Health Canada Santé Canada

Guidelines for Canadian Drinking Water Quality:

Supporting Documentation

Trichloroethylene

Prepared by the
Federal-Provincial-Territorial Committee on Drinking Water
of the
Federal-Provincial-Territorial Committee on Health and the Environment

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Other supporting documents in the Canadian Guidelines for Drinking Water Quality can be found on the Water Quality and Health Bureau web page at http://www.hc-sc.gc.ca/hecs-sesc/water/dwgsup.htm.

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Trichloroethylene

1.0 Guideline

The maximum acceptable concentration (MAC) for trichloroethylene in drinking water is 0.005 mg/L (5 µg/L).

2.0 Executive Summary

Trichloroethylene (TCE) is a volatile solvent that is used extensively in the automotive and metals industries for vapour degreasing and cold cleaning of metal parts. TCE is not manufactured in Canada, and its use is regulated under the *Canadian Environmental Protection Act, 1999*. Canadians can be exposed to TCE through its presence in drinking water, air and food. Certain segments of the population could be exposed via contaminated soil or occupational settings.

This supporting document focuses on the health risks associated with TCE in drinking water, including multiple routes of exposure — ingestion as well as inhalation and skin absorption from showering and bathing. It assesses all identified health risks, taking into account new studies and approaches, and incorporates appropriate safety factors. The guideline of 0.005 mg/L will protect humans from both cancer and non-cancer health risks.

2.1 Health Effects

Animal studies have shown links between exposure to TCE and kidney and testicular tumours in rats and pulmonary and liver tumours in mice. Studies in humans seem to support these links, but further studies are needed to confirm them, in part because other chemicals were also present. Based on the evidence from both animal and human studies, TCE has been classified as probably carcinogenic to humans. Research is ongoing in this area.

Animal and human studies have shown a small increase in the rate of reproductive effects (heart malformations in fetuses) above what can be expected under normal circumstances. The data from the human studies came from people who had been exposed to very high levels of TCE and other solvents through contaminated groundwater. Further studies are required to confirm these developmental effects as well as their long-term significance to human health.

2.2 Exposure

TCE evaporates readily from surface water but may occasionally be found in groundwater. TCE is not a concern for the majority of Canadians who rely on surface water as their source of drinking water. TCE is not a widespread problem in Canada, affecting only some groundwater supplies; where TCE is detected in Canadian drinking water supplies, levels are generally less than 0.001 mg/L. TCE can be introduced into groundwater as a result of industrial effluents or spills or leaking from old dump sites.

2.3 Treatment

Options to reduce exposure to TCE include finding an alternative source of drinking water; enhancing treatment to reduce the level of TCE in the drinking water to below the proposed guideline in municipal systems relying on groundwater supplies; and using drinking water

treatment devices where individual households obtain drinking water from private wells. Health Canada recommends that consumers use certified treatment devices. Point-of-entry systems are preferred for volatile organic compounds (VOCs) such as TCE because they reduce exposure through inhalation and dermal absorption by providing treated water for bathing and showering. Although certified point-of-use treatment devices are currently available for the reduction of VOCs, including TCE, certified point-of-entry treatment devices cannot be purchased off the shelf; however, systems can be designed and constructed with certified materials.

3.0 Identity, Use and Sources in the Environment

Trichloroethylene ($CHCl=CCl_2$; relative molecular mass 131.4), also known as TCE and trichloroethene, is a colourless liquid with a sweet odour. Its odour thresholds are 546–1092 mg/m^3 in air and 0.31 mg/L in water (Amoore and Hautala, 1983; Ruth, 1986). At room temperature, TCE is a volatile, non-viscous liquid with a boiling point of 86.7°C. TCE is moderately soluble in water (1.1–1.4 g/L) and has a low *n*-octanol/water partition coefficient (log K_{ow} 2.29–2.42), a high vapour pressure (8.0–9.9 kPa at 20–25°C; McNeill, 1979; ATSDR, 1989) and a Henry's law constant of 1.1 $kPa \cdot m^3/mol$ at 25°C (Hine and Mookerjee, 1975). In air, 1 ppm is equivalent to 5.41 mg/m^3 at 20°C and 101.3 kPa (Verschueren, 1983). Under conditions of normal use, TCE is considered non-flammable and moderately stable, but it requires the addition of stabilizers (up to 2% v/v) in commercial grades.

