Supplement*, 8:6–17.

Elcombe CP, Rose MS, Pratt IS (1985) Biochemical, histological, and ultrastructural changes in rat and mouse liver following administration of trichloroethylene: Possible relevance to species differences in hepatocarcinogenicity. *Toxicology and Applied Pharmacology*, 79:365–376.

Environment Canada (1986) *Toxic organic data summary*. Ottawa, Ontario, Environment Canada, Environmental Protection Service, Pollution Measurement Division.

Epstein DL et al. (1992) Cardiopathic effects of dichloroacetate in the fetal Long-Evans rat. *Teratology*, 46: 225–235.

Epstein DL et al. (1993) Cardiopathic effects of dichloroacetate in the Long-Evans rat fetus. *Teratology*, 47: 529.

Fahrig R, Madle S, Baumann H (1995) Genetic toxicology of trichloroethylene (TCE). *Mutation Research*, 340:1–36.

Fan AM (1988) Trichloroethylene: water contamination and health risk assessment. *Reviews of Environmental Contamination and Toxicology*, 101:55–92.

Ferencz C, Loffredo CA, Correa-Villasenor A (1997) *Perspectives in pediatric cardiology*. Vol. 5. *Genetic and environmental risk factors of major cardiovascular malformation. The Baltimore–Washington infant study 1981–1989*. New York, NY, Futura Publishing.

Fernandez JG et al. (1975) Exposition au trichloréthylène. Bilan de l'absorption, de l'excrétion et du metabolisme sur des sujets humains. *Archives des Maladies Professionnelles de Medecine du Travail et de Securité Sociale (Paris)*, 36:397–407.

Fernandez JG et al. (1977) Trichloroethylene exposure simulation of uptake, excretion, and metabolism using a mathematical model. *British Journal of Industrial Medicine*, 34:43–55.

Fisher JW et al. (1987) Kinetics of trichloroethylene in pregnant and lactating rats and rat pups. *Toxicology*, 47:206–207.

Fisher JW, Whittaker TA, Taylor DH (1989) Physiologically based pharmacokinetic modeling of the pregnant rat: a multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. *Toxicology and Applied Pharmacology*, 99:395–414.

Fisher JW et al. (1991) Physiologically based pharmacokinetic modeling with trichloroethylene and its metabolite, trichloroacetic acid, in the rat and mouse. *Toxicology and Applied Pharmacology*, 109:183–195.

Fisher JW et al. (2001) Trichloroethylene, trichloroacetic acid, and dichloroacetic acid: Do they affect fetal rat heart development? *International Journal of Toxicology*, 20:257–267.

Forkert PG et al. (2003) Identification of trichloroethylene and its metabolites in human seminal fluid of workers exposed to trichloroethylene. *Drug Metabolism and Disposition*, 31(3):306–311.

Fronk CA (1987) Destruction of volatile organic contaminants in drinking water by ozone treatment. *Ozone Science and Engineering*, 9(3):265–288.

Fukuda K, Takemoto K, Tsuruta H (1983) Inhalation carcinogenicity of trichloroethylene in mice and rats. *Industrial Health*, 21:243–254.

Garabrant DH et al. (1988) Mortality of aircraft manufacturing workers in southern California. *American Journal of Industrial Medicine*, 13:686–693.

Goeptar AR et al. (1995) Metabolism and kinetics of trichloroethylene in relation to toxicity and carcinogenicity. Relevance of the mercapturic acid pathway. *Chemical Research in Toxicology*, 8:3–21.

Goldberg SJ et al. (1990) An association of human congenital cardiac malformations and drinking water contaminants. *Journal of the American College of Cardiology*, 16:155–164.

Goldsworthy TL, Popp JA (1987) Chlorinated hydrocarbon-induced peroxisomal enzyme activity in relation to species and organ carcinogenicity. *Toxicology and Applied Pharmacology*, 86:225–233.

Goldsworthy TL et al. (1988) Potential role of α-2μ-globulin, protein droplet accumulation, and cell replication in the renal carcinogenicity of rats exposed to trichloroethylene, perchloroethylene, and pentachloroethane. *Toxicology and Applied Pharmacology*, 96:367–379.

Green T (2000) Pulmonary toxicity and carcinogenicity of trichloroethylene: species differences and modes of action. *Environmental Health Perspectives*, 108(Suppl. 2):261–264.

