PUBLIC HEALTH GOALS FOR CHEMICALS IN DRINKING WATER

TRICHLOROETHYLENE

July 2009

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Public Health Goal for TRICHLOROETHYLENE In Drinking Water

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PREFACE

Drinking Water Public Health Goals

Pesticide and Environmental Toxicology Branch

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This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (Health and Safety Code, Section 116365) requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and adopt PHGs for contaminants in drinking water based exclusively on public health considerations. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances that may cause chronic disease shall be based solely on health effects and shall be set at levels that OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider potential adverse effects on members of subgroups that comprise a meaningful proportion of the population, including but not limited to infants, children, pregnant women, the elderly, and individuals with a history of serious illness.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. OEHHA shall consider additive effects of exposure to contaminants in media other than drinking water, including food and air, and the resulting body burden.
7. In risk assessments that involve infants and children, OEHHA shall specifically assess exposure patterns, special susceptibility, multiple contaminants with toxic mechanisms in common, and the interactions of such contaminants.

8. In cases of insufficient data for OEHHA to determine a level that creates no significant risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
9. In cases where scientific evidence demonstrates that a safe dose response threshold for a contaminant exists, then the PHG should be set at that threshold.
10. The PHG may be set at zero if necessary to satisfy the requirements listed above in items seven and eight.
11. PHGs adopted by OEHHA shall be reviewed at least once every five years and revised as necessary based on the availability of new scientific data.

PHGs are not regulatory requirements, but instead represent non-mandatory goals. Using the criteria described above, PHGs are developed for use by the California Department of Public Health (DPH) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Thus, PHGs are not developed as target levels for cleanup of ground or ambient surface water contamination, and may not be applicable for such purposes, given the regulatory mandates of other environmental programs.

Whereas PHGs are to be based solely on scientific and public health considerations, drinking water standards adopted by DPH are to consider economic factors and technical feasibility. Each primary drinking water standard adopted by DPH shall be set at a level that is as close as feasible to the corresponding PHG, with emphasis on the protection of public health. Each primary drinking standard adopted by DPH is required to be set at a level that is as close as feasible to the corresponding PHG, with emphasis on the protection of public health. MCLs established by DPH must be at least as stringent as the federal MCL, if one exists.

Additional information on PHGs can be obtained at the OEHHA Web site at www.oehha.ca.gov.

TABLE OF CONTENTS

PUBLIC HEALTH GOAL FOR TRICHLOROETHYLENE IN DRINKING WATER

SUMMARY

An updated Public Health Goal (PHG) of 1.7 parts per billion (ppb) is established for trichloroethylene (TCE) in drinking water, which is derived from the same studies as the existing PHG of 0.8 ppb, published in 1999. The public-health protective concentrations calculated in the previous PHG and in this document are based on the occurrence of hepatocellular carcinomas and adenocarcinomas in mice in three studies, in both sexes, by inhalation and oral routes of administration, and a linear dose-response approach. However, different, updated cancer models were employed in the current re-analysis. As in the previous PHG calculation, the cancer slope factor (CSF) was based on a geometric mean of four values using a pharmacokinetic dose metric of metabolized dose of TCE. The PHG was set at a level providing a *de minimis* theoretical lifetime excess individual cancer risk of one in one million (10^{-6}) through ingestion of tap water, plus an allowance for inhalation and dermal exposures to TCE via showering, flushing of toilets, and other typical household uses of tap water.

A health-protective value for noncancer toxicity of 1 part per million (ppm) was also calculated, based on the benchmark dose (BMD_{10}) for kidney nephropathy in an oral chronic study in rats and a 100-fold uncertainty factor. The current federal and California Maximum Contaminant Level (MCL) for TCE in drinking water is 0.005 mg/L (5 ppb), based on cancer risk.

In the current document, the Office of Environmental Health Hazard Assessment (OEHHA) completed an extensive review of the literature since the publication of the first PHG (OEHHA, 1999) and the pertinent findings are presented below. The animal and epidemiological studies include new data on the ototoxicity, neurotoxicity, reproductive and developmental toxicity, immunotoxicity, nephrotoxicity, and other toxicological effects associated with TCE exposure. Several reviews and risk assessments on TCE, published after the 1999 document was completed, are available. These reviews analyzed the toxicological and epidemiological data, and discussed issues associated with methodologies and confounding factors involved in determining a correlation between cancer and TCE exposure. Overall, each of the cancer studies contributes to the toxicity assessment of TCE. Although the epidemiological data alone do not demonstrate direct causation of cancer due to exposure to TCE and many of the studies have some confounding issues, the findings suggest a consistency across studies. These studies in conjunction with the animal data do provide evidence that TCE should be considered a “known human carcinogen.” The U.S. Environmental Protection Agency (U.S. EPA) has not completed its TCE reassessment, but has characterized the chemical as "highly likely to produce cancer in humans" (U.S. EPA, 2001). The International Agency for Research on Cancer (IARC) has designated TCE as Group 2A, probably carcinogenic to humans (IARC, 1995).

Upon review of the recent literature, no pertinent new toxicity data for TCE were found that appear to be more appropriate than that used to calculate the original PHG. However, the newer cancer models provided a lower CSF and thus a new public-health-protective concentration. Several issues (i.e., selection of appropriate dose metrics, role of peroxisome proliferator activation in rodent carcinogenesis, and use of TCE cancer epidemiology in the risk assessment) concerning the TCE risk assessment continue to be actively debated. OEHHA will continue to monitor developments in these areas.

INTRODUCTION

The purpose of this document is to review any new data regarding the toxicity of TCE that are relevant to estimation of a health-protective level in drinking water, and propose changes in the previous risk assessment, if warranted. This document is based on earlier OEHHA risk assessments for TCE in drinking water (DHS, 1988; OEHHA, 1999) and air (DHS, 1990a).

Trichloroethylene is a volatile organic compound (VOC) that has been extensively used as a metal degreaser, a solvent in adhesives, textile manufacturing, paint stripping, and dry cleaning, etc. Production in the United States was estimated as about 130,000 metric tons/year (ATSDR, 1997). There are currently two U.S. manufacturers of TCE with a combined capacity of 145,000 metric tons/year (ATSDR, 1997). Due to widespread use, TCE is a common environmental contaminant. The primary public health concern from chronic low-level exposures via contaminated drinking water is the cancer risk.

As described in previous PHG document (OEHHA, 1999), rodent cancer bioassays have shown that oral administration of TCE in corn oil leads to an increased incidence of hepatocellular carcinoma in $B6C3F_1$ mice (NCI, 1976; NTP, 1983, 1988, 1990; Maltoni *et al.*, 1986). In parallel studies, TCE did not induce hepatocellular carcinoma in Osborne-Mendel rats (NCI, 1976), Fischer 344 rats (NTP, 1983), and Sprague-Dawley rats (Maltoni *et al.*, 1988). Fukuda *et al.* (1983) reported lung tumors in $B6C3F_1$ mice after TCE inhalation exposure. The metabolism of TCE is thought to play a key role in its carcinogenic effects (U.S. EPA, 2005a,b,c; Buben and O'Flaherty, 1985). The TCE metabolites chloral hydrate (CH), trichloroacetic acid (TCA), and dichloroacetic acid (DCA) induced hepatocellular tumors in $B6C3F_1$ mice when administered in drinking water (Herrn-Freund *et al.*, 1987; Bull *et al.*, 1993; DeAngelo *et al.*, 1991; Daniel *et al.*, 1992; Pereira, 1996). Similar administration of TCA and DCA to rats did not result in liver tumors (Herrn-Freund *et al.*, 1987; Bull *et al.*, 1990; Daniel *et al.*, 1992). Other metabolites such as trichloroethanol (TCOH), its glucuronide (TCOG), and related metabolites may also play a role in the overall mode of action.

Epidemiological studies are suggestive of potential cancer risks at multiple sites, but are far from conclusive (U.S. EPA, 2001; Scott and Chiu, 2006).

CHEMICAL PROFILE

Chemical Identity, Properties, and Uses

Trichloroethylene (TCE) is a volatile, colorless, chlorinated hydrocarbon compound that has been widely used as a degreasing solvent. Its structure is shown below in Figure 1, and relevant properties are summarized in Table 1.

Figure 1. Trichloroethylene Structure

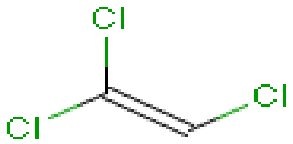


Table 1. Physical and Chemical Properties of Trichloroethylene[1]

Property	**Value**
CAS No.	79-01-6
Physical state	Liquid
Molecular weight	131.39
Melting point	-84.7 ^{o}C
Boiling point	87.2 ^{o}C
Density	1.464
Solubility in water	1.07 g/L at 20 °C
Solubility in organic solvents	Soluble in ethyl alcohol, chloroform, ethyl ether, benzene
Vapor Pressure	50 mm Hg at 20 °C
Henry's Law constant	0.011 atm-m^3/mol at 25 °C
Octanol-water partition coefficient (Log K_{ow})	2.61
Conversion factor:	1 ppm = 5.37 mg/m^3

[1] ATSDR (1997), HSDB (2006).

TCE is widely used as an industrial solvent, primarily for the vapor degreasing and cold cleaning of fabricated metal parts. It is also used in textile cleaning and solvent extraction processes. Of the total TCE used in the U.S. in 1982, 66 percent was used in

vapor degreasing, 22 percent for export, 7 percent as chemical intermediates, and 5 percent for miscellaneous uses (ATSDR, 1997).

Contaminants found in commercial TCE include epichlorohydrin, carbon tetrachloride, 1,2-dichloroethane, cis- and trans-1,2-dichloroethylene, pentachloroethane, 1,1,1,2- and 1,1,2,2-tetrachloroethane, 1,1,1- and 1,1,2-trichloroethane, 1,1-dichloroethylene, tetrachloroethylene, bromodichloromethane, bromodichloroethylene, chloroform , and benzene (Verschueren, 1983; IARC, 1995).

Degradation products, dichloroethylene (DCE) in the form of *cis*- and *trans*-l, 2-DCE, and relative degradation rates, half-life of approximately 1 to 1.5 yr, have been observed in several field studies (ATSDR, 1997). The extent of degradation varies with the amount and type of local microorganisms.

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

TCE released to the environment tends to partition to the atmosphere. It has been estimated that 60 percent to 90 percent of the annual world production of TCE is released to the environment (WHO, 1985). The environmental degradation of TCE primarily involves atmospheric photooxidation. The key properties of TCE that affect its movement in the environment are its high vapor pressure and relatively low solubility in water.

In surface waters TCE volatilizes rapidly into the atmosphere while degradation occurs slowly in groundwater by microbial action. Wind speed, agitation of the water, and water and air temperatures affect evaporation rates. Photodegradation and hydrolysis are slow decay processes and do not appear to be important in the overall removal of TCE (McNeill, 1979). The U.S. EPA (1985) summarized several studies of surface waters, in which the half-life of TCE in the Rhine River was 1 to 4 days compared to a half-life of 3 hr for rapidly moving shallow streams to 10 hr or longer for ponds or lakes. TCE concentrations in drinking water supplies from surface waters have been measured in 133 cities through federal surveys (U.S. EPA, 1985). Thirty-two percent of the treated drinking waters contained TCE concentrations ranging from 0.06 to 3.2 μg/L and averaging 0.47 μg/L. Thirty percent of all systems sampled contained a TCE level below 1 μg/L and the remaining 2 percent contained TCE concentrations between 1 and 4 μg/L.

TCE concentrations in groundwater have been measured extensively in California. Between 1984 and 2001, TCE was detected 859 times out of 15,447 water samples taken (DHS, 2005). Between 2005 and 2008, the MCL for TCE was exceeded in 133 sources and the PHG was exceeded in 323 sources (DPH, 2009). TCE has the most frequently exceeded California MCL for an organic chemical. The counties that most often had sources that exceeded the MCL were Los Angeles (201 sources), Fresno (15), San Bernardino (13), and Riverside (11).

METABOLISM AND PHARMACOKINETICS

Pertinent information is summarized below, including several studies published since the development of the previous PHG. In general, TCE is rapidly absorbed across the surface of the lungs and gastrointestinal tract. Although some TCE can be absorbed through the skin, the rate and extent of dermal absorption are limited, in contrast with more lipophilic xenobiotic compounds. Once absorbed, TCE distributes to all parts of the body and accumulates in fat and other tissues. The major routes of TCE elimination from the body are metabolism and exhalation of the parent chemical. Oxidation by the microsomal mixed-function oxidases (P450s) and conjugation with glutathione (GSH) by glutathione-S transferases are the primary metabolism pathways. For pharmacokinetics, several studies were found and are summarized below. In addition, several investigators describe modes of action (MOA) for TCE.

Absorption

Poet *et al.* (2000) evaluated the dermal absorption characteristics of TCE in rats and humans. The authors reported that rat skin was significantly more permeable than human skin following topical application of a patch containing TCE in water or soil (rats and humans) or immersion of hands in contaminated water. Estimates for permeability coefficient (K_P) in a water matrix were 0.31 +/- 0.01 cm/h and 0.015 +/- 0.003 cm/h in rats and humans, respectively. K_P estimates were more than three times higher from water than soil matrices in both species. K_P values calculated using the standard Fick's Law equation were strongly affected by exposure length and volatilization of TCE. In comparison, K_P values estimated using noninvasive real-time breath analysis coupled with the physiologically-based pharmacokinetic (PBPK) model were consistent, regardless of volatilization, exposure concentration, or duration.

The amount of TCE expired after inhalation and dermal shower exposures have been suggested to be nearly equivalent internal doses for these two exposure routes (Weisel and Jo, 1996). For the dermal shower exposure, the amount of TCE in expired air was 0.03 ± 0.011 μg and after the inhalation exposure it was 0.074 ± 0.08 μg in eleven subjects.

Distribution

In brief, TCE diffuses readily through blood and into body tissues. Studies with experimental animals have found TCE or its metabolites in blood, brain, testes, vas deferens, seminal vesicles, prostate, epididymis, adrenals, skeletal muscle, fat, liver, kidney, lungs, and heart of most species (Barrett *et al.*, 1939; Zenick *et al.*, 1984). The highest concentrations were measured in the adipose tissue and the lowest in the muscle. Blood and tissue levels were reported to increase in a dose-dependent manner (Pfaffenberger *et al.*, 1980; Zenick *et al.*, 1984). A combination of experimental and theoretical data indicates that TCE becomes widely distributed within the human body with bioaccumulation also occurring (Fernandez *et al.*, 1977; De Baere *et al.*, 1997).

Recently, Forkert *et al.* (2003) reported that TCE and its metabolites, including chloral, trichloroethanol (TCOH), trichloroacetic acid (TCA), and dichloroacetic acid (DCA) were present in seminal fluid and urine samples from eight mechanics diagnosed with clinical infertility and exposed to TCE occupationally. In addition, TCE and its metabolites were also observed in epididymis and testis of mice exposed to TCE (1,000 ppm) by inhalation for 1 to 4 weeks. Earlier, Forkert *et al.* (2002) found the epididymis of mice to metabolize TCE at higher levels than testis and that the cytochrome enzyme CYP2E1 was also present at higher levels in the epididymis of mice. Mice were treated with 1,000 ppm TCE, 6 hrs/day for 5 days a week for up to 19 days.

Metabolism

The postulated metabolism of TCE begins with the cytochrome P450-dependent mixed function oxygenases (MFO) transforming TCE to an intermediate electrophilic epoxide, trichloroethylene oxide (TCE-oxide) (Byington and Leibman, 1965; Bonse *et al.*, 1975; Henschler, 1977; ATSDR, 1997). The TCE-oxide then rearranges spontaneously to chloral that is rapidly hydrated to form chloral hydrate. Chloral hydrate undergoes oxidation to trichloroacetic acid (TCA), a short-lived intermediate metabolite, and some of the TCA contributes to the formation of dichloroacetic acid (DCA) and carbon dioxide (CO_2). Alternatively, chloral hydrate can be reduced to trichloroethanol (TCOH), which upon reaction with glucuronyl transferase forms trichloroethanol glucuronide (urochloralic acid, TCOG) (Barrett *et al.*, 1939; Powell, 1945,1947; Butler, 1948; Byington and Leibman, 1965; Nomiyama and Nomiyama, 1971; Muller *et al.*, 1972; Kimmerle and Eben, 1973; Cole et al., 1975; Hathaway, 1980; Ikeda *et al.*, 1980; Miller and Guengerich, 1982, 1983; Parchman and Magee, 1982; Stott *et al.*, 1982; Costa and Ivanetich, 1984; Dekant *et al.*, 1984; Green and Prout, 1985; Prout *et al.*, 1985).

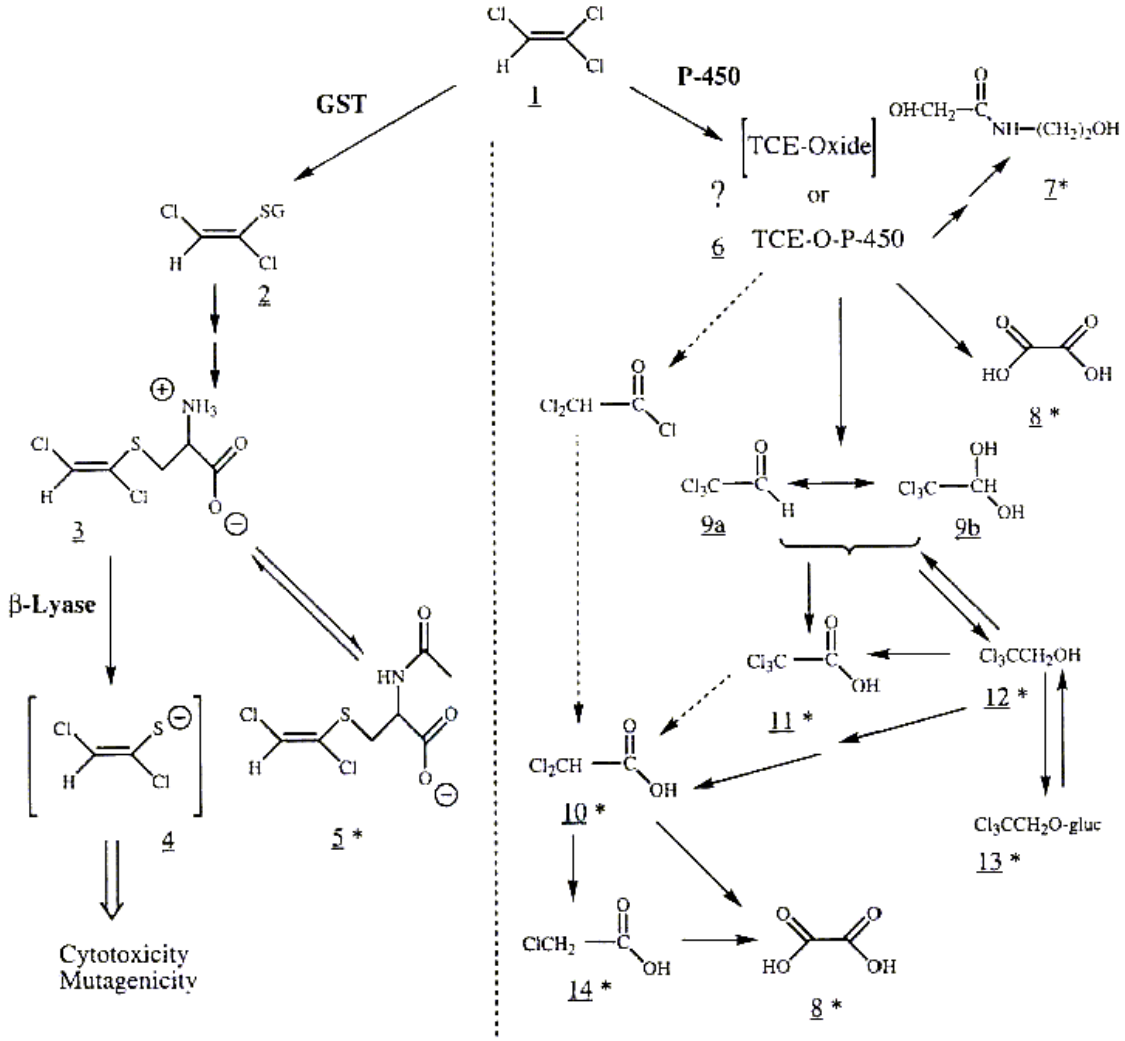
TCA, TCOH, and TCOG are the principal metabolites, but other minor metabolites have been reported. Under certain conditions, TCE-oxide forms dichloroacetyl chloride, then rearranges to DCA or can undergo oxidation and hydrolysis to form formic acid, glyoxylic acid, oxalic acid, CO_2, and carbon monoxide (Kline and Van Duuren, 1977; Hathaway, 1980; Dekant *et al.*, 1984). The other minor metabolites are monochloroacetic acid and *N*-(hydroxyacetyl)-aminoethanol (HAAE) (Soucek and Vlachova, 1960; Bartonicek, 1962; Ogata and Saeki, 1974; Bonse *et al.*, 1975; Traylor *et al.*, 1977; Fetz *et al.*, 1978; Hathaway, 1980; Miller and Guengerich, 1982, 1983; Dekant *et al.*, 1984; Green and Prout, 1985).