TCE use has declined sharply in industrialized countries since 1970 (McNeill, 1979). In Canada, 90% of the TCE consumed is used in metal degreasing operations, and the balance is used in miscellaneous applications, including textile solvents, paint removers, coatings and vinyl resins. TCE may also be present in household and consumer products, such as typewriter correction fluids. Production in Canada ceased in 1985; however, TCE is still imported into the country. Over the period 1995–1999, total annual Canadian demand averaged 220 tonnes. More recently, the demand for TCE has decreased. This may be due to several factors, including the use of other solvents for metal degreasing, a decline in the number of companies conducting metal degreasing and an increase in solvent recovery/recycling by users (CPI, 2000). Reporting facilities to Environment Canada's National Pollutant Release Inventory indicated that approximately 17% of TCE was recycled over the period 1996–2000 (Environment Canada, 2000).

Most of the TCE used for degreasing is believed to be emitted to the atmosphere (U.S. EPA, 1985a). TCE may, however, 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. 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. The biodegradation of another volatile organic pollutant, tetrachloroethylene (or perchloroethylene, PCE), in groundwater may also lead to the formation of TCE (Major *et al*., 1991).

4.0 Exposure

Canadians can be exposed to TCE through its presence in drinking water, air and food. In addition, certain segments of the population can be exposed via contaminated soil, through the use of specific consumer products or in occupational settings. Since TCE has been detected in

human milk, nursing infants could potentially be exposed (U.S. EPA, 2001b). Although some exposure data are available, they are considered insufficient to justify modifying the default allocation factor for drinking water of 20%.

4.1 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/L). 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/L (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/L or less, and the maximum level was 9 µg/L (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/L. The maximum concentration was 23.9 µg/L (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/L. The maximum concentration was 81 µg/L. Of those samples with detectable TCE concentrations, most were from groundwater (Alberta Department of Environmental Protection, 2002; New Brunswick Department of Health and Wellness, 2002; Ontario Ministry of Environment and Energy, 2002; Yukon Department of Health and Social Services, 2002; Ministère de l'Environnement du Québec, 2003).

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/L) (Yuen and 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/L and 1680 µg/L, 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/L), 3.6%

had a maximum concentration of <1 µg/L, 1.4% had a maximum of 1–10 µg/L, 0.43% had a maximum of 10–100 µg/L and 1.3%* had a maximum of >100 µg/L (Raven and Beck Environmental Ltd., 1995).

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/L), 48.1% had a maximum of 1–10 µg/L, 2.1% had a maximum of 10–100 µg/L and 0.8% had a maximum of >100 µg/L. 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 TCE/L 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).

4.2 Multi-route 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. These contributions are expressed in litre equivalents per day (Leq/day). For example, an inhalation exposure of 1.7 Leq/day means that the daily exposure to TCE via inhalation is equivalent to a person drinking an extra 1.7 L 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 Leq/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 U.S. age-specific consumption rates, and the dermal number was derived using a generic dermal absorption coefficient value for VOCs, rather than a TCE-specific value. In addition to the shower scenario, these authors quantified exposure via household air when determining the Leq/day value for the inhalation route.

Weisel and 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 Leq/day values for the various routes are not easily achieved.

Lindstrom and 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/L, these authors calculated that the ingested dose was more important than the inhaled dose for a 10-minute shower, which in turn was greater than the dermal dose.

Krishnan (2003) determined Leq/day values for dermal and inhalation exposures of adults and children (6-, 10- and 14-year-olds) to TCE (5 µg/L) in drinking water for a 10-minute shower and a 30-minute bath on the basis of the methodological approach of Lindstrom and Pleil (1996), the use of physiologically based pharmacokinetic (PBPK) models and consideration of the

* 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/L.

fraction absorbed (Laparé *et al.*, 1995; Lindstrom and 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 hours. 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).

Leq/day values for the inhalation and dermal routes were higher for the 30-minute bath scenario than for the 10-minute shower for all subpopulations based on the longer exposure time. The highest value was 3.9 Leq/day (1.5 L ingestion, 1.7 L inhalation, 0.7 L dermal) for adults. The 3.9 Leq/day value (which can be rounded to 4.0 Leq/day) is considered to be conservative, since most Canadians do not take a 30-minute bath on a daily basis. In the event that individuals spend more than 10 minutes in a shower or are exposed to TCE via other household activities, the calculated 4.0 Leq/day value (which includes inhalation and dermal exposure from a 30-minute bath) should be adequate.