Green T, Mainwaring GW, Foster JR (1997) Trichloroethylene-induced mouse lung tumors: Studies of the mode of action and comparisons between species. *Fundamental and Applied Toxicology*, 37:125–130.

Green T et al. (1998) Formic acid excretion in rats exposed to trichloroethylene: a possible explanation for renal toxicity in long-term studies. *Toxicology*, 127:39–47.

Gu ZW et al. (1981a) Effets du trichloroéthylène et de ses métabolites sur le taux d'échanges de chromatides-soeurs. *Annales de* Génétique, 24:105–106.

Gu ZW et al. (1981b) Induction d'échanges entre les chromatides soeurs (SCE) par le trichloréthylène et ses métabolites. *Toxicological European Research*, 111(2):63–67.

Haag-Gronlund M, Fransson-Steen R, Victorin K (1995) Application of the benchmark method to risk assessment of trichloroethylene. *Regulatory Toxicology and Pharmacology*, 21:261–269.

Hand DW et al. (1994) Synthetic adsorbent versus GAC for TCE removal. *Journal of the American Water Works Association*, 86(8):64–72.

Hansch C, Leo AJ (1985) *MedChem Project*. Claremont, CA, Pomona College (Issue No. 26).

Health Canada (1998) *Exposure factors for assessing total daily intake of priority substances by the general population of Canada*. Ottawa, Ontario, Health Canada, Bureau of Chemical Hazards, Priority Substances Section.

Health Canada (2003a) *Unit risks for TCE in drinking water*. Ottawa, Ontario, Health Canada, Healthy Environments and Consumer Safety Branch, Biostatistics Unit, March.

Health Canada (2003b) *Benchmark dose for TCE in drinking water*. Ottawa, Ontario, Health Canada, Healthy Environments and Consumer Safety Branch, Biostatistics Unit, April.

Healy TFJ, Poole TR, Hopper A (1982) Rat fetal development and maternal exposure to trichloroethylene at 100 p.p.m. *British Journal of Anaesthesiology*, 54:337–341.

Henschler DH et al. (1980) Carcinogenicity study of trichloroethylene by long-term inhalation in three animal species. *Archives of Toxicology*, 43:237–248.

Henschler D et al. (1995a) Increased incidence of renal cell tumors in a cohort of cardboard workers exposed to trichloroethylene. *Archives of Toxicology*, 69:291–299.

Henschler D et al. (1995b) Increased incidence of renal cell tumors in a cohort of cardboard workers exposed to trichloroethylene [responses to comments on Henschler et al., 1995a]. *Archives of Toxicology*, 70:131–133.

Hine J, Mookerjee PK (1975) The intrinsic hydrophilic character of organic compounds. Correlations in terms of structural contributions. *Journal of Organic Chemistry*, 40:292–303.

Holmberg PC, Nurminen M (1980) Congenital defects of the central nervous system and occupational factors during pregnancy: a case–referent study. *American Journal of Industrial Medicine*, 1:167–176.

Holmberg PC et al. (1982) Oral clefts and organic solvent exposure during pregnancy. *International Archives of Occupational and Environmental Health*, 50:371–376.

Howe RB (1995) *THC: A computer program to compute a reference dose from continuous animal toxicity data using the benchmark dose method.* Ruston, LA, ICF Kaiser Engineers, Inc.

IARC (1995) Trichloroethylene. In: *Drycleaning, some chlorinated solvents, and other industrial chemicals*. Lyon, International Agency for Research on Cancer, pp. 75–158 (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 63).

IPCS (1985) *Trichloroethylene*. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 50).

IPCS (1994) *Assessing human health risks of chemicals: derivation of guidance values for health-based exposure limits*. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 170).

Jakobson I et al. (1982) Uptake via the blood and elimination of 10 organic solvents following epicutaneous exposure of anesthetized guinea pigs. *Toxicology and Applied Pharmacology*, 63:181–187.

Johnson PD, Dawson BV, Goldberg SJ (1998a) A review: trichloroethylene metabolites: potential cardiac teratogens. *Environmental Health Perspectives*, 106(Suppl. 4):995–999.

Johnson PD, Dawson BV, Goldberg SJ (1998b) Cardiac teratogenicity of trichloroethylene metabolites. *Journal of the American College of Cardiology*, 32:540–545.

Johnson PD et al. (2003) Threshold of trichloroethylene contamination in maternal drinking waters affecting fetal heart development in the rat. *Environmental Health Perspectives*, 111(3):289–292.