Glutathione (GSH) conjugates can be formed non-enzymatically or be catalyzed by cytosolic and microsomal GSH *S*-transferases. S-(1,2-dichlorovinyl)glutathione (DCVG) is formed and goes through further metabolism to yield various mercapturic acid isomers (*N*-Acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine (1,2-DCVC-Nac), and N-Acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine (2,2-DCVC-Nac)) (Birner *et al.*, 1993; Goeptar *et al.*, 1995).

Recently, Forkert *et al.* (2003) reported that TCE is metabolized in the epididymis of the mouse and monkey based on *in vitro* incubation studies with epididymal microsomes and presence of parent and metabolite *in vivo*. Cummings *et al.* (2000, 2001) also reported CYP2E1 involvement in the metabolism of TCE by proximal tubular cells along with

CYP2E1 metabolism in both the hepatic and renal microsomes. Of interest, Lash *et al.* (2000a) and U.S. EPA (2005a,b) provide a more comprehensive review on the metabolism of TCE. These articles reviewed the metabolic processes following exposure to TCE. Specifically, key metabolites, pathways, and organs involved in metabolism of TCE were identified. A postulated scheme for the pathways of TCE metabolism is shown in Figure 2, which is consistent with the most recent summary of TCE pharmacokinetics and metabolism (U.S. EPA, 2005a,b).

Figure 2. Major Trichloroethylene Metabolism Pathways (adapted from Lash *et al.* 2000a).



Metabolites marked with an asterisk are known urinary metabolites. Metabolites: 1 = TCE; 2 = DCVG; 3 = DCVC; 4 = 1,2-dichlorovinylthiol; 5 = NAcDCVC; 6 = TCE-P450 or TCE-oxide intermediate; 7 = *N*-(hydroxyacetyl)-aminoethanol; 8 = oxalic acid; 9a = chloral; 9b, chloral hydrate; 10 = dichloroacetic acid; 11 = trichloroacetic acid; 12 = trichloroethanol; 13 = trichloroethanol glucuronide; 14 = monochloroacetic acid.

In the U.S. EPA papers, key issues associated with the formation of DCA and GSH conjugates from TCE exposure are described (U.S. EPA, 2005a,b, and references within). Both metabolic processes are postulated to be involved in liver and kidney tumor induction. Highlighted in the U.S. EPA reviews were the uncertainties associated with formation of DCA and GSH conjugates. The major issue discussed was whether the present information on metabolism within and across species was sufficient to quantify rates of TCE metabolism and the factors that influence differential flux through the various metabolic pathways. For example, some researchers (e.g., Barton *et al.*, 1999) have suggested that DCA levels are too low in mice and humans after TCE exposure to play a significant role in TCE toxicity. Yet, other researchers (e.g., Bull *et al.*, 2002) have noted that DCA could not be detected *in vivo* in some DCA-induced toxicities. Similar tribulations are noted for the GSH pathway metabolites. Lash *et al.* (1999, 2001) reported the detection of DCVG that would lead to dichlorovinylcysteine (DCVC) and mercapturates in blood. However, Bloemen *et al*. (2001) was not able to detect DCVC in blood and the mercapturates were only sporadically detected in urine. Although these studies provide a better understanding of complex metabolic pathways, the current available information is limited for developing a firm quantitative understanding of relative rates of *in vivo* processing. Other issues addressed were the potential importance of the lung and male reproductive system in the formation of bioactive metabolites.

Other articles reviewed which evaluated metabolism of TCE in animals were De Smet *et al.* (2000) and Kumar *et al.* (2002).

In humans, Bloemen *et al.* (2001) evaluated the contribution of the mixed function oxidase and GSH metabolic pathways in the metabolism of TCE. The authors analyzed the urine collected from human volunteers exposed to TCE, using repeated 15 min exposures at 50 and 100 ppm (internal doses of 1.30 and 2.40 mmol of TCE, respectively) or from occupationally exposed workers [exposure levels of 0.4 and 21 ppm [8-h time-weighted average (TWA)]. None of the GSH metabolites were found in urine of volunteers or were below levels of detection in urine of workers. The authors concluded that GSH-mediated metabolism is of minor importance in humans exposed to TCE. In addition to the low human metabolic activity for the GSH pathway, Snawder and Lipscomb (2000) described an interindividual variance in cytochrome P450 forms in human hepatic microsomes. The authors re-affirm the role of CYP2E1 and CYP2B in the metabolism of TCE. CYP2E1 was strongly correlated with chloral hydrate formation and CYP2B displayed the strongest correlation with trichloroethanol formation.

Excretion

Data on the metabolism and excretion of TCE were summarized in an extensive review by U.S. EPA (1985). Animal inhalation studies have shown urinary excretion of the major metabolite of TCA, while unmetabolized TCE is excreted by exhalation through the lungs. In Wistar rats injected intravenously with 3 to 15 mg/kg of TCE, blood concentrations of TCE were found to decrease in blood and perirenal fat with half-life ($t_{1/2}$) values of about 4 min and 3.6 hr, respectively (Withey and Collins, 1980). TCE blood peak concentrations and half-life values were higher and longer in rats than in mice

treated (orally) with 1000 mg/kg or higher (Prout *et al.*, 1985). Peak blood concentrations were 1 hr and 3 hr for mice and rats, respectively, while the half-life values were 1 and 3 hr, respectively. The faster metabolism rate in the mouse resulted in higher levels of metabolite concentrations than in the rat. Prout *et al*. (1985) reported that higher concentrations of TCA were maintained for over 30 hours in rats compared to mice when treated with the same dose.

In humans, the primary metabolites of TCE, TCA, TCOH and TCOG, are excreted in urine for the most part. Kostrzewski *et al.* (1993) proposed a bi-exponential relation with $t_{1/2}$ values of 0.5 and 21.7 hr for the early (first few hours) and later phases of elimination, respectively. Excretion also occurs via exhalation. The kinetics for exhalation excretion of TCE parallel depletion of TCE concentration in blood after exposure cessation. In addition, a small amount of metabolized TCE is excreted in bile (Bartonicek, 1962) and in exhaled air as TCOH (Monster *et al.*, 1976) in humans.

Pharmacokinetics & PBPK Models

In general, PBPK modeling has been applied in many chemical risk assessments to correct for metabolic saturation of high bioassay doses, to derive improved internal dose metrics, and to improve inter-route and inter-species extrapolations. Several PBPK models for TCE have recently been published. The more recent models were developed to estimate target tissue doses in neurotoxicity; estimate target tissue doses for the principal animal tumors (liver, lung, and kidney) associated with TCE exposure; assess possible metabolic interactions within chemical mixtures; or further validate and describe TCE dynamics within specific compartments. The main focus of these models has been on the oxidative metabolic pathway and the major oxidative metabolites TCA, TCOH, and TCOG, which reflects the limited quantitative understanding for the other metabolic pathways, DCA and GSH conjugates (U.S. EPA, 2005a,b, and references within).

A recent article (Clewell and Andersen, 2004) applied the mouse tumor data for TCE to the current U.S. EPA guidelines as an example on how to conduct a carcinogen risk assessment using PBPK models. The authors reanalyzed the mouse data and determined the lowest points of departure (lower bound estimates of the exposure associated with 10 percent tumor incidence) for lifetime human exposure to TCE based on the mouse liver tumors. A mode of action primarily involving mitogenicity of the metabolite TCA was assumed. The authors reported linear unit risk estimates for mouse liver tumors as 1.5×10^{-6} for lifetime exposure to 1 μg TCE per cubic meter in air and 0.4×10^{-6} for lifetime exposure to 1 μg TCE per liter in drinking water. For these calculations, Clewell and Andersen did not consider species differences in carcinogenic effects of TCA in the liver between mice and man. When the species differences were considered, the authors used a margin-of-exposure (MOE) approach and recalculated a risk estimate. Applying an MOE of 1,000, the authors determined that environmental exposures below 66 μg TCE per cubic meter in air and 265 μg TCE per liter in drinking water were unlikely to present a carcinogenic hazard to human health.

Isaacs *et al.* (2004) found that TCE inhibited the enzyme parameters evaluated in a competitive manner as they attempted to determine the mechanism of metabolic

interactions occurring during simultaneous exposures to the organic solvents chloroform and TCE. Dobrev *et al.* (2001, 2002) also developed a model to predict the individual kinetics of TCE, perchloroethylene (PERC), and methylchloroform (MC) in humans exposed to different mixtures of the three solvents. The authors first calibrated the single-compound PBPK models using published data and described metabolic interactions within the chemical mixtures using kinetic constants estimated in rats. Model simulations indicated that, among these three chemicals, the inhibition was competitive. The authors then calculated interaction thresholds for binary and ternary mixtures of TCE, PERC, and MC by measuring two common biomarkers of exposure, peak TCE blood levels and total amount of TCE metabolites generated, in rats and humans. The authors reported that increases in the TCE blood levels, as the result of the competitive inhibition, led to higher availability of the parent compound for GSH conjugation. This simulated change in production rates of toxic conjugative metabolites exceeded 17 percent for a corresponding 10 percent increase in TCE blood concentration, assuming that a 10 percent change in the biomarkers corresponds to a significant health effect. Their conclusion indicated a nonlinear risk increase due to combined exposures because of the higher levels of parent compound in the blood as a result of the competitive inhibition in metabolism. Such an approach for mixtures was also reported by Haddad *et al.* (2000).

Lipscomb *et al.* (2003) reported that amounts of TCE oxidized in the liver by CYP2E1 differ by 2 percent or less under extreme values (5^{th} and 95^{th} percentiles) of CYP2E1 expression and activity, based on simulations of an 8-hour inhalation exposure to 50 ppm and an oral exposure to 5 μg TCE/L in 2 L drinking water. The authors conclude that differences in enzyme expression and TCE oxidation among the central 90 percent of the adult human population account for approximately two percent of the difference in production of the risk-relevant pharmacokinetic outcome for TCE-mediated liver injury.

Keys *et al.* (2003) expanded and validated tissue dosimetry of a rat PBPK model for TCE during and after inhalation exposure to 50 or 500 ppm TCE, following oral administration of 8 mg/kg TCE, or following intra-arterial injection of 8 mg/kg TCE. The expanded model (fat compartment modified to be diffusion-limited; a deep liver compartment added) did not significantly affect predictions of TCE concentrations in the liver, fat, or venous blood in rat. The additional deep liver compartment improved simulations in the mouse model. Albanese *et al.* (2002) also focused on the role of the fat compartment in the overall systemic disposition of TCE. The authors applied three different characteristics of TCE in fat: perfusion-limited and diffusion-limited compartmental models and an axial-dispersion type model for the adipose tissue. The latter model better captured key physiological heterogeneities of fat tissue, including widely varying fat cell sizes, lipid distribution, and blood flow properties.

Simmons *et al.* (2003) established a model to explore the relationship between measures of internal dose of TCE and neurotoxic outcome and that was specific to the Long Evans (LE) rat. The authors used five compartments (brain, fat, slowly perfused tissue, rapidly perfused viscera, and liver), determined partition coefficients for blood, fat, muscle, brain, and liver in LE rats, and used vapor uptake data from LE rats for estimation of V_{max}. As blood flow values for LE rats were not available, values from Sprague-Dawley (SD) and

Fischer-344 (F344) rats were used in separate simulations. The resulting values of V_{maxc} were used to simulate tissue (blood, liver, brain, fat) TCE concentrations, which were measured during (5, 20, 60 min) and after (60 min of TCE followed by 60 min of air) flow-through inhalation exposures of LE rats to 200, 2,000, or 4,000 ppm TCE. Earlier, Boyes *et al.* (2000) reported a good correlation of acute effects of TCE on behavior and visual function with estimated concentration values (AUC) of TCE in blood of LE rats. However, equal cumulative exposures, which were defined as same overall TCE exposure as in acute exposure but administered over a longer period of time, did not result in a good correlation. The authors concluded that the risk of acute effects would be overestimated when extrapolating from shorter to longer duration exposures, and underestimated when extrapolating from longer to shorter duration exposures.

Other articles reviewed that described development or refinement of PBPK models are those by Barton and Clewell (2000), Bois (2000a,b), Clewell *et al.* (2000), Fischer *et al.* (2000), Greenberg *et al.* (1999), and Lee *et al.* (2000a,b). The key point found within these and the articles above is that the model parameters have not yet been agreed upon. Issues such as species differences, metabolic pathways, large uncertainties about exposures, large interindividual variability, and mode of action, will be critical in the development of the most appropriate model.

The new studies and issues associated with TCE model development were recently summarized by U.S. EPA (2005a) and provided to the National Academy of Sciences for their guidance on developing an appropriate model for TCE. In the interim model, the U.S. EPA and the U.S. Air Force attempted to combine the common elements in the currently available TCE PBPK models (Bois 2000a,b; Clewell *et al.*, 2000; Fisher, 2000). The interim model results fit a variety of data evaluated for TCE and its major oxidative metabolites in a single model structure. This work provides an advancement in TCE PBPK modeling because the model was evaluated against a larger database of kinetic data than done before. However, the authors reported that a number of uncertainties remain as to model assumptions, structure, and parameters. For example, it is not clear whether differences noted in metabolism or enterohepatic recirculation after oral or inhalation exposure are due to inherent variability or to structural misspecification of the model. The validity of the assumption used to describe compartmental concentrations, that physiological compartments are well mixed over the time-scales of blood flow, is also not certain.

TOXICOLOGY

An extensive review of the literature was conducted. The summary below provides descriptions of earlier studies from the previous PHG (OEHHA, 1999) as well as the more recent studies which have been located. These are followed by our current conclusions on the toxicological endpoints listed below.

Toxicological Effects in Animals

Acute Toxicity

Boyes *et al.* (2000, 2003) evaluated electrophysiological parameters in acute inhalation studies with TCE in rats. Adult, male Long-Evans rats were exposed to trichloroethylene (TCE) vapor in a head-only exposure chamber using five exposure conditions designed to administer 0 ppm for 4 hr, 4,000 ppm-hr as 1,000 ppm for 4 hr, 2,000 ppm for 2 hr, 3,000 ppm for 1.3 hr, or 4,000 ppm for 1 hr (n = 9-10/concentration). Pattern onset/offset visual evoked potentials (VEPs) were recorded during exposure. The authors reported that the amplitude of the VEP frequency double component (F2) was decreased significantly by exposure and that the decrease was related to concentration but not to time or to the concentration x time product. A PBPK model was used to estimate the concentrations of TCE in the brain achieved during each exposure condition. The F2 amplitude of the VEP decreased monotonically as a function of the estimated peak brain concentration but was not related to the AUC of the brain TCE concentration. A linear form of Haber's rule did not accurately predict the effects of acute exposure to TCE, nor did an estimate of AUC of brain TCE. Haber's rule theorizes that similar effects should be obtained when the same dose of a chemical is given over varying time periods, i.e., that acute effects are related to concentration times time (in inhalation studies).

Shih *et al.* (2001) reported that large doses of TCE differentially alter the susceptibility to chemically induced convulsions in mice. Mice were pretreated with TCE (250-2000 mg/kg, i.p.) and then exposed to pentylenetetrazol (PTZ), picrotoxin (PIC), bicuculline (BIC), strychnine (STY), 4-aminopyridine (4-AP), or N-methyl-D-aspartate (NMDA). The pretreatment caused a significant increase in PTZ-, PIC-, BIC-induced convulsion thresholds and lethal doses compared to STY-, 4-AP-, and NMDA-induced convulsion thresholds and lethal doses. This is likely related to the direct sedative and anesthetic effect of TCE.

Ohta *et al.* (2001) reported that TCE affected long-term potentiation (LTP) in mice exposed to 300 mg/kg or 1000 mg/kg TCE for 24 hours. Slices of the hippocampi of treated mice had smaller post-per-pre ratio of the population spike, and optical signals and response area compared to control animal hippocampi. The authors suggest that the impairment of LTP is one of the mechanisms of the impairment of immediate memory after acute exposure to TCE in humans.

Subchronic Toxicity

Literature prior to 1999 shows that TCE was able to cause hepatic, renal, neurochemical, or hematological changes in various animal species exposed from 30 days to 6 months. Inhalation of 150 ppm TCE for 30 days caused statistically significant increases in liver weights of mice, rats, and gerbils, and statistically significant increases in kidney weights in gerbils (Kjellstrand *et al.*, 1981, 1983a,b). Statistical significant liver weight increases were also observed with inhalation of 75 ppm in male and female mice (Kjellstrand *et al.*, 1983b). In a 90-day inhalation study done by Prendergast *et al*. (1967), no effects were

reported in dogs, guinea pigs, rats, rabbits, and monkeys exposed to 189 mg/m^3 (35 ppm) TCE for 8 hr/d, 5 d/wk. However, all animals exposed to 3,815 mg/m^3 (710 ppm) had non-specific inflammatory changes of the lung; some rats and guinea pigs also developed lung congestion (Prendergast *et al*., 1967). Inhalation exposures of 400 ppm TCE to rats did not cause any changes in neurotransmitter levels following one month of treatment, while 800 ppm TCE did cause a statistically significant ($p < 0.05$) decrease in acetylcholine content of the striatum and in norepinephrine content of the cortex and hippocampus (Honma *et al*., 1980).