4.3 Air

Studies conducted in the 1980's and 1990's have detected TCE 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).

More recent U.S. 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 (U.S. 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/L) increased levels of TCE in bathroom air from <0.5 to 67–81 mg/m^3 in less than 30 minutes (Andelman, 1985). However, it should be noted that TCE concentrations in Canadian water supplies are usually less than 1 μg/L. Therefore, the Leq/day values outlined above appear reasonable.

4.4 Food

The U.S. EPA (2001a) 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 bw per day and from 0.01 to 0.04 μg/kg bw per day, respectively (Department of National Health and Welfare, 1993). These numbers were based on TCE

concentrations from U.S. 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.

5.0 Analytical Methods

Several methods can be used to measure TCE in drinking water. Due to TCE's volatility, analytical methods for the chemical are based on purge and trap or head space gas chromatography using photoionization or mass spectrometric detection. TCE can also be captured by liquid–liquid extraction followed by gas chromatography with electrolytic conductivity detection.

Four methods for measuring TCE in drinking water have been approved by the U.S. 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/L (U.S. 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/L (U.S. 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/L (U.S. 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/L (U.S. EPA, 1999b).

For the determination of TCE in water, the practical quantitation limit (PQL) considered to be achievable by most good laboratories is 5 µg/L.

6.0 Treatment Technology

6.1 Municipal

Municipal water filtration plants that rely on conventional water treatment techniques (coagulation, sedimentation, precipitative softening, filtration and chlorination) have been found to be ineffective in reducing concentrations of TCE in drinking water (Robeck & Love, 1983). Two common water treatment technologies that, when combined, are effective in removing TCE are air stripping and activated carbon.

Air stripping has been found to be effective in removing VOCs such as TCE from groundwater. Air stripping is effective in stripping large quantities of TCE from water, but at low rates of removal (Russell *et al.*, 1992).

Adsorption onto activated carbon is widely used to remove synthetic organic compounds such as TCE from drinking water, if the activated carbon filter bed is deep enough (Russell *et al.*, 1992).

Combining air stripping and activated carbon into a two-step treatment train has been shown to improve TCE removal. In a municipal-scale treatment plant combining these processes, air stripping is used in the first step for bulk removal of the majority of the TCE from the water, and activated carbon is used in the second step to remove most residual TCE from the water.

TCE levels below 1 µg/L can be achieved in municipal drinking water supplies using these methods (U.S. EPA, 1985b).

6.2 Residential

Generally, it is not recommended that drinking water treatment devices be used to provide additional treatment to municipally treated water. In cases where individual households obtain drinking water from private wells or the drinking water is contaminated by low concentrations of TCE, private residential water treatment devices may be an option for removing TCE from drinking water.

A number of residential treatment devices from various manufacturers are available that are affordable and can remove TCE from drinking water to make it compliant with the applicable guidelines or regulations. Filtration systems may be installed at the faucet (point of use) or where water enters the home (point of entry). Point-of-entry systems are preferred for VOCs such as TCE because they provide treated water for bathing and laundry as well as for cooking and drinking. Certified point-of-use treatment devices are currently available for the reduction of VOCs, including TCE. Although certified point-of-entry treatment devices cannot be purchased off the shelf, systems can be designed and constructed with certified materials. Periodic laboratory testing should be conducted on both the water entering a treatment device and the water it produces to verify that the treatment device is effective.

Health Canada does not recommend specific brands of drinking water treatment devices, but it strongly recommends that consumers look for a mark or label indicating that the device has been certified by an accredited certification body as meeting the appropriate NSF International (NSF)/American National Standards Institute (ANSI) standards. These standards have been designed to safeguard drinking water by helping to ensure the material safety and performance of products that come into contact with drinking water. Certification organizations provide assurance that a product or service conforms to applicable standards. In Canada, the following organizations have been accredited by the Standards Council of Canada (www.scc.ca) to certify drinking water devices and materials as meeting NSF/ANSI standards:

- Canadian Standards Association International (www.csa-international.org);
- NSF International (www.nsf.org);
- Water Quality Association (www.wqa.org);
- Underwriters Laboratories Inc. (www.ul.com);
- Quality Auditing Institute (www.qai.org); and
- International Association of Plumbing & Mechanical Officials (www.iapmo.org).