Kaneko T, Wang P, Sato A (1997) Assessment of health effects of trichloroethylene. *Industrial Health*, 35:301–324.

Katz R et al. (1981) Dichloroacetate, sodium: 3-month oral toxicity studies in rats and dogs. *Toxicology and Applied Pharmacology*, 57:273–287.

Kerbey AL et al. (1976) Regulation of pyruvate dehydrogenase in rat heart. *Biochemical Journal*, 154:327–348.

Kimmerle G, Eben A (1973) Metabolism, excretion and toxicology of trichloroethylene after inhalation. 1. Experimental exposure on rats. *Archiv für Toxikologie*, 30:115–126.

Kjellstrand P et al. (1981) Trichloroethylene: effects on body and organ weights in mice, rats, and gerbils. *Toxicology*, 21:105–115.

Kjellstrand P et al. (1983a) Effects of continuous trichloroethylene inhalation on different strains of mice. *Acta Pharmacologica et Toxicologica*, 53:369–374.

Kjellstrand P et al. (1983b) Trichloroethylene: further studies of the effects on body and organ weights and plasma butyrylcholinesterase activity in mice. *Acta Pharmacologica et Toxicologica*, 53:375–384.

Kleinfeld M, Tabershaw IR (1954) Trichloroethylene toxicity — Report of five fatal cases. *Archives of Industrial Hygiene and Occupational Medicine*, 10:141–143.

Konietzko H et al. (1978) [Chromosome studies on trichloroethylene workers.] *Archives of Toxicology*, 40:201–206 (in German).

Krishnan K (2003) *Evaluation of the relative importance of dermal and inhalation routes of exposure for trichloroethylene*. Contract report submitted to the Water Quality and Health Bureau, Safe Environments Programme, Health Canada, Ottawa, Ontario, pp. 1–19.

Kruithof JC, Koppers HMM (1989) Experiences with groundwater treatment and disposal of the eliminated substances in the Netherlands. *Aqua*, 38:207–216.

Kruithof JC et al. (1985) Removal of organic contaminants from drinking water. *Aqua*, 35(2):89–99.

Kusakabe K et al. (1991) Destruction rate of volatile organochlorine compounds in water by ozonation with ultraviolet radiation. *Water Research*, 25(10):1199–1203.

Lagakos SW, Wessen BJ, Zelen M (1986) An analysis of contaminated well water and health effects in Woburn, Massachusetts. *Journal of the American Statistical Association*, 81:583–596.

Laparé S, Tardif R, Brodeur J (1995) Effect of various exposure scenarios on the biological monitoring of organic solvents in alveolar air. II. 1,1,1-Trichloroethane and trichloroethylene. *International Archives of Occupational and Environmental Health*, 67:375–394.

Lash L, Parker J, Scott C (2000) Modes of action of trichloroethylene for kidney tumorigenesis. *Environmental Health Perspectives*, 108(Suppl. 2):225–240.

Leong KJ, Schwetz BA, Gehring PJ (1975) Embryo and fetotoxicity of inhaled trichloroethylene, perchloroethylene, methylchloroform and methylene chloride in mice and rats. *Toxicology and Applied Pharmacology*, 33:136–137.

Lindstrom AB, Pleil JD (1996) A methodological approach for exposure assessment studies in residences using volatile organic compound-contaminated water. *Journal of the Air and Waste Management Association*, 46(11):1058–1066.

Major DW, Hodgins EW, Butler BJ (1991) Field laboratory evidence of *in situ* biotransformation of tetrachloroethene to ethene and ethane at a chemical transfer facility in North Toronto. In: Hinchee RE, Olfenbuttel RF, eds. *On-site bioreclamation: Processes for xenobiotic and hydrocarbon treatment*. Columbus, OH, Battelle Memorial Institute; Boston, MA, Butterworth-Heinmann, pp. 147–171.

Maltoni C, Lefemine G, Cotti G (1986) *Archives of research on industrial carcinogenesis*. Vol. V. *Experimental research on trichloroethylene carcinogenesis*. Princeton, NJ, Princeton Scientific Publishing Co.

Maltoni C et al. (1988) Long-term carcinogenic bioassays on trichloroethylene administered by inhalation to Sprague-Dawley rats and Swiss and B6C3F1 mice. *Annals of the New York Academy of Sciences*, 534:316–351.