Oral administration of TCE also caused statistically significant increases in kidney and liver weights in mice and rats. Rats given 1,100 mg/kg of TCE by gavage for 5 days a week for 3 weeks caused statistically significant ($p < 0.01$) increases in liver weight and in hepatic DNA synthesis (Stott *et al.*, 1982). In mice, oral administration also caused statistically significant increases in liver weight ($p < 0.05$) following administration of 100 mg/kg-day or greater for 5 days a week for 3 weeks (Tucker *et al.*, 1982, Buben and O'Flaherty, 1985). Statistically significant increases ($p < 0.05$) in kidney weights were noted in mice that received 5.0 mg/mL TCE in drinking water for 4 to 6 months (Tucker *et al.*, 1982). Other liver effects reported with exposure to TCE are lipid peroxidation, hepatocellular proliferation, and enzyme induction (Channel *et al.*, 1998). In the study by Channel *et al.* (1998), male B6C3F$_1$ mice were dosed orally once daily 5 days/wk for 8 wk at 0, 400, 800, or 1,200 mg/kg-day TCE in corn oil. Effects (e.g., lipid peroxidation) were observed in all doses within 35 days of exposure, and cell and peroxisomal proliferation was observed during the same period in the 1,200 mg/kg-day group.

More recent inhalation studies have also found renal, hepatic, and neurological changes following TCE exposure. In a study by Kaneko *et al*. (2000), dose-response gross histological changes in spleen and liver were reported in MRL-lpr/lpr mice, which are genetically labile to autoimmune diseases, when dosed with 500 ppm TCE or higher for 4 hrs/day, 6 days/week for 8 weeks. Immunotoxicological effects, as indicated by measuring changes in serum antibody titers to B cells and T cell subsets, were also reported in these animals only with 1,000 ppm TCE (suppression of IgG production) and 2,000 ppm TCE (both T and B cell effects).

For the neurotoxicity effects, only a brief summary is provided here and a more detailed summary is provided in the Neurotoxicity Section below. TCE is a central nervous system (CNS) depressant, can cause behavioral changes, inhibited learning ability, result in hearing loss, and a decrease in wakefulness. Rats exhibited an inhibition in learning ability when exposed to 560 ppm TCE or greater for 4 hrs/day for 10 days (Goldberg *et al.*, 1964a) or 2,600 ppm TCE or higher for 30 min/day 6 days/week for 80 days (Battig and Grandjean, 1963). Rats exposed by inhalation to 50 ppm TCE or higher for 8 hrs/day 5 days/week for 6 weeks resulted in decreases in wakefulness indicative of CNS toxicity (Arito *et al*., 1994). Inhalation exposure to high concentrations of TCE has also been shown to damage hearing in the mid-frequency range in the rat (Crofton *et al*., 1994; Crofton and Zhao, 1997; Fechter *et al.*, 1998; Muijser *et al.*, 2000). Crofton *et al.* (1994) reported that 3,500 ppm TCE (N = 7-8 animals per group, 8 hr/day for 5 days) caused increased reflex modification audiometry thresholds for the mid-frequency tones (e.g., 8 and 16 kHz) in rats following 5 or 8 weeks of inhalation exposure. Crofton and Zhao

(1997) further observed that the hearing loss occurred only following high dose exposures (1 day = 4,000-8,000 ppm, 1 week = 1,000-4,000 ppm, 4 weeks = 800-3,200 ppm, and 13 weeks = 800-3,200 ppm), irrespective of exposure duration. The hearing loss in rats exposed to 4,000 ppm TCE (6 hr/day for 5 days) was due to loss of spiral ganglion cells in the middle turn, but not in the basal turn (Fechter *et al.*, 1998). Also, Muijser *et al*. (2000) reported that hearing loss in the animals exposed to the combination of TCE (3,000 ppm 5 days/week for 3 weeks) and noise (95 dB SPL) resulted in larger auditory threshold changes than that produced by either TCE alone or noise. More recently, Albee *et al*. (2006) reported that a no-observable-adverse-effect level (NOAEL) in their 13-week inhalation study in Fischer 344 rats was 800 ppm based on ototoxicity found at 2,500 ppm. The ototoxicity effects reported were mild frequency-specific hearing deficits and focal loss of hair cells in the upper basal turn of the cochlea. The authors found no other treatment-related lesions during the neurohistopathologic examination besides the cochlear damage.

Chronic Toxicity and Carcinogenicity

Previous reviews on the chronic effects of TCE in rodents have reported that TCE is a liver and renal toxicant through oral, inhalation, and dermal exposure (OEHHA, 1999; ATSDR, 1997). Several studies report carcinogenic effects. Other effects reported with chronic exposure to TCE are dermal toxicity, neurotoxicity, immunotoxicity. The latter effects are reported in more detail in their own sections below. Key studies were those of the National Cancer Institute (NCI, 1976), Henschler *et al.* (1980), Fukuda *et al.* (1983), Maltoni *et al*. (1986), and the National Toxicology Program (NTP, 1983, 1988).

The NCI (1976) study was the first major long-term cancer bioassay of TCE. TCE was administered by gavage 5 d/wk for 78 wk to groups of 50 $B6C3F_1$ mice and 50 Osborne-Mendel rats of both sexes, with 20 animals/group in the matched control groups. The industrial grade of TCE used contained 1,2-epoxybutane (0.19 percent) ethyl acetate (0.04 percent), epichlorohydrin (0.09 percent), N-methylpyrrole (0.02 percent), and diisobutylene (6.03 percent) as stabilizers. Following the dosing period, the animals were observed for 12 wk and sacrificed in the 90th (mice) or 110th (rats) wk. Investigators also reported the tumor incidence in groups of colony controls. Male mice received initial daily doses of 1,000 or 2,000 mg/kg of body weight; dose levels were increased during the course of the study resulting in time-weighted average (TWA) doses of 1,169 or 2,339 mg/kg. Initial doses to female mice were 700 or 1,400 mg/kg, and the corresponding TWA doses were 869 or 1,739 mg/kg. Dose levels to rats were lowered during the study because of poor survival and decreasing body weights. Initial doses to both sexes were 650 or 1,300 mg/kg, and TWA doses were 549 or 1,097 mg/kg.

The incidences of hepatocellular carcinoma in female mice were 0/20, 4/50, and 11/50 ($p < 0.01$) for control, mid and high dose respectively. For male mice the incidences were 1/20, 26/50 ($p < 0.001$), and 30/50 ($p < 0.001$), respectively. Tests for linear trend on age-adjusted data were highly significant for hepatocellular carcinoma in males ($p < 0.001$) and females ($p < 0.002$). Metastases of the liver cancer to the lung were observed in four low-dose and three high-dose males. The first hepatocellular carcinoma was

observed among the high-dose males at 27 wk and among the low-dose males at 81 wk. In contrast, tumor incidences in rats showed no significant difference in specific or total tumors between treated and control groups. High-dose male rats exhibited significantly ($p = 0.001$) decreased survival relative to that of controls.

Questions were raised about the possible impact of the epichlorohydrin impurity in the TCE used in these bioassays. This contaminant may have directly contributed to tumor induction because it is a direct-acting, mutagenic alkylating agent (Kucerova *et al.*, 1977; Bridges, 1978; Laskin *et al.*, 1980; Konishi *et al*., 1980; Kawabata, 1981). However, the estimated doses of epichlorohydrin in the NCI study are much less than those observed to cause cancer in other studies, and the tumor sites are different.

Henschler *et al.* (1980) exposed three species of rodents (Han:NMRI mice, Han:WIST rats, and Syrian hamsters) to concentrations of pure TCE at 100 and 500 ppm for 6 hr/d, 5 d/wk, for 78 wk. Surviving mice and hamsters were sacrificed at the 130th week, and rats at the 156th week. Neither rats, hamsters nor male mice were observed to have significantly increased tumor incidence. Dosed female mice, however, exhibited significantly ($p < 0.05$) higher incidences of malignant lymphoma relative to the controls (100 ppm, 17/30; 500 ppm, 18/28; controls, 9/29). The time-to-tumor occurrence also decreased in a dose-related fashion. Henschler *et al.* (1980) cited three studies that describe a high spontaneous incidence of malignant lymphoma in female NMRI mice. The authors referenced several studies that attribute the development of murine lymphoma to immunosuppressive agents that allow lymphoma induction by specific in-born viruses. In its review of this study, the U.S. EPA also suggests that immunosuppression by TCE or some other nonspecific agent provides a possible interpretation of the positive results of this study (U.S. EPA, 1985a).

In a study by Fukuda *et al.* (1983), female Sprague-Dawley rats and female ICR mice were exposed by inhalation to concentrations of 50, 150, or 450 ppm of reagent grade TCE for 7 hr/d, 5 d/wk, for 104 weeks. The surviving animals were killed in the 107th week. Animals were 7 weeks old when placed on the study. Size of the test groups varied between 49 and 51. The test sample contained TCE (99.824 percent), carbon tetrachloride (0.128 percent), benzene (0.019 percent), epichlorohydrin (0.019 percent), and l,l,2-trichloroethane (0.010 percent) in the vapor phase. The incidence of lung adenocarcinomas among mice in the two higher exposure groups was significantly greater ($p < 0.05$) (150 ppm, 8/50; 450 ppm, 7/46) than that in the low dose (3/50) or controls (1/49). The incidence of total lung tumors (adenomas and adenocarcinomas combined) in exposed mice was not significantly different (6/50, 13/50, 11/46) from that of the controls (6/49). Statistical analysis of the tumor incidences among rats showed no significant increases or trends.

In order to clarify the question of contaminant effects in the 1976 NCI mouse study, NTP (1983) initiated a repeat series of carcinogenicity studies in B6C3Fl mice and F344/N rats. Dosing began when the animals were 8 weeks of age. The TCE contained no epichlorohydrin and was stabilized with 8 ppm diisopropylamine. Treated mice and high-dose rats received 1,000 mg/kg by gavage 5 d/wk; low-dose rats received 500 mg/kg 5 d/wk. The dosing period lasted for 103 wk, and survivors were killed within 4 wk after treatment. The incidences of renal tubular-cell adenocarcinoma in male rats dosed with

either 500 mg/kg (0/49) or 1,000 mg/kg (3/49) were not significantly different from controls (3/49). However, high-dose male rats that survived until the end of the experiment exhibited a statistically significant higher incidence (3/16) of renal tubular-cell adenocarcinoma than that of the controls (0/33) ($p = 0.028$) using the "Life Table" or "Incidental Tumor" tests referenced in NTP (1983). These kidney tumors are considered uncommon occurrences in F344/N rats. Only three of 748 (0.4 percent) male rats from historical vehicle gavage control groups have exhibited such tumors. The incidence of mesotheliomas of the peritoneum among the low dose rats (5/50, 10 percent) significantly ($p<0.05$) exceeded concurrent (1/50, 2 percent) and historical controls (16/752, 2.1 percent). However, toxic nephrosis was considered to make these results equivocal and "inadequate to evaluate the presence or absence of a carcinogenic response" of the rats to TCE (NTP, 1983).

A significantly higher incidence of hepatocellular carcinoma in dosed male mice (13/49, $p < 0.05$) relative to controls (8/48 and 2/48, respectively) confirmed the positive results of the 1976 NCI mouse study. Dosed female mice were also found to have a statistically significant ($p< 0.05$) increase in the incidence of hepatocellular adenomas (8/49) relative to that of controls (2/48) (NTP, 1983).

Henschler *et al.* (1984) tested different samples of TCE with or without epichlorohydrin and/or 1,2-epoxybutane for carcinogenicity in groups of 50 male or female ICR/Ha-Swiss mice. Treated animals received TCE with or without epoxides (males: 2,400 mg/kg; females: 1,800 mg/kg) by corn oil gavage 5 d/wk for 18 months. Dosing was interrupted during weeks 35 to 40, 65, and 69 to 78. All doses were reduced to half the initial amount after the 40th week. We calculated experimental TWA daily doses of 1,900 mg/kg for males and 1,400 mg/kg for females by dividing the sum of the product of the number of days dosed and the administered dose by the number of days dosed. After the dosing period (61 doses in 78 weeks), the mice were observed for 26 weeks and then sacrificed during the 104th week. Mice dosed with purified, amine-stabilized TCE did not exhibit a statistically significant increase in the incidence of any tumor type. The administration of TCE with 0.6 percent epichlorohydrin (equivalent to lifetime TWA epichlorohydrin doses of 8.1 and 6.0 mg/kg-day for males and females, respectively), or both 0.25 percent epichlorohydrin and 0.25 percent 1,2-epoxybutane was associated with a significant ($p<0.05$) increase in forestomach papillomas or carcinomas in both sexes. Incidence of these tumors in the control mice was 1/50 in both sexes versus 8/50 in males and 12/50 in females exposed to epichlorohydrin-stabilized TCE. The response in dosed females was the most significant ($p = 0.0002$, using an age-adjusted chi-squared test). The administration of TCE with 1,2-epoxybutane (0.8 percent) was associated with a significant ($p < 0.05$) increase in squamous cell carcinomas in males.

Maltoni *et al.* (1986) reported the results of a series of eight TCE carcinogenicity experiments performed between 1976 and 1983. This project employed nearly 4,000 mice and rats that were observed until spontaneous death. Inhalation was the primary route of administration. The statistically and biologically significant results of these bioassays (BT301, 302, 303, 304, 304-bis, 305, 306, and 306-bis) are summarized below.

BT301 was the only non-inhalation experiment in the project. TCE was administered by stomach tube to 30 male and female Sprague-Dawley rats/group at doses of 50 or 250

mg/kg, 4 to 5 d/wk, for 52 wks. Dosing began when the rats were 13 weeks old. The TCE was epoxide free and contained 50 ppm or less each of 1,2-dichloroethylene, chloroform, carbon tetrachloride and 1,1,2-trichloroethane. A dose-related higher frequency of leukemia was observed in treated males, but this increase was not statistically significant. Beginning exposure at 13 weeks would provide no indication of the carcinogenic potential in developing animals, and the exposure duration (39 weeks) was well below a lifetime.

Bioassays BT302 (Sprague-Dawley rats) and BT303 (Swiss mice) were even shorter-term inhalation studies. The animals were exposed to 100 or 600 ppm TCE for 7 hr/d 5 d/wk for 8 wks. Treated male mice exhibited an increase in the incidence of hepatomas over that of the controls, but the increase was not statistically significant at a 95 percent confidence level. No statistically significant effects were observed.

BT304 and BT304-bis were both similar long-term inhalation experiments whose results were combined and evaluated together. Sprague-Dawley rats were exposed to 100, 300, or 600 ppm TCE for 7 hr/d 5 d/wk for 104 wks. A statistically significant exposure-related increase in the incidence of testicular Leydig cell tumors was observed in treated rats: 31/130 (23.8 percent) at 600 ppm ($p < 0.01$), 30/130 (23.1 percent) at 300 ppm ($p < 0.01$), 16/130 (12.3 percent) at 100 ppm ($p < 0.05$), and 6/135 the (4.4 percent) in the control group.

In experiment BT305, Swiss mice (90/sex/dose group) were exposed to TCE at a concentration of 100, 300, or 600 ppm for 7 hr/d 5 d/wk for 78 wk. Males exposed to the two higher levels showed pulmonary tumors (27/90 at 600 ppm, $p < 0.01$; 23/90 at 300 ppm, $p < 0.05$) relative to that of the control group (11/90). The increased incidence of pulmonary tumors included a slight increase in adenomas and adenocarcinomas, but the statistical significance of the increase in pulmonary tumors was clearly due to an increase in the number of animals with adenomatous hyperplasia or early adenomas (i.e., borderline adenomas). Males exposed to 600 ppm TCE also had a higher frequency of hepatomas (13/90, $p < 0.05$) than that of controls (4/90). Females did not show any significant response to TCE exposure in this bioassay.

BT306 and BT306-bis were both conducted with $B6C3F_1$ mice (90/sex/dose group) under similar conditions. BT306-bis was added due to early high mortality in BT306 males. Animals were exposed to 100, 300, or 600 ppm TCE for 7 hr/d 5 d/wk for 78 wk. Total malignant tumors were increased in female mice at all three dose levels: 64.4 percent at 600 ppm ($p < 0.01$); 58.9 percent at 300 ppm ($p < 0.01$); 57.8 percent at 100 ppm ($p < 0.05$); and 46.7 percent in the controls. A dose-related increase in the incidence of pulmonary tumors (primarily adenomas) was observed in females, but was significant ($p < 0.01$) only at 600 ppm (14/87) relative to that in the control group (2/90). If males and females are considered together, a slight increase in the incidence of hepatomas was observed in treated animals, significant ($p < 0.01$) at 600 ppm (15/180; controls: 4/180). Males in BT306 (poor survival group) showed significant increases in hepatoma (1/59 control, 1/31, 3/38, 6/37, $p < 0.01$). Males in BT306-bis (normal survival) didn't show significant increases in hepatoma (17/77 control, 19/47, 27/67, 21/63). Combined males from these two groups showed significant differences in hepatoma at all dose levels by

Fisher's exact test and a significant Mantel-Haenszel trend test [18/136 control, 20/78 ($p = 0.02$), 30/105 ($p = 0.0027$), 27/100 ($p = 0.0066$), trend ($p = 0.0024$)].

In summary, Maltoni *et al.* (1986) reported statistically significant increases in pulmonary tumors (benign and malignant combined) and hepatomas (malignant) in treated mice and testicular tumors (benign and malignant combined) in treated rats. An increased incidence of renal tubular cell adenocarcinoma was also observed in treated rats. Although the incidence of this neoplasia was not statistically significant, Maltoni *et al*. (1986) considered the appearance of these tumors to be biologically significant because of their rarity in control animals (0/460).

In the NTP (1988) study, four strains of rat (ACI, August, Marshall and Osborne-Mendel) received 500 or 1,000 mg/kg daily doses of TCE in corn oil by gavage 5 d/wk for 103 wk. The TCE contained no epichlorohydrin. Test groups consisted of 50 animals of each sex. Male Osborne-Mendel rats exhibited a statistically significant higher incidence of renal cell adenomas (6/50) at the lower dose vs. controls (0/50) ($p = 0.007$). Male Marshall rats exhibited a statistically significant higher incidence of testicular interstitial cell tumors at the higher dose (32/48) vs. the controls (17/46) ($p = 0.002$). The incidence of these proliferative testicular lesions was also high in control groups of ACI rats (36/49), but ACI rats showed a (nonsignificant) decrease in incidence (23/49 in the low-dose rats, 17/49 in the high-dose rats). Therefore, the biological significance of the dose-related increase observed in this study is in question (DHS, 1990a; Bogen *et al.*, 1988).

Consistent negative dose-response trends were observed in the incidence of adrenal pheochromocytomas in male ACI, female Marshall, and male and female August and Osborne-Mendel rats in this study. Results of audits conducted in the Fall of 1983 and the Spring of 1984 (the in-life portion of the study was completed many years before the final report was issued) revealed that the documentation of animal breeding, animal identity, clinical observations, environmental conditions, and analytical chemistry data were inadequate to support any meaningful interpretation of the reported tumor incidence data (NTP, 1988).