Treatment devices to remove TCE from untreated water (such as from a private well) should be certified for the removal of either TCE or VOCs. In the case of TCE, these treatment devices are certified to reduce TCE levels from an average influent (challenge) concentration of 0.3 mg/L to a maximum finished effluent concentration of 0.005 mg/L or less (NSF International, 2005). Treatment devices that are certified to remove TCE or VOCs incorporate some type of adsorption technology, usually activated carbon, or utilize reverse osmosis, usually in combination with one or more adsorption-type filters.

7.0 Kinetics and Metabolism

7.1 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 variabilities in TCE absorption following all routes of exposure have 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 bw, implying that the rate of absorption was higher at low doses than at high doses in both species (Daniel, 1963; Parchman and Magee, 1982; Dekant and Henschler, 1983; Dekant *et al.*, 1984; Buben and O'Flaherty, 1985; Mitoma *et al.*, 1985; Prout *et al.*, 1985; Rouisse and 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 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 and 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 10 or 600 ppmv over a 6-hour 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 minutes 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 and Dodd, 1964; Sato and Nakajima, 1978); however, variability between individuals precludes any meaningful interpretation of these data.

7.2 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).

7.3 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 P-450 and conjugation with glutathione (GSH) by glutathione-S-transferases (GSTs) (OEHHA, 1999; Lash *et al.*, 2000). In the liver, TCE is metabolized by cytochrome P-450 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 sulphur 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 P-450 (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 by GST also occurs primarily in the liver, 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 P-450-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; U.S. EPA, 2001a).

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 have recently been discussed (Chen, 2000). A recent paper (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, however, apparent that while DCA may be formed during the metabolism of TCE, it is very unlikely to be produced in significant amounts at environmental levels of exposure to TCE.

7.4 Excretion

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 (DCVCNac) (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 and

Nomiyama, 1971; Muller *et al.*, 1974; Fernandez *et al.*, 1975; Sato *et al.*, 1977; Monster and 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 and Magee, 1982; Stott *et al.*, 1982; Dekant *et al.*, 1984; Buben and O'Flaherty, 1985; Mitoma *et al.*, 1985; Prout *et al.*, 1985; Rouisse and Chakrabarti, 1986). In humans, interindividual heterogeneity was seen in the metabolism and elimination of TCE (Nomiyama and Nomiyama, 1971; Fernandez *et al.*, 1975; Monster *et al.*, 1976).

8.0 Health Effects

8.1 Effects in 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 in 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 sister chromatid exchange (SCE) 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[**] 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 and 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 about their exposure to TCE. Although it is generally 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), 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.

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

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

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 United States (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's lymphoma seen in cohort studies. A marginally increased risk for non-Hodgkin's 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's 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, vs. 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 multi-functional enzymes that catalyse several reactions between GST and elecrophilic 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 and 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 and Blot, 1997). Although McLaughlin and 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 byWartenberg *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's lymphoma (RR = 1.5, 95% CI = 0.9–2.3), as well as for cervical cancer, Hodgkin's 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 affect 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 von Hippel Landau (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.

Overall, although several studies have indicated a positive association between solvent exposure 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).

8.2 Effects on Experimental Animals and *In Vitro*

Many studies of a wide range of toxic endpoints 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.

8.2.1 Acute Toxicity

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 bw in mice (Tucker *et al.*, 1982) and 4920 mg/kg bw in rats (Smyth *et al.*, 1969; IPCS, 1985; ATSDR, 1993, 1997). The 4-hour inhalation LC_{50} was calculated to be 12 500 ppm in rats (Siegel *et al.*, 1971) and 8450 ppm in mice (Fan, 1988). A review of studies of dermal exposure of TCE in rabbits indicates that skin irritation occurs after 24 hours at 0.5 mL and degenerative skin changes occur within 15 minutes 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.