Mann Testing Laboratories Ltd (1983) *GC/MS analysis of 51 volatile pollutants in raw and treated water, Phase II.* Report submitted to Environmental Health Directorate, Department of National Health and Welfare, Ottawa, Ontario, July.

McKinnon RJ, Dykesen JE (1984) Removing organics from groundwater through aeration plus GAC. *Journal of the American Water Works Association*, 76(5):42–47.

McLaughlin JK, Blot WJ (1997) A critical review of epidemiology studies of trichloroethylene and perchloroethylene and risk of renal-cell cancer. *Archives of Occupational and Environmental Health*, 70:222–231.

McNeill WC (1979) Trichloroethylene. In: *Kirk-Othmer encyclopedia of chemical technology*, 3rd ed. Vol. 5. New York, NY, John Wiley & Sons, pp. 745–753.

MDPH (1994) *Final report of the Woburn Environmental and Birth Study*. Vol. 1. *Analysis of reproductive outcomes and environmental exposures in Woburn, MA.* Boston, MA, Massachusetts Department of Public Health, Bureau of Environmental Health Assessment (draft for public comment).

Melnick RL et al. (1987) Application of microencapsulation for toxicology studies. *Fundamental and Applied Toxicology*, 9:432–442.

Mitoma C et al. (1985) Metabolic disposition study of chlorinated hydrocarbons in rats and mice. *Drug and Chemical Toxicology*, 8:183–194.

Monster AC (1979) Difference in uptake, elimination, and metabolism in exposure to trichloroethylene, 1,1,1-trichloroethane and tetrachloroethylene. *International Archives of Occupational and Environmental Health*, 42:311–317.

Monster AC, Houtkooper JM (1979) Estimation of individual uptake of trichloroethylene, 1,1,1-trichloroethane and tetrachloroethylene from biological parameters. *International Archives of Occupational and Environmental Health*, 42:319–323.

Monster AC, Boersma C, Duba WC (1976) Pharmacokinetics of trichloroethylene in volunteers, influence of workload and exposure concentration. *International Archives of Occupational and Environmental Health*, 38:87–102.

Monster AC, Boersma C, Duba WC (1979) Kinetics of trichloroethylene in repeated exposure of volunteers. *International Archives of Occupational and Environmental Health*, 42:283–292.

Moore MM, Harrington-Brock K (2000) Mutagenicity of trichloroethylene and its metabolites: Implications for risk assessment of trichloroethylene. *Environmental Health Perspectives*, 108(Suppl. 2):215–225.

Muller G, Spassovski M, Henschler D (1974) Metabolism of trichloroethylene in man. II. Pharmacokinetics of metabolites. *Archives of Toxicology*, 32:283–295.

Muller G, Spassovski M, Henschler D (1975) Metabolism of trichloroethylene in man. III. Interaction of trichloroethylene and ethanol. *Archives of Toxicology*, 33:173–189.

Nagaya T, Ishikawa N, Hata H (1989) Sister-chromatid exchanges in lymphocytes of workers exposed to trichloroethylene. *Mutation Research*, 222:279–282.

Nakai JS et al. (1999) Penetration of chloroform, trichloroethylene, and tetrachloroethylene through human skin. *Journal of Toxicology and Environmental Health*, 58(3):157–170.

Nakajima T et al. (1993) Cytochrome P450-related differences between rats and mice in the metabolism of benzene, toluene and trichloroethylene in liver microsomes. *Biochemical Pharmacology*, 45:1079–1085.

Narotsky MG, Kavlock RJ (1995) A multidisciplinary approach to toxicological screening. II. Developmental toxicity. *Journal of Toxicology and Environmental Health*, 45:145–171.

Narotsky MG et al. (1995) Nonadditive developmental toxicity in mixtures of trichloroethylene, di(2-ethylhexyl) phthalate, and heptachlor in a 5 × 5 × 5 design. *Fundamental and Applied Toxicology*, 27:203–216.

NCI (1976) *Carcinogenesis bioassay of trichloroethylene*. Bethesda, MD, US Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Cancer Institute (NCI-CGTR-2, NIH 76-802).

Nomiyama K, Nomiyama H (1971) Metabolism of trichloroethylene in humans. Sex difference in urinary excretion of trichloroacetic acid and trichloroethanol. *Internationales Archiv für Arbeitsmedizin*, 28:37–48.