NTP (1990) also conducted carcinogenesis bioassays of epichlorohydrin-free TCE by corn oil gavage in groups of 50 male and 50 female F344/N rats and $B6C3F_1$ mice. Dose levels were 0, 500 and 1,000 mg/kg for rats and 1,000 mg/kg for mice. TCE was administered five days/week for 103 weeks, and surviving animals were sacrificed between weeks 103 and 107. Groups of 50 male and 50 female rats were also used as untreated controls. Survival of treated animals was less than that of vehicle controls. Mean body weights of treated animals were also lower than control animals, except for female mice. Cytomegaly (toxic nephrosis) of the kidney was seen in 96/98 male and in 97/97 female rats given TCE, and in none of the vehicle controls. Cytomegaly was observed in 45/50 male mice and 48/49 female mice given TCE, and in none in the vehicle controls. Renal tubular cell adenocarcinomas were found in three high-dose male rats killed at the end of the study (0/33, 0/20, and 3/16, 19 percent, $p < 0.05$). Renal cell adenocarcinomas are considered uncommon in F344/N rats with a 0.4 percent historical incidence in vehicle gavage controls. Additional renal tumors in TCE-treated male rats included one transitional cell carcinoma of the renal pelvis at the high dose and two tubular cell adenomas in low dose and one carcinoma of the renal pelvis in a high dose

animal. No renal neoplasms were seen in vehicle control rats. In female rats, one tubular cell adenocarcinoma was found in the high dose group. The results in male rats were considered equivocal for detecting a carcinogenic response because both TCE-treated groups showed significantly reduced survival compared to the vehicle control (35/50, 70 percent; 20/50, 40 percent; 16/50, 32 percent) and because 20 percent of the animals in the high dose group were lost due to gavage dosing errors.

TCE administration to mice caused increased incidences of hepatocellular carcinomas in males (8/48 vs. 31/50, $p < 0.001$) and in females (2/48 vs. 13/49, $p < 0.005$). Hepatocellular carcinomas metastasized to the lungs in five dosed male mice and one control male; none was observed in female mice. The incidence of hepatocellular adenomas was increased in male mice (7/48 vs. 14/50) and in female mice (4/48 vs. 16/49, $p < 0.05$). Under the conditions of the studies, TCE caused renal tubular cell neoplasms in male F344/N rats, produced toxic nephrosis in both sexes, and shortened survival time of males. The significance of these findings is compromised by inadequate survival. No evidence of carcinogenicity was seen in female F344/N rats. TCE was carcinogenic for B6C3F1 mice, causing increased hepatocellular carcinomas in males and females and increased hepatocellular adenomas in females (NTP, 1990).

Since the OEHHA (1999) and Agency for Toxic Substances and Disease Registry (ATSDR) (1997) reviews, the hepatotoxicity, renal toxicity and ototoxicity have been reviewed by Kumar *et al.* (2001b) and Mensing *et al*. (2002).

Kumar *et al.* (2001b) reported similar findings in the liver of rats exposed through inhalation of TCE (376 ppm for 4 hr/d 5 d/wk) for 8, 12 and 24 weeks in a whole body inhalation chamber. The findings included increase in liver weight, appearance of necrotic lesion with fatty changes and marked necrosis, lysosomal rupture along with reduced GSH content and total increased sulfhydryl content in liver tissue. Mensing *et al.* (2002) evaluated the long term kidney effects of TCE in Long Evans rats following inhalation of 500 ppm TCE for 6 months, 6 hr/d 5 d/wk. The authors reported that TCE caused the release of biomarkers indicative of nephrotoxicity, with alterations preferably in the proximal tubules of the exposed rats.

Wang *et al.* (2002) coadministered TCE with chloroform, 1,1-dichloroethane, 1,1-dichloroethylene, 1,1,1-trichloroethane, and tetrachloroethylene to ICR male and female mice in drinking water for 16 and 18 months, respectively. Three doses of the mixture were administered. Marginal increases of liver and lung weights, blood urea nitrogen, and serum creatinine levels in male mice were observed in the mid and high dose groups. Increased liver, kidney, and uterus and ovary total weights were observed in the female high dose group without effects on serum biochemistry parameters. The administered mixtures had no effects on the total GSH content or the level of glutathione S-transferase activity in the livers and kidneys of male and female mice. In male mice, the mixture produced a trend of increasing frequency of hepatocellular neoplasms and induced a significantly higher incidence of mammary adenocarcinoma in female mice. The odds ratios for mammary adenocarcinoma in female mice induced by low-, medium-, and high-dose mixtures were 1.14, 1.37, and 3.53 versus the controls, respectively.

Genetic Toxicity

The genotoxicity of TCE or its metabolites has been evaluated in bacteria, fungi, yeast, plants, insects, rodents, and humans using many different assays. The genetic endpoints measured by these assays include: forward and reverse mutation, sister chromatid exchanges, gene conversion, chromosomal aberrations, micronuclei formation, and mitotic recombination. In a review of the early genotoxicity literature by U.S. EPA (1985), positive responses reported included reverse mutation and gene conversion in *Saccharomyces cerevisiae*, gene mutation in the plant *Tradescantia* sp., cell transformation in rat embryo cells *in vitro*, mouse spot test and micronucleus test *in vivo*, and unscheduled DNA synthesis (UDS) and sister chromatid exchange (SCE) in human lymphocytes *in vitro*. Fahrig *et al*. (1995) also reviewed the genetic toxicity of TCE and concluded that TCE has a very specific genotoxic activity, which is not typical of a genotoxic carcinogen. The authors found that TCE was weakly active *in vitro* in the micronucleus tests using mammalian cell cultures and induced *in vitro* and *in vivo* sister chromatid exchanges and aneuploidies while not being able to induce gene mutations or structural chromosomal aberrations (Fahrig *et al.*, 1995).

More recent *in vivo* studies have produced similar findings. Giver *et al.* (2001) reported that mouse aneuploid cells in the hematopoietic stem cells compartment increased four- to eight-fold in a dose- and schedule-independent manner six days after topical application of 7, 14, or 28 g/kg TCE (100, 200, or 400 μL). A similar induction of aneuploidies at high concentration only was reported earlier (Fahrig *et al.*, 1995). Giver *et al.* (2001) also found that aneuploid lymphoid and myeloid cells from TCE-treated mice were approximately the same as in controls. In 2002, Mensing *et al.* (2002) could not detect any DNA-strand breaks using the comet assay in kidney cells of Long Evans rats inhaling 500 ppm TCE for 6 months, 6 hr a day, 5 days a week. More recently, Robbiano *et al.* (2004) evaluated the ability of TCE to induce DNA fragmentation and formation of micronuclei in primary cultures of rat and human kidney cells, and in the kidney of intact rats, as measured by the Comet assay. The authors reported significant dose-dependent increases in the frequency of DNA single-strand breaks and alkali-labile sites as well as in micronuclei frequency in primary kidney cells from male rats and humans of both sexes. The concentration of TCE used in the *in vitro* studies was 1 to 4 mM. *In vivo* results included statistically significant increases in the average frequency of both DNA breaks and micronucleated cells in the kidney of rats given a single oral dose (1/2 LD_{50}) of TCE.

Developmental and Reproductive Toxicity

Coleman *et al.* (1999) evaluated the potential neurodevelopment changes in offspring of rats treated with TCE (7,000 ppm for 1 hr for 3 days during the last week of pregnancy). The authors reported longer gestation periods; smaller number of litters delivered; fewer live pups per litter; impaired ability to perform the inverted screen, negative geotaxis, and vertical screen tests; less forelimb grip strength; and reduced locomotor activity for the TCE treated group.

Johnson *et al.* (2003) reported a dose-related increased incidence of abnormal hearts in offspring of Sprague Dawley rats treated during pregnancy with 0, 2.5 ppb, 250 ppb, 1.5 ppm, and 1,100 ppm TCE in drinking water (0, 0.00045, 0.048, 0.218, and 128.52 mg/kg-day, respectively). The NOAEL for the Johnson study was reported to be 2.5 ppb (0.00045 mg/kg-day) in this short exposure (22 days) study. The percentage of abnormal hearts in the control group was 2.2 percent, and in the treated groups was 0 percent (low dose), 4.5 percent (mid dose 1), 5.0 percent (mid dose 2), and 10.5 percent (high dose). The number of litters with fetuses with abnormal hearts was 16.4 percent, 0 percent, 44 percent, 38 percent, and 67 percent for the control, low, mid 1, mid 2, and high dose, respectively. The reported NOAEL is separated by 100-fold from the next higher dose level. The data for this study were not used to calculate a public-health protective concentration since a meaningful or interpretable dose-response relationship was not observed. These results are also not consistent with earlier developmental and reproductive toxicological studies done outside this lab in mice, rats, and rabbits: The other studies did not find adverse effects on fertility or embryonic development, aside from those associated with maternal toxicity (Hardin *et al.*, 2004).

Mechanistically, Boyer *et al.* (2000) evaluated potential cardiac defects in an *in vitro* chick-AV canal culture model using concentrations of 50 to 250 ppm. The authors reported that TCE might cause cardiac valvular and septal malformations by inhibiting endothelial separation and early events of mesenchymal cell formation in the heart.

However, Fisher *et al.* (2001) did not report any increased incidence of abnormal fetal hearts in the offspring of female Sprague Dawley rats treated with TCE (500 mg/kg), TCA (300 mg/kg), or DCA (300 mg/kg) once per day on days 6 through 15 of gestation. Fetal hearts were first examined in situ by cardiovascular stereomicroscopy on gestation day (GD) 21. After this, a microscopic dissection and examination of the formalin-fixed heart was conducted. The authors reported a decrease in maternal weight gain during gestation (3 percent to 8 percent) and a decrease of 8 percent and 9 percent in fetal weights on GD 21 in the TCA and DCA treatment groups, respectively, compared to the water control group. No differences in the incidence of heart malformation (3 percent to 5 percent) were observed in fetuses from the TCE-, TCA-, and DCA-treated dams compared to control values (2.9 percent-water control and 6.5 percent-soybean control) on a per fetus or per litter basis.

Other recent studies evaluated the effect and metabolic characteristics of TCE in the male reproductive organ, following up on reports of sperm abnormalities and decreased fertility in humans exposed to TCE.

Kumar *et al.* (2000) reported that testosterone biosynthesis was impaired in rats treated with TCE by inhalation. They reported a significant decrease ($p < 0.05$) in total epididymal sperm count, sperm motility, specific activities of enzymes glucose 6-phosphate dehydrogenase and 17 beta hydroxy steroid dehydrogenase with a concomitant decrease in serum testosterone concentrations in TCE-treated rats. Kumar *et al.* (2001a) reported that male rats exposed to TCE by inhalation for 12 and 24 weeks had a significant reduction in absolute testicular weight, altered marker testicular enzyme activity associated with spermatogenesis and germ cell maturation, along with marked histopathological changes showing depletion of germs cells and spermatogenic arrest.

Forkert *et al.* (2002) found the epididymis of mice to metabolize TCE at higher levels than testis and that CYP2E1 was also present at higher levels in the epididymis of mice. Mice were treated with 1,000 ppm TCE, 6 hrs/day for 5 days a week for up to 19 days.

DuTeaux *et al.* (2003) investigated the effects of TCE in male rat sexual tissues. Using western blot analysis, they confirmed the observation of Forkert *et al.* (2002) of CYP2E1 in the epididymis of the rat and determined that rat epididymal microsomes could generate TCE metabolites. These findings suggest that TCE and its metabolites, being at the site of action, could lead to decreased fertilizing ability.

In 2004, DuTeaux *et al.* (2004) followed up these findings by showing that TCE administered to male rats at 0.2 percent or 0.4 percent in their drinking water for 14 days (doses of about 180 or 360 mg/kg-day) decreased the fertilizing capacity of their sperm. Immunochemical staining of sperm also revealed halos of oxidized proteins around the sperm head and midpiece. The authors postulate that this effect may explain the diminished fertilizing capacity of the sperm.

A diminished fertilizing capacity in animals treated with TCE was also reported by Xu *et al.* (2004). The authors found that exposure of male mice to TCE (1,000 ppm by inhalation 6 hr/day, 5 days/week up to 6 weeks) leads to impairment of sperm fertilizing ability *in vivo* and *in vitro*. *In vivo*, the percentages of eggs fertilized were significantly decreased after 2 and 6 weeks of TCE exposure and only slightly at 4 weeks. Xu *et al.* (2004) also showed that sperm-egg binding *in vitro* was significantly decreased when sperm were pretreated with chloral hydrate (0.1-10 μg/mL) and trichloroethanol (0.1-10 μg/mL). They interpreted the latter results as evidence that the decreased fertility effects of TCE were likely a result of the direct effects of these TCE metabolites on sperm.

Immunotoxicity

Earlier immunotoxicity studies evaluated the potential of TCE to affect humoral immunity, cell-mediated immunity, hepatic or splenic lymphocytic activity, or autoimmunity (Sanders *et al.*, 1982; Wright *et al.*, 1991; Kahn *et al.*, 1995). In male mice treated for 4 to 6 months, humoral immunity was inhibited with 2.5 mg/mL TCE or higher in drinking water while cell-mediated immunity and bone-marrow-stem cell colonization were inhibited with 0.1 mg/mL or higher (Sanders *et al.*, 1982). Wright *et al.* (1991) reported decreased splenocyte counts and inhibition of hepatic natural killer cell, natural cytotoxic cell activities in mice and rats given 10 and 5 mmol/kg TCE, respectively, i.p. for 3 days. Kahn *et al.* (1995) reported that TCE and its metabolite dichloroacetyl chloride (DCAC) induced or accelerated autoimmune responses (i.e., increased serum IgG levels) in female autoimmune prone mice (MRL +/+) when mice were injected (i.p.) with 10 mmol/kg of TCE or 0.2 mmol/kg DCAC for 6 weeks. Increases in serum IgG levels were also observed in Brown Norway rats administered TCE (500 mg/kg) 5 days a week for an 8-week period by oral gavage (NTP, 1997).

Gilbert *et al.* (1999) treated autoimmune disease-prone MRL +/+ mice with TCE in the drinking water (0, 2.5, and 5.0 mg/mL) for up to 22 weeks. At 4 weeks of treatment, TCE was found to promote the expansion of CD4+ T cells that expressed a

memory/activation phenotype (i.e., CD44hi CD45RBlo) and secreted high levels of IFN-gamma, but not IL-4. The authors conclude that TCE is capable of promoting autoimmunity in genetically predisposed individuals. In a follow-up study (Blossom *et al.*, 2004), the metabolites of TCE, trichloroacetaldehyde hydrate (TCAH) and TCA, were also found to cause immunoregulatory effects in MRL+/+ mice following administered of TCAH and TCA in the drinking water for 4 weeks. Both TCAH and TCA CD4+ T cells from -treated MRL+/+ mice, unlike CD4+ T cells from control mice, demonstrated functional and phenotypic signs of activation, as evidenced by increased IFN-gamma production in association with the increased percentage of CD62L(lo) CD4+ T cells.

Kaneko *et al.* (2000) also used MRL-lpr/lpr mice in order to study the immunoregulatory effect of TCE. Kaneko *et al.* (2000) exposed mice to TCE concentrations of 500, 1,000, or 2,000 ppm through inhalation 4 hr/day, 6 days/week, for 8 weeks. They reported that only IgG production capacity was suppressed; no changes were observed in T cell subsets at concentrations up to 1,000 ppm. At 2,000 ppm, changes were noted in both T and B cell functions. The spleen and liver showed a dose-response in morphological changes observable by light microscopy at all concentrations.

More recently, Peden *et al.* (2006) evaluated the immunotoxicity potential of TCE in young animals. Newborn $B6C3F_1$ mice were exposed to TCE via drinking water (0, 1,400, 14,000 ppb) from gestation day 0 to either 3 or 8 weeks of age. At 3 and 8 weeks of age, male mice had decreases in SRBC-specific IgM production with 1,400 ppb TCE or higher while female mice had decreases in SRBC-specific IgM production in animals treated with 1,400 ppb TCE at 8 weeks of age and with 14,000 ppb TCE at both ages. Other findings reported were decreased splenic B220+ cells at 3 weeks of age with 14,000 ppb TCE, increases in T-cell thymic subpopulations (CD4+, CD8+, CD4+/CD8+, and CD4-/CD8-) at 8 weeks of age, and delayed-typed hypersensitivity increases in females at both TCE levels and in males at the high dose only.

Neurotoxicity

Evidence from human and animal studies suggest that TCE induces a variety of adverse effects on the central nervous system (CNS). TCE is a CNS depressant, can result in nausea, headache, loss of appetite, weakness, dizziness, ataxia, and tremors under occupational settings, and cause irreversible nerve damage and death following acute exposure to high concentrations of TCE.

Bushnell and Oshiro (2000) reported that Long Evans rats inhaling TCE had a progressive attenuation of impairment of signal detection behavior across several weeks of intermittent exposure. The authors suggested that the animals developed tolerance. The authors published two more articles on this hypothesis (Oshiro *et al.*, 2001, 2004). These studies found that TCE exposure (1,600 or 2,400 ppm, 6 hr/day for 20 days) through inhalation caused a persistence of effects on sustained attention in adult male Long-Evans rats.

Waseem *et al.* (2001) exposed rats to 350, 700 and 1,400 ppm of TCE in drinking water for 90 days and to 376 ppm of TCE through inhalation for 4 hr/day, 5 days/week for 180

days. No significant effects on spontaneous locomotor activity or cognitive ability were reported following oral exposure, whereas inhalation exposure resulted in an increase in locomotor activity at day 30 but a decrease at day 60 of exposure. The time spent in ambulatory and stereotypic movements as well as the number of stereotypic movements were enhanced significantly only at day 30. The learning ability of the rats was not affected significantly up to day 180.

Nunes *et al.* (2001) evaluated the potential for a synergistic or additive neurotoxicity effect when lead carbonate (2,000 mg/kg for 16 days) and TCE (2,000 mg/kg for the last 7 days) are given concurrently to male rats. The authors reported that the toxicities of lead carbonate and TCE are "expressed only as though one toxicant was given," i.e., no additive or synergistic effects were noted.

More recently, Shafer *et al*. (2005) evaluated the potential of TCE (as well as toluene and perchloroethylene) to perturb voltage-sensitive calcium channels in neurons. TCE caused a reversible, concentration-dependent inhibition of the whole-cell calcium current (ICa) in nerve growth factor-differentiated pheochromocytoma (PC12) cells. Potency for inhibition (IC_{50}) for TCE was 1,525 μM. In addition to inhibiting whole-cell calcium current, TCE also caused changes in inactivation kinetics (i.e., from a single- to double-exponential function) with voltage activation/inactivation shifts. The authors conclude that the observed changes in the PC12 cells following *in vitro* exposure to TCE could contribute to the acute neurotoxicity of TCE.

Toxicological Effects in Humans

Acute Toxicity

Reports of acute toxicity resulting from accidental or occupational exposure have been reported as early as 1950 (Cotter, 1950; Kleinfeld and Tabershaw, 1954; Joron *et al.*, 1955; Gutch *et al.*, 1965; Secchi *et al.*, 1968; Baerg and Kimberg, 1970; U.S. EPA, 1985; World Health Organization, 1985; Bruning *et al.*, 1998; Baelum, 1999; Morimatsu *et al*., 2006). The toxicity associated with acute exposure to TCE consists of general effects (e.g., drowsiness, headache, vomiting, fever, chills, abdominal pain, general motor restlessness, loss of consciousness), cardiotoxicity (e.g., cardiac arrest, atrial and ventricular extrasystole, tachycardia, and ventricular fibrillation), hepatotoxicity (e.g., abnormal hepatic function, hyperglobulinemia, hypercalcemia hepatitis, jaundice, and hepatic necrosis), renal damage (e.g., abnormalities of the glomeruli and tubular degeneration, elevated blood urea nitrogen levels, and acute renal failure), and death. These effects have been observed following inhalation or ingestion of TCE. Although the effects have been described in some detail, the concentration of TCE and the duration of exposure that caused these effects are not clearly understood. Some investigators have attempted to establish tissue concentrations with pharmacokinetic modeling. Ford *et al*. (1995) gave model predictions of TCE exposure concentrations in the 500-10,000 ppm range with blood TCE values of 174 mg/L and brain TCE levels of 809 mg/kg. Perbellini

et al. (1991) and Ford *et al.* (1995) reported values of 3-110 mg/L and 2-270 mg/kg, respectively, in two fatality cases.

