OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Air Toxics Hot Spots Program

Perchloroethylene Inhalation Cancer Unit Risk Factor

Technical Support Document for Cancer Potency Factors

Appendix B

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Air, Community, and Environmental Research Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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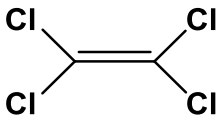
List of Acronyms

AIC	Akaike information criterion	MCL	Mononuclear cell leukemia
AUC	Area under the concentration curve	MCMC	Markov Chain Monte Carlo method
BMD	Benchmark Dose	mg-hr/(L-d)	Milligram-hours per liter per day
BMDL	Estimation of the BMD 95% lower	mg/kg-d	Milligram per kilogram per day
BMDS	Benchmark Dose Software	$\mu g/m^3$	Microgram per cubic meter
BW	Body weight	MLE	Maximum likelihood estimate
CDPH	California Department of Health Services	MOA	Mode of action
CYP450	Cytochrome P450	N-AcTCVC	N-acetyl-S-(1,2,2-trichlorovinyl)-cysteine
DCA	Dichloroacetic acid	NAT	N-acetyl transferase
DEHP	Diethyl hexyl phthalate	NCI	National Cancer Institute
DNA	Deoxyribose nucleic acid	NRC	National Research Council
DCVG	S-(1,2-dichlorovinyl)-glutathione	NTP	National Toxicology Program
FMO3	Flavin-containing mono-oxygenase 3	PBPK	Physiologically-based pharmacokinetic
GLP	Good Laboratory Practice standards	PCE	Perchloroethylene
GSH	Glutathione	PHG	Public Health Goal
GST	Glutathione-S-transferase	PPARα	Peroxisome proliferator-activated receptor-α
HEC	Human equivalent concentration	ppb	Parts per billion
IARC	International Agency for Research on Cancer	ppm	Parts per million
IRIS	Integrated risk information system (US EPA)	TAC	Toxic Air Contaminant
JBRC	Japan Bioassay Research Center	TCA	Trichloroacetic acid
JISHA	Japan Industrial Safety and Health Association	TCE	Trichloroethylene
LGLL	Large granular lymphocyte leukemia	TCVC	S-(trichlorovinyl)cysteine
		TCVG	S-(1,2,2-trichlorovinyl)-glutathione
		TSD	Technical Support Document
		URF	Unit risk factor
		US EPA	U.S. Environmental Protection Agency
		VOC	Volatile organic compound

PERCHLOROETHYLENE



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1. INTRODUCTION

The Office of Environmental Health Hazard Assessment (OEHHA) develops potency values for carcinogenic substances that are candidate Toxic Air Contaminants (TACs) (Health and Safety Code Section 39660) or are listed under the Air Toxics Hot Spots Act (Health and Safety Code Section 44321). These values are used in the Air Resources Board's (ARB's) air toxics control programs and also by other State regulatory bodies, to estimate cancer risk in humans.

Perchloroethylene (PCE), also commonly referred to as tetrachloroethylene, was officially placed on the TAC list by the ARB in 1991. In support of that decision, the California Department of Health Services evaluated the toxicology of PCE and determined that it was a potential carcinogen in humans, besides displaying other forms of toxicity (CDHS, 1991). Shortly thereafter, OEHHA derived inhalation potency values for PCE using dose-response data from a National Toxicology Program (NTP) study of the chemical's carcinogenic effects in rodents (OEHHA, 1992; NTP, 1986). OEHHA's potency values were based upon the induction of liver tumors in male mice and incorporated a simple pharmacokinetic model to estimate internal metabolized doses.

The present document updates the dose-response analysis for inhalation exposure to PCE and derives a cancer unit risk factor (expressed as $(\mu g/m^3)^{-1}$) and corresponding cancer slope factor (expressed in $(mg/kg\text{-}d)^{-1}$) using OEHHA's current Air Toxics Hot Spots program risk assessment guidelines (OEHHA, 2009), as well as research made available since our last PCE review in 1992. In particular, OEHHA has identified an additional well-conducted, lifetime rodent inhalation study (JISHA, 1993); also, a refined physiologically-based pharmacokinetic (PBPK) model for PCE has been published (Chiu and Ginsberg, 2011). Both of these studies were used in the update. Where appropriate, the current analysis draws upon material from previous OEHHA evaluations, as well as recent toxicological assessments published by the US Environmental Protection Agency (US EPA, 2012a) and the International Agency for Research on Cancer (IARC, 2014).

2. SUMMARY OF DERIVED VALUES

OEHHA's revised potency values for PCE are based on the elevated incidence of several tumor types observed in male mice and rats in relation to PCE-metabolized doses calculated with a simplified adaptation of the Chiu and Ginsberg (2011) model. For dose-response calculations, OEHHA used US EPA's Benchmark Dose Software (BMDS) (US EPA, 2015) and its implementation of the multi-stage cancer model. BMDS was also

used to evaluate the multi-site tumor risks. After considering several issues related to data quality and analytical uncertainty, the geometric mean of 4 dose-response values was chosen as the best estimate of carcinogenic potency. The potency values for PCE, in terms of external exposure, are:

Unit Risk Factor $(\mu g/m^3)^{-1}$	6.1E-06
Slope Factor $(mg/kg\text{-}day)^{-1}$	2.1E-02

3. MAJOR SOURCES AND USES

PCE is a dense volatile liquid with an ether-like odor. It is used mainly as a chemical intermediate, solvent, and cleaning agent. The total US demand for PCE in 2004 was 355 million pounds (Dow, 2008). In the US, 60 percent of PCE use was for chemical production (e.g., to make hydrofluorocarbon alternatives to chlorofluorocarbons), 18 percent was used in surface preparation and cleaning, 18 percent in dry-cleaning and textile processing, and 4 percent for miscellaneous other uses (*ibid.*). Total air emissions of PCE in California for 2010 were estimated by ARB to be 3832 tons per year (ARB, 2012).

4. SELECTED PHYSICAL AND CHEMICAL PROPERTIES OF PCE

Molecular weight	165.83
Boiling point	121 ^oC
Melting point	-19 ^oC
Vapor pressure	18.47 mm Hg @ 25 ^oC
Air concentration conversion	1 ppm = 6.78 mg/m^3 @ 25 ^oC

(HSDB, 2010)

5. NATIONAL AND INTERNATIONAL HAZARD EVALUATIONS

According to the National Toxicology Program (NTP) 13th Report on Carcinogens (RoC), PCE is "reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals" (NTP, 2014). The RoC found that PCE exposure produced tumors in multiple tissue types of both sexes of mice and rats. For inhalation exposure, the tumor types cited by NTP were: mononuclear-cell leukemia in rats, tubular-cell kidney tumors in male rats and liver tumors in mice. Additionally, NTP noted increased liver tumors in mice exposed to PCE by ingestion.

IARC found that PCE is "probably carcinogenic to humans," citing limited epidemiological findings (primarily increased bladder cancer in dry cleaning workers) and sufficient evidence in experimental animals (IARC, 2014). For rodents, in addition to the tumor types listed by NTP, IARC notes an increased incidence of: hemangioma and hemangiosarcoma of the liver in mice, spleen and Harderian gland tumors in male mice,

brain and testicular tumors in male rats, and skin tumors in mice dermally exposed to the PCE metabolite, tetrachloroethylene oxide.

US EPA states that PCE is “likely to be carcinogenic in humans by all routes of exposure,” based upon suggestive epidemiologic data (bladder cancer, non-Hodgkin’s lymphoma, and multiple myeloma) and conclusive evidence from carcinogenicity studies in rodents (referring to the same set of tumors as above) (US EPA, 2012b).

PCE has been listed on California’s Proposition 65 list since 1988 as a chemical "known to the state to cause cancer." California’s Public Health Goal for drinking water is based on PCE-induced carcinogenicity (OEHHA, 2001).

6. TOXICOKINETICS

PCE is readily absorbed through the lungs and gastrointestinal tract, and can also be absorbed to a lesser extent through the skin. The blood-air partition coefficients of PCE in humans and rodents are in the range of about 15 to 20 (Chiu and Ginsberg, 2011). These values indicate the ratio by which the PCE concentration in blood will be greater than its concentration in air at equilibrium. Humans breathing air containing 100 ppm PCE over 8 hours absorbed approximately 70 percent of inhaled PCE after the first hour, and 50 percent of the PCE intake at the end of the exposure period (Fernandez, *et al.*, 1976). Once in the body, PCE disperses into all tissues, concentrating preferentially in fatty tissues. For example, in rats inhaling 500 ppm PCE for 2 hours, the area under the concentration curve (AUC) after 72 hours, in milligram-minutes per milliliter of tissue, was: 1493 (fat), 33 (brain), 31 (liver), 26 (kidney), and 8.4 (blood) (Dallas, *et al.*, 1994).

PCE has a relatively low rate of metabolism in rodents and humans and is primarily eliminated unchanged via exhalation. In rats exposed to 150 ppm PCE in drinking water for 12 hours and monitored for an additional 72 hours, approximately 88% of the body burden was eliminated unmetabolized by exhalation (Frantz and Watanabe, 1983). Ohtsuki, *et al.* (1983) monitored occupationally exposed dry-cleaning workers and estimated that at the end of an 8-hour exposure to 50 ppm, about 38% of absorbed PCE was exhaled unchanged and 2% metabolized and excreted in urine.

PCE Metabolites

The metabolism of perchloroethylene has been studied mostly in mice, rats, and humans. Detailed reviews of this literature have been published (Lash and Parker, 2001; Anders *et al.*, 1988; Dekant, 1986). Briefly, rodent studies have identified the following urinary metabolites:

- trichloroacetic acid (TCA)
- N-trichloroacetyl aminoethanol
- oxalic acid
- N-oxalylaminoethanol
- dichloroacetic acid (DCA)

- S-(1,2,2-trichlorovinyl)glutathione (TCVG)
- N-acetyl-S-(1,2,2-trichlorovinyl)cysteine (N-AcTCVC)

Trichloroacetic acid and N-AcTCVC have also been observed in the urine of exposed humans. The aminoethanol derivatives, N-trichloroacetyl aminoethanol and oxalyl aminoethanol, are thought to arise from the reaction of the intermediate acyl chlorides with phosphatidyl ethanolamine present in biological membranes (Dekant, *et al.*, 1986). Carbon dioxide has also been found as an exhaled metabolite. Trichloroethanol has been detected in urine samples in some studies, but not in others, and it is unclear whether it was produced from co-exposure to trichloroethylene (in occupational exposures), or in other cases, if it was an artifact of the analytical methods employed (Lash and Parker, 2001). More recent work (e.g., Yoshioka, *et al.*, 2002) has not detected trichloroethanol and supports the conclusion that it is not a significant PCE metabolite (US EPA, 2012a).

Metabolic Pathways

A simplified metabolic scheme for PCE is presented in Figure 1. Two main pathways of metabolism have been identified. The first, referred to here as the "oxidative pathway," involves initial oxidation of PCE by Cytochrome P450 (CYP450) enzymes. The second "GST pathway" is initiated by glutathione-S-transferase (GST)-catalyzed conjugation of PCE with glutathione (GSH).

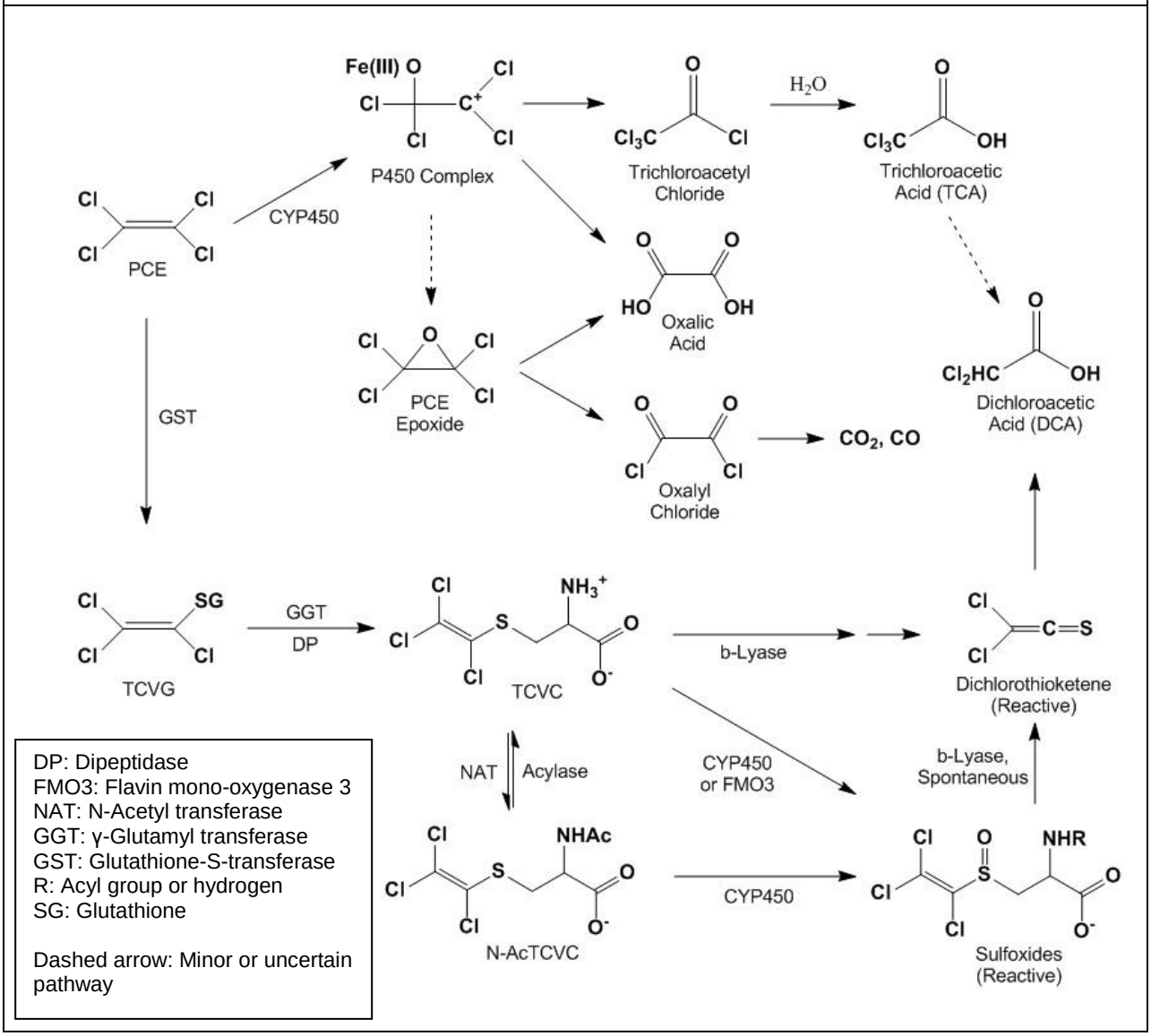
In the oxidative pathway the primary CYP450 isoform involved is thought to be CYP2E1, based upon analogy with other small halogenated molecules, with additional participation of isoforms 2B1/2, and 3A (Lash and Parker, 2001). The main, chemically stable metabolic product of oxidation is trichloroacetic acid (TCA), formed by hydrolysis of the reactive intermediate trichloroacetyl chloride, the latter of which appears to be formed by molecular rearrangement of the substrate-CYP450 complex (Guyton, *et al.*, 2014). A secondary product is the reactive tetrachloroethylene oxide (PCE epoxide), which decomposes to oxalyl chloride and then to carbon monoxide and carbon dioxide (Yoshioka, *et al.*, 2002). Oxalic acid may also form from decomposition of PCE epoxide or directly from the substrate-enzyme complex (Guyton *et al.*, 2014).