NTP (1983) *NTP technical report on the carcinogenesis studies of trichloroethylene (without epichlorohydrin) (CAS No.79-01-6) in F344/N rats and B6C3F1 mice (gavage studies). Draft report.* Research Triangle Park, NC, US Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Toxicology Program (NIH Publication No. 83-1799).

NTP (1985) *Trichloroethylene: reproduction and fertility assessment in CD-1 mice when administered in the feed.* Research Triangle Park, NC, US Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Toxicology Program (NIH Publication No. 86-068).

NTP (1986) *Trichloroethylene: reproduction and fertility assessment in F344 rats when administered in the feed. Final report.* Research Triangle Park, NC, US Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Toxicology Program (NIH Publication No. 86-085).

NTP (1988) *Toxicology and carcinogenesis studies of trichloroethylene (CAS No. 79-01-6) in four strains of rats (ACI, August, Marshall, Osborne-Mendel) (gavage studies).* Research Triangle Park, NC, US Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Toxicology Program (NTP Technical Report Series No. 273; NIH Publication No. 88-2525).

NTP (1990) *Carcinogenesis studies of trichloroethylene (without epichlorohydrin) (CAS No. 79-01-6) in F344/N rats and B6C3F1 mice (gavage studies).* Research Triangle Park, NC, US Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Toxicology Program (NTP Technical Report Series No. 243).

Odum J, Foster JR, Green T (1992) A mechanism for the development of Clara cell lesions in the mouse lung after exposure to trichloroethylene. *Chemico-Biological Interactions*, 83:135–153.

OEHHA (1999) *Public health goal for trichloroethylene in drinking water*. Sacramento, CA, California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, 102 pp.

Otson R, Williams DT, Bothwell PD (1982) Volatile organic compounds in water at thirty Canadian potable water treatment facilities. *Journal of the Association of Official Analytical Chemists*, 65:1370.

Otson R, Fellin P, Whitmore R (1992) *A national pilot study on occurrence of airborne VOCs in residences — design and progress.* Presented at the 1992 US Environmental Protection Agency / Air and Waste Management Association Symposium on "Measurement of Toxic and Related Air Pollutants," 4–8 May 1992, Durham, NC [cited in Department of National Health and Welfare, 1993].

Pailard H, Brunet R, Dore M (1987) Application of oxidation by a combined ozone/ultraviolet radiation system to the treatment of natural water. *Ozone Science and Engineering*, 9(4):391–418.

Parchman LG, Magee PN (1982) Metabolism of ^{14}C-trichloroethylene to $^{14}CO_2$ and interaction of a metabolite with liver DNA in rats and mice. *Journal of Toxicology and Environmental Health*, 9:797–813.

Pastino GM, Yap WY, Carroquino M (2000) Human variability and susceptibility to trichloroethylene. *Environmental Health Perspectives*, 108(Suppl. 2):201–204.

Pellizzari ED, Hartwell TD, Harris BS (1982) Purgeable organic compounds in mother's milk. *Bulletin of Environmental Contamination and Toxicology*, 28:322–328.

Pirbazari M (1992) MF-PAC for treating waters contaminated with natural and synthetic organics. *Journal of the American Water Works Association*, 84(12):95–103.

Poet TS et al. (2000) Assessment of the percutaneous absorption of trichloroethylene in rats and humans using MS/MS real-time breath analysis and physiologically based pharmacokinetic modeling. *Toxicological Sciences*, 56:61–72.

Prout MS, Provan WM, Green T (1985) Species differences in response to trichloroethylene. Pharmacokinetics in rats and mice. *Toxicology and Applied Pharmacology*, 79:389–400.

Qi S et al. (1992) Using isotherms to predict GAC's capacity for synthetic organics. *Journal of the American Water Works Association*, 84(9):113–120.

Rasmussen K et al. (1988) A genotoxic study of metal workers exposed to trichloroethylene. Sperm parameters and chromosome aberrations in lymphocytes. *International Archives of Occupational and Environmental Health*, 60:419–423.

Raunio H et al. (1995) Diagnosis of polymorphism in carcinogen-activating and inactivating enzymes and cancer susceptibility — a review. *Gene*, 159:113–121.

Raven and Beck Environmental Ltd (1995) *Survey of tetrachloroethylene and trichloroethylene occurrences in Canadian groundwater.* Report prepared for Monitoring and Criteria Division, Health Canada, Ottawa, Ontario.

Rothman KJ (1986) *Modern epidemiology*. Boston, MA, Little Brown and Company.