Subchronic Toxicity

Similar to the acute exposure, subchronic exposures to TCE can also result in liver and renal effects. McCunney (1988) reported various effects, including hepatic and renal toxicity, following TCE exposures varying from a few days to 18 months. The author reported that dermal exposure resulted in encephalopathy characterized by impaired short term memory and a sense of inebriation. Irritability and personality changes developed after low dose exposure in a few cases. Landrigan *et al.* (1987) also observed behavioral changes (i.e., drowsiness, dizziness, or mental confusion) in workers following a short-term occupational exposure to TCE. McCunney (1988) found 5 of 288 cases of industrial poisoning by TCE to result in hepatic toxicity. The incidence of hepatic toxicity increased when TCE was used as an anesthetic. Hepatitis has also been attributed to the inhalation of spot remover that contained 45 percent TCE. In addition to the liver and neurological effects associated with TCE exposure, renal impairment (McCunney, 1988) and systemic scleroderma have also been associated with occupational TCE exposures (Nakayama *et al.*, 1988; Yanez Diaz *et al.*, 1992).

Chronic Toxicity

Chronic effects noted in early studies include effects on the nervous system, liver, and kidneys as well as cardiovascular, immunological, and cancer (ATSDR, 1997). The major target organs for chronic toxic effects are the liver and to some extent, the kidney. Specific liver effects include liver enlargement and increases of serum levels of liver enzymes. The kidney effects include increased *N*-acetyl-β-D-glucosaminidase. The information on chronic human exposure to TCE comes from inhalation exposure in the workplace or chronic ingestion of contaminated water.

Since the OEHHA (1999) and ATSDR (1997) reviews, Anagnostopoulos *et al.* (2004) and Pantucharoensri *et al.* (2004) reported on three individuals, a shoemaker working in a poorly ventilated basement and two metal cleaners from a watch factory, who were diagnosed with hepatitis following long term exposure to TCE-containing products. In both articles, the disease appeared to result from poor working conditions, which led to high TCE exposures. One individual died from liver failure two weeks after the first symptom appearance. The other two individuals recovered.

Long-term exposure to TCE can also lead to dermatological conditions. Rascu *et al.* (2003) and Goon *et al.* (2001) found that TCE caused dermal sensitization in humans. In the report presented by Rascu *et al.* (2003), cutaneous effects were first seen and then respiratory (obstructive respiratory syndrome) changes occurred.

Sulkowski *et al.* (2002) reported that industrial solvents like TCE caused a higher incidence (47 percent vs. 5 percent) of hearing and vestibular disorders in 61 workers exposed to a mixture of organic solvents at a paint and varnish production facility, compared to a control group (40 age-matched non-exposed subjects).

Green *et al.* (2004) investigated the renal toxicity potential of TCE in a currently exposed population; the average duration of exposure was 4.1 years with a range of 1 to 20 years for the 70 workers in the study. The authors measured urinary metabolites of TCE, nephrotoxicity markers (e.g., N-acetylglucosaminidase and albumin), and the mode of action marker, formic acid. Although significant differences were observed in marker levels between exposed and control populations, there was no correlation between marker level and magnitude or duration of exposure to TCE.

Genetic Toxicity

The potential for TCE-induced genetic toxicity in humans remains largely inconclusive. Several studies of sister chromatid exchange (SCE) tests in peripheral lymphocyte cultures from exposed workers show no or only minor effects on SCE frequencies (Konietzko *et al.*, 1978; Nagaya *et al.*, 1989; Brandom *et al*., 1990; Seiji *et al*., 1990; Gu *et al*., 1981a,b cited in Fahrig *et al.*, 1995). However, Robbiano *et al*. (2004) reported that five of six chemicals known to induce kidney tumors gave significant dose-dependent increases in the frequency of DNA single-strand breaks and in micronuclei frequency in primary kidney cells from both male rats and humans of both genders with subtoxic concentrations of the test chemicals, including TCE. *In vivo* results included statistically significant increases in the average frequency of both DNA breaks and micronucleated cells in the kidney of rats given a single oral dose (1/2 LD_{50}) of TCE. These results provide evidence that TCE may be a genotoxicant.

Moore and Harrington-Brock (2000) and Harth *et al.* (2005) evaluated the potential of TCE to induce tumors in humans via a mutagenic mode of action. In the review by Moore and Harrington-Brock (2004), the authors used a weight of the evidence approach to suggest that definitive conclusions as to whether TCE will induce tumors in humans via a mutagenic mode of action could not be made based on the available information. Harth *et al.* (2005) went one step further with the use of a weight of evidence approach. Harth *et al.* (2005) concluded, based on the findings of experimental, mechanistic, and epidemiologic studies, that TCE can be considered a human carcinogen; specifically, a carcinogen with a threshold in which no relevant carcinogenic effect is to be expected at doses below the threshold. Harth *et al*. (2005) report that in the studies reviewed, low TCE exposure (not higher than the current Occupational Exposure Limits) is not associated with an increased risk of malignancy, but the risk of renal cell cancer is significantly elevated in persons reporting long-term (several years) and high (with prenarcotic episodes) occupational exposure to TCE.

Developmental and Reproductive Toxicity

A survey of 80,938 live births and 594 fetal deaths in an area of New Jersey with TCE-contaminated public drinking water (mean concentration of 55 ppb) found an association between TCE concentrations >10 ppb and oral clefts, CNS defects, neural tube defects, and major cardiac defects (Bove *et al.*, 1995).

Chia *et al.* (1996) evaluated semen parameters of workers exposed to TCE. Semen volume and sperm density, viability, motility, and morphology were analyzed. TCA was

assayed in urine. Personal monitoring of 12 subjects showed a mean air TCE concentration of 29.6 ppm (range 9-26 ppm plus one individual at 131 ppm). The mean urine TCA was 22.4 mg/g creatinine (range 0.8-136.4). No differences were observed between "high"- and "low"-exposure groups in volume, motility, and morphology of sperm. One of the sperm parameters to show a significant difference between low (<25 mg/g urine TCA) and high exposure subjects was sperm density, at 56.9 $x10^6$/mL for the "high" group vs. 63.6 $x10^6$/mL for the "low" group ($p = 0.044$). The authors also report an apparent dose-response relation between TCA in urine and hyperzoospermia or sperm densities >120 $x10^6$/mL. The prevalence ratios vs. the low exposure group were: 50 to <75, 2.36 (0.92-6.07); 75 to <100, 3.00 (1.00-9.02); $\geq$100 mg TCA/g creatinine, 3.58 (1.09-11.80). Hyperzoospermia has been implicated in infertility but no additional information has been reported about TCE and hyperzoospermia.

Rodenbeck *et al.* (2000) reported a non-statistically significant association between maternal exposure to TCE via drinking water and very-low-birth-weight babies (i.e., < 1,501 grams) (odds ratio (OR) = 3.3; 95 percent confidence interval (CI) = 0.5-20.6; and Wald chi-square p value = 0.2), based on data obtained from an environmental dose-reconstruction study and the Arizona Birth Information Tapes. However, no association was found between maternal exposure to TCE via drinking water and low birth weight or full-term low-birth-weight infants (gestational period > 35 wk and < 46 wk).

Lorente *et al.* (2000) report an association between occupational exposures of mothers who worked during the first trimester of pregnancy to TCE and orofacial clefts (OR 6.7, 95 percent CI 0.9-49.7). The total number of participants in the study was 851, including 100 mothers of babies with oral clefts and 751 mothers of healthy referents. All the women were part of a multicenter European case-referent study conducted using six congenital malformation registers between 1989 and 1992. However, due to the limited number of subjects and lack of statistical significance of this result, caution is recommended when interpreting the results.

In a review of reports including their own earlier study, Bove *et al.* (2002) did not find any clear evidence for an association between adverse birth outcomes and TCE exposure through drinking water. Nevertheless, the authors recommended that a follow up evaluation is needed due to the findings of excess neural tube defects, oral clefts, cardiac defects, and choanal atresia in studies that evaluated TCE-contaminated drinking water.

Cardiac and other developmental effects due to TCE exposure have also been evaluated more recently. Yauck *et al.* (2004) tested the hypothesis that the odds of maternal residence close to TCE-emitting sites would be greater among infants with congenital heart defects (CHDs) than among infants without CHDs. In their case-control study, 4,025 infants born from 1997 to 1999 to Milwaukee, Wisconsin mothers were included in the study. When adjusted for other variables, CHD risk was over three-fold greater among infants of older, exposed mothers compared to infants of older, non-exposed mothers (adjusted OR, 3.2; 95 percent CI, 1.2-8.7). The authors suggest that maternal age and TCE exposure interact to increase CHD risk but residence close to TCE sites alone was not a risk.

Immunotoxicity

The immunotoxicity potential of TCE was also evaluated in humans. Lehmann *et al.* (2002) reported that maternal exposure to TCE (via a home renovation) may have an influence on the immune status of the newborn child. The authors evaluated the cytokine secretion profile of cord-blood T cells in a randomly selected group of 85 neonates. The odds ratio for an association between TCE exposure and immune status at birth was 4.9 (CI 1.40-16.7).

Iacovoli *et al.* (2005) reported the first quantitative immune changes induced by occupational exposure to low TCE levels. The authors measured levels of interleukin-2, interleukin-4, and interferon-gamma in sera obtained from workers exposed to TCE and compared them to those of internal and external control subjects. A significant increase in sera interleukin-2 and interferon-gamma levels and a reduction in interleukin-4 concentrations are observed in the workers compared with those of the internal and external control groups. The workers were exposed to a mean environmental TCE level of 35 +/- 14 mg/m and had a mean urinary TCA concentration of 13.3 +/- 5.9 mg/g creatinine.

Neurotoxicity

TCE is a CNS depressant and has been used as an anesthetic and analgesic. TCE's popularity as an anesthetic declined with the accumulation of evidence that documented severe (and occasionally fatal) neurotoxic effects (Atkinson, 1960; Thierstein *et al.*, 1960; Defalque, 1961; Nomura, 1962; Tomasini and Sartorelli, 1971). Occupational exposure to TCE has resulted in nausea, headache, loss of appetite, weakness, dizziness, ataxia, and tremors (Longley and Jones, 1963; Milby, 1968; Mitchell and Parsons-Smith, 1969; Okawa and Bodner, 1973). In general, these effects are reversible. On the other hand, acute exposure to high concentrations of TCE has caused irreversible nerve damage (Buxton and Hayward, 1967; Mitchell and Parsons-Smith, 1969; Barret *et al.*, 1982) and death (Kleinfeld and Tabershaw, 1954; James, 1963; Buxton and Hayward, 1967). Evidence from experimental human and animal studies indicates that TCE induces a variety of adverse effects on the CNS.

More recently, Reif *et al.* (2003) administered a Neurobehavioral Core Test Battery (NCTB), tests of visual contrast sensitivity, and the profile of mood states (POMS) to a population-based sample of 143 residents of a community in which the municipal water supply had been contaminated with TCE and related chemicals from several adjacent hazardous waste sites between 1981 and 1986. The authors reported that TCE exposure >15 ppb six years following peak concentrations of TCE in municipal drinking water was associated with poorer performance on the digit symbol, contrast sensitivity C test, and contrast sensitivity D test, and higher mean scores for confusion, depression, and tension. In addition, they found evidence of a strong interaction between exposure to TCE and alcohol consumption.

Kilburn (2002) also compared neurobehavioral functions, POMS, frequencies of 35 symptoms, and questionnaire responses provided by 236 residents from an exposure zone

of chlorinated solvents. Solvents noted to be involved were TCE, 1,1-dichloroethane (1,1-DCA), 1,2-dichloroethane (1,2-DCA), 1,1,1-trichloroethane, tetrachloroethylene (PCD), and vinyl chloride (VC). Estimates of exposure were based on analyses of the ground-water plume of these chemicals. Concentration of TCE in well water was reported to be 0.2 ppb to more than 10,000 ppb. The concentrations of the other chemicals were in the range of 0.2 ppb to 330 ppb (VC), 1,600 ppb (1,2-DCE, 6,900 (1,1-DCE), and 260,000 ppb trichloroethane. Exposed resident results were compared with responses provided by 161 unexposed regional referents and by 67 residents of Phoenix, Arizona who lived outside the exposure zone. The authors reported that individuals who lived in the exposure zone had neurobehavioral impairments ($p < 0.05$; delayed simple and choice reaction times, impaired balance, delayed blink reflex latency R-1, and abnormal color discrimination), reduced pulmonary functions, elevated POMS scores, and excessive symptom frequencies. The results reported in this study confirm the observations of an earlier study done in Tucson, Arizona, by Kilburn and Warshaw (1993). Although similar observations are made in both studies, the significance of the findings is confounded by the exposure to a mixture of chlorinated solvents.

Carcinogenicity

Earlier reviews (OEHHA, 1999; ATSDR, 1997; U.S. EPA, 2001) have described an association between TCE exposure and several types of cancers in humans, especially kidney, liver, cervix, and lymphatic system. The International Agency for Research on Cancer (IARC) has designated TCE as Group 2A, probably carcinogenic to humans (IARC, 1995). Consistency across epidemiological studies is strongest for an association between TCE exposure and kidney cancer. In several more recent articles (Vamvakas *et al.*, 1998; Dosemeci *et al.*, 1999; Dumas *et al.*, 2000; Wartenberg *et al.*, 2000, 2002; Hansen *et al.*, 2001; Ojajarvi *et al.*, 2001; Stern *et al.*, 2001; Costas *et al.*, 2002; Bruning *et al.*, 2003; Chang *et al.*, 2003a,b; Lee *et al.*, 2003; Raaschou-Nelsen *et al.*, 2003; Hansen, 2004; U.S. EPA, 2005d; Scott and Chiu, 2006), an association between TCE and cancers of the kidney, pancreas, liver, cervix, and lymphatic system is further delineated. However, other investigators report no increased incidence of cancer associated with exposure to TCE (Boice *et al.*, 1999; Pesche *et al.*, 2000, 2004; Hansen *et al.*, 2001; Morgan and Cassady, 2002; Wong, 2004).

Vamvakas *et al.* (1998) reported an association between renal cell cancer and long-term exposure to TCE (OR = 10.80; 95 percent CI: 3.36-34.75) when 58 patients with renal cell cancer were evaluated and compared to 98 individuals as controls. The authors compared the exposure conditions for patients and control subjects as well as considered several risk factors (i.e., age, obesity, high blood pressure, smoking and chronic intake of diuretics) in their analysis. When the authors analyzed the exposure levels for potential renal cell cancer risk, a statistically significant increase ($p < 0.05$) in odds ratios was observed: Low level category = OR 6.61 (95 percent CI 0.50-87.76); Mid-level category = OR 11.92 (CI 2.55-55.60); High level category = OR 11.42 (CI 1.96-66.79). The authors also combined their data with the data of previously conducted studies in order to evaluate the relationship between risk of renal cell cancer and exposure to TCE. Although the overall OR for all studies increased slightly (1.47, CI 1.17-1.86, to 1.59, CI

1.27-2.00), the present study adds to the weight of evidence that TCE has carcinogenic activity in long-term and severely exposed humans.

Dosemeci *et al.* (1999) reported that the risk of renal cell carcinoma was significantly elevated among women, not men, exposed to all organic solvents combined (OR = 2.3, 95 percent CI 1.3-4.2), to all chlorinated aliphatic hydrocarbons (CAHC) combined (OR = 2.1, CI 1.1-3.9), and to TCE (OR = 2.0, CI 1.0-4.0). The potential risk of renal cell carcinoma was evaluated using an a priori job exposure matrix developed by NCI in a population-based case-control study in Minnesota. A total of 438 renal cell cancer cases (273 men and 165 women) and 687 controls (462 men and 225 women) were interviewed. The authors note that the observed gender differences in risk of renal cell carcinoma may be explained by chance based on small numbers, or by the differences in body fat content, metabolic activity, the rate of elimination of xenobiotics from the body, or by differences in the exposure level between men and women.

Boice *et al.* (1999) reported that slight to moderately increased rates of non-Hodgkin's lymphoma were found among workers exposed to TCE or perchloroethylene, but none was significant. Overall, the authors concluded that there was no clear evidence that occupational exposures of 77,965 workers at an aircraft-manufacturing factory to TCE, perchloroethylene or other chemicals resulted in increases in the risk of death from cancer or other diseases. The workers were followed up over a period of three decades and the exposure assessment was described in Marano *et al.* (2000).

Dumas *et al.* (2000) reported an association between rectal cancer and TCE. The authors conducted a population-based case-control study to determine if an association between occupational exposure to 294 substances, 130 occupations and industries, and various cancers existed. Interviews were carried out with 3,630 histologically confirmed cancer cases (257 of these had rectal cancer) and with 533 population controls. Some of the confounders were determined and regression analyses adjusted for age, education, cigarette smoking, beer consumption, and body mass index. The authors report several substances showed some association with rectal cancer: rubber dust, rubber pyrolysis products, cotton dust, wool fibers, rayon fibers, a group of solvents (carbon tetrachloride, methylene chloride, TCE, acetone, aliphatic ketones, aliphatic esters, toluene, styrene), polychloroprene, glass fibers, formaldehyde, extenders, and ionizing radiation. However, the independent effect of many of these substances could not be discerned from one another since the effects were highly correlated with each other.

The groups of Hansen *et al.* (2000) and Raschou-Nielson *et al.* (2003) examined the association between TCE exposure and liver, biliary tract, kidney, cervical, lung, and testicular cancers. Hansen *et al.* (2001) and Hansen (2004) reported that TCE exposure was associated with increased standardized incidence ratio (SIR) for non-Hodgkin's lymphoma (SIR = 3.1; CI = 1.3-6.1), esophageal adenocarcinomas (SIR = 4.0; CI = 1.5-8.7), and cervical cancer (SIR = 3.8; CI = 1.04-2.8) in a cohort of 806 Danish subjects followed from 1968 to 1996. However, dose response was not found in the small sample group. No support for TCE-exposure and risk of lung (CI = 0.8; CI = 0.5-1.3), testis (SIR = 0.7; CI = 0.01-0.4), and kidney (SIR = 1.1; CI = 0.3-2.8) cancer was reported.

Wartenberg *et al.* (2000, 2002) found evidence of excess cancer incidence among occupational cohorts and kidney cancer (relative risk [RR] = 1.7, 95 percent CI 1.1-2.7), liver cancer (RR = 1.9, CI 1.0-3.4), non-Hodgkin's lymphoma (RR = 1.5, CI 0.9-2.3), cervical cancer, Hodgkin's disease, and multiple myeloma in 80+ published papers and letters reviewed. The authors report that their findings support the overall summary of the epidemiological evidence as being consistent with previous conclusions of some evidence of liver cancer and non-Hodgkin's lymphoma following exposure to TCE.

The meta-analysis by Ojajarvi *et al.* (2001) found a weak excess of pancreatic cancer (meta-relative risk (MRR) = 1.24; 95 percent CI 0.79-1.97) for TCE exposed workers but failed to reveal trends in renal cancer. The authors analyzed occupational exposures to chlorinated hydrocarbon solvents and pancreatic cancer, primarily based on studies that addressed exposure directly (agent studies) and secondarily on studies that reported data without verification of individual chlorinated hydrocarbon exposures (job title studies). Besides a suggestive weak excess for TCE, the authors found other chemicals to have a weak excess: polychlorinated biphenyls (MRR = 1.37; CI 0.56-3.31), methylene chloride (MRR = 1.42; CI 0.80-2.53), and vinyl chloride (MRR = 1.17; CI 0.71-1.91) but not for carbon tetrachloride. The authors also note that confounding may have been insufficiently controlled in the studies they reviewed. In addition, the analysis done by the authors addressed interactions between environmental and occupational agents, lifestyle factors, and genetic susceptibility.