8.2.2 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 bw per day in female rats and up to 2000 mg/kg bw per day in male rats, or up to 6000 mg/kg bw 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 bw per day. Pulmonary vasculitis involving small veins was reported in female rats at 1000 mg/kg bw per day. Mild to moderate cytomegaly and karyomegaly of the renal tubular epithelial cells occurred in rats at 1000 mg/kg bw per day (females) or 2000 mg/kg bw per day (males). The no-observed-adverse-effect level (NOAEL) in rats was reported as 1000 mg/kg bw

per day (males) and 500 mg/kg bw per day (females). Among the mice, there were decreases in survival in both sexes and body weight gain in males at 750 mg/kg bw per day and above. Doses of 3000 mg/kg bw 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 bw per day for mice.

Exposure to TCE through drinking water has been evaluated in subchronic 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/L (equivalent to 0, 18.4, 216.7, 393 or 660 mg/kg bw per day) for 4 or 6 months. Females at 5.0 mg/L and males at and above 2.5 mg/L 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/L. In addition, at 5.0 mg/L, 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/L, there was enlargement of the liver and an increase in urinary protein and ketone levels in males. Inhibition of humoral immunity, cell-mediated immunity and bone marrow stem cell colonization was seen among females at 2.5 mg/L and greater. The lowest-observed-adverse-effect level (LOAEL) was considered to be 2.5 mg/L, 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/L (equivalent to 216.7 mg/kg bw 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, 49, 175 or 330 ppmv TCE for 4 hours per day, 5 days per week, for 14 weeks. Another group was exposed to 55 ppmv TCE for 8 hours 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 and Eben, 1973). In a study in which mice, rats and gerbils (unspecified strains) were exposed to TCE continuously by inhalation at 150 ppmv 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 150 ppmv atmospheres of TCE continuously for 30 days had increased ($p < 0.05$) kidney weight. NMRI mice exposed to 37, 75, 150 or 300 ppmv TCE continuously for 30 days had significantly increased ($p < 0.05$) kidney weight at 75 ppmv in males and above 150 ppmv in females. No kidney effects were evident in the remaining strains of mice (Kjellstrand *et al.*, l983a).

8.2.3 Long-term Exposure and Carcinogenicity

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 bw per day (rats) and 0 or 1000 mg/kg bw 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 renal tubular epithelium, occurred in rats at 500 mg/kg bw per day and above and in mice at 1000 mg/kg bw per day. LOAELs of 500 mg/kg bw per day in rats and 1000 mg/kg bw per day in mice were defined for long-term effects. A NOAEL was not determined (NTP, 1990).

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 (U.S. EPA, 2001a). 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 ($p < 0.05$) 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) (Table 1). There were no treatment-related liver tumours in rats.The male rats at 1000 mg/kg bw 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; Table 1). These kidney tumours were considered biologically significant, given the rarity of kidney tumours in that rat strain.

Table 1: Carcinogenicity assay in rodents administered TCE by gavage[a,b]

Dose (mg/kg bw per day)	B6C3F1 mice[c]		F344/N rats[c]
	Males, incidence of hepatocellular carcinomas	Females, incidence of hepatocellular adenomas	Males, incidence of renal tubular cell adenocarcinomas
0	8/48	2/48	0/49
500	n/a	n/a	0/49
1000	13/49	8/49	3/49

[a] From NTP (1983).
[b] Epichlorohydrin-free TCE stabilized with 8 ppm diisopropylamine by gavage, 5 days per week for 103–107 weeks.
[c] $n = 50$ per sex per dose.

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 (Table 2). The incidence of testicular interstitial cell tumours was also increased in the male Marshall rats (Table 2). 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.

Table 2: Carcinogenicity assay in rodents administered TCE by gavage[a,b]

Dose (mg/kg bw per day)	Incidence[c] of kidney tumours in Osborne-Mendel rats (male)	Incidence[c] of testicular interstitial cell tumours in Marshall rats (male)
0 (untreated control)	0/46	16/46
0 (vehicle control)	0/47	17/46
500	6/44	21/33
1000	2/33	32/39

[a] From NTP (1988).
[b] Epichlorohydrin-free TCE administered in corn oil by gavage, 5 days per week for 103 weeks.
[c] $n = 50$ per sex per dose, adjusted for survival.

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 carcinoma and adenomas ($p < 0.05$) in female mice (Table 3). 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 and were considered toxicologically significant due to the rarity of kidney tumours in the rats (Table 3).