The liver is considered the main site of metabolism for the oxidative pathway, although other tissues with appropriate CYP450 activity, e.g., lung, kidney, brain, and lymphocytes,[1] may oxidize PCE to a smaller extent.

The initial step in the GST pathway produces the conjugate, S-(trichlorovinyl)glutathione (TCVG). The tripeptide glutathione moiety of TCVG can then be degraded via enzymatic cleavage of its glycine and glutamine units, producing S-(trichlorovinyl)-cysteine (TCVC). TCVC may be subsequently transformed as follows:

[1] Lymphocyte microsomes from male Wistar rats have been found to contain CYP450 2B, 2E, and 3A activity at 20, 4, and 2.4 percent of liver microsomal activity. Lymphocyte CYP450 content can also be chemically induced, resulting in 2 to 4-fold increases in activity (Hannon-Fletcher and Barnett, 2008).

Figure 1: Simplified Metabolic Scheme for PCE [(a)]



(a) From Guyton *et al.* (2014), US EPA (2012a), and Lash and Parker (2001).

- The free amino group of TCVC may be acylated by N-acetyl transferase, forming N-acetyl-S-(trichlorovinyl)cysteine (N-AcTCVC) which passes into urine; this process may also be reversed by acylases, regenerating TCVC.
- The sulfur atom of TCVC and N-AcTCVC may be oxidized by CYP450 or flavin-containing mono-oxygenase 3 (FMO3); this process forms reactive α,β-unsaturated sulfoxides that can bond with nucleophilic biological molecules or spontaneously decompose to dichlorothioketene, itself a reactive metabolite.
- The carbon-sulfur bond of TCVC may be cleaved by β-lyase, releasing an unstable trichlorovinyl thiol that spontaneously decomposes to dichlorothioketene.

Dichloroacetic acid, believed to arise mainly by hydrolysis of dichlorothioketene, was found in rat but not human urine. Evidence for this mechanism comes from the detection of a covalent protein adduct, N-(dichloroacetyl)-L-lysine, in rat kidney cells (Birner *et al.*, 1994).

Multi-Organ Metabolism in the GST Pathway

The GST pathway involves a series of enzymatic transformations with cycling of metabolic intermediates mainly between the liver and kidney, and including some entero-hepatic processing. The initial glutathione conjugation step occurs primarily in the liver, forming TCVG which is transported to the blood and bile. The kidney epithelium actively absorbs the circulating conjugate from blood for further processing and excretion. As noted above, this involves cleavage of TCVG by gamma glutamyl transferase (GGT) and dipeptidase (DP) to form TCVC. The amino group of TCVC can then be acylated to form mercapturate N-AcTCVC in the kidney, or TCVC may recirculate back to the liver for acylation (Lash and Parker, 2001).

In some species, such as rabbit and guinea pig, significant intrahepatic processing of glutathione conjugates may occur, with formation of TCVC from TCVG by the bile-duct epithelium, followed by reabsorption into hepatocytes and subsequent acylation. Additionally, TCVG excreted via the bile can be converted to TCVC in the intestinal lumen and undergo entero-hepatic cycling (Hinchman and Ballatori, 1994; Irving and Elfarra, 2013).

The kidney is viewed as the main site for formation of genotoxic metabolites by β-lyase cleavage of TCVC since β-lyase activity is relatively high in this organ. Smaller amounts of β-lyase have been found in other organs, such as the liver, brain, and spleen (Rooseboom, *et al.*, 2002), raising the possibility that reactive dichlorothioketene may be generated and produce genetic damage in other tissues independent of its production in the kidney. Although the liver contains a form of β-lyase, enzymatic cleavage of TCVC does not appear to be toxicologically significant in this organ. For example, in rats treated with the PCE-conjugate analogues, dichlorovinyl glutathione (DCVG) and dichlorovinyl cysteine (DCVC), significant pathology was observed in the kidney, but no tissue damage was seen in the liver (Lash and Parker, 2001).

Oxidation of TCVC and N-AcTCVC to the reactive α,β-unsaturated sulfoxides can occur in the liver and kidney, as well as other organs that contain flavin mono-oxygenase 3 (FMO3) or CYP450 3A activity. As noted above, the sulfoxides are reactive Michael acceptors and can bond with nucleophilic sites on biological molecules. Discussing the metabolism of trichloroethylene (TCE), Irving and Elfarra (2012) noted that the α,β-unsaturated sulfoxides formed in the GST pathway may be further conjugated with glutathione, but that this process could also be reversible (by retro-Michael addition). This would create a mechanism by which the reactive sulfoxides could circulate in a stabilized form through the blood to other organs where they may be regenerated. The mechanism would likely be operative for PCE as well.

Pharmacokinetic Model

Numerous physiologically based pharmacokinetic (PBPK) models have been proposed for PCE over the course of several decades. Reddy (2005), Clewell (2005), and US EPA (2012a) have reviewed this body of research. Although the models are reasonably consistent in estimating PCE blood concentrations, they differ widely in their predictions of metabolized PCE at lower exposure concentrations. For example, at an inhaled concentration of 1 ppb, some models predict about 1 or 2 percent metabolism, while others predict metabolism in the range of 20 to 35 percent, and perhaps as high as 60 percent (Chiu and Ginsberg, 2011). Since PCE's carcinogenic potency is likely to depend upon the formation of genotoxic metabolic products, the wide range of estimated PCE metabolism among models has been a recognized problem for assessing the cancer risk from low-level PCE exposure.

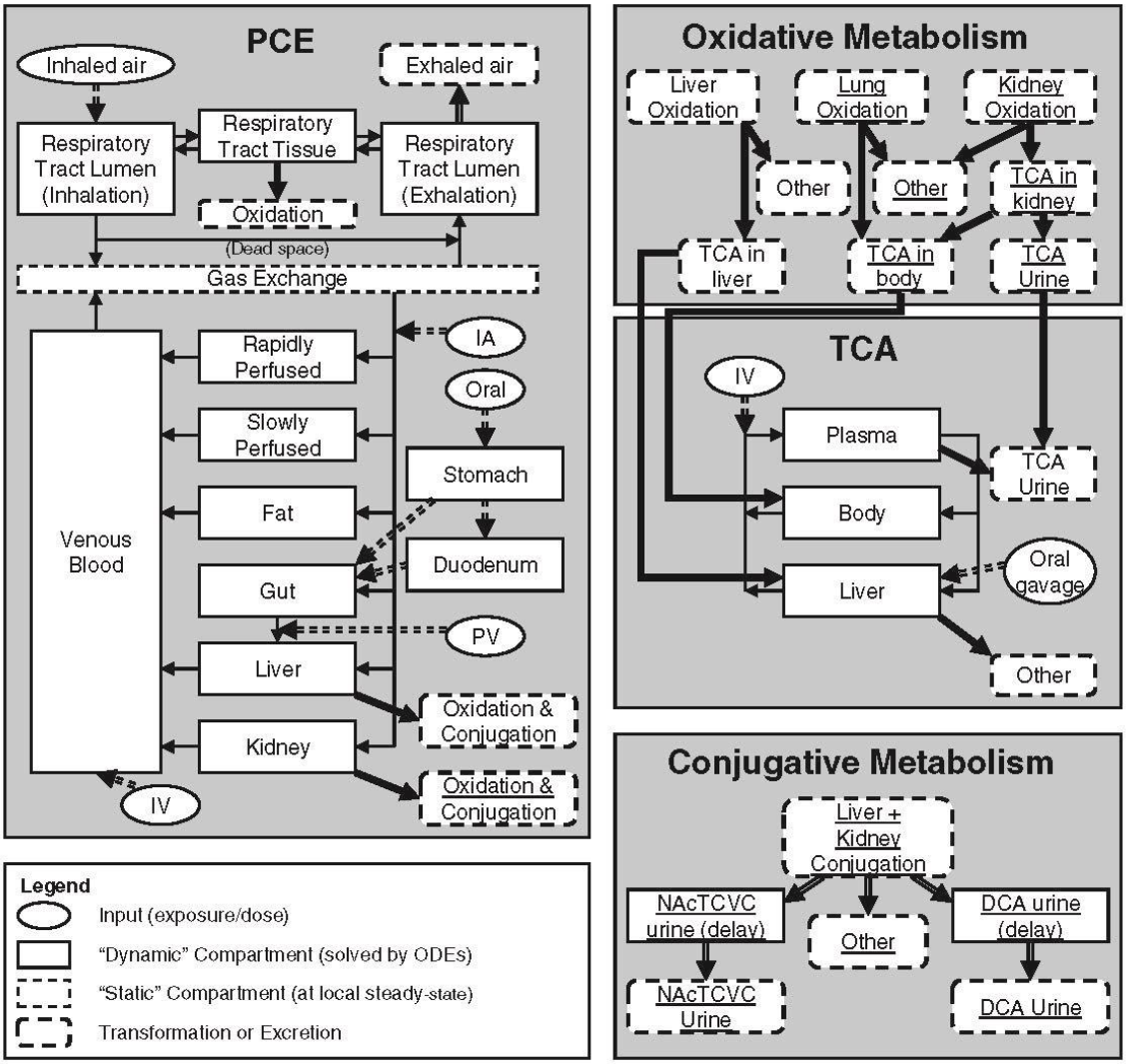
The most recent and comprehensive PBPK model for PCE is that of Chiu and Ginsberg (2011). It was developed following the recommendations of the National Research Council (NRC, 2010) that the available models for PCE be integrated into a single harmonized model incorporating various improvements. The Chiu and Ginsberg (2011) model incorporates lung, liver, kidney, fat, and venous blood compartments, and lumped compartments for rapidly and slowly perfused tissues. It has components for simulating inhalation, oral, and injection exposures.[2] Absorption-desorption of PCE in the upper respiratory tract (i.e., the "wash-in/wash-out" effect) is also taken into account. The rate of PCE oxidation is modeled in liver, kidney and lung, and GSH conjugation is modeled in the liver and kidney. The model can estimate (for example): concentrations of PCE in exhaled air, blood concentrations of PCE and TCA, and urinary excretion of TCA and the GSH-conjugation metabolites, N-AcTCVC and DCA. A graphical representation of the Chiu and Ginsberg PBPK model is provided in Figure 2.

US EPA (2012a) used the Chiu and Ginsberg (2011) model to estimate internal dose metrics in its recent PCE cancer potency factor update, which included the development of a URF for inhalation exposures. The most important improvements of the Chiu and Ginsberg (2011) model, as discussed by the US EPA (2012a), are:

- It uses Bayesian Markov Chain Monte Carlo (MCMC) methodology to determine the most likely values (posterior modes) for key metabolic constants.
- The model considered all the available toxicokinetic data for PCE in mice, rats, and humans, and is calibrated using a wide range of *in vivo* toxicokinetic data.
- It is the first model to include a separate glutathione conjugation pathway.
- It incorporates recent information on TCA toxicokinetics from trichloroethylene modeling studies.

[2] Note that the Chiu and Ginsberg model does not include dermal absorption of PCE vapor, which is considered to be relatively small in rodents and humans. For example, McDougal et al. (1990) measured dermal absorption in rats exposed to 12,500 ppm PCE vapor and found that dermal uptake was about 3.5% of inhalation + dermal uptake. Percutaneous penetration of PCE vapor in humans exposed to 600 ppm was approximately 1% of pulmonary absorption (Riihimaki and Pfaffli, 1978).