Hansen *et al.* (2001) evaluated cancer occurrence among 803 Danish workers exposed to TCE, using historical files of individual air and urinary measurements of TCE-exposure. The authors found an elevated SIR for non-Hodgkin's lymphoma (SIR = 3.5; n = 8) and cancer of the esophagus (SIR = 4.2; n = 6) in men exposed to TCE while a significant increase (SIR = 3.8; n = 4) in cervical cancer was reported for women exposed to TCE. However, the author concluded that no overall increase in cancer risk, including kidney, was observed among TCE-exposed workers in Denmark. Dose assessment or exposure for their study population was established earlier (Raaschou *et al.*, 2001; 2002).

Costas *et al.* (2002) reported an association with childhood leukemia among children whose mothers were likely to have consumed water from wells contaminated with TCE in Woburn, MA (OR 8.3, 95 percent CI 0.8-94.7). The authors reported a statistically significant exposure-response relationship was identified for the period during pregnancy after adjusting data to control for maternal smoking during pregnancy, maternal age at birth of child, and breast-feeding using a composite covariate. However, no association was found between a child's exposure from birth to diagnosis and the risk of leukemia.

Morgan and Cassady (2002) reported that no significant differences between observed and expected numbers were found for all cancers (SIR 0.97; 99 percent CI, 0.93 to 1.02), thyroid cancer (SIR, 1.00; 99 percent CI, 0.63 to 1.47), or 11 other cancer types from 1988 to 1998 in a California community. Like Costas *et al*. (2002), Morgan and Cassidy (2002) did not find any relationship between leukemia risk and a child's exposure from birth to diagnosis. However, the authors did not investigate critical time periods of susceptibility.

Raaschou-Nielsen *et al.* (2003) reported association between total cancers, non-Hodgkin's lymphoma, renal cell carcinoma, and esophageal adenocarcinoma in a cohort of 40,049 blue-collar workers in 347 Danish companies with documented TCE use. The authors report SIRs of 1.1 (95 percent CI 1.04-1.12) for total cancer in men and 1.2 (CI 1.14-1.33) in women; SIR of 1.2 (CI 1.0-1.5) for non-Hodgkin's lymphoma; SIR of 1.2 (CI 0.9-1.5) for renal cell carcinoma; and SIR of 1.8 (CI 1.2-2.7) for esophageal adenocarcinoma. The earlier papers of Raaschou-Nielsen *et al.* (2001, 2002) describe exposure assessment methods for this cohort study. The finding of esophageal adenocarcinomas was unexpected and no major confounders are known for adenocarcinomas of the esophagus (Hansen, 2004).

In a German study, Bruning *et al.* (2003) found a significant excess risk associated with the longest-held job in a TCE-exposing industry with renal cell carcinomas (odds ratio 1.8, 95 percent CI 1.01-3.2). The authors reviewed 134 cases of renal cell carcinomas and 401 controls from hospitals or nursing homes in Germany. The cases were matched by sex and age and adjusted for smoking.

Lee *et al.* (2003) found an association between exposure to chlorinated hydrocarbons, where source of exposure was an electronic factory upstream from water wells, and liver cancer in males (mortality odds ratio = 2.6, 95 percent CI 1.2-5.5). In addition, the authors did not find an association between other cancer sites and residency in the village containing solvent-contaminated well water. Several weaknesses of the study were the lack of individual information on groundwater exposure, non-consideration of potential confounding with hepatitis viral infection status, or potential misclassification due to the inclusion of secondary liver cancer among the case series.

Pesche *et al.* (2000, 2004) did not find a statistically significant increase or exposure-response relationship between TCE exposure and renal cell carcinoma in 935 cases with 4,298 controls identified from five regions of Germany (OR = 1.3). Controls were adjusted for age, study center, and the number of pack/years of smoking. Exposure information was obtained from participant interviews and not from interviews with a relative.

Wong (2004) reviewed epidemiologic studies of TCE manufacturing workers, metal polishers and platers, aerospace manufacturing and maintenance workers, electronic factory workers, jewelry workers, and nearby community residents who consumed TCE-contaminated groundwater from the United States, Taiwan, or Europe. The author reported no causal association between exposure to TCE and an increased risk of any site-specific cancer, including cancer of the liver and biliary passages and non-Hodgkin's lymphoma.

Alexander *et al.* (2006) concluded in their meta-analysis that the data did not support an etiologic association between occupational TCE exposure and risk of multiple myeloma or leukemia. The authors used random effects models to calculate summary relative risk estimates (SRRE) in the eight cohort or case–control studies they identified. An SRRE of 1.05 (95 percent CI 0.80–1.38; $P = 0.94$) for multiple myeloma and a SRRE of 1.11 (CI 0.93–1.32; $P = 0.50$) for leukemia was calculated based on TCE-exposed subgroup meta-analyses. Study-specific relative risk estimates for multiple myeloma ranged between

0.57 and 1.62, while the relative risk estimates for leukemia ranged between 1.05 and 1.15 in five studies.

Summary of cancer data

In the recent literature, several issues (e.g., confounding exposure to other solvents and other risk factors, lack of documentation of TCE exposure, methods for data collection or sample size) that complicate interpretation of the epidemiological data are found. The studies of Chang *et al.* (2003a,b) and Stern *et al.* (2001) provide no information to gauge the percentage of the cohort with potential TCE exposure. Several weaknesses were noted in the Lee *et al.* (2003) study: lack of individual information on groundwater exposure, non-consideration of potential confounding with hepatitis viral infection status, and potential misclassification due to the inclusion of secondary liver cancer among the case series. Hansen *et al.* (2001) cited the small number of observed cases found and the lack of dose-related effects in those cancer sites where excesses were noted in their study as the reason for their final conclusion as not being conclusive. The sample size for Dosemeci *et al.* (1999) was also small so that the observed gender differences in risk of renal cell carcinoma may be a chance occurrence based on small numbers. The same concern exists with the study of Vamvakas *et al.* (1998). Wartenberg *et al.* (2000) mentioned that only a few of the 80 studies reviewed described a clear exposure to TCE while the remainder had multiple solvent exposures. Dumas *et al.* (2000) found the other chemicals present also to be highly correlated with the rectal cancer reported. In some studies (Costas *et al.*, 2002), confounding factors like hepatitis viral infections status were not well addressed.

Even when most confounding factors are addressed, some investigators have reported no association between TCE exposure and increased incidence of cancer. Morgan and Cassidy (2002) did not find any relationship between leukemia risk and a child's exposure from birth to diagnosis. Wong (2004) reported no causal association between exposure to TCE and an increased risk of any site-specific cancer, including cancer of the liver and biliary passages, and non-Hodgkin's lymphoma. Pesche *et al.* (2000, 2004) also did not find a statistically significant increase or exposure-response relationship between TCE exposure and renal cell carcinoma. Finally, Alexander *et al*. (2006) did not find an association between occupational TCE exposure and risk of multiple myeloma or leukemia in their meta-analysis.

However, several investigators have reported associations between TCE exposure and malignancy. For example, Raaschou-Nielsen *et al.* (2003) reported associations between total cancers, non-Hodgkin's lymphoma, renal cell carcinoma, and esophageal adenocarcinoma. No major confounders are known for esophageal adenocarcinomas according to Hansen (2004). Also, Bruning *et al.* (2003) found a significant excess risk associated with the longest-held job in a TCE-exposing industry with renal cell carcinomas for which cases were matched by sex, age, and adjusted for smoking. Scott and Chiu, in reviewing TCE epidemiology studies, concluded that recently published studies appear to provide further support for association of TCE exposure with excess

risk of cancer at several sites, with modestly elevated site-specific risks (mostly between 1.5 and 2.0).

Another recent comprehensive review of the current issues associated with epidemiological studies and TCE exposure was provided by U.S. EPA (2005d). An association is shown between TCE exposure and several cancers, including several at the same sites seen in animal bioassays. The U.S. EPA review was provided as background information for the National Academy of Science (NAS) scientific panel, to help provide U.S. EPA with guidance on the scientific issues for TCE health risks. The NAS panel concluded that "the evidence on carcinogenic risk and other health hazards from exposure to trichloroethylene has strengthened since 2001" (when U.S. EPA completed its previous TCE risk assessment) (NAS, 2006). The panel concluded that the relevance of the kidney cancer data to humans is stronger than that for other sites, including liver, based on mechanism of action interpretations.

According to the NAS panel, the meta-analyses conducted by Wartenberg *et al.* (2000) and Kelsh *et al*. (2005) should not be used for hazard characterization in the TCE risk assessment due to several limitations. The panel recommended that a new meta-analysis of the TCE cancer epidemiologic data be performed with improved techniques (e.g., documenting essential design features, exposure, and results; excluding studies in which exposure conditions are unclear; classifying studies in terms of objective characteristics; combining case-control and cohort studies unless the study introduces substantial heterogeneity; testing of heterogeneity; and performing sensitivity analysis in which each study is excluded from analysis to determine whether any single study significantly influences the findings). U.S. EPA plans to utilize feedback from the NAS scientific panel and the public to develop a TCE human health risk assessment with the most recent available data.

Overall, each of the cancer studies contributes to the toxicity assessment of TCE. Although the epidemiological data do not demonstrate direct causation of cancer due to exposure to TCE, and many of the studies have confounding issues, the findings suggest a consistency across studies as just summarized. These studies in conjunction with the animal data do provide evidence that TCE can be considered a human carcinogen.

MODE OF ACTION

An extensive review of the literature was conducted. Plausible modes of actions were summarized in the previous PHG for TCE (OEHHA, 1999) as well as by Green (2000), Lash *et al.* (2000b), Bull (2000), and NAS (2006). Production of liver, renal cell carcinoma, and lung tumors by TCE have complex modes of action with contributions from: (1) non-genotoxic processes related to cytotoxicity; (2) possibly receptor-mediated mitogenic stimulation; (3) genotoxic metabolites such as chloral, DCVC and related metabolites, and; (4) possibly reactive oxygen species related to peroxisomal induction in the liver. Support for each path can be found in the literature. For example, cytotoxicity seems to play an important role in TCE-induced liver cancer in rodents, but it is uncertain what role cytotoxicity plays in human cancers induced by TCE at exposure levels below

those expected to cause frank toxicity. Demonstration of linear dose-dependent DNA adduct formation by relevant doses of TCE in mice *in vivo* tend to support a genotoxic MOA. Harth *et al.* (2005) proposed that a threshold effect exists for TCE renal cell tumorigenesis: low exposure to TCE is not associated with increased risk of malignancy but the risk of renal cell cancer is significantly elevated in persons reporting long-term (several years) and high (with prenarcotic episodes) occupational exposures to TCE.

Several mutagenic or carcinogenic metabolites of TCE can also contribute to an increased incidence of renal cell carcinoma. Of the compounds reviewed, only TCE, TCA, and chloral hydrate cause liver tumors in the mouse, while DCA causes liver tumors in both mice and rats. TCE and two metabolites (DCA and TCA) have been shown to induce peroxisome proliferation in rodents via the peroxisome proliferator-activated receptor alpha (PPARα), and cause oxidative damage, and lead to initiation of carcinogenesis. In the end, the main issues in evaluating these data are whether the mechanism for carcinogenicity is linear or non-linear, and what, if any, is the relevance of the MOA to humans.

Klaunig *et al.* (2003) and Cohen *et al.* (2003) reviewed the relationship between the MOA for PPARα agonists and the human relevance of related animal tumors. These authors found that substantial species differences exist, with rodents being more susceptible than primates in response to peroxisome proliferators *in vivo*. Exposure of rats and mice to TCE and other chemicals can result in both males and females developing tumors. Based on the MOA data from three different rodent tissues (i.e., rat and mouse liver, rat pancreas, and rat testis), PPARα activation may be a causal first step in the development of the tumors. There are several different postulated MOAs, some beginning with PPARα activation as a causal first step. Using the new human relevance framework (HRF), case studies involving PPARα-related tumors in each of these three tissues produced a range of outcomes, depending partly on the quality and quantity of MOA data available from laboratory animals and related information from human data sources.

U.S. EPA (2005c) provided a summary and uncertainties associated with the peroxisome proliferation mode of action for liver tumorigenesis as background for an NAS expert panel to consider in the development of advice for U.S. EPA. The U.S. EPA report discusses the variety of divergent perspectives regarding the role of PPARα agonists, primarily TCE, which could affect their toxicity and human health risks. Key issues or questions that U.S. EPA wanted the NAS panel to consider are whether effects from PPARα agonism may be involved in TCE-induced rodent liver tumors and what human health risks may be posed by PPARα agonists.

The NAS review (2006) makes several inferences on species differences and on uncertainty of the relationship between TCE and kidney/liver carcinogenesis in humans based on the animal data. TCE was noted as being a complete kidney carcinogen in animals, where "(t)he committee ruled out the accumulation of $\alpha_{2\mu}$-globulin, peroxisome-proliferator activated receptor α (PPARα) agonism, and formic acid production as modes of action for the production of renal tumors in rodents" (NAS, 2006). For liver tumors, the committee concluded that the mode of action of TCE and its metabolites in liver

carcinogenesis in the rodent studies is "principally as a liver peroxisome proliferator and agonist of PPARα rather than as a genotoxicant." However, the NAS review goes on to state that "species differences in susceptibility and phenotypic differences in tumors derived from TCE and its metabolites suggest that there are mechanistic differences in the way these chemicals (peroxisome proliferators) cause tumors that cannot be fully explained by peroxisome proliferation."

Keshava and Caldwell (2006) reiterate this point in their review of the role of peroxisomal proliferation in TCE toxicity. They conclude that given the many responses to PPARα activation that occur in both rodents and humans, it would be premature to conclude that the potential effects in humans can be predicted from the animal data. This would include whether the toxic mechanisms of TCE in humans are mediated through PPARα activation. Keshava and Caldwell state that "recent data also suggest that even for liver tumor induction, extraperoxisomal effects such as changes in mitochondria and activation of Kupffer cells may play an important role, so inferences based on [peroxisomal proliferation] or purified hepatocyte cultures alone may be misleading."

Klaunig *et al*. (2007) emphasize that the toxic effects of TCE are complex, and are not all related to or dependent upon peroxisomal proliferation. More recent studies on the many metabolic effects associated with PPAR receptors in both animals and humans further complicate the assessment of the significance of this mechanism of action of various compounds (Yang *et al.*, 2007; Gonzalez and Shah, 2008; Scatena *et al*., 2008).

Given the uncertainty whether humans are more or less susceptible to the carcinogenic effects of TCE than other animals, as described in the NAS TCE review and subsequent PPARα studies, OEHHA considers the linear extrapolation default approach as the better approach at this time. The key events critical to tumor induction of TCE and the cross-species relevance of these key events have yet to be identified. The receptor appears to be pleiotropic in its actions, and responses appear to be chemical, gender, age, and concentration dependent. The overall relevance of liver tumors induced by peroxisome proliferation to human risk is controversial and remains to be resolved.

In addition, species and gender differences observed following exposure to TCE have been presumed to result from differences in cytochrome P450 and glutathione-dependent metabolism. The sensitive species, the mouse, has a greater capability for generating high levels of toxic metabolites with a lower ability to eliminate them. Peak blood concentrations of TCE occurred at 1 hr and 3 hr for mice and rats, respectively. The half-life values are also about 1 hr in mice and 3 hr in rats, while in humans the half-lives are 0.5 hr for the fast phase and 21 hr for the slow phase. Although removal of TCE from mouse blood occurred more rapidly than from rat blood, blood concentrations of the TCE metabolite, TCA, were about 10 times greater in mice than in rats over a 48-hr post-exposure observation period. In addition to the biochemical differences, morphological differences of key cell types (e.g., Clara cells) also exist between mouse and human. Green *et al.* (2000) reported that the human lung has approximately 600-fold less ability to metabolize TCE than the mouse. Thus, the large quantitative differences between the metabolic capacity of the mouse together with the species differences in the number and morphology of target cells suggest that the risks to humans may be lower than in mice.

DOSE-RESPONSE ASSESSMENT

Non-Carcinogenic Effects

Repeated exposure of humans to TCE in the workplace appears to have some toxic effects on kidney or liver as well as nervous system, cardiovascular, immunological, and cancer effects (ATSDR, 1997). Available data show no consistent effect of TCE on the human reproductive system. Chia *et al.* (1996) did report an apparent dose-response relation between TCA in urine and hyperzoospermia or sperm densities. Although hyperzoospermia has been implicated in infertility, no additional information has been reported about TCE and hyperzoospermia. Since exposure assessment is not clear in most human studies and the effects observed are often associated with confounding factors, the data used in the derivation of the public-health protective concentration is based on the findings of the animal studies.

TCE is primarily a liver and renal toxicant through oral, inhalation, and dermal exposure in animals (ATSDR, 1997). Other effects reported with repeated exposures to TCE are dermal toxicity, ototoxicity, neurotoxicity, and immunotoxicity. TCE is a more potent peroxisome proliferator in livers of mice than of rats. In animals, Johnson *et al.* (2003) reported a dose-related increased incidence of abnormal hearts in offspring of Sprague Dawley rats treated during pregnancy with 0, 0.00045, 0.048, 0.218, or 128.5 mg/kg-day TCE in drinking water. The NOAEL for the Johnson study was reported to be 2.5 ppb (0.00045 mg/kg-day) in this short exposure (22 days) study, but this is too imprecise to use in a risk estimate since this dose differs by more than 100-fold from the lowest observed adverse effect level (LOAEL). The results of this study are also not consistent with earlier developmental and reproductive toxicological studies done outside this lab in mice, rats, and rabbits. The other studies did not find adverse effects on fertility or embryonic development, aside from those associated with maternal toxicity (Hardin *et al.*, 2004). More appropriate endpoints for derivation of a non-cancer health-protective value were derived by Haag-Gronlund *et al.* (1995) and Barton and Das (1996) based on the data from Henschler *et al*. (1980), Maltoni *et al*. (1988), NTP (1988), and Narotsky *et al*. (1995).

Haag-Gronlund *et al.* (1995) and Barton and Das (1996) used the LOAEL of 10 mg/kg-day and the benchmark dose of 82 mg/kg-day for liver effects to calculate safe drinking water concentrations of 1 and 10 ppm, respectively. The kidney LOAEL and developmental NOAEL gave similar values. Haag-Gronlund *et al*. (1995) applied the benchmark dose method to a TCE risk assessment, using polynomial models for both quantal and continuous data sets. Toxicity data on liver, kidney, CNS effects, and tumor data were evaluated. Benchmark doses (BMD) were estimated at the 1, 5, and 10 percent response levels. For kidney effects in the NTP (1988) rat study, BMD_{10} values for cytomegaly ranged from 11-24 mg/kg-day for different sexes and strains of rat. The nephropathy BMD_{10}s ranged from 50-210 mg/kg-day. These values are lower than the study lowest observed effect level of 500 mg/kg-day. The lowest BMD_{10} values for liver, kidney, and CNS effects in inhalation experiments (Henschler *et al.*, 1980; Fukuda *et al.*, 1983; Maltoni *et al.*, 1988) were 23, 122, and 10 ppm respectively. Overall, all no-effect

levels were higher than the BMD_{01}, and 42 percent of the no-effect levels and 93 percent of the lowest observed effect levels were higher than the BMD_{10}. The authors noted that the polynomial regression models often failed to fit the experimental data at the desired level of significance in the X^2 "goodness of fit" test ($p > 0.05$).