Table 3: Carcinogenicity assay in rodents administered TCE by gavage[a,b]

Dose (mg/kg bw per day)	Incidence[c] of combined hepatocellular carcinomas and adenomas in B6C3F1 mice		Incidence[c] of tubular cell adenomas and adenocarcinomas in male F344/N rats
	Male	Female	
0 (untreated control)	14/48	6/48	0/48
0 (vehicle control)	n/a	n/a	0/46
500	n/a	n/a	2/46
1000	39/50	22/49	3/33

[a] From NTP (1990).
[b] Epichlorohydrin-free TCE administered in corn oil by gavage, 5 days per week for 103–107 weeks.
[c] $n = 50$ per sex per dose, adjusted for survival.

In a long-term carcinogenicity study by the inhalation route (Maltoni *et al.*, 1986), the increased incidence of renal tubular adenocarcinomas in male rats was statistically significant ($p < 0.05$) when adjusted for survival (U.S. EPA, 2001a) (Table 4). The authors noted a lack of statistical significance, but 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).

Table 4: Carcinogenicity assay in Sprague-Dawley rats administered TCE by inhalation[a,b]

Dose (mg/m^3)	Incidence[c] of renal tubular adenocarcinomas in male rats
0	0/120
112.5	0/118
337.5	0/116
675	4/122

[a] From Maltoni *et al.* (1986).
[b] Administered for 7 hours per day, 5 days per week, for 104 weeks.
[c] n = 120 per sex per dose, adjusted for survival.

In summary, 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.

8.2.4 Mutagenicity/Genotoxicity

A range of assays, covering a wide spectrum of genetic endpoints, 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 endpoints measured by these assays include forward and reverse mutation, 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 for 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 for 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 and Carere, 1989; Fahrig *et al.*, 1995). TCE was also observed to induce increased DNA synthesis and mitosis in mouse liver *in vivo* (Dees and 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 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 and 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.

In summary, 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.

8.2.5 Reproductive and Developmental Toxicity

The reproductive, embryo-fetotoxic and teratogenic effects of TCE have been studied in several species (Smith *et al.*, 1989; Dawson *et al.*, 1990, 1993; Johnson *et al.*, 1998a,b). In an inhalation reproductive toxicity study, Long-Evans rats were exposed to TCE at 1800 ppmv for 6 hours per day, 5 days per week, for 12 weeks before mating; for 6 hours per day, 7 days per week, during pregnancy through gestation day 21; or for 6 hours per day, 5 days per week, for 2 weeks before mating and for 6 hours 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 bw 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 generations, 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 bw 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 by inhalation to 300 ppm TCE for 7 hours 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 (300 ppmv), 7 hours 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 100 ppmv TCE for 4 hours 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 ppm (equal to 0, 0.18 or 132 mg/kg bw 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 bw per day) in dams exposed before and during pregnancy and only at the high (132 mg/kg bw per day) dose (10.4% vs. 3% in controls) in animals exposed only during pregnancy. The LOAEL was set at 0.18 mg/kg bw 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 bw 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 bw 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 ppm, or 129 mg/kg bw 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 Evan 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 number of litters with abnormal hearts ranged from 0 to 66.7%, while 16.4% of control litters had abnormal hearts (Table 5). Although this study appears to suggest the presence of a dose–response, with the effects beginning to manifest at a dose of 250 µg/L (0.048 mg/kg bw per day) and a NOAEL at 2.5 µg/L (0.00045 mg/kg bw per day), the dose–response is not as clear as might first appear on closer examination of the data.

Table 5: Incidence of heart malformations in the litters of Sprague-Dawley rats exposed to TCE for the duration of pregnancy[a]

TCE concentration in drinking water (µg/L)	TCE dose (mg/kg bw per day)	% rat litters with abnormal hearts	% abnormal hearts
0[b]	0[b]	16.4	2.2
2.5	0.00045	0	0
250	0.048	44.4	4.5
1500	0.218	38.5	5
1 100 000	129	66.7	10.5

[a] From Johnson *et al.* (2003).
[b] Distilled water was used in the control.

While the study authors' conclusion that their data support the cardiac teratogenicity of TCE seem quite reasonable, their assertion that the threshold is below 250 µg/L 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 endpoint, which results from very short term (acute) exposure, deserves close scrutiny and is chosen as the critical endpoint on the basis of the currently available data.