Figure 2: Chiu and Ginsberg (2011) PBPK Model for PCE [(a)]



(a) Figure adapted from Chiu and Ginsberg (2011). IV = intravenous, IA = intra-arterial, PV = portal vein.

Chiu and Ginsberg (2011) used a hierarchical Bayesian population approach to obtain estimates of the posterior modes[3] for a subset of important PBPK model parameters including: the pulmonary ventilation rate, metabolic constants for oxidation and conjugation of PCE, and urinary excretion of metabolites. Other model parameters, such as partition coefficients and most of the physiological parameters, were fixed at baseline values chosen from the literature. Inclusion of several intake routes (e.g., inhalation, oral, and intravenous) allowed the model to be calibrated and evaluated against a wide variety of experimental *in vivo* data.

[3] These are also the maximum likelihood estimates (MLEs) since flat prior distributions were used in the model.

In the MCMC analysis, sampling variation was characterized by running multiple chains of length 5000 (retaining every 10th value) using randomly chosen starting conditions for each chain. For the rodent PBPK models, 24 independent MCMC chains were run, each producing a chain-specific, posterior mode estimate. The parameter set with highest overall posterior probability of all the chains was selected as the posterior mode of the optimized PBPK model. For the human model, 48 independent chains were used since preliminary analysis indicated a potential for multiple maxima.

Table 1 shows a summary of predictions for several types of dose metrics based on the optimized model for inhalation exposures, reported by Chiu and Ginsberg (2011). With respect to the PCE AUC and PCE oxidation metrics, the range of chain-specific values was less than 40% of the overall posterior mode estimates. For example, in the mouse model at 1 ppm exposure, the overall posterior mode for percent of PCE oxidized was 17.4% of intake, and the range of chain-specific posterior modes was 11.5% to 17.9%.[4]

The estimates for PCE conjugation were more variable (with the exception of the rat model). In mice exposed at 1 ppm, for example, the model predicts that 0.016% of PCE intake will be conjugated with a range of 0.0068% to 0.43%. In the human model, the overall posterior mode indicates that 9.4% of PCE intake is metabolized by GSH conjugation, with a range of 0.003% to 10%. The human model displayed an apparent bimodal distribution for the rate of GSH conjugation. Nonetheless, the most probable posterior mode was at the high end of estimated conjugation rates. Regarding the results for humans, Chiu and Ginsberg (2011) note that:

> "The parameter optimization procedure revealed two distinct modes in the rate of GSH conjugation — one with 'high' GSH conjugation (the overall posterior mode) and one with 'low' GSH conjugation (a number of the alternative posterior modes). The log-likelihood for the overall posterior mode with high GSH conjugation is 38 units higher than the alternative posterior modes with low GSH conjugation, which would be significant by any classical statistical test."

Chiu and Ginsberg (2011) were not able to determine how much of the spread in the human conjugation model was due to uncertainty or population variation, but pointed out that the distribution could represent actual variability given the large differences in GST activities displayed by humans. On the other hand, a high level of variability was not observed in metabolic studies of trichloroethylene (TCE). Lash *et al.* (1999) looked at rates of GSH conjugation of TCE in 40 ethnically and age-diverse, male and female human liver samples and found less than a 10-fold variation.

As noted above, US EPA (2012a) used Chiu and Ginsberg's model results to derive its updated PCE potency factors. However, because of the large range of model estimates for PCE conjugation, US EPA prioritized the dose metrics based on oxidative metabolism and PCE AUC in their final analysis.

[4] Ranges of MCMC chain-specific posterior modes are from Table S-8 of Chiu and Ginsberg, 2011.

Table 1: PCE Internal Dose Metrics from the Chiu and Ginsberg (2011) PBPK Model (and reproduced by the OEHHA model extract) [(a)]

Constant Inhalation Doses (posterior mode estimates)

Dose metric	Exposure Concentration (ppm)					Prediction Range (at 1 ppm)
	0.01	***1***	***10***	***100***	***1000***	
PCE AUC Blood	*(mg-hr)/(L-d) per ppm*					
Mouse	2.1	2.2	2.4	2.6	2.7	2.2-2.4
Rat	2.25	2.25	2.25	2.25	2.4	2.25-2.27
Human	2.0	2.0	2.0	2.0	2.0	2.0-2.4
PCE Oxidation	*Percent of intake that is oxidized*					
Mouse	18.8	17.4	11.8	7.3	6.6	11.5-17.9
Rat	4.2	4.2	4.1	3.3	1.1	3.9-4.2
Human	0.98	0.98	0.98	0.98	0.98	0.69-1.0
PCE Conjugation	*Percent of intake that is conjugated*					
Mouse	0.015	0.016	0.021	0.025	0.026	0.0068-0.43
Rat	0.31	0.31	0.31	0.32	0.335	0.20-0.50
Human [(b)]	9.4	9.4	9.4	9.4	9.3	0.003-10.0 (bimodal)[(b)]

(a) Values are from Chiu and Ginsberg (2011), Tables S-6 through S-8, and are also reproduced by OEHHA's inhalation-only model extract, at the presented level of significance.
(b) Values presented are for the most probable posterior mode.

Use of Chiu and Ginsberg (2011) Harmonized PBPK Model

Although there are unresolved issues related to the Chiu and Ginsberg model predictions for PCE's GST pathway, OEHHA considers the model to be the best available methodology for estimating dose metrics in the dose-response assessment. Regarding uncertainty in GSH conjugation, the Office evaluated the effect of including the GST pathway in the dose metric on the overall cancer potency analysis (see the following section).

The full Chiu and Ginsberg (2011) model contains large portions of code designed to perform the Bayesian MCMC simulation, which determined the posterior mode estimates for key PBPK parameters. Once obtained, the posterior modes can be used to forecast the most likely values for internal doses at various exposure concentrations.

For the inhalation potency evaluation, OEHHA relied on Chiu and Ginsberg's optimized PBPK model results. Since only dose metrics for inhalation exposures needed to be

estimated, the inhalation-relevant portion of the Chiu and Ginsberg (2011) model was extracted. Specifically, OEHHA: (1) identified the main inhalation components of the MC-Sim program obtained from the authors, (2) extracted the relevant equations and inputs from the model code and translated them from the MC-Sim language into Berkeley Madonna code, (3) ran the code using the optimized, Bayesian posterior mode parameters and other baseline values developed by Chiu and Ginsberg (2011), and (4) tested the output against the original model dose estimates reported in the Chiu and Ginsberg (2011) paper.

A graphic depicting OEHHA's inhalation-only model is presented in Figure 3. As in the original Chiu and Ginsberg model, it includes lung, liver, kidney, fat, and venous blood compartments, and lumped compartments for rapidly and slowly perfused tissues. The first transformation in the oxidative pathway is modeled in the lung, liver, and kidney, and the first step of the GST pathway is included for liver and kidney. Absorption-desorption of PCE in the upper respiratory tract is also included. The model adequately reproduced the predictions of the original Chiu and Ginsberg model for inhalation exposures: OEHHA's model extract reproduces the internal dose-metric values obtained by Chiu and Ginsberg (2011), as presented in Table 1.[5] The Berkeley Madonna model code for mouse, rat, and human is provided in Appendix A.

Uncertainty and/or Variation in the Model Estimates

Additional discussion of the uncertainty related to GSH conjugation, particularly in the human model, is provided here to support the choice of dose metric (presented later, in Section 9). Four issues are addressed as follows.

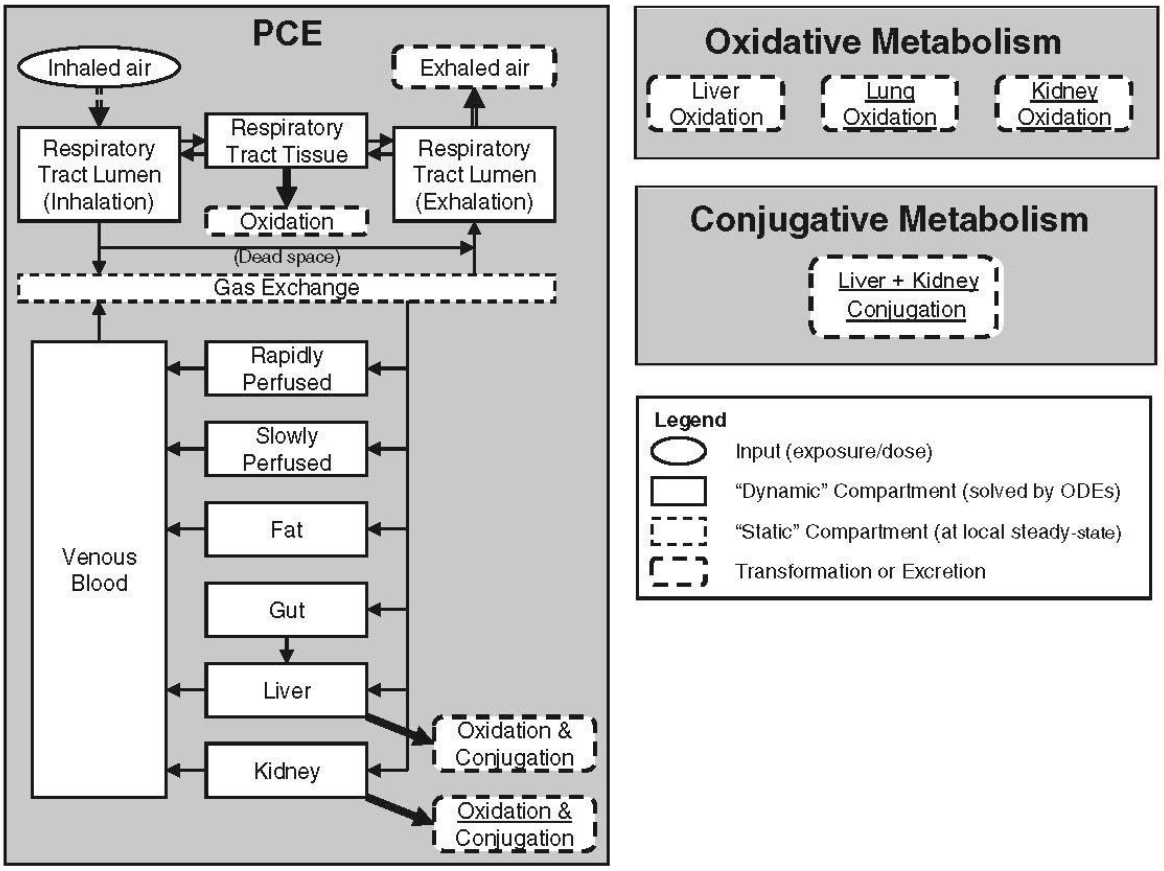
First, as noted above, the modeled rate of GSH conjugation in humans displayed a relatively high amount of uncertainty and/or variation: 0.003 -10%, with the overall posterior mode at 9.4% of intake. Commenting on this large range, Chiu and Ginsberg (2011) noted that the *in vivo* data available for model calibration were "inadequate to constrain the flux through this pathway, either extreme providing plausible fits to the data."

The overall posterior mode for PCE conjugation is, however, consistent with the *in vitro* rates for TCE and other halogenated VOCs that have been reported in the literature (e.g., Lash *et al.*, 1998; and Wheeler, *et al.*, 2001). The low value for PCE conjugation is consistent with the low-end of *in vitro* activity obtained for PCE by Dekant *et al.* (1998), which were below the analytical method detection limits.[6]

[5] OEHHA's model extract reproduced the dose-metric estimates reported in Tables S-6 through S-8 of the original Chiu and Ginsberg (2011) paper, typically to 3 significant figures.

[6] It should be noted that the *in vitro* GSH-conjugation data was not used to calibrate the model.

Figure 3: Inhalation-Only PBPK Model for PCE [(a)]



(a) Figure adapted from Chiu and Ginsberg (2011).

The large prediction range obtained in the human conjugation model raises a question - particularly with regard to interspecies dose extrapolation - of whether the model's GSH conjugation estimates should be used along with the PCE oxidation rates in a "total metabolized dose" metric. The alternative would be to define an internal dose metric using only the less-variable model predictions for PCE oxidation, as was done by US EPA (2012a).[7]

The impact of PBPK model uncertainty in this case is muted when both PCE oxidation and GSH conjugation are included together as a total metabolized dose, increasing the potency estimate by about one order of magnitude (much less than the range observed in the MCMC analysis).

[7] Omitting GSH-conjugation from the internal dose metric is similar to using the lower-likelihood mode for human GSH-conjugation in a total metabolized dose. With the lower mode, the rates of conjugation for humans, rats, and mice would all be small relative to PCE oxidation rates, and thus have little impact on both the dose-response calculations using the rodent data and interspecies dose extrapolation using the human PBPK model.

In order to demonstrate this, OEHHA compared the results of interspecies dose extrapolation using the human PBPK model with the two alternative dose metrics, i.e., using either total metabolism (GSH-conjugation + PCE oxidation) or PCE oxidation-only metabolism. We used the model to calculate human equivalent concentrations (HECs) from a range of example benchmark doses that could be obtained from the dose-response modeling of PCE exposure in rodents. As can be seen from the PBPK-derived HEC values presented in Table 2, the total metabolism dose metric produces HECs that are about 11-fold smaller than HECs obtained using an oxidation-only dose metric (Note that smaller HECs result in larger cancer potency factors).