Rouisse L, Chakrabarti SK (1986) Dose-dependent metabolism of trichloroethylene and its relevance to hepatotoxicity in rats. *Environmental Research*, 40:450–458.

RTECS (1993) *Registry of Toxic Effects of Chemical Substances online database*. Bethesda, MD, US Department of Health and Human Services, National Library of Medicine, National Toxicology Information Program.

Ruth JH (1986) Odor threshold and irritation levels of several chemical substances. A review. *American Industrial Hygiene Association Journal*, 47:148–151.

Sanders VM et al. (1982) Humoral and cell-mediated immune status in mice exposed to trichloroethylene in the drinking water. *Toxicology and Applied Pharmacology*, 62:358–368.

Sato A, Nakajima T (1978) Differences following skin or inhalation exposure in the absorption and excretion kinetics of trichloroethylene and toluene. *British Journal of Industrial Medicine*, 35:43–49.

Sato A et al. (1977) A pharmacokinetic model to study the excretion of trichloroethylene and its metabolites after an inhalation exposure. *British Journal of Industrial Medicine*, 34:56–63.

Schwetz BA, Leong BKJ, Gehring PJ (1975) The effect of maternally inhaled trichloroethylene, perchloroethylene, methyl chloroform, and methylene chloride on embryonal and fetal development in mice and rats. *Toxicology and Applied Pharmacology*, 32:84–96.

Seiji K et al. (1990) Sister chromatid exchanges in peripheral lymphocytes of workers exposed to benzene, trichloroethylene, or tetrachloroethylene, with reference to smoking habits. *International Archives of Occupational and Environmental Health*, 62:171–176.

Semmens MJ, Qin R, Zander A (1989) Using a microporous hollow-fiber membrane to separate VOCs from water. *Journal of the American Water Works Association*, 81(4):162–167.

Siegel J et al. (1971) Effects on experimental animals of acute repeated and continuous inhalation exposures to dichloroacetylene mixtures. *Toxicology and Applied Pharmacology*, 18:168–174.

Simon P, Mitchell J (1992) The aeration removal of volatile organochlorine compounds from a well-water site near London. *Aqua*, 41(6):322–329.

Slagle D (1990) Ozonation as an attractive option for groundwater systems. *Public Works*, 121(3):45–46.

Smith MK et al. (1989) Teratogenic activity of trichloroethylene in the rat. *Teratology*, 40:445–451.

Smith MK, Randall JL, Read EJ (1992) Developmental toxicity of dichloroacetate in the rat. *Teratology*, 46:217–223.

Smyth HF et al. (1969) Range-finding toxicity data. VII. *American Industrial Hygiene Association Journal*, 30:470–476.

Spirtas R et al. (1991) Retrospective cohort mortality study of workers at an aircraft maintenance facility. I. Epidemiological results. *British Journal of Industrial Medicine*, 48:515–530.

Stacpoole PW, Moore GW, Kornhauser DM (1979) Toxicity of chronic dichloroacetate. *New England Journal of Medicine*, 300:372.

Statistics Canada (1994) *Human activity and the environment 1994*. Ottawa, Ontario, Minister of Industry, Science and Technology, 300 pp. [cited in Raven and Beck Environmental Ltd, 1995].

Stenner RD et al. (1997) Enterohepatic recirculation of trichloroethanol glucuronide as a significant source of trichloroacetate in the metabolism of trichloroethylene. *Drug Metabolism and Disposition*, 25:529–535.

Stenner RD et al. (1998) Physiologically-based pharmacokinetic model for trichloroethylene considering enterohepatic circulation of major metabolites. *Risk Analysis*, 18:261–269.

Stewart PA, Lee JS, Marano DE (1991) Retrospective cohort mortality study of workers at an aircraft maintenance facility: II. Exposures and their assessment. *British Journal of Industrial Medicine*, 48:531–537.

Stewart RD, Dodd HC (1964) Absorption of carbon tetrachloride, trichloroethylene, tetrachloroethylene, methylene chloride, and 1,1,1-trichloroethane through human skin. *Journal of the American Industrial Hygiene Association*, 25:439–446.

Stott WT, Quast JF, Watanabe PG (1982) The pharmacokinetics and macromolecular interactions of trichloroethylene in mice and rats. *Toxicology and Applied Pharmacology*, 62:137–151.