8.2.6 Mode 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 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 (PP) in the liver by TCE metabolites. Although PP has been correlated with carcinogenesis, the actual mechanism of carcinogenesis as it relates to PP is unknown (Bull, 2000). PP is more substantial in mice than in rats (Bogen and Gold, 1997). The prevailing view of TCE-induced mouse liver carcinogenesis has been that these tumours arise in parallel with PP in the liver by TCE metabolites (Elcombe, 1985; Elcombe *et al.*, 1985; Goldsworthy and Popp, 1987; Melnick *et al.*, 1987; DeAngelo *et al.*, 1989; Cattley *et al.*, 1998). However, the role of PP has been questioned as a mechanism of action for human liver carcinogenesis. As PP 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, is 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 PP 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 PP 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 and 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 plays 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 and 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 100 ppm chloral in air 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, PP and altered cell signalling; genotoxic processes,

such as the production of genotoxic metabolites, including 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 effects of endocrine disruption in human populations exposed to TCE are an area for more 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 and 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 ppm) 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.

9.0 Classification and Assessment

9.1 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 endpoints 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, some of the TCE metabolites excreted by tumour-bearing animals are similar to metabolites excreted by humans with similar cancers (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, TCE can be classified in Group II (probably carcinogenic to humans). This categorization has been confirmed by the International Agency for Research on Cancer (IARC, 1995), which now lists 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) method 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 allometric scaling factor was applied to the final unit risks, assuming a rat weighs 0.35 kg and a human weighs 70 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 8.11×10^{-4} $(\text{mg/kg bw per day})^{-1}$ in males and 5.82×10^{-4} $(\text{mg/kg bw per day})^{-1}$ in females, while the unit risks for renal tubular adenocarcinomas in rats following inhalation exposure for 104 weeks (Maltoni *et al.*, 1986) were 1.20×10^{-4} $(\text{mg/m}^3)^{-1}$ in males and 8.1×10^{-5} $(\text{mg/m}^3)^{-1}$ in females. The unit risk value of 8.11×10^{-4} $(\text{mg/kg bw per day})^{-1}$ 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, assuming a "de minimus" (essentially negligible) cancer risk level of 10^{-6}, the maximum acceptable concentration (MAC) for TCE in drinking water can be calculated as follows:

$$\text{MAC} = \frac{70\ \text{kg} \times 10^{-6}}{8.11 \times 10^{-4}\ (\text{mg/kg bw per day})^{-1} \times 4.0\ \text{Leq/day}} \approx 0.022\ \text{mg/L}\ (22\ \mu\text{g/L})$$

where:

- 70 kg is the average body weight of an adult
- 10^{-6} is the de minimis level of theoretical lifetime excess individual cancer risk

- 8.11×10^{-4} (mg/kg bw per day)$^{-1}$ is the unit risk calculated using the LMS model[***]
- 4.0 Leq/day is the daily volume of water consumed by an adult, accounting for multi-route exposure (see "Exposure" section).

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 guideline values, which were then compared with the value obtained using the reproductive-developmental endpoint below. Overall, even with the use of the probably more conservative LMS method, the guideline values based on carcinogenicity were above that determined for the reproductive-developmental endpoint.

9.2 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 endpoint (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) endpoint. 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; U.S. EPA, 1995) due to its many advantages over the NOAEL/LOAEL/uncertainty factor methodology. For example, the

[***] The potency estimates were converted to human equivalence (in (mg/kg bw per day)$^{-1}$) using an allometric scaling factor $(0.35/70)^{1/4}$ for scaling from rat to adult 70-kg human.

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 and Das, 1996; Clewell *et al.*, 2000). A lower confidence limit of the benchmark dose (BMDL) has been suggested as an appropriate replacement of the NOAEL (Crump, 1984; Barton and 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 and 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 mg/L and 1100 mg/L (0, 0.18 and 132 mg/kg bw 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 bw 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 and 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 bw per day was chosen to best represent the NOAEL.