Table 2: Impact of Internal Dose Metric Choice on Interspecies Conversion Calculations

Example Benchmark Doses [(a)] (mg/kg-d)	PBPK-Derived Human Equivalent Concentration (HEC; ppm)		Ratio of HECs
	Oxidative + GSH Conjugation	Oxidative Metabolism Only	
0.1	0.61	6.5	10.7
1.0	6.1	65.0	
3.0	18.2	195.0	

(a) Since oxidative metabolism is significantly greater than GSH conjugation in rodents, both dose metrics will produce similar benchmark doses in the rodent dose-response models. A rough HEC comparison can therefore be made on a single benchmark dose for both dose-metric scenarios.

Thus, it appears that using a dose-metric incorporating a "high-end" value for human GSH conjugation, as opposed to using an oxidation-only dose metric which effectively sets GSH-conjugation to zero, adds a relatively small amount of "conservatism" to the dose-response analysis. OEHHA has determined that inclusion of the GST pathway in a total metabolized dose metric ensures that the resulting potency values are adequate to protect public health, per the recommendations of our current Air Toxics Hotspots program risk assessment guidelines (OEHHA, 2009).

A second issue is whether using the model's uncertain estimate for glutathione conjugation in mice could have a large impact upon the dose-response calculation. As above, this question is addressed by looking at the difference between using either total metabolism or oxidation-only metabolism as the dose metric. In this case, the impact would be low. From Table 1, the model's posterior mode estimates of PCE oxidation and conjugation in mice indicate that oxidation dominates conjugation by factors of 290-1250, such that both dose metrics (total and oxidation-only) reflect mainly PCE oxidation, and should produce similar benchmark doses in a dose-response model.

Third, there is an unresolved disagreement regarding the large variation in the results of key *in vitro* studies that have estimated glutathione conjugation of PCE and TCE in rodent and human tissues. In its IRIS PCE review, the US EPA (2012a) pointed out:

> "The GSH pathway for tetrachloroethylene was originally demonstrated only in rodents, and interpretation of the then-existing data led some scientists to conclude that the pathway was not operative in humans (Green *et al.*, 1990). More recent data clearly demonstrate the existence of the pathway in humans (Schreiber *et al.*, 2002; Völkel *et al.*, 1998; Birner *et al.*, 1996) [...]
>
> "There are discrepancies regarding reported rates of tetrachloroethylene GSH metabolism (Lash *et al.*, 2007; Lash and Parker, 2001; Dekant *et al.*, 1998; Lash *et al.*, 1998; Green *et al.*, 1990). These differences may be due, in part, to different chemical assay methodology or to problems resulting from the stability of the chemical product being measured or both (Lash and Parker, 2001)."

Some of the *in vitro* studies predict relatively high TCVG (and DCVG) production rates in humans (e.g., Lash *et al.*, 1998; Lash *et al.*, 1999), while others indicate very low conjugation. For example, with TCE, Green *et al.* (1997) measured DCVG formation at 0.19 picomole per minute per milligram protein (pmol/min/mg) using human liver cytosol from 4 individuals. Conversely, Lash *et al.* (1999) measured TCE conjugation at 5,770 (pmol/min/mg) in human cytosolic protein pooled from 20 individuals. This large difference in measured GSH conjugation rates is reflected in the uncertainty/variability displayed by the Chiu and Ginsberg human model (and to a lesser extent, the mouse model).

Several commentators have raised doubts regarding the accuracy of the PCE and TCE conjugation rates reported by Lash *et al.* (1998, 1999, 2007), pointing to potential issues with the chemical analysis methods used by the laboratory. On the other hand, the apparent chemical instability of the GSH conjugates raises questions for studies that have measured low conjugate levels. However, no work has apparently been done to determine the true source of the discrepancy among the various divergent study results. The Lash laboratory has published several papers following Lash *et al.* (1998) involving the analysis of TCVG and DCVG, and has described various quality controls used ensure analytical accuracy.[8] Consistent results were generally obtained in these studies. On the other hand, Lash, *et al.* (2006) measured DCVG levels in blood and tissue samples of rats orally exposed to TCE and obtained a mixture of high and unexpectedly low values. However, the higher values obtained by Lash *et al.* (2006) were generally consistent with blood DCVG concentrations found in orally exposed mice by Kim *et al.* (2009), and with mouse tissue and serum concentrations measured by Yoo, *et al.* (2015), both using a different method of analysis.

Discrepancies in measured conjugation rates in humans might also be due to variable quality of the tissue samples used, and it is possible that some samples were not representative of the known variation in human GST activities. Thus, OEHHA does not

[8] For example, see Lash *et al.* (2007) and Lash, *et al.* (1999).

find convincing evidence to discount the high-end *in vitro* values for human glutathione conjugation of PCE, and estimated by the Chiu and Ginsberg (2011) PBPK model as well.

Finally, as Chiu and Ginsberg (2011) noted, a large part of the spread in estimated human conjugation rates may be due to biologic variation in the human population. Three important classes of GST enzyme have been identified in human liver cytosol: GSTA, GSTM, and GSTT. Both GSTM and GSTT have isoforms, GSTM-1 and GSTT-1, that are absent in a relatively large fraction of the population as a result of genetic variation (in this case due to gene deletion). Moyer *et al.* (2007) investigated the prevalence of GSTM-1 and GSTT-1 null individuals in the population and found frequencies of 50.5 - 78%, and 33.5 - 73.5%, respectively. Ginsberg *et al.* (2009) reported that some ethnic groups have high percentages of members that are null in both the GSTM-1 and GSTT-1 isoforms. For example, more than 30% of ethnic Chinese people appear to lack both enzymes.

It is currently not known which GSTs are most active in conjugating PCE. However, it appears that some low molecular weight halogenated hydrocarbons, such as dichloromethane, are primarily conjugated by GSTT-1. If PCE is a substrate mainly of GSTT-1 or GSTM-1, then the presence of many individuals lacking these enzymes would produce a large range of variability in the rate of PCE conjugation.

7. GENOTOXICITY AND CARCINOGENICITY

Genotoxicity

A large number of studies have tested the genotoxicity of PCE, and less frequently its metabolites, in microorganisms, mammalian cells, and in *Drosophila* and rodents. There have also been a few occupational exposure studies looking at genetic abnormalities in lymphocytes. This literature has recently been reviewed in detail by IARC (2014) and US EPA (2012a). Selected results based on these reviews and the literature are presented below.

PCE was not mutagenic in the Ames test with *S. typhimurium* or *E. coli* in the presence or absence of S9 metabolic activation. It was mutagenic, however, in *S. typhimurium* when tested with purified rat-liver GST, glutathione, and rat kidney fractions, where TCVG would be formed (Vamvakas, *et al.*, 1989). Most studies looking at chromosomal aberrations, micronuclei formation, or sister chromatid exchange have been negative, but micronuclei induction was seen in Chinese hamster ovary cells (Wang *et al.*, 2001) and human lymphoblastoid cells expressing CYP450 enzymes (White *et al.*, 2001). Genetic alterations have also been observed in rapidly growing yeast cell cultures (US EPA, 2012a).

Other types of tests, such as DNA strand break assays, DNA-adduct and cell transformation studies, and *Drosophila* mutation tests have provided mixed results. Positive findings include: Elevated DNA single-strand breaks in mouse liver and kidney *in vivo* (Walles, 1986), and DNA-adduct formation in mouse and rat tissues *in vivo* (Mazzullo, *et al.*, 1987).

Results from occupational studies have also been mixed. Ikeda *et al.* (1980) tested ten factory workers exposed to high (92 ppm PCE) or low (10-40 ppm) and found no evidence of cytogenetic damage to lymphocytes or altered cell cycle kinetics. No increase in sister chromatid exchanges in lymphocytes was found in a study of 27 subjects exposed to 10 ppm (geometric mean) of PCE (Seiji *et al.*, 1990). A decrease (not increase) of 8-hydroxy-deoxyguanosine, a marker of oxidative DNA damage, was observed in leukocytes of 38 female dry cleaners exposed to average concentrations of less than 5 ppm PCE (Toraason *et al.*, 2003). On the other hand, a study of 18 dry-cleaning workers exposed to 3.8 ppm PCE (average) found evidence of short-term genetic damage to peripheral blood lymphocytes, indicated by an increase in acentric chromosomal fragments (Tucker *et al.*, 2011).

Genotoxicity testing of various PCE metabolites includes the following positive results:

- TCA exhibited genotoxicity in several *in vivo* tests, for example: DNA strand breaks, chromosomal abnormalities, and micronucleus formation in mice; and chromosomal aberrations in chicken bone marrow (IARC, 2014; US EPA, 2012a).
- Genotoxicity has been demonstrated with DCA in the Ames test, micronucleus induction test, a mouse lymphoma assay, and *in vivo* cytogenetic tests; DCA has also been shown to cause DNA strand breaks *in vivo* in mouse and rat liver (*ibid.*).
- Trichloroacetyl chloride vapor tested positive in the Ames test with and without metabolic activation (DeMarini, *et al.*, 1994).
- PCE epoxide was mutagenic without metabolic activation in the Ames test with *S. typhimurium* TA1535 at the lower doses tested; toxicity occurred at higher doses (Kline *et al.*, 1982).
- TCVG incubated with rat kidney protein containing γ-glutamyl transpeptidase (GGT) and dipeptidases was mutagenic in the Ames test (Vamvakas, *et al.*, 1989).
- TCVC and N-AcTCVC tested positive in the Ames test without metabolic activation (Dekant *et al.*, 1986; Vamvakas, *et al.*, 1987).
- TCVC sulfoxide was mutagenic in the Ames test with *S. typhimurium* TA 100, but was 30-fold less potent than TCVC (Irving and Elfarra, 2013).

The GST-pathway metabolites, TCVG and TCVC, appear to be relatively potent mutagens based upon the available genotoxicity data (Lash and Parker, 2001).

In addition, several metabolites have been tested for carcinogenicity in animals. Dermal exposure of mice to PCE epoxide induced skin tumors (Van Duuren, *et al.,* 1983). Several long-term drinking-water bioassays of TCA and DCA in mice, with limited pathologic analysis of tissues other than the liver, found increases in hepatocellular tumors. Initiation–promotion studies with TCA or DCA in mice also demonstrated that

they promote liver tumors following initiation by other carcinogens (IARC, 2014; Guyton *et al.*, 2014).

Cancer Epidemiology

Numerous epidemiologic studies of PCE have been published, including more than 25 larger cohort and case-control studies since OEHHA's last toxicity review (*circa* 2000, for our PHG for drinking water). Several detailed reviews of the literature have recently been published (Guyton, *et al.*, 2014; IARC, 2014; and US EPA, 2012a).

Epidemiologic studies of PCE have all relied on semi-quantitative measures of exposure such as high/medium/low, ever/never exposed, or job categories. As such, the exposure data in this body of research are not of sufficient quality for use in quantitative dose-response analysis. However, it provides evidence that PCE causes cancer in humans and qualitatively supports the development of a unit risk value from animal studies. US EPA (2012a) evaluated the results of the cohort and case-control studies that developed more precise exposure assessments and concluded that PCE increases the risk of three types of cancer in humans: bladder cancer, non-Hodgkin's lymphoma (NHL), and multiple myeloma. IARC (2014) agreed with US EPA regarding bladder cancer, but concluded that the evidence for PCE inducing other cancers in humans was insufficient given the conflicting results across various studies. With non-Hodgkin's lymphoma, for example, "three cohort studies showed an increased risk based on small numbers, and the largest study with the best control of potential confounders did not. Case-control studies on non-Hodgkin lymphoma did not find significant associations" (*ibid.*).

A recent meta-analysis of bladder cancer risk in dry-cleaning workers (Vlaanderen, *et al.*, 2014) integrated the results of seven studies and 139 exposed cases, and found an overall relative risk level of about 1.5 for exposed versus non-exposed groups (with a 95% confidence level of 1.16 to 1.85).

Animal Studies of PCE

Increased tumor incidence was found in mice and rats in three long-term carcinogenicity studies of PCE. An oral study was conducted by the National Cancer Institute (NCI,1977), where $B6C3F_1$ mice and Osborne-Mendel rats were administered PCE in corn oil by gavage, 5 days/week for 78 weeks with additional follow-up of 32 weeks for rats and 12 weeks for mice. PCE caused a significant increase of hepatocellular carcinomas in mice of both sexes, and the tumors appeared considerably sooner in treated mice than in controls. Survival in the high dose groups was much lower than the control group at 40 to 45 weeks, and toxic nephropathy was observed in 93% of mice exposed. In rats, a high level of early mortality occurred in all treated groups, which obscured conclusions regarding carcinogenicity.

Two lifetime inhalation bioassays of PCE have also been published and are described as follows.

A lifetime inhalation cancer study was conducted by the Japan Bioassay Research Center (JBRC) of the Japan Industrial Safety and Health Association (JISHA, 1993). Good Laboratory Practice (GLP) standards were used in the conduct of the study. Dose-response data was analyzed by standard statistical procedures and study results were thoroughly documented in a manner similar to NTP rodent cancer study reports.

The study was conducted using F344/DuCrj rats and Crj:BDF_1 mice. Groups of 50 male and 50 female rats were exposed to PCE (99.0% pure) at 50, 200 or 600 ppm, and similar groups of mice were exposed to 10, 50, or 250 ppm, for 6 hours per day, 5 days per week, and 104 weeks. During the study period, the general status, body weight, and food consumption of the animals were monitored. Urinalyses, hematological, and blood chemistry tests were performed near the end of exposure for the surviving animals. Upon death, animals were necropsied and organ weights were determined. Histopathologic examination of all major tissue types was performed on all animals. Survival was good for both sexes of rats and mice in all dose categories: more than 80 percent of rats and 70 percent of mice were alive at week 92. Nonetheless, survival was significantly reduced at the highest exposure levels when compared with control groups.