Barton and Das (1996) conducted a similar assessment for chronic non-cancer effects from oral exposures to TCE. Four dose response models for quantal data and one for continuous data were employed. The analysis considered liver effects, kidney toxicity, and developmental effects. For liver endpoints, the BMD_{05} values ranged from 82 to 289 mg/kg-day. The lowest value was based on liver weight/body weight ratio changes (LW/BW) in $B6C3F_1$ mice (Elcombe *et al.*, 1985) and the highest value on LW/BW in F344 rats (Melnick *et al.*, 1987). For kidney, toxicity data from NTP (1988) and Maltoni *et al*. (1986) were evaluated. For rat kidney cytomegaly, BMD_{05} values with superior fits ($p > 0.1$) ranged from 0.14 to 24 mg/kg-day for 8 data sets. For toxic nephrosis, BMD_{05}s ranged from 5 to 276 mg/kg-day. For eye defects (Narotsky *et al.*, 1995) in rats, BMD_{05}s ranged from 231 to 308 mg/kg-day and the BMD_{01}s ranged from 48 to 60 mg/kg-day. The authors noted high model dependence in the BMD values generated. They used the LOAEL of 10 mg/kg-day and the BMD of 82 mg/kg-day for liver effects in a calculation of safe drinking water concentrations of 1 and 10 ppm, respectively. The kidney LOAEL and developmental NOAEL gave similar values.

Carcinogenic Effects

In its recent review of TCE carcinogenicity for U.S. EPA, the NAS panel (NAS, 2006) recommended:

- "Several points of departure should be considered and compared when performing point-of-departure-based dose-response assessments for cancer and non-cancer end points.
- When modeled estimates are used as points of departure in cancer and non-cancer risk assessments, it is important that (1) criteria are established for determining which data sets are suitable for modeling, (2) the selected response level is justified or multiple response levels are modeled and compared, (3) dose-response models are clearly described, (4) different dose metrics are considered and compared to assess whether the choice of metric substantially affects the dose-response assessment, and (5) when animal data are modeled, the methods for estimating human-equivalent doses are specified.
- Toxicologic data should be used to fit the primary dose-response model(s), and the available epidemiologic data should be used only for validation. Because the available information is insufficient to determine the best dose-response model for trichloroethylene, the default linear extrapolation procedure suggested in EPA's cancer guidelines can be applied but should first be explicitly defined."

OEHHA believes that this approach is logical and consistent with our past practice. The preferred approach for dose response assessment is one based on a relevant biologically-based model or a case-specific model for tumor responses in both the observed range and

in the extrapolated range (U.S. EPA, 2005e). When a specific model is not available, the default procedure is to fit the data in the observed range with a curve-fitting model using a nonlinear extrapolation, a linear extrapolation, or a combination of linear and nonlinear based on the mode of action (MOA). If the MOA indicates linearity through direct alterations in DNA, for example, then the linear model is used. Alternatively, a nonlinear approach is used when adequate data on the MOA show that linearity is not the most reasonable judgment and there is sufficient evidence to support a nonlinear MOA. For example, the non-linear approach could be used when a nonlinear overall dose response or sharp reduction in tumor incidence with decreasing dose exists, accompanied by strong evidence against a genotoxic mechanism. In the latter case, the default approach would employ either a margin of exposure (MOE) analysis using a ten percent tumor response level (LED_{10}) or related biological response data as the point of departure.

The data sets selected for dose response analysis were the liver tumors in the NCI (1976) study in $B6C3F_1$ mice, the liver and lung tumors from Maltoni *et al.* (1986) in $B6C3F_1$ mice, and the lung tumors from Fukuda *et al.* (1983) in ICR mice. The NCI study used oral gavage administration of TCE while the other two studies used inhalation exposure. Each of these data sets had at least two non-zero dose groups.

For the dose response assessment of TCE in mice the PBPK modeling results of Abbas and Fisher (1997) were employed to estimate the following dose metrics: area under the curve (AUC) of chloral hydrate (CH) for lung tumors and AUC of TCA+DCA for liver tumors. AUC (blood or tissue concentrations integrated over time) is considered to be a better metric of exposure than applied dose or peak concentration for tissue-reactive carcinogens because the common mechanisms of carcinogenic action most likely depend on the time-integrated availability of chemical to the tissue. Abbas and Fisher's results are based on male $B6C3F_1$ mice dosed orally with 300, 600, 1,200, and 2,000 mg/kg TCE. The model predictions for CH in lung and the dose metric of AUC CH in mg-hr/L-d were regressed against applied dose to give the relation AUC CH = 0.05496 (mg/kg-day) - 3.46, R= 0.9976. The AUC values were estimated by triangulation from the tabular Cmax values and the graphs of CH lung concentration vs. time in the paper as AUC (mg hr/L-day) = Cmax - 0.1 Cmax mg x 20 hr/2 L-day. A similar process on the liver TCA+DCA AUC produced the following relation: AUC TCA+DCA = 0.476 (mg/kg-day) + 191.2, R = 0.92. For values below 300 mg/kg-day the relation was forced through the origin with a slope of 0.575 (mg/kg-day)/(mg-hr/L-day). Using these relationships, bioassay doses could be converted to AUC dose metrics adjusted for lifetime exposure, converted back to oral equivalents and fitted to the tumor incidence data. The results of the analysis are shown in Table 2.

The U.S. EPA Benchmark Dose software (BMDS version 1.4.1) (U.S. EPA, 2008) is currently our preferred statistical package for cancer potency analysis. Typically, various statistical modules are evaluated for a cancer data set, and the best-fit approaches are chosen.

Using the AUC TCA+DCA metric (Cronin *et al.*, 1995), the human equivalent LED_{10} values for liver tumors in both sexes in two studies by two administration routes vary by over 50-fold, i.e., 9.4-486 mg/kg-day. For lung tumors with the AUC CH metric the LED_{10} values from two inhalation studies varied by less than two, i.e., 101-155 mg/kg-

day. The geometric mean (Gmean) cancer slope factor (CSF), using the U.S. EPA BMDS quantal linear or multistage cancer models for the four liver data sets is 0.0021 (mg/kg-day)$^{-1}$ and for the two lung data sets is 0.0008 (mg/kg-day)$^{-1}$. If the total TCE metabolism dose metric (AMET) (Cronin *et al.*, 1995) is used, the LED_{10} values are lower, at 2.7-72.3 mg/kg-day (CSFs of 0.0125, 0.0365, 0.0020, 0.0014 in Table 2), corresponding to a higher geometric mean CSF of 0.0059 (mg/kg-day)$^{-1}$. The four geometric mean CSFs and Q_1*s vary by less than a factor of 1.5. Both the AMET and AUC TCA+DCA dose metrics gave adequate fits on 5/6 data sets ($p > 0.1$). However, the AMET gave marginally better fits and a closer agreement of Gmeans. In addition, the Cronin *et al*. data set has the further advantage of attempting to simulate the actual bioassay doses.

Table 2. Carcinogenic Dose Response Estimates for TCE Based on PBPK Dose Metrics and Mouse Bioassays

Study, Sex, Route, Site	**Applied Doses** [a]	**AUC or AMET Lifetime Dose Metrics**	**Quantal Tumor Incidence; X^2, p, k** [d]	**ED_{10}/LED_{10} mg/kg-day Human equivalent** [e]	**CSF** [f] **q1*** [g] **(mg/kg-day)$^{-1}$**
NCI, 1976 female, gavage, liver	0, 869, 1739 mg/kg-day	[b] 0, 243.9*, 156.7 mg hr/L d	0/20, 4/50, 11/50; 8.21, 0.02, 2	37.8/21.18	**0.0047** **0.0276**
		[c] 0, 73.57, 54.26 mg/kg-day	6.63, 0.04, 2	13.64/8.01	**0.0125** **0.0095**
male, gavage, liver tumors	0, 1169, 2339 mg/kg-day	[b] 0, 314.06*, 479.84, mg hr/L d	1/20, 26/50, 30/50; 0.4, 0.53, 2	11.98/9.36	**0.0107** **0.0264**
		[[c]] 0, 91.99, 113.46 mg/kg-day	0.01, 0.93, 2	3.49/2.74	**0.0365** **0.038**
Maltoni *et al*., 1986, female, inhalation, liver tumors (Fisher & Allen, 1993)	0, 100, 300, 600 ppm	[[b]] 0, 203.74, 175.5, 271.14 mg hr/L d;	2/90, 3/90, 4/89, 9/87; 0.80, 0.67, 3	64.87/53.62	**0.0019** **0.0020**
		[c] 0, 60.89, 125.68, 143.1 mg/kg-day	1.08, 0.58, 3	62.70/51.17	**0.0020** **0.0017**
(Bogen & Gold, 1997)		[b] 0, 203.74, 175.5, 271.14 mg hr/L d	3/88, 4/89, 4/88, 9/85; 0.46, 0.79, 3	308.6/84.77	**0.0012** **0.0018**
		[c] 0, 60.89, 125.68, 143.1 mg/kg-day	1.32, 0.52, 3	66.88/52.59	**0.0019** **0.0017**
male, inhalation, liver tumors (Fisher &	0, 100, 300, 600 ppm	[b] 0, 242.47, 689.16, 456.72 mg hr/L d	1/85, 1/86, 3/88, 6/88; 0.0, 0.94, 3	767.4/486.4	**0.00021** **0.00073**

Study, Sex, Route, Site	**Applied Doses** [a]	**AUC or AMET Lifetime Dose Metrics**	**Quantal Tumor Incidence; X^2, p, k** [d]	**ED_{10}/LED_{10} mg/kg-day Human equivalent** [e]	**CSF** [f] **q1*** [g] **$(mg/kg\text{-}day)^{-1}$**
Allen, 1993)					
		[c] 0, 59.13, 160.59, 179.05 mg/kg-day	0.43, 0.81, 3	88.96/72.34	**0.0014** **0.00099**
(Bogen & Gold, 1997)		[b] 0, 242.47, 689.16, 456.72 mg hr/L d	1/59, 1/31, 3/38, 6/37; 3.39, 0.18, 3	342.9/122.76	**0.00082** **0.0020**
		[c] 0, 59.13, 160.59, 179.05 mg/kg-day	0.57, 0.45, 3	64.16/39.58	**0.0025** **0.0027**
female, inhalation, lung tumors	0, 100, 300, 600 ppm	0, 4.75, 19.1, 40.8 mg hr/L d	2/90, 6/90, 7/89, 14/87; 1.24, 0.54, 3	233.4/155.4	**0.00064** **0.00067**
Fukuda *et al.*, 1983, female, inhalation, lung tumors	0, 50, 150, 450 ppm	0,1.52*, 9.8, 34.9 mg hr/L d	1/49, 3/50, 8/50, 7/46; 4.1, 0.13, 3	173.6/101.2	**0.00099** **0.0010**
Gmean Liver AUC[b]					**0.0021** **0.0057** **0.0026** **0.0072**
Gmean Liver AMET[c]					**0.0059** **0.0050** **0.0068** **0.0064**
Gmean Lung AUC[b]					**0.00080** **0.00083**

[a] Unadjusted applied doses.

[b] AUC from linear regressions of values for CH (lung) or TCA+DCA (liver) vs. oral mg/kg-day (Abbas and Fisher, 1997); inhalation applied doses in mg/kg-day adjusted to lifetime average daily AUC doses (DHS, 1990b). Lifetime AUCs converted back to oral mg/kg-day using regression (* in three low doses the slope was forced through zero) and oral equivalent AUC doses fit to the tumor incidence data.

[c] AMET dose metric from Cronin *et al.* (1995) is the total TCE metabolized, in mg/kg-day. NCI (1976) studies adjusted for premature termination by multiplying potencies by $(104/90)^3$.

[d] Quantal tumor responses for respective doses, fit statistics using quantal linear or multistage-cancer models given in boldface type.

[e] ED_{10}/LED_{10} human equivalents using $(body\ weight)^{3/8}$ interspecies scaling (i.e., scaling factors used for CSF and q_1* were $(70/0.027)^{1/8}$ and $(70/0.034)^{1/8}$ for female and male mice respectively).

[f] Carcinogen slope factor (CSF) calculated as $0.1/LED_{10}$.

[g] q_1* carcinogenic potency determined by linearized multistage model; in some cases mid or top doses were removed to achieve model fit. For Maltoni *et al.* (1986) data sets quantal tumor responses as reported by Fisher and Allen (1993) and by Bogen and Gold (1997) were included in separate Gmeans.

Based on these latter metrics, the CSF of 0.0059 $(mg/kg\text{-}day)^{-1}$, is considered the most appropriate for use in calculating possible PHG values. While carcinogenic potencies based on the linearized multistage model (LMS) varied somewhat from the CSFs depending on the specific data set, the geometric mean potency estimates for liver tumors with the AMET dose metric were very similar to those derived from the CSFs. With these good data fits, use of the slightly more health-protective (higher) CSF appears justified.

Different quantal responses have been published for some of the data sets, particularly for the Maltoni *et al*. (1986) liver tumor data. The calculations noted above were performed on the Maltoni mouse liver tumor values noted by Bogen and Gold (1997) and Fisher and Allen (1993) for both dose metrics. While the latter are used in the current analysis, the Bogen and Gold values gave only slightly higher CSFs of 0.0068 $(mg/kg\text{-}day)^{-1}$ for the AMET based Gmean and 0.0026 $(mg/kg\text{-}day)^{-1}$ for the AUC based Gmean.

Not included in Table 2 are the dose response results from the combined male mouse groups from Maltoni *et al.* (1986) (BT306 and BT306-bis). For the AMET dose metric, this combined data set gave a CSF of 0.01 $(mg/kg\text{-}day)^{-1}$ with adequate fit statistics. The AUC metric did not give an adequate fit with this data set (chi squared = 8.24, p = 0.02). These are not considered significant differences from the values given above in the context of this risk assessment.

Recently, Hack *et al.* (2006) attempted to refine the TCE PBPK modeling. The dosimetry from the Hack model generally gives better fits with the tumor data than the earlier published values of Cronin *et al.* (1995) but there does not appear to be an adequate mass balance for the TCA submodel. This may be an artifact since there is no problem with the main TCE model or other submodels. Since the difference in the risk estimates is relatively small, we have chosen to use the revised Cronin dosimetry noted above until we can further evaluate the Hack model or it is replaced with a better version.

CALCULATION OF PHG

Non-Carcinogenic Effects

For estimation of a health-protective concentration of TCE in drinking water, an acceptable daily dose (ADD) of the chemical from all sources will first be calculated. This involves incorporation of appropriate estimates of uncertainty in the extrapolation of the critical toxic dose from human or animal studies to the estimation of a lifetime ADD that is unlikely to result in any toxic effects. For this purpose, the following equation can be used:

$$\text{ADD} = \frac{\text{NOAEL/LOAEL/BMD in mg/kg-day}}{\text{UF}}$$

where,

ADD = estimated maximum daily dose which can be consumed by humans for an entire lifetime without toxic effects;

NOAEL/LOAEL/BMD = no-observed-adverse-effect level, lowest-observed-adverse-effect level, or benchmark dose used in the critical study;

UF = uncertainty factor(s).

Table 3 lists more relevant benchmark dose estimates and LOAELs documented in the literature with the corresponding endpoint in the mouse or rat considered for determination of the health-protective level for non-carcinogenic effects of TCE. The newer evaluations either used much higher doses for their exposures or used a similar dose but for a shorter duration compared to the key studies used to calculate the previous non-cancer value. Table 4 provides a list of the corresponding doses for the new studies. The one exception was the study done by Johnson *et al.* (2003), which was not considered appropriate for derivation of a health-protective level because appropriate dose-response relationships were not maintained and the findings are not consistent with other developmental and reproductive toxicological studies (Hardin *et al.*, 2004).

Table 3. TCE Benchmark Dose Estimates and LOAELs for Noncancer Endpoints

Study	Species, Study Duration, Route	Toxicity Endpoint	BMD or LOAEL/NOAEL
Haag-Gronlund *et al.*, 1995	Rat, chronic (52 weeks), oral	Kidney nephropathy	50 mg/kg-day [a]
Barton & Das, 1996	Mouse, subchronic (6 weeks/6 months), oral	Liver effects, LW/BW	82 mg/kg/d [a]
Barton & Das, 1996	Rat, subacute (6-15 days), oral	Eye defects	101 mg/kg-day [b]

[a] BMD_{10};
[b] LOAEL.

Table 4. Reported NOAELs and LOAELs for TCE Noncancer Endpoints

Study	Species, Study Duration, Route	Toxicity Endpoint	NOAEL or LOAEL
Johnson *et al.*, 2003	Rat, 22 day, oral	Diminished fertility	0.00045 mg/kg-day or 2.5 ppb [a]
Xu *et al.*, 2004	Mouse, 6 weeks, oral	Diminished fertility	1000 ppm [b]
DuTeaux *et al.*, 2004	Rat, 14 day, oral	Diminished fertility	180 mg/kg-day [b]

Study	Species, Study Duration, Route	Toxicity Endpoint	NOAEL or LOAEL
Forkert *et al.*, 2002	Rat, 19 day, inhalation	Diminished fertility	1000 ppm [b]
Albee *et al.*, 2006	Rat, 13 weeks (6 hr/day, 6 d/wk), inhalation	Ototoxicity	800 ppm [a]
Kumar *et al.*, 2001b	Rat, 24 weeks (4 hr/day, 5 d/wk), inhalation	Increased liver weight	376 ppm [b]
Mensing *et al.*, 2002	Rat, 26 weeks (6 hr/day, 5 d/wk), inhalation	Kidney effects	500 ppm [b]
Peden *et al.*, 2006	Mice, 8 weeks, oral	Immunological (SRBC-specific IgM decreases)	1400 ppb [b]
Kaneko *et al.*, 2000	Mice, 8 weeks (4 hr/day, 6 d/wk), inhalation	Immunological (IgG changes)	500 ppm [b]
Oshiro *et al.*, 2001 & 2004	Rat, 20 days (6 hr/day), inhalation	Neurological	1600 ppm [b]

[a] NOAEL;
[b] LOAEL.

For TCE, the benchmark dose of 50 mg/kg-day for kidney nephropathy from the study of Haag-Gronlund *et al.* (1995) is considered the most appropriate endpoint for derivation of a non-cancer health-protective value. To this is applied an uncertainty factor of 10 for inter-species extrapolation and 10 for intraspecies variation, including potential susceptible subpopulations. Thus,

$$\text{ADD} = \frac{50 \text{ mg/kg-day}}{100} = 0.5 \text{ mg/kg-day}$$

Estimation of a health-protective concentration from this dose requires consideration of exposure terms for body weight, drinking water consumption, and other potential sources of TCE. The standard default values used for the body weight and potential source of contribution were used for the calculation of the health-protective concentration. For body weight, the default adult human body weight is 70 kg. Since the exposure and calculation assume a life-time exposure, the use of the 70 kg body weight is a conservative value. Similarly, the relative source contribution (RSC) chosen for TCE is

the 20 percent default, indicating significant anticipated exposure via other sources besides drinking water, notably ambient air and food. A more detailed exposure assessment could possibly result in a higher RSC value, but it is judged that the data are inadequate for a comprehensive evaluation.