The TDI for TCE can be calculated as follows:

$$\text{TDI} = \frac{0.146 \text{ mg/kg bw per day}}{100} = 0.00146 \text{ mg/kg bw per day (1.46 µg/kg bw per day)}$$

where:

- 0.146 mg/kg bw 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, the MAC can be calculated as follows:

$$\text{MAC} = \frac{0.00146 \text{ mg/kg bw per day} \times 70 \text{ kg} \times 0.2}{4.0 \text{ Leq/day}} = 0.00511 \text{ mg/L } (5.11 \text{ µg/L})$$

where:

- 0.00146 mg/kg bw per day is the TDI, as derived above
- 70 kg is the average body weight of an adult
- 0.2 is the default allocation factor for drinking water
- 4.0 Leq/day is the daily volume of water consumed by an adult, accounting for multi-route exposure (see "Exposure" section).

The alternative, more traditional approach would have been to derive a value using the concentrations in the 1993 Dawson *et al.* study (0, 1.5 and 1100 ppm) and converting them to doses which reflect actual consumptions (Tardif, 2004). Using the reported quantities ingested (TCE in µL/day), an average body weight of 306 g per rat (average weight gain of 112 g during treatment) and the TCE density of 1.44 g/ml, the concentrations of 0, 1.5 and 1100 ppm would then correspond to doses of 0, 1.18 and 70 mg/kg bw per day, respectively. Using a LOAEL of 1.18 mg/kg day, and an appropriate uncertainty factor of 1000, would yield a value of 4.13 µg/L. The use of this approach would provide a value that is consistent with the recommended MAC of 5 µg/L.

10.0 Rationale

Both cancer and non-cancer endpoints were considered in the derivation of the MAC of 0.005 mg/L (5 µg/L) for TCE in drinking water.

Animal studies have shown links between TCE exposure and various types of tumours in both rats (kidney and testicular) and mice (pulmonary and liver). Evidence for carcinogenicity resulting from the ingestion of TCE through drinking water is further supported by epidemiological studies that suggest a positive correlation between exposure to TCE and cancer in humans, although the presence of other chemicals was a confounding factor in confirming the association. However, further research is needed to better identify the specific agent(s) that confer this health risk and to estimate the magnitude of this risk. TCE has been classified as "probably carcinogenic to humans," since the evidence of carcinogenicity observed in animal and epidemiological studies suggests a positive association between TCE exposure and cancer.

The cancer risk assessment for TCE is based on kidney tumours observed in male and female rats. Such tumours have also been observed in some epidemiological studies in occupationally exposed industrial workers. The LMS method was used to calculate unit risks for the kidney tumour types observed in rats. A MAC for TCE in drinking water of 0.022 mg/L (22 µg/L) can be derived based on the cancer risk assessment. This assessment assumes a "de minimis" cancer risk level of 10^{-6}, which is considered to be "essentially negligible."

Choice of the developmental toxicity study for the non-cancer risk assessment was based on several factors: 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 endpoint (heart malformations) and existing evidence for similar effects (e.g., cardiac anomalies) from epidemiological studies; and the observation of similar malformations resulting from exposure to metabolites of TCE. The BMD approach was used to estimate the NOAEL, which takes into account the LOAEL observed in the key study. A MAC for TCE in drinking water of 0.005 mg/L (5 µg/L) can be derived based on the developmental effect observed.

The lower of the two calculated MACs (0.005 mg/L) is selected as the guideline value, as it is protective for both cancer and non-cancer endpoints. The MAC is measurable by available analytical methods and achievable by both municipal-scale and residential-scale treatment technologies.

11.0 References

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Appendix A: List of Acronyms

ANSI	American National Standards Institute
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
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	N-acetyl-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
EPA	Environmental Protection Agency (USA)
GSH	glutathione
GST	glutathione-S-transferase
kg bw	kilogram body weight
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
Leq	litre equivalent
LMS	linearized multistage
LOAEL	lowest-observed-adverse-effect level
LOEL	lowest-observed-effect level
MAC	maximum acceptable concentration
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level

NSF	NSF International
OR	odds ratio
PBPK	physiologically based pharmacokinetic
PCE	perchloroethylene (tetrachloroethene)
PP	peroxisome proliferation
PPAR	peroxisome proliferator activated receptor
ppb	parts per billion
ppm	parts per million
ppmv	parts per million by volume
PQL	practical quantitation limit
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
VHL	von Hippel Landau
VOC	volatile organic compound