Additional findings related to tumorigenesis are (see also Table 3):

- For exposed male and female rats, the only tumor type that was found to be elevated was mononuclear cell leukemia (MCL). A statistically significant dose-response trend was found by the Cochran-Armitage and exact trend tests (in males) or a life-table test (in females). In addition, for males, the highest dose category displayed a significant increase when compared to controls by the Fisher exact test.

- In exposed mice, an increased incidence of hepatocellular adenoma and carcinoma was found in both sexes as indicated by significant dose-response trends and pair-wise comparison of the high dose category against controls. In the males, there was also an increase in all-organ, hemangioma or hemangiosarcoma (mostly in the spleen and liver), and Harderian gland tumors.

NTP (1986) conducted a study where B6C3F1 mice and F344/N rats, in groups of 50, were exposed to PCE (99.9% pure) by inhalation, 6 hours/day, 5 days/week for 103 weeks. Mice were exposed to concentrations of 100 or 200 ppm, and rats to 200 or 400 ppm, in addition to controls. The general status and body weight of the animals were monitored during the study. Upon death, animals were necropsied and histopathologic examination of all relevant tissues was performed on all animals. Approximately 70 percent or more of both sexes of mice and rats were alive at week 90 of the study. Survival was significantly reduced in male rats at the higher exposure level when compared with controls. Survival was decreased in both dose levels in male mice and in the high dose group of female mice.

As shown in Table 4, PCE significantly increased the rate of hepatocellular carcinomas in mice of both sexes. The combined incidence of liver adenoma or carcinoma was also

increased, although the incidence of liver adenomas separately was not. In female and male rats, PCE also produced significant increases in mononuclear cell leukemia (MCL). Male rats additionally exhibited apparent increases in tumor incidence in the kidney, brain, and testes. Statistical tests for increases in renal tubular-cell adenomas and adenocarcinomas appeared to be dose-related, but did not reach customary significance levels. However, the historical incidence of these tumors is low (0.4%) at the laboratory and increased incidence has been found with other chlorinated ethanes and ethylenes. Thus renal tubular-cell tumors were judged to be related to PCE exposure. Brain glioma, another rare tumor type in F344 rats, was observed in one male control rat and in four male rats at 400 ppm exposure. This increase was not statistically significant. However, because the historical incidence of these tumors is 0.8% for the laboratory, the increased brain tumor incidence in this study was also carried though the analysis. Testicular interstitial cell tumors showed significant dose-responses in both life table and incidental tumor tests calculated by NTP. This tumor type was therefore included in the dose-response evaluation, but was considered to be more uncertain, given the high background rate of testicular tumors in F344 rats (both historically and in the NTP study controls)

Primary Studies for the Dose-Response Assessment

Both the NTP (1986) and JISHA (1993) inhalation studies were judged to be of high quality and suitable for the development of an inhalation potency factor. The studies used different strains of mice (Crj:BDF_1 vs. B6C3F_1) and different substrains of F344 rats. They displayed variability of outcome with respect to the tissues affected, as well as the strength of the dose-response relationships for various tumor types, and differing incidence rates in the control groups. Some of this variability could be due, in the case of the rat models, to the fact that the different substrains used may have genetic and phenotypic variation as a result of mechanisms such as genetic drift.

For example, Tiruppathi *et al.* (1990) and Thompson *et al.* (1991) reported that the Japanese and German substrains of the Fischer 344 (F344) rat, but not the US substrain, were deficient in dipeptidyl dipeptidase-4 activity in the kidney and liver. This enzyme has been implicated in the degradation of collagen, blood clotting, immunomodulation, and metabolism of hormonal peptides (Tiruppathi, *et al.*, 1990). While this particular enzymatic variation may not be directly relevant to PCE metabolism, it indicates that F344 rat substrains can display significantly divergent biological traits. With regard to the mice, the genetic variation issue is accentuated by the use of two different mouse hybrid strains, not substrains.

Table 3: Primary Tumor Incidence in Mice and Rats Exposed to PCE Rates at Exposure Concentrations in PPM (JISHA, 1993)

Mice (Crj:BDF$_1$)

Tumor Type	Sex	Adjusted Rates[(a)(b)] (at 0-250 ppm)				Rate Percent (at 0-250 ppm)			
		0	10	50	250	0	10	50	250
Hepatocellular adenoma or carcinoma	M	13/46**	21/47	19/47	40/49**	28.3	44.7	40.4	81.6
	F	3/44**	3/41	7/40	33/46**	6.8	7.3	17.5	71.7
Hemangioma or hemangiosarcoma (All sites)	M	4/46*	2/47	7/47	9/49*	8.7	4.3	14.9	18.4
Harderian gland adenoma	M	2/41**	2/45	2/37	8/39	4.9	4.4	5.4	20.5

Rats (F344/DuCrj)

Tumor Type	Sex	Adjusted Rates[(a)(b)] (at 0-600 ppm)				Rate Percent (at 0-600 ppm)			
		0	50	200	600	0	50	200	600
Mononuclear cell leukemia	M	11/50**	14/48	22/50	27/49*	22.0	29.2	44.0	55.1
	F	10/50[(c)]	17/50	16/50	19/50	20.0	34.0	32.0	38.0

(a) Tumor-incidence denominator adjusted by excluding animals dying before the first corresponding tumor type observed in each study.

(b) Statistical test indicators: (*) P-value < 0.05; (**) P-value < 0.005. Fisher exact test results are as reported by JISHA, except that mouse, all-site hemangioma/hemangiosarcoma values were calculated by OEHHA. The control group column indicates the results of trend tests. Both the Cochran-Armitage trend test (reported by JISHA) and the exact trend test calculated by OEHHA gave the same indications of significance.

(c) A significant trend was found in a life-table test reported by JISHA, P-value = 0.049.

Table 4: Primary Tumor Incidence in Mice and Rats Exposed to PCE Rates at Exposure Concentrations in PPM (NTP, 1986)

Mice ($B6C3F_1$)

Tumor Type	Sex	Adjusted Rates[a][b] (at 0-200 ppm)			Rate Percent (at 0-200 ppm)		
		0	100	200	0	100	200
Hepatocellular adenoma or carcinoma	M	17/49**	31/47**	41/50**	34.7	70.0	82.0
	F	4/44**	17/42**	38/47**	9.1	40.5	80.9

Rats (F344/N)

Tumor Type	Sex	Adjusted Rates[a][b] (at 0-400 ppm)			Rate Percent (at 0-400 ppm)		
		0	200	400	0	200	400
Mononuclear cell leukemia	M	28/50*	37/48*	37/50*	56.0	77.1	74.0
	F	18/49*	30/50*	29/50*	36.1	60.0	58.0
Renal tubule adenoma or carcinoma	M	1/47[c]	3/42	4/40	2.1	7.1	10.0
Brain glioma	M	1/44[c]	0/37	4/35	2.3	0.0	11.4
Testicular interstitial cell	M	35/49[c]	39/46	41/50	71.4	84.8	82.0

(a) Tumor-incidence denominator adjusted by excluding animals dying before the first corresponding tumor type observed in each study.

(b) Statistical test indicators: (*) P-value < 0.05; (**) P-value < 0.005. Fisher exact test results are as reported by NTP. The control group column indicates the results of trend tests. Both the Cochran-Armitage trend test (reported by NTP) and the exact trend test calculated by OEHHA gave the same indications of significance.

(c) Although testicular tumors and brain glioma did not appear to be significantly increased by the Fisher exact and trend tests, life table tests conducted by NTP did show significant increases in trends of <0.001, and 0.039 respectively. In addition, NTP's incidental tumor tests showed increased testicular tumors by both trend and pair-wise comparisons. The life table trend test for kidney tumors was nearly significant at 0.054.

Although it cannot be determined whether the different outcomes for mice and rats observed by NTP (1986) and JISHA (1993) resulted from differences in animal biology, the data suggest that each study provides non-redundant information for the analysis.

The JISHA dataset offers the advantage of an additional dose category for each species, as well as the use of several lower exposure concentrations. Moreover, the control rate of MCL incidence in the F344/DuCrj rats used in the Japanese study (22 and 20%) was significantly lower than for the F344/N rats used in the NTP study (56 and 36%), and is expected to improve the precision of the fitted model. The NTP study, nonetheless, provides important additional data on tumor development in the kidney, brain, and testes of F344/N rats, and supporting data on the dose-response rate for MCL.

Based on the above considerations, OEHHA chose both the JISHA (1993) and NTP (1986) bioassays as primary studies for the dose-response analysis. The dose-response data and results of statistical tests are presented in Tables 3 and 4. Given the availability of two acceptable inhalation studies, the oral NCI (1977) study was not used in the quantitative analysis.

Relevance of MCL to Humans

Some concerns about the propriety of using the rat MCL data for human risk assessment were raised by an NRC expert panel (without consensus) during a review of US EPA's PCE IRIS evaluation (NRC 2010). One issue brought up by the panel was that MCL is a common tumor in aging F344 rats that lacks a corresponding tumor in humans. Panel members also questioned the statistical significance of the MCL dose-response data in light of the elevated historical and control-group incidence rates for MCL. This section briefly addresses both questions.

Regarding the issue of tumor-site concordance, current research in cancer biology indicates that the basic cellular mechanisms of carcinogenesis are similar among mammals. However, this does not imply that exposure to a chemical carcinogen will always produce cancer in the same organ in different species (US EPA, 2005). In the case of human leukemias and lymphomas that are known to be induced by specific carcinogens, rodents develop different types of leukemia and lymphoma (US EPA, 2012c). The sites of induced cancer may not be the same because of differing toxicokinetics and tissue susceptibilities. For leukemia and lymphoma, variation in susceptibility could be related to differences in hematopoiesis and immune surveillance. Accordingly, there is no expectation—in general or specifically for MCL—of tumor-site concordance when using animal studies to predict human cancer risk (OEHHA, 2009).

Notwithstanding this general principle, there is evidence that rat MCL corresponds to at least one form of human leukemia. The specific cell type and biological mechanisms that give rise to rat MCL are not known, but it appears to arise from a lymphocyte or monocyte lineage, and it is thought that the cell of origin resides in the spleen or undergoes neoplastic transformation in the spleen (Thomas *et al.*, 2007). One reasonable hypothesis is that rat MCL is a form of Large Granular Lymphocyte Leukemia (LGLL), a cancer that develops in the spleen and is phenotypically and functionally similar to human LGLL (IARC, 1990; Thomas *et al.*, 2007). Human LGLL derives from either T-cell or natural killer (NK) cell lineages (Sokol and Loughran, 2006). Additional support for linking rat MCL to

human LGLL is provided by a study using the F344 rat MCL as a model for human NK-LGLL, which observed similar cellular responses in samples of the two tumor-cell types (Liao *et al.*, 2011).

Exposure of humans and animals to relatively low doses of PCE produces adverse effects upon blood and the immune system (e.g., see: Marth, 1987; Kroneld, 1987; and Emara *et al.*, 2010) that could plausibly give rise to a variety of carcinogenic response in different species. In addition to human LGLL, rat MCL may correspond to other types of human leukemia or lymphoma.

Regarding statistical issues arising from the elevated incidence of MCL in control groups, an NTP workshop focusing on the high background incidences of MCL and other tumors in the F344 rat noted that, “From a statistical perspective, high background rates of such tumors in control animals will generally decrease the ability to detect an exposure-related effect. In addition, when a statistically significant tumor effect is found in test animals relative to concurrent controls, the effect may not be considered exposure-related if it falls within the range observed in historical controls” (King-Herbert and Thayer, 2006). The foregoing statement focuses on the problem of false negative test results. However, since US EPA found MCL incidence to be significantly elevated in PCE-exposed rats, NRC panel members were concerned with the potential for false positive test results. On this issue, OEHHA agrees with the Massachusetts Department of Environmental Protection (MDEP), who reviewed the historical background rates of MCL in the NTP and JISHA study laboratories and found that,

> "For both the NTP (1986) and JISHA (1993) studies, the background rate of MCL in the same study control group was greater than or equivalent to the historical control rates for the same lab, same sex. Thus, the controls in both studies did not exhibit anomalously low MCL, which could, had it occurred, lead to false positive responses in the treatment groups." (MDEP, 2014)

Indeed, for the JISHA male rat MCL data, where the incidence in study controls was 22%, the historical incidence was 6-22%, and the Cochran-Armitage test for trend was highly significant, having a p-value of less than 0.0005.

8. MODE(S) OF ACTION

PCE's carcinogenic modes of action (MOA) likely involve the genotoxicity of one or more of its oxidative- or GST-pathway metabolites, although the precise mechanisms are unknown.

Several PCE metabolites, e.g., PCE epoxide, oxalyl chloride, trichloroacetyl chloride, dichlorothioketene, and the TCVC sulfoxides, are reactive compounds and expected to have short half-lives in the nucleophile-rich cellular environment.[9] These substances will tend to react chemically and enzymatically with cellular components near their site of production. The relatively stable metabolites, such as: TCA, DCA, TCVC, and N-AcTCVC, are more likely to circulate throughout the body where they may be further metabolized

[9] For example, the high reactivity of PCE epoxide is indicated by its 2.6-minute half-life in a neutral aqueous buffer solution at 37° C (Yoshioka, *et al.*, 2002).

and impact tissues other than the liver and kidney.

Both trichloroacetic acid (TCA) and dichloroacetic acid (DCA) have independently been found to increase tumor formation in mice. Since TCA is a major metabolite of PCE, US EPA (2012a) evaluated whether it could be the primary source of PCE's carcinogenicity in mouse liver. Using dose-response data from the JISHA (1993) and NTP (1986) PCE studies and a drinking water study of TCA in mice (DeAngelo, *et al.*, 2008), US EPA found that metabolically-generated TCA could contribute from 12 to 100 percent of the increased risk of liver tumors. This large range is not highly informative, and leaves open the possibility that other reactive metabolites may contribute significantly to the production of liver tumors in mice.

There are several non-genotoxic MOAs that may contribute to PCE's carcinogenicity, though in as yet poorly understood ways. These have been discussed at length by US EPA (2012a), and include: cytotoxicity with subsequent cellular proliferation, oxidative stress-induced cellular transformation, and dysregulation due to altered DNA methylation. Two specific MOAs that are potentially relevant for evaluating PCE involve α2u-globulin nephropathy in the male rat, and PPARα activation[10] for mouse liver tumors. In both cases, the biological bases for these MOAs in rodents are thought to be muted or absent in humans, indicating that the particular tumor-types may not be useful for human risk assessment.

α2u-Globulin Nephropathy

The α2u-globulin MOA in male rats is defined by: accumulation of α2u-globulin-containing hyaline droplets in the proximal tubules of the kidney, cytotoxicity with tubular cell proliferation, exfoliation of epithelial cells into the proximal tubular lumen and formation of granular casts, papillary mineralization, hyperplastic foci, and renal tumors (US EPA, 1991).

Green *et al.* (1990) found accumulation of α2u-globulin in the proximal tubules of F344 rats exposed by inhalation to 1000 ppm of PCE for 10 days, or given 1500 mg/kg PCE by gavage for 42 days. However a 400 ppm inhalation exposure for 28 days did not produce protein droplets or other signs of toxicity. For chemicals known to cause α2u-globulin toxicity, the formation of protein droplets in the kidney occurs rapidly upon exposure (frequently after a single dose), and further indications of tissue damage begin to appear in 3 to 4 weeks (Lehman-McKeeman, 2010; Green *et al.*, 1990). Thus, the absence of α2u-globulin accumulation after a 28-day exposure suggests that 400 ppm of PCE will not result in α2u-globulin toxicity upon long-term exposures.

The NTP (1986) study provided additional evidence along these lines. Karyomegaly and cytomegaly were observed in the kidneys of rats exposed to 200 or 400 ppm for 2 years, but indicators of α2u-globulin nephropathy (e.g., hyaline droplets, mineralization, and cast formation) were not found. The NTP protocol at the time was not designed to detect hyaline droplets or α2u-globulin accumulation (US EPA 2012a) but would have observed other markers of α2u-globulin toxicity if this MOA had been in effect. Moreover, comparable toxicity was observed in female rats in the NTP study, and PCE caused

[10] PPARα = "peroxisome proliferator-activated receptor-α."

similar kidney damage in rats and mice of both sexes in the NCI (1977) gavage study. This suggests that PCE's nephrotoxicity is neither sex nor species specific, as would be expected with an α2u-globulin MOA.

PPARα Activation

The PPARα MOA involves activation of the PPARα nuclear receptor, which is hypothesized to cause alterations in cell proliferation and apoptosis, and clonal expansion of initiated cells. The proposed indicators for this mode of action are: (1) PPARα activation with associated peroxisome proliferation, or (2) PPARα-activation plus increased liver weight and effects such as increased peroxisomal β-oxidation, CYP4A, or acyl CoA oxidase (Klaunig, *et al.*, 2003).

Numerous studies have been carried out to verify the PPARα MOA. The evidence obtained from this body of research has been mixed, and it currently remains unclear whether this hypothetical MOA is a major causal factor in mouse-liver tumor formation. The US EPA has published several detailed reviews of the PPARα MOA in its IRIS program toxicity reviews for PCE and TCA (US EPA 2012a, 2011). The main conclusions of these reviews are:

- PPARα activators can produce multiple effects in addition to peroxisome proliferation, including genotoxicity, oxidative stress, hypomethylation of DNA, and activation of other nuclear receptors.
- Peroxisome proliferation and the associated markers of PPARα activation are poor predictors of hepatocarcinogenesis in mice and rats. Studies with various PPARα activators show that the correlation between *in vitro* PPARα activation potency and tumorigenesis is weak and this relationship does not appear to be due to differences in pharmacokinetics. This suggests the involvement of carcinogenic mechanisms other than PPARα-activation.
- Studies of the PPARα-agonist, diethyl hexyl phthalate (DEHP) in transgenic mouse strains, although not fully conclusive, have cast doubt on whether the key events in the PPARα MOA (receptor activation, hepatocellular proliferation, and clonal expansion) are sufficient to cause liver tumors. The studies suggest that DEHP can induce tumors in a PPARα-independent manner (Ito *et al.*, 2007a), and that PPARα activation in hepatocytes is insufficient to cause tumorigenesis (Yang *et al.*, 2007). This again indicates that other mechanisms, either independently or in combination with PPARα-activation, are necessary to induce tumors.
- PCE exposure leads to PPARα-activation and modest levels of peroxisome proliferation, predominantly through its metabolite TCA. There is conflicting evidence as to whether this causes cellular proliferation in animals exposed to PCE: the peroxisome proliferation caused by PCE lacks specificity and consistency with respect to tissue, species, dose, and sequence of events. Also, there is little evidence indicating that PCE can induce clonal expansion of initiated cells. The available information for PCE is insufficient to demonstrate that the PPARα MOA plays a significant causative role in mouse hepatocarcinogenesis.

Conclusion on PCE's Mode(s) of Action

Given the limited understanding of the various non-genotoxic MOAs that may modify or add to the tumorigenic effects of PCE's genotoxic metabolites, there are insufficient grounds to evaluate PCE as primarily a non-genotoxic carcinogen using a non-linear model.

9. DOSE-RESPONSE ASSESSMENT

Dose Metrics

Much of the following information has already been presented, but is briefly restated here because of its relevance to choosing metrics for the dose-response calculations:

- The liver is the main site of oxidative PCE-metabolite formation, but other tissues with CYP 450 2E1, 2B, and 3A activity may also contribute to the oxidative-pathway. TCA is a relatively stable metabolite that has been found to increase liver tumors in mice via oral exposure. TCA's cancer potency in other tissues has not been adequately examined.
- Of the two metabolic pathways, oxidation is the main pathway in rodents. For example, at 10 ppm exposure, the PBPK model indicates that the ratio of oxidation to glutathione conjugation is 600 in mice and 19.5 in rats.
- Saturation of the oxidative pathway begins to occur between 1 and 10 ppm exposure in mice, and between 10 and 100 ppm exposure in rats (see Table 1). Saturation causes the ratio of oxidized to absorbed PCE to decrease at higher exposure concentrations. The smaller amount of metabolism that occurs via the GST pathway, on the contrary, increases somewhat at higher exposure concentrations in rodents.
- Although most GST conjugation of PCE takes place in the liver, the kidney is likely to be the main site for production of reactive GST-pathway metabolite dichlorothioketene. Other metabolites such as: TCVC, N-AcTCVC, and TCVC sulfoxide are formed in both the liver and kidney, and may circulate to other metabolizing tissues as well.
- It is not known which PCE metabolites, or even which of the two main metabolic pathways produces the most carcinogenic risk. However, genotoxicity tests suggest that some GST-pathway metabolites are relatively potent genotoxicants.
- The PBPK model for the GST pathway in humans involves a large variability or uncertainty, with two possible values (posterior modes) for the rate of PCE conjugation that differ by a factor of approximately 3000. However, as was discussed earlier in Section 6, the impact of the human PBPK model uncertainty/variability upon the overall dose-response evaluation is several orders of magnitude lower than this. It is not known how much of the model variability is due to the wide range of GST activities that have been observed in the human population, but it is reasonable to assume that some segment of the population could be efficient metabolizers while other segments (e.g., individuals who are homozygous in GST-null variants) could be much less efficient. It is currently unclear which GST isoforms are most active with regard to PCE conjugation.

- The more probable and larger of the two values indicates that glutathione conjugation predominates over oxidation in humans, the ratio of PCE conjugation to oxidation being about 10.

OEHHA considered the advantages and disadvantages of using several dose metrics for the dose-response calculations. These are briefly discussed below.

- Applied air concentration: This would be the simplest approach in that it does not rely upon the output of complicated PBPK modeling calculations. However, given the large body of evidence indicating that PCE's metabolites are likely to be responsible for its tumorigenic properties, using applied concentration as the dose metric may reduce the accuracy of the dose-response analysis, especially for the mouse, where the dose-response data indicate significant metabolic saturation in the oxidative pathway at the higher PCE exposure concentrations tested.

- PCE blood concentration: This dose metric does make use of the PBPK modeling estimates but has the same weakness as using the applied air concentration, since blood concentrations of the parent compound may not be directly related to concentrations of the potentially carcinogenic metabolites of PCE. Blood concentrations of PCE may even be less accurate than applied concentrations, since PCE blood concentrations are expected be inversely related to metabolite concentrations (For example, see Table 1 entries for the mouse dose-metrics where "PCE AUC per ppm exposure" increases and "percent oxidation/ppm" decreases) as one moves to higher exposure concentrations).

- Pathway specific metabolized dose: Defining a dose metric based upon either the oxidation or GST conjugation pathway would be better in terms of focusing on the production of PCE's carcinogenic metabolites instead of the parent compound. However, using either of the two pathways alone would be problematic, since each pathway produces several genotoxic substances that could be important for PCE's overall tumorigenicity. From Table 1 it can be seen that for mice, the quantity of oxidative metabolites produced with increasing exposure appears to be inversely related to the quantity of conjugation metabolites. Furthermore, if humans are more efficient conjugators than rodents, using an oxidation-only dose metric could underestimate the dose-response function. On the other hand, using glutathione conjugation alone has the problem of large model uncertainties with larger impacts upon the overall dose-response assessment (note that this impact is muted for total metabolism, as discussed above in Section 6).

- Choosing one or more metabolites: Using a subset of concentrations of one or more metabolites for the dose metric has similar problems as using pathway specific metabolism. For example, in Section 8 we briefly discussed US EPA's evaluation of TCA, a major metabolite generated in the oxidation pathway, where it was estimated that TCA might be responsible for as little as 12 percent of liver tumor risk in mice. An added issue is that the available PBPK models only incorporate a few of the various metabolites, such as TCA and DCA.

- Total PCE metabolized dose: Using total metabolism for the dose metric accounts for toxicokinetic differences across species and provides a dose adjustment for saturation effects in the oxidative pathway. It has the advantage of taking into account both pathways generating potentially carcinogenic metabolites. However, it involves assuming that carcinogenic potency is proportional to the combined rate of the first step of metabolism in each pathway. This assumption is simplistic but unavoidable given the many unknowns involved in PCE's toxicokinetics and toxicodynamics. As noted above, total metabolized dose has an advantage over using either oxidative or glutathione conjugation alone. Using oxidation-only may not be adequately protective of human health given the potential genotoxicity of metabolites formed in the conjugation pathway. Total metabolized dose is also advantageous compared with using the GST-pathway metabolites alone, since the PBPK modeling uncertainties have relatively little impact upon the dose-response assessment using total metabolism as the metric.

Considering all of the above factors, total metabolism (using the high-end, and more health-protective model estimate for GST conjugation in humans) was chosen as the best dose metric for the dose-response analysis of all the tumor types identified in the primary mouse and rat studies.[11] The PBPK-estimated, total metabolized doses used in the dose-response analysis are presented in Appendix B.

Dose-Response Model

Based upon its metabolic profile and the genotoxic activity of some of the metabolites formed, OEHHA considers PCE to be a genotoxic carcinogen. This information supports the assumption that the dose-response relationship approaches linearity at low doses and the use of the multistage cancer model to estimate the potency factor. This is consistent with OEHHA risk assessment guidelines, which indicate that use of the multistage model (and assuming low-dose linearity) is reasonable under such circumstances (OEHHA, 2009). In the traditional, linearized-multistage model, cancer potency is estimated as the upper 95% confidence bound, (q_1*), on the linear coefficient (q_1) in the following expression relating lifetime probability of cancer (p) to dose (d):

$$p = q_0 + (1 - q_0)\left(1 - exp\left[-\left(q_1 d + q_2 d^2 + \cdots\right)\right]\right)$$

In the above equation, (*d*) represents the average daily dose resulting from a uniform, continuous exposure over the nominal lifetime of the animal (two years for both mice and rats); (q_0) is the tumor incidence in the non-exposed group. For studies where the exposures vary in time, they are averaged over the entire study period and modeled as if they were uniform and continuous. Prior to fitting the dose-response model to the study data, an adjustment is made to the incidence rates to account for inter-current mortality, which decreases the pool of animals at risk of developing tumors throughout the study.

The latest version of BMDS (Version 2.6.0.1, US EPA, 2015) was used to carry out the

[11] In using total metabolized dose as the preferred dose metric, OEHHA considered the uncertainty in the available scientific information and, in contrast to US EPA (2012a), has chosen a modeling approach that will produce a more health-protective potency estimate. This is consistent with OEHHA's cancer risk assessment guidelines (OEHHA 2009), which establish a policy of developing cancer potency factors that are adequate to protect public health.

necessary dose-response calculations. The BMDS dichotomous multi-stage cancer model was run for all allowed degrees of the approximating polynomial, with a benchmark risk (BMR) of 5 percent. Instead of (q_1*) the software calculates benchmark doses (BMDs) and their 95% lower confidence levels (BMDLs). When multiplied by the BMR, the reciprocal of the BMDL gives a unit risk factor that is generally close in value to, and is used in place of (q_1*). For each tumor site, the model with the lowest value of AIC (Akaike's Information Criterion) was chosen, as long as its p-value for goodness-of-fit was above 0.1 and the absolute value of the scaled residual for the dose near the BMD was less than 2.0. The optimal model typically resulted from fitting a polynomial of 1 or 2 degrees, and the models with the lowest AIC also had the highest p-values (signifying the best fit to the data).

Interspecies extrapolation from experimental animals to humans was based on body weights (*bw*) raised to three-quarters power (US EPA, 2005; Anderson *et al.*, 1983), which for BMDLs, may be expressed in terms of body weight raised to one-quarter power, as follows:

$$BMDL_{(Human)} = BMDL_{(Animal)}\ x \left(\frac{bw_{(Animal)}}{bw_{(Human)}}\right)^{1/4}$$

The above equation is presumed to account for the toxicokinetic and toxicodynamic differences between species. Toxicokinetic modeling can sometimes eliminate the need for toxicokinetic scaling between animals and humans. This would be the case, for example, if the dose metric used in the analysis was the AUC of a directly carcinogenic metabolite. The remaining toxicodynamic differences would then be addressed, according to OEHHA practice, by scaling according to the one-eighth power of the body weight ratio.[12] Using the rate of PCE metabolism as a dose metric, on the other hand, does not account for the toxicokinetics of other downstream biological processes that determine tissue concentrations of the relevant carcinogenic species. In this case, the full cross-species scaling factor is used (US EPA, 1992).

Since PCE induced tumors at multiple sites in male mice (JISHA study) and male rats (NTP study), the combined cancer potency was also estimated for these groups using the multi-site tumor module provided in BMDS. The BMDS procedure for summing risks over several tumor sites uses the profile likelihood method. In this method, the maximum likelihood estimates (MLEs) for the multistage model parameters (q_i) for each tumor type are added together $(i.e., \sum q_0, \sum q_1, \sum q_2)$, and the resulting model is used to determine a combined BMD. Then a confidence interval for the combined BMD is calculated by computing the desired percentile of the chi-squared distribution associated with a likelihood ratio test having one degree of freedom.

Once the organ-specific and multi-site BMDLs were obtained and scaled by body-weight, the toxicokinetic model was used to estimate the continuous 24-hour air concentration that would produce the same daily metabolized dose for an adult human (i.e., the human equivalent concentration or "HEC"). The cancer potency values were then calculated by dividing the BMR of 0.05 by the HEC. Table 5 provides the calculated BMDs, BMDLs, and the interspecies-adjusted BMDLs for individual and combined tumor sites. Potency values

[12] US EPA risk assessment guidelines (2005) suggest "retaining some of the cross-species scaling factor (e.g., using the square root of the cross-species scaling factor)," when toxicokinetic modeling is used without toxicodynamic modeling.

derived from the primary studies are presented in Table 6 as unit risks factors (URFs) with units of reciprocal µg/m^3.

Inhalation Potency Value for PCE

The updated carcinogenic potency value for PCE is based on the following observations and rationale:

- Tissue-specific URF values calculated from the JISHA study are of similar magnitude to the corresponding URFs obtained from the NTP study, though somewhat lower. For mouse liver tumors, the ratio of the JISHA URF to the NTP URF was about 0.8 in both females and males. For rat MCL the corresponding ratios were 0.4 for females and 0.6 for males. The smaller URF values from the JISHA data may be due in part to the higher precision obtained by the study having used lower doses and an additional dose group.
- In both studies, the males of both species appeared to be more sensitive than the corresponding females to the tumorigenic effects of PCE.
- The URF values from both studies ranged from 2.8E-06 to 1.6E-05 (per µg/m^3), within a factor of 6. (The compared values included the multi-tumor risks for male NTP rats and male JISHA mice, as well as tissue-specific risks for other organs in mice and rats of both sexes.) Looking only at males of each species, the URFs range from 4.0E-06 to 1.6E-05.
- The highest URF was obtained from the combined site (i.e., multi-tumor) risk in male rats in the NTP study. This value was obtained by including MCL, brain, testicular, and renal tumors in the multi-tumor calculation.
- The URF values for mouse liver tumors and rat MCL were judged by OEHHA to be more certain in view of the qualitative and quantitative agreement between the two primary studies; mouse liver tumors were also found in the NCI (1977) oral exposure study.
- The unique tumors seen in the NTP study, including kidney tumors, are important to consider. The kidney is one site where the GST-pathway may contribute substantially to the cancer potency. Moreover, there is reasonable evidence that the GST-pathway may also contribute to tumorigenesis in other organ systems.
- Although it appears likely that PCE exposure increased the rate of testicular tumors in rats, the relatively high risk value obtained for testicular tumors in NTP rats may be more uncertain, given the high tumor incidence seen in the control group (71%).

Considering the above points, and also that the set of calculated values is clustered in a narrow range, the geometric mean of the male mouse and rat URFs from both studies was chosen as the best estimate of PCE cancer potency. Specifically, the geometric mean was calculated using the URF values shown in Table 7. The resulting URF, when rounded to two significant figures, is 6.1E-06 (µg/m^3)$^{-1}$. A cancer potency factor of 2.1E-02 (mg/kg-day)$^{-1}$ was also calculated from the URF using an adult body weight of 70 kg and an inspiration rate of 20 (m^3/day).

10. COMPARISON WITH U.S. EPA (2012) URF for PCE

In this section we briefly compare US EPA's 2012 URF to the OEHHA value, indicating the primary methodological differences that have resulted in differing estimates of cancer potency. The URF developed by US EPA (2012a) for inhalation exposure to PCE is 2.6E-07 $(\mu g/m^3)^{-1}$. This value is approximately 23 times less potent than OEHHA's URF, or about 20 times smaller based on US EPA's rounded URF of 3E-07 $(\mu g/m^3)^{-1}$.

The main sources of this difference are:

- US EPA used oxidation-only liver metabolism (metabolized dose) to calculate internal doses relevant to male mouse liver tumors. OEHHA used the total metabolized dose, consisting of PCE oxidation plus conjugation, in its calculations. The impact of this difference can be seen by comparing US EPA's URF, 2.6E-07 $(\mu g/m^3)^{-1}$, to OEHHA's URF for liver tumors in JISHA (1993) male mice: 3.5E-06 $(\mu g/m^3)^{-1}$ (see Table 6). OEHHA's mouse liver URF is about 13 times larger than US EPA's value. As already discussed, using total metabolism versus total oxidative metabolism (i.e., oxidation in liver, kidney and lung) is responsible for a factor of about 11 difference in the two URFs. An additional factor of roughly 1.2 is due mainly to the fact that US EPA used oxidation in liver only.
- US EPA used male mouse liver tumor data from the JISHA (1993) study as the most representative tumor-type for estimating cancer risk in humans; OEHHA used multiple tumor types in male mice and rats and both the JISHA (1993) and NTP (1986) studies, and we calculated multi-site risks for JISHA mice and NTP rats. OEHHA used a mid-range value (geometric mean) of URFs based upon all elevated tumor-types found in mice and rats in both studies. Had we followed US EPA by using only male mouse data from JISHA (1993), OEHHA's URF would have been 3.5E-06 $(\mu g/m^3)^{-1}$. Thus, using the larger body of tumor data from both studies is responsible for a factor of about 1.7 difference between the OEHHA and US EPA URFs.

Finally, it is interesting to note that US EPA (2012a) evaluated the consistency of its animal-based URF with exposure-response data for humans reported in a study that modeled increased laryngeal cancer in PCE-exposed workers (van Wijngaarden and Hertz-Picciotto, 2004). While cautioning that the human exposure information used in the exercise was subject to bias, US EPA estimated a range of URFs of 2E-06 to 8E-06 $(\mu g/m^3)^{-1}$. Although these values should be viewed as having increased uncertainty, they show greater consistency with OEHHA's URF than with US EPA's URF.

Table 5: BMDS Modeling Results for the Primary Studies

Study	Sex	Tumor Type	P-value for multi-stage model fit	Scaled residual for dose near the BMD	BMD (mg/kg-day)	BMDL (mg/kg-day)	Animal BW (kg)	BW-Scaled BMDL (mg/kg-day)
Results from Mouse Studies								
JISHA	M	Hepatocellular adenoma or carcinoma	0.22	1.17	3.06	2.16	0.048	0.350
		Harderian gland	0.99	-0.06	38.56	12.34	0.048	1.997
		Hemangioma or Hemangiosarcoma	0.35	0.94	26.61	12.98	0.048	2.100
		Combined site			2.73	1.85	0.048	0.300
	F	Hepatocellular adenoma or carcinoma	0.77	-0.23	10.33	3.84	0.035	0.574
NTP	M	Hepatocellular adenoma or carcinoma	0.85	0.03	2.46	1.79	0.037	0.272
	F	Hepatocellular adenoma or carcinoma	0.82	0.05	11.27	3.15	0.025	0.432
Results from Rat Studies								
JISHA	M	Mononuclear cell leukemia	0.79	0.07	1.34	0.89	0.45	0.251
	F	Mononuclear cell leukemia	0.37	1.05	3.99	1.84	0.30	0.472
NTP	M	Mononuclear cell leukemia	0.23	-0.31	0.92	0.51	0.44	0.144
		Testicular interstitial cell	0.35	-0.26	1.06	0.48	0.44	0.136
		Renal adenoma or carcinoma	0.93	0.07	6.76	3.24	0.44	0.913
		Brain glioma	0.15	0.62	9.45	5.07	0.44	1.426
		Combined site			0.46	0.28	0.44	0.078
	F	Mononuclear cell leukemia	0.25	-0.30	1.24	0.72	0.32	0.188

Table 6: Unit Risk Factors from Primary Studies					
Study	**Sex**	**Tumor Type**	**BW-Scaled BMDL (mg/kg-day)**	**HEC based on PBPK Model (ppm)**	**Unit Risk Factor (URF) per µg/m^3**
Results from Mouse Studies					
JISHA	M	Hepatocellular adenoma or carcinoma	0.350	2.14	3.5E-06
		Harderian gland	1.997	12.20	6.0E-07
		Hemangioma or Hemangiosarcoma	2.100	12.83	5.7E-07
		Combined site	0.300	1.83	4.0E-06
	F	Hepatocellular adenoma or carcinoma	0.574	3.51	2.1E-06
NTP	M	Hepatocellular adenoma or carcinoma	0.272	1.66	4.4E-06
	F	Hepatocellular adenoma or carcinoma	0.432	2.64	2.8E-06
Results from Rat Studies					
JISHA	M	Mononuclear cell leukemia	0.251	1.53	4.8E-06
	F	Mononuclear cell leukemia	0.472	2.88	2.6E-06
NTP	M	Mononuclear cell leukemia	0.144	0.88	8.4E-06
		Testicular interstitial cell	0.136	0.83	8.9E-06
		Renal adenoma or carcinoma	0.913	5.57	1.3E-06
		Brain glioma	1.426	8.71	8.5E-07
		Combined site	0.078	0.47	1.6E-05
	F	Mononuclear cell leukemia	0.188	1.15	6.4E-06

Table 7: URFs Used to Calculate Mean		
Species	**Study**	**URF** $(\mu g/m^3)^{-1}$
Male Mouse	JISHA (Multiple tumor)	4.02E-06
	NTP (Liver)	4.44E-06
Male Rat	JISHA (MCL)	4.81E-06
	NTP (Multiple tumor)	1.57E-05
	Geometric Mean	6.06E-06

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APPENDIX A

PBPK Model Code for Simplified, Inhalation-Only Adaptation of Chiu and Ginsberg (2011) PCE Model, for Mice, Rats, and Humans (Written in Berkeley Madonna)

{ Inhalation-Only Adaptation of Chiu and Ginsberg (2010) PCE Model for MICE }

```
METHOD RK4
STARTTIME = 0
STOPTIME=504
DT = 0.002

ppm=10      {inhaled conc in ppm}
CInh=If (Mod(Time,24)<=6 AND Mod(Time,168)<=120) Then (ppm*165.83/24450) Else 0

; BW=0.037 {NTP Male}
; BW=0.048 {JISHA Male}
; BW=0.025 {NTP Female}
  BW=0.035 {JISHA Female}

QC=11.6*BW^0.75
QP=QC*2.5*exp(0.325015)
QM=QP/0.7   {minute volume, L/h}
DResp=QP*exp(0.203)
; Intake=QM*Cinh*24/BW

QGut=0.141*QC
QLiv=0.02*QC
QKid=0.091*QC
QFat=0.07*QC
QRap=0.461*QC
QSlw=0.217*QC

PB=18.6
PResp=79.1/PB
PGut=62.1/PB
PLiv=48.8/PB
PKid=79.1/PB
PRap=62.1/PB
PSlw=79.1/PB
PFat=1510.8/PB

VResp=0.0007*BW
VRespEff=VResp*PResp*PB
VRespLum=0.004667*BW
VGut=0.049*BW
VLiv=0.055*BW
VKid=0.017*BW
VRap=0.1*BW
VFat=0.07*BW
VBld=0.049*BW
VSlw=(0.8897*BW)-(VResp+VGut+VLiv+VKid+VRap+VFat+VBld)

{ Metabolic Constant Calculations }
{=================================}
KMo=                88.6
lnKMC=              -5.35885
ClCo=               1.57
lnClC=              3.18103
lnKM2C=             15
lnCl2OxC=           -1.20051
KmKidLivo=          0.616
```

```
ClKidLivo=          0.0211
VMaxLungLivo=       0.07
VMaxTCVGo=          35.3
lnVMaxTCVGC=        10.2
ClTCVGo=            0.656
lnClTCVGC=          -9.17006
VMaxKidLivTCVGo=    0.15
ClKidLivTCVGo=      0.24

KM=KMo*exp(lnKMC)
VMax= KM*ClCo*VLiv*exp(lnClC)

KM2=KM*exp(lnKM2C)
VMax2=KM2*(VMax/KM)*exp(lnCl2OxC)

KMKid=KM*KMKidLivo
VMaxKid=(VMax/KM)*KMKid*(VKid/VLiv)*ClKidLivo

KMClara=KM*PLiv/(PB*PResp)
VMaxClara=VMax*VMaxLungLivo

VMaxTCVG=VMaxTCVGo*VLiv*exp(lnVMaxTCVGC)
KmTCVG=VMaxTCVG/(ClTCVGo*exp(lnClTCVGC))

VMaxKidTCVG=VMaxTCVG*(VKid/VLiv)*VMaxKidLivTCVGo
KmKidTCVG=VMaxKidTCVG/(ClKidLivTCVGo*(VKid/VLiv)*(VMaxTCVG/KMTCVG))
{==================================}

Init AGut=0             Limit AGut>=0
Init AResp=0            Limit AResp>=0
Init AExhResp=0         Limit AExhResp>=0
Init AInhResp=0         Limit AInhResp>=0
Init ALiv=0             Limit ALiv>=0
Init AKid=0             Limit AKid>=0
Init ARap=0             Limit ARap>=0
Init ASlw=0             Limit ASlw>=0
Init AFat=0             Limit AFat>=0
Init ABld=0             Limit ABld>=0

{Respiratory Model Concentrations}
CInhResp=AInhResp/VRespLum          {conc resp lumen during inh, mg/L}
CResp=AResp/VRespEff                {conc resp tract tissue, mg/L}
CExhResp=AExhResp/VRespLum          {conc resp lumen during exh, mg/L}

{Blood Concentrations}
CVGut=(AGut/VGut)*(1/PGut)
CVLiv=(ALiv/VLiv)*(1/PLiv)
CVKid=(AKid/VKid)*(1/PKid)
CVRap=(ARap/VRap)*(1/PRap)
CVSlw=(ASlw/VSlw)*(1/PSlw)
CVFat=(AFat/VFat)*(1/PFat)
CVBld=(ABld/VBld)
CArt=(QC*CVBld+QP*CInhResp)/(QC+(QP/PB))

{Metabolism: P450 Oxidation}
RAMetLng=(VMaxClara*CResp)/(KMClara+CResp)
RAMetLiv1=(VMax*CVLiv)/(KM+CVLiv)+(VMax2/KM2)*CVLiv
RAMetKid1=(VMaxKid*CVKid)/(KMKid+CVKid)
```

```
{Metabolism: GST Conjugation}
RAMetLiv2=(VMaxTCVG*CVLiv)/(KMTCVG+CVLiv)
RAMetKid2=(VMaxKidTCVG*CVKid)/(KMKidTCVG+CVKid)

{Respiratory Model Mass Balance Equations}
AInhResp'=QM*CInh+DResp*(CResp-CInhResp)-QM*CInhResp
AResp'=DResp*(CInhResp+CExhResp-2*CResp)-RAMetLng
AExhResp'=QM*(CInhResp-CExhResp)+QP*((CArt/PB)-CInhResp)+DResp*(CResp-CExhResp)

{Other Mass Balance Equations}
AGut'=QGut*(CArt-CVGut)
ALiv'=(QLiv*CArt)+(QGut*CVgut)-((QLiv+QGut)*CVLiv)-RAMetLiv1-RAMetLiv2
AKid'=QKid*(CArt-CVKid)-RAMetKid1-RAMetKid2
ARap'=Qrap*(CArt-CVRap)
ASlw'=QSlw*(CArt-CVSlw)
AFat'=QFat*(CArt-CVFat)
ABld'=(QFat*CVFat)+((QGut+QLiv)*CVLiv)+(QSlw*CVSlw)+(QRap*CVRap)+(QKid*CVKid)-
(QC*CVBld)

init MetCum=0           Limit MetCum>=0
init LivOxCum=0         Limit LivOxCum>=0

MetTot=RAMetLng+RAMetLiv1+RAMetKid1+RAMetLiv2+RAMetKid2
MetCum'=If TIME>=336 Then (MetTot/(7*BW)) Else 0
LivOxCum'=If TIME>=336 Then (RAMetLiv1/(7*BW)) Else 0
```

{ Inhalation-Only Adaptation of Chiu and Ginsberg (2011) PCE Model for RATS }

```
METHOD RK4
STARTTIME = 0
STOPTIME=504
DT = 0.002

ppm=50      {inhaled conc in ppm}
CInh=If (Mod(Time,24)<=6 AND Mod(Time,168)<=120) Then (ppm*165.83/24450) Else 0

; BW=0.44   {NTP Male}
  BW=0.45   {JISHA Male}
; BW=0.32   {NTP Female}
; BW=0.30   {JISHA Female}

QC=13.3*BW^0.75
QP=QC*1.9*0.61643
QM=QP/0.7   {minute volume, L/h}
DResp=QP*exp(1)
; Intake=QM*Cinh*24/BW

QGut=0.153*QC
QLiv=0.021*QC
QKid=0.141*QC
QFat=0.07*QC
QRap=0.279*QC
QSlw=0.336*QC

PB=15.1
PResp=32.7/PB
PGut=40.6/PB
PLiv=50.3/PB
PKid=32.7/PB
PRap=40.4/PB
PSlw=21.6/PB
PFat=1489.3/PB

VResp=0.0005*BW
VRespEff=VResp*PResp*PB
VRespLum=0.004667*BW
VGut=0.032*BW
VLiv=0.034*BW
VKid=0.007*BW
VRap=0.088*BW
VFat=0.07*BW
VBld=0.074*BW
VSlw=(0.8995*BW)-(VResp+VGut+VLiv+VKid+VRap+VFat+VBld)

{ Metabolic Constant Calculations }
{=================================}
KMo=              69.7
lnKMC=            -0.805889
ClCo=             0.36
lnClC=            2.02965
KMKidLivo=        1.53
ClKidLivo=        0.0085
VMaxLungLivo=     0.0144
```

```
VmaxTCVGo=        93.9
lnVMaxTCVGC=      10.2
ClTCVGo=          2.218
lnClTCVGC=        -6.99311
VMaxKidLivTCVGo=  0.15
ClKidLivTCVGo=    0.098

KM=KMo*exp(lnKMC)
VMax=KM*ClCo*VLiv*exp(lnClC)

KMKid=KM*KMKidLivo
VMaxKid=(VMax/KM)*KMKid*(VKid/VLiv)*ClKidLivo

KMClara=KM*PLiv/(PB*PResp)
VMaxClara=VMax*VMaxLungLivo

VMaxTCVG=VMaxTCVGo*VLiv*exp(lnVMaxTCVGC)
KmTCVG=VMaxTCVG/(ClTCVGo*exp(lnClTCVGC))

VMaxKidTCVG=VMaxTCVG*(VKid/VLiv)*VMaxKidLivTCVGo
KmKidTCVG=VMaxKidTCVG/(ClKidLivTCVGo*(VKid/VLiv)*(VMaxTCVG/KMTCVG))
{================================}

Init AGut=0             Limit AGut>=0
Init AResp=0            Limit AResp>=0
Init AExhResp=0         Limit AExhResp>=0
Init AInhResp=0         Limit AInhResp>=0
Init ALiv=0             Limit ALiv>=0
Init AKid=0             Limit AKid>=0
Init ARap=0             Limit ARap>=0
Init ASlw=0             Limit ASlw>=0
Init AFat=0             Limit AFat>=0
Init ABld=0             Limit ABld>=0

{Respiratory Model Concentrations}
CInhResp=AInhResp/VRespLum    {conc resp lumen during inh, mg/L}
CResp=AResp/VRespEff          {conc resp tract tissue, mg/L}
CExhResp=AExhResp/VRespLum    {conc resp lumen during exh, mg/L}


{Blood Concentrations}
CVGut=(AGut/VGut)*(1/PGut)
CVLiv=(ALiv/VLiv)*(1/PLiv)
CVKid=(AKid/VKid)*(1/PKid)
CVRap=(ARap/VRap)*(1/PRap)
CVSlw=(ASlw/VSlw)*(1/PSlw)
CVFat=(AFat/VFat)*(1/PFat)
CVBld=(ABld/VBld)
CArt=(QC*CVBld+QP*CInhResp)/(QC+(QP/PB))

{Metabolism: P450 Oxidation}
RAMetLiv1=(VMax*CVLiv)/(KM+CVLiv)
RAMetKid1=(VMaxKid*CVKid)/(KMKid+CVKid)
RAMetLng=(VMaxClara*CResp)/(KMClara+CResp)

{Metabolism: GST Conjugation}
RAMetLiv2=(VMaxTCVG*CVLiv)/(KMTCVG+CVLiv)
RAMetKid2=(VMaxKidTCVG*CVKid)/(KMKidTCVG+CVKid)
```

```
{Respiratory Model Mass Balance Equations}
AInhResp'=QM*CInh+DResp*(CResp-CInhResp)-QM*CInhResp
AResp'=DResp*(CInhResp+CExhResp-2*CResp)-RAMetLng
AExhResp'=QM*(CInhResp-CExhResp)+QP*((CArt/PB)-CInhResp)+DResp*(CResp-CExhResp)

{Other Mass Balance Equations}
AGut'=QGut*(CArt-CVGut)
ALiv'=(QLiv*CArt)+(QGut*CVgut)-((QLiv+QGut)*CVLiv)-RAMetLiv1-RAMetLiv2
AKid'=QKid*(CArt-CVKid)-RAMetKid1-RAMetKid2
ARap'=Qrap*(CArt-CVRap)
ASlw'=QSlw*(CArt-CVSlw)
AFat'=QFat*(CArt-CVFat)
ABld'=(QFat*CVFat)+((QGut+QLiv)*CVLiv)+(QSlw*CVSlw)+(QRap*CVRap)+(QKid*CVKid)-
(QC*CVBld)

init MetCum=0     Limit MetCum>=0

MetTot=RAMetLng+RAMetLiv1+RAMetKid1+RAMetLiv2+RAMetKid2
MetCum'=If TIME>=336 Then (MetTot/(7*BW)) Else 0
```

{ Inhalation-Only Adaptation of Chiu and Ginsberg (2011) PCE Model for HUMANS }

```
METHOD RK4
STARTTIME=0
STOPTIME=840
DT = 0.0002

ppm=10      {inhaled conc in ppm}
CInh=ppm*165.83/24450

BW=70
QC=16*BW^0.75
QP=0.96*1.28*QC
QM=QP/0.7   {minute volume, L/h}
DResp=QP*exp(-5.06)
; Intake=QM*Cinh

QGut=0.19*QC
QLiv=0.065*QC
QKid=0.19*QC
QFat=0.05*QC
QRap=0.285*QC
QSlw=0.22*QC

PB=14.7
PResp=58.6/PB
PGut=59.9/PB
PLiv=61.1/PB
PKid=58.6/PB
PRap=59.9/PB
PSlw=70.5/PB
PFat=1450/PB

VResp=0.00018*BW
VRespEff=VResp*PResp*PB
VRespLum=0.002386*BW
VGut=0.02*BW
VLiv=0.025*BW
VKid=0.0043*BW
VRap=0.088*BW
VFat=0.199*BW
VBld=0.077*BW
VSlw=(0.8560*BW)-(VResp+VGut+VLiv+VKid+VRap+VFat+VBld)

{ Metabolic Constant Calculations }
{=================================}
KMo=              55.8
lnKMC=            6.9
ClCo=             0.202
lnClC=            0.2501
KMKidLivo=        1.04
ClKidLivo=        0.0125
lnClKidLivC=      4.57452
VMaxLungLivo=     0.0128
VMaxTCVGo=        0.665
lnVMaxTCVGC=      10.2
ClTCVGo=          0.0196
```

```
lnClTCVGC=        5.59162
VMaxKidLivTCVGo=  0.15
ClKidLivTCVGo=    0.14

KM=KMo*exp(lnKMC)
VMax=KM*ClCo*VLiv*exp(lnClC)

KMKid=KM*KMKidLivo
VMaxKid=(VMax/KM)*KMKid*(VKid/VLiv)*ClKidLivo*exp(lnClKidLivC)

KMClara=KM*PLiv/(PB*PResp)
VMaxClara=VMax*VMaxLungLivo

VMaxTCVG=VMaxTCVGo*VLiv*exp(lnVMaxTCVGC)
KmTCVG=VMaxTCVG/(ClTCVGo*exp(lnClTCVGC))

VMaxKidTCVG=VMaxTCVG*(VKid/VLiv)*VMaxKidLivTCVGo
KmKidTCVG=VMaxKidTCVG/(ClKidLivTCVGo*(VKid/VLiv)*(VMaxTCVG/KMTCVG))
{================================}

{Metabolism: P450 Oxidation}
RAMetLiv1=(Vmax*CVLiv)/(KM+CVLiv)
RAMetKid1=(VMaxKid*CVKid)/(KMKid+CVKid)
RAMetLng=(VMaxClara*CResp)/(KMClara+CResp)

{Metabolism: GST Conjugation}
RAMetLiv