Styles JA, Wyatt I, Coutts C (1991) Trichloroacetic acid: studies on uptake and effects on hepatic DNA and liver growth in mouse. *Carcinogenesis*, 12:1715–1719.

Tao L et al. (2000a) Hypomethylation and overexpression of *c-jun* and *c-myc* protooncogenes and increased DNA methyltransferase activity in dichloroacetic and trichloroacetic acid-promoted mouse liver tumors. *Cancer Letters*, 158:185–193.

Tao L et al. (2000b) Effect of trichloroethylene and its metabolites, dichloroacetic acid and trichloroacetic acid, on the methylation and expression of *c-jun* and *c-myc* protooncogenes in mouse liver: Prevention by methionine. *Toxicological Sciences*, 54:399–407.

Topudurti K et al. (1994) Field evaluation of perox-pure chemical oxidation technology. *Water Science and Technology*, 30(7):95–104.

Tsuruta H (1978) Percutaneous absorption of trichloroethylene in mice. *Industrial Health*, 15:145–151.

Tucker AN et al. (1982) Toxicology of trichloroethylene in the mouse. *Toxicology and Applied Pharmacology*, 62:351–357.

Urano K (1991) Adsorption of chlorinated organic compounds on activated carbon from water. *Water Research*, 25(12):1459–1464.

US EPA (1985a) *Health assessment document for trichloroethylene.* Washington, DC, US Environmental Protection Agency, July (EPA/600/8-82/006F).

US EPA (1985b) National primary drinking water regulations, volatile synthetic organic compounds. US Environmental Protection Agency. *Federal Register*, 50(219):46902.

US EPA (1995) *The use of the benchmark dose approach in health risk assessment*. Washington, DC, US Environmental Protection Agency, Risk Assessment Forum (EPA/630/R-94/007).

US EPA (1999a) *AIRDATA*. Research Triangle Park, NC, US Environmental Protection Agency, Office of Air Quality Planning and Standards.

US EPA (1999b) *UCMR List 1 and List 2 chemical analytical methods and quality control manual*. Washington, DC, US Environmental Protection Agency, Office of Water.

US EPA (2001) *Trichloroethylene health risk assessment: synthesis and characterization.* Washington, DC, US Environmental Protection Agency, Office of Research and Development, August (external review draft; EPA/600/P-01/002A).

Vaidya VS et al. (2003) Renal injury and repair following *S*-1,2-dichlorovinyl-L-cysteine administration to mice. *Toxicology and Applied Pharmacology*, 188(2):110–121.

Vamvakas S, Dekant W, Henschler D (1993) Nephrocarcinogenicity of haloalkenes and alkynes. In: Anders MW et al., eds. *Renal disposition and nephrotoxicity of xenobiotics*. San Diego, CA, Academic Press, pp. 323–342.

Vamvakas S et al. (1998) Renal cell cancer correlated with occupational exposure to trichloroethene. *Journal of Cancer Research and Clinical Oncology*, 124:374–382.

Verschueren K (1983) *Handbook of environmental data on organic chemicals*, 2nd ed. New York, NY, Van Nostrand Reinhold Company.

Wartenberg D, Reyner D, Scott CS (2000) Trichloroethylene and cancer. Epidemiologic evidence. *Environmental Health Perspectives*, 108(Suppl. 2):161–176.

Weisel CP, Jo W-K (1996) Ingestion, inhalation, and dermal exposures to chloroform and trichloroethene from tap water. *Environmental Health Perspectives*, 104:48–51.

WHO (2004) *Guidelines for drinking-water quality*, 3rd ed. Vol. 1. Recommendations. Geneva, World Health Organization.

Wilson PD et al. (1998) Attributable fraction for cardiac malformations. *American Journal of Epidemiology*, 148:414–423.

Windholz M et al., eds. (1976) *The Merck index: an encyclopedia of chemicals and drugs*. Rahway, NJ, Merck.

Yount EA et al. (1982) Comparison of the metabolic and toxic effects of 2-chloropropionate and dichloroacetate. *Journal of Pharmacology and Experimental Therapeutics*, 222:501–508.

Yuen W, Zimmer J (2001) *Manitoba First Nation community water supplies 2000*. Report prepared for First Nations & Inuit Health Branch, Health Canada, by Saskatchewan Research Council, Regina, Saskatchewan (SRC Publication No. 10497-1C01).

Zenick H et al. (1984) Effects of trichloroethylene exposure on male reproductive function in rats. *Toxicology*, 31:237–250.