For estimating the water intake value, exposures from multiple routes were used to calculate a combined liter-equivalent (Leq) value. The multiroute tap water intake value of 7.1 Leq/day (Bogen *et al.*, 1988, 1992) was used to account for ingestion, dermal and inhalation exposures from typical household uses of TCE contaminated tap water. This value was based on age and sex-averaged exposure per unit body weight and activity level to give equivalent lifetime daily fluid intake by a 70 kg adult of the sum of 2.2 L ingestion absorption, 2.9 Leq inhalation absorption, and 2.0 Leq dermal absorption. Bogen *et al*. (1988) also give upper-bound estimates of 3.8 L, 11.9 Leq, and 2.6 Leq respectively for a total upper-bound exposure of 18.3 Leq/day. Note that the earlier study of Weisel and Jo (1996) measured showering and bathing exposures only and not other household exposures which would be expected to add to the inhalation exposure. Their results provided lower estimates of 2 L, 3 Leq and 3 Leq (8 Leq total) and upper estimates of 2 L, 6 Leq, and 6 Leq (14 Leq total) for ingestion, inhalation and dermal intakes, respectively. An alternative analysis was performed using CalTOX©, a multimedia total exposure model (v. 1.5, DTSC, 1994). For tap water containing 5 ppb TCE and a lifetime exposure of 70 years, the CalTOX© model predicts lower dermal and inhalation doses compared to Bogen *et al*. (1988), relative to ingestion dose, namely 10 percent dermal, 32 percent inhalation, and 58 percent ingestion at 0.022 L/kg-day tap water ingestion (1.54 L/day for a 70 kg adult). Overall the estimates of Bogen *et al.* (1988) seem best suited to use in this calculation because the values are based on averaged specific parameters for age, sex, activity level, and unit body weight. In addition, the values obtained by Bogen *et al.* (1988) gave a more conservative estimate of exposure.

Calculation of a public health-protective concentration (C, in mg/L) for TCE in drinking water uses the following equation for noncarcinogenic endpoints:

$$C = \frac{\text{ADD mg/kg-day} \times \text{BW in kg} \times \text{RSC}}{\text{L/day}}$$

where,

BW = the default adult human body weight of 70 kg;

RSC = relative source contribution (usually 20 to 80 percent (0.20 to 0.80), and the lower default value of 0.2 in this case;

L/day = an estimated total equivalent water consumption rate of 7.1 Leq/day.

Therefore,

$$C = \frac{0.50 \text{ mg/kg-day} \times 70 \text{ kg} \times 0.2}{7.1 \text{ L/day}} = 1 \text{ mg/L, or } 1{,}000 \text{ ppb}$$

The calculated value is judged to be protective of all individuals from non-carcinogenic, acute as well as chronic, effects of TCE in their drinking water. However, since the concentration based on carcinogenic effects is much lower, the PHG is based on the carcinogenic effects.

Carcinogenic Effects

For carcinogens, the following general equation was used to calculate the public health-protective concentration (C) for a carcinogenic chemical in drinking water (in mg/L):

$$C = \frac{BW \times R}{CSF \times W} = mg/L$$

where,

BW = adult body weight (a default of 70 kg);

R = *de minimis* level for theoretical lifetime excess individual cancer risk (a default of 10^{-6});

CSF = cancer slope factor, CSF, is a human equivalent potency derived from the lower 95 percent confidence limit on the 10 percent tumor dose (LED_{10}), CSF = 0.1/ LED_{10}, using $BW^{3/4}$ scaling from the animal data;

W = daily volume of water consumed in Leq/day, which accounts for multi-route exposures to volatile organic chemicals.

Potency estimates are provided for the LMS model in addition to estimates with the LED_{10} method (U.S. EPA, 2005e) for perspective. The LMS model focuses on the linear low dose extrapolation while the LED_{10} method places a higher premium on fitting the observed data to estimate the ED_{10} and the 95 percent lower bound (LED_{10}), the point from which the low dose extrapolation is made (U.S. EPA, 2005e).

Using the Gmean CSF from the AMET dose metric for mouse liver tumors of 0.0059 $(mg/kg\text{-}day)^{-1}$ from the NCI (1976) and the Maltoni *et al.* (1986) studies (Table 2), and a value of 7.1 Leq/day for water consumption (Bogen *et al.*, 1988), the water concentration corresponding to a negligible cancer risk, C, is calculated as follows:

$$C = \frac{70\ kg \times 10^{-6}}{0.0059\ (mg/kg\text{-}d)^{-1} \times 7.1\ Leq/day} = 0.00167\ mg/L = 1.7\ ppb$$

If we assumed that the dose response is nonlinear we could treat the LED_{10} for liver tumors as a chronic LOAEL. In this case appropriate uncertainty factors would be 10 for LOAEL to NOAEL, 3 for interspecies extrapolation (since the LED_{10} estimate incorporates an interspecies adjustment), 10 for interindividual variation, and 10 for severity of effect, possibly cancer, giving a total UF of 3,000. Note that the relative source contribution (RSC) is included in this calculation.

$$C = \frac{7.9 \text{ mg/kg-d} \times 70 \text{ kg} \times 0.2}{3{,}000 \times 7.1 \text{ Leq/day}} = 5.2 \times 10^{-3} \text{ mg/L} = 5 \text{ ppb}$$

Since the values for the cancer endpoint are lower than the values calculated for noncancer endpoints, the PHG is based on the more health-protective cancer endpoint. Of the dose response approaches for the cancer endpoint, the linear default is appropriate to use for TCE because of the evidence for genotoxic mechanisms of action of TCE and its metabolites. In view of the mandate in the California Safe Drinking Water Act, namely, in cases when "the currently available scientific data is insufficient to determine the amount of a contaminant that creates no significant risk to public health, the public health goal shall be set at a level that is protective of public health," OEHHA rejects the nonlinear approach to cancer risk estimation in this case. Thus, 1.7 ppb is hereby established as the PHG for TCE.

The public health-protective concentration of 1.7 ppb is lower than the current state and federal MCLs of 5 ppb, but higher than the PHG established in 1999 of 0.8 ppb. The public health-protective concentration is based on a re-analysis of the data sets used to calculate the previous published PHG (OEHHA, 1999) and the current OEHHA oral and inhalation potencies for TCE of 0.015 and 0.01 $(\text{mg/kg-day})^{-1}$ respectively (OEHHA, 2005). Re-analysis of the data using the more current versions of the cancer models resulted in a lower cancer potency of 0.0059 $(\text{mg/kg-day})^{-1}$, which in the end doubled the calculated PHG.

For TCE, emerging new science, expansion of limited data, and appropriate methods of risk assessment are being widely discussed. The outcome of these discussions may ultimately lead to changes in models or assumptions used for the TCE risk assessment. The PHG will be further revised as needed to respond to improvements in the science.

RISK CHARACTERIZATION

The calculated health-protective level of 1.7 ppb obtained from the CSF of 0.0059 $(\text{mg/kg-day})^{-1}$ and based on the geometric mean of CSF values of liver tumors from the NCI (1976) and Maltoni *et al.* (1986) studies using the AMET dose metric, is twice the published PHG. A *de minimis* theoretical excess individual cancer risk level of 10^{-6} was assumed in calculating this value for the PHG. The corresponding levels for cancer risk levels of 10^{-5} or 10^{-4} are 17 and 170 ppb, respectively.

Some of the uncertainties involved in this assessment of TCE are as follows:

- CSF based on the LED_{10} vs. the q_1^* based on the linearized multistage model. Since the Gmeans for the tumor site and dose metric of choice, i.e., liver by AMET, are similar, there is no difference between the methods. For lung tumors the CSF is 7 percent lower and for liver tumors using AUC metric the CSF is 23 percent higher. For the sum of lung and liver by AUC the CSF is 5 percent higher than a LMS based potency. Typically CSF and q_1^* values differ by 5-10 percent.

- Use of the AMET dose metric based CSF vs. the AUC-based values. The difference is a 2.5-fold greater estimate of theoretical extra lifetime risk for a single site. The AUC values are based on estimates made from published graphs of blood or tissue concentrations of metabolites versus time and are thus less accurate than the published AMET values. The AUC values are probably underestimated by 10 percent or more.
- Use of an average value vs. a CSF based on the most sensitive tumor site. The highest CSF calculated was 0.0365 and the lowest was 0.00021 $(mg/kg\text{-}day)^{-1}$, a 174-fold range. The selected value was six-fold less than the highest individual value and twenty eight-fold higher than the lowest individual value.
- Use of $(body\ weight)^{3/4}$ interspecies scaling vs. $(body\ weight)^{2/3}$ scaling, no scaling, or the use of a human PBPK model. Scaling from mouse to human using the ¾ power increases the CSF value about 6.5-fold vs. no scaling, whereas scaling using the 2/3 power would increase the value by 13-fold. The scaling factor is used to account for metabolic differences between rodents and humans where humans are generally considered the more sensitive species. Fisher (1993) used a human PBPK model and the AMET dose metric described above to estimate a safe drinking water TCE concentration of 7 ppb (see also Fisher and Allen, 1993; Allen and Fisher, 1993). The methods of Fisher and Allen addressed pharmacokinetic differences in the interspecies extrapolation but not possible pharmacodynamic differences or significant interindividual differences (see Fisher *et al.*, 1998).
- Use of linear dose response for cancer risk assessment vs. a nonlinear approach. Controversy over the quantitative treatment of hepatic tumorigenesis continues. If instead of the linear approach we applied the nonlinear approach as described above we would obtain a value of 5 ppb or three-fold higher than the PHG value (1.7 ppb). In this calculation an uncertainty factor of 3,000 was applied to the LED_{10} of 7.9 mg/kg-day (see above).
- Use of animal data vs. human data. The estimates based on human data including sensitive subpopulations with specific glutathione S-transferase (GST) polymorphisms range from three-fold higher to two-fold lower CSFs compared to the mouse-based values. The human exposure estimates are too uncertain to rely on fully, but are in fair agreement with the mouse-based CSF. In view of the uncertainty in the human exposure data, it is an open question whether mouse liver-based CSF estimates adequately protect humans against TCE-induced renal cancer. In general, tumor concordance between species is poor. In addition, the GST polymorphisms have only been studied in limited subpopulations and there may be other sensitivity factors besides GST polymorphisms related to age and disease conditions.

Several other points have been considered in the assessment, including synergistic or antagonist interactions, volatility of TCE, and relative source contribution for TCE. Regarding the synergistic or antagonistic interactions TCE may have with other chemicals (e.g., diethylhexyl phthalate or 1,1-DCE), these effects were noted at higher doses but were considered unlikely to be relevant to drinking water exposures to TCE. However

this is an area of limited data and further studies need to be conducted with other common water contaminants.

Regarding volatility, TCE is a volatile organic chemical (VOC) and can be expected to result in oral and dermal doses related to showering, bathing, flushing toilets and other typical household uses of tap water. An estimate of such exposures is included in the calculation of the PHG. The range of estimates from Bogen *et al*. (1988) is 2.2 L/day for ingestion of water only, to 18.3 Leq/day for the upper bound on the sum of ingestion (3.8 L/day), inhalation, and dermal absorption. Predictions of the CalTOX™ program ranged from total intakes of 4.2 Leq/day for average exposure to 26.5 Leq/d for high exposure. The 7.1 Leq/day value derived by Bogen *et al.* (1988) appeared to be the best-justified and most appropriate multi-route exposure estimate.

The RSC chosen for TCE is the 20 percent default, indicating significant anticipated exposure via other sources besides drinking water, notably ambient air and food. A more detailed exposure assessment could possibly result in a higher RSC value, but it is judged that the data are inadequate for a comprehensive evaluation.

For PHGs, our use of the RSC has, with some exceptions, followed U.S. EPA drinking water risk assessment methodology. For noncarcinogens, the U.S. EPA reference doses (RfDs, in mg/kg-day), drinking water equivalent levels (DWELs, in mg/L) and maximum contaminant level goals (MCLGs, in mg/L) are calculated using uncertainty factors (UFs), body weights and water consumption rates (L/day) and the RSC respectively. The RSC defaults are 20 percent for agents with expected non-water sources, but could be up to 80 percent for agents whose anticipated exposure is judged to be almost exclusively from water; other values may be used depending on the strength of scientific evidence supporting them.

For approaches that use low-dose extrapolation based on quantitative risk assessment, U.S. EPA does not factor in an RSC. The use of low-dose extrapolation is considered by U.S. EPA to be adequately health-protective without the additional source contributions. In developing PHGs, we have adopted the assumption that RSCs should not be factored in for carcinogens grouped in U.S. EPA categories A and B, and for C carcinogens for which we have calculated cancer potency based on low-dose extrapolation. This is an area of uncertainty and scientific debate and it is not clear how this assumption impacts the overall health risk assessment.

Based on the available animal and human data, the best approach and limitations for each tumor type have been evaluated, considering in-depth the studies published since the previous PHG review. In 2000, up-to-date reviews on the carcinogenicity of TCE and MOA were provided in a supplemental issue of Environmental Health Perspectives (Bull, 2000; Chen, 2000; Green, 2000; Moore and Harrington-Brock, 2000; Rhomberg, 2000; Wartenburg *et al.*, 2000, etc.), summarized by Scott and Cogliano (2000). Moore and Harrington-Brock (2000) addressed whether TCE or its metabolites caused a direct gene expression or DNA mutations in order to achieve the tumorigenic endpoint. Quantitative dose-response issues important to the statistical modeling of both noncarcinogenic and carcinogenic effects were also updated in these reviews (Barton and Clewell, 2000; Bois, 2000a,b; Boyes *et al.*, 2000; Chen, 2000; Clewell *et al.*, 2000; Fisher, 2000; and

Rhomberg, 2000). Chen (2000) used a biologically based model to explore the relationship between TCE and two of its oxidative metabolites (DCA and TCA) under the hypothesis that these chemicals induce liver tumors in mice through promotion of preexisting initiated cells. The authors suggest that DCA alone could be responsible for all the carcinomas in liver of $B6CF_1$ mice and that low-dose risk estimates to humans would be overestimated unless the different background rates between mice and humans are properly taken into account.

In 2001, U.S. EPA reviewed the existing animal and human data on TCE, and provided a draft risk assessment for TCE (U.S. EPA, 2001). This document underwent peer review and received extensive comments from the public. As a result of the issues raised by the panel of independent scientists through U.S. EPA's Science Advisory Board and public comments, U.S. EPA in cooperation with the Department of Defense, Department of Energy, and the National Aeronautics and Space Administration requested a consultation on these issues with an expert panel convened by the NAS Board on Environmental Studies and Toxicology in 2004.

The charge to the NAS Expert Panel committee was to highlight issues critical to the development of an objective, realistic, and scientifically balanced TCE health risk assessment. The committee was to focus on the issues of hazard characterization/mode of action for TCE toxicity; possible approaches to synthesize epidemiological data in informing the hazard characterization of TCE; differential susceptibility in different subpopulations or life-stages; the evidence for effects from TCE exposures alone compared with that for effects from mixtures of chemicals that include TCE; PBPK modeling; dose-response assessment; and quantitative assessment of both cancer and non-cancer risks. Special attention was to be given to the availability of appropriate data and methods to implement the committee's advice, as well as the distinction between data analysis and data generation. The committee was to distinguish between issues that can be addressed through short-term analyses and issues that are more appropriately addressed through medium- or long-term research projects. U.S. EPA provided four papers on these issues that gave an overview of the latest information in order to assist the NAS Panel (U.S. EPA, 2005a,b,c,d).

The NAS panel concluded in its report (NAS, 2006) that U.S. EPA (and other federal agencies) should "finalize their risk assessment with currently available data so that risk management decisions can be made expeditiously." The panel stated that "*The default linear extrapolation procedure suggested in the EPA cancer risk assessment guidance can be applied but should first be explicitly defined.*" After receiving the advice from the NAS, along with comments from the U.S. EPA Science Advisory Board and the public, as well as recently published scientific literature, U.S. EPA plans to incorporate the findings into a revised risk assessment of TCE. The revised TCE risk assessment will then undergo both external peer review and public comment.

OEHHA is following a similar process with the present risk assessment. We acknowledge the active research in hepatotoxic mechanisms relevant to TCE and other chemicals. In addition, OEHHA will keep abreast of the ongoing process at U.S. EPA and incorporate any useful new insights or data into subsequent revisions of our PHG for TCE.

OTHER REGULATORY STANDARDS

No changes to other regulatory standards were found. The U.S. EPA has a maximum contaminant level (MCL) of 0.005 mg/L (5 ppb) and a maximum contaminant level goal (MCLG) of zero mg/L for TCE, based on cancer risk. U.S. EPA has also set a drinking water equivalent level (DWEL) of 0.3 mg/L and a $1x10^{-4}$ cancer risk level of 0.3 mg/L. In their draft Health Risk Assessment document, an oral reference dose (RfD) of $3x10^{-4}$ mg/kg-d was developed based on critical effects in the liver, kidney, and developing fetus (U.S. EPA, 2001). An inhalation reference concentration (RfC) of $4x10^{-2}$ mg/m^3 was developed based on critical effects in the central nervous system, liver, and endocrine system. In addition, several cancer slope factors were developed, with most between $2x10^{-2}$ and $4x10^{-1}$ (mg/kg-d)$^{-1}$.

However, the document has not been finalized. U.S. EPA is currently reassessing TCE for oral and inhalation reference doses, cancer classification, etc. U.S. EPA plans to incorporate the advice from the NAS, along with comments from the U.S. EPA Science Advisory Board, the public and recent published scientific literature, into a revised TCE health risk assessment. This revised assessment will then undergo both external peer review and public comment prior to being completed.

The California Department of Health Services (DHS) promulgated a California primary drinking water standard (MCL) of 0.005 mg/L (5 ppb) for TCE in 1989 (Section 64444, California Health and Safety Code). Under Proposition 65, risk specific intake levels of 50 μg/day for ingestion and 80 μg/day for inhalation have been established for TCE (DHS, 1990a; OEHHA, 2007). Although the MCL is over 6 times higher than the existing PHG, the DHS position, as stated in the "MCL Evaluation for Trichloroethylene" (December, 2001; www.dhs.ca.gov/ps/ddwem/chemicals/PHGs/TCEMCLreport12-01.pdf), was: "Depending on the particular exposure scenario, animal study, and tumor site selected in the PHG risk analysis, the range of drinking water concentrations within the *de minimis* level (10^{-6}) ranges from 0.1 to 64 ppb. Since the 5 ppb MCL is within that range, it meets the acceptable risk level of 10^{-4} to 10^{-6} that Federal and State regulatory agencies use for establishing drinking water MCLs to protect public health." However, DHS (now the Department of Public Health, or DPH) subsequently developed a draft cost benefit analysis of possible MCL revisions. This effort was suspended in 2004 when OEHHA announced its initiation of re-review for the PHG (DPH, 2008).

The Agency for Toxic Substances and Disease Registry has derived an acute duration inhalation minimal risk level (MRL) of 2 ppm with an uncertainty factor of 30 for TCE based on neurological effects in humans (Stewart *et al.*, 1970), and an intermediate duration MRL of 0.1 ppm with an uncertainty factor of 300, based on neurological effects in rats (Arito *et al.*, 1994). An acute duration oral MRL of 0.2 mg/kg-day with an uncertainty factor of 300 was derived based on developmental effects in mice (Fredriksson *et al.*, 1993; ATSDR, 1997).

The International Agency for Research on Cancer (IARC) designated TCE as Group 2A, probably carcinogenic to humans (IARC, 1995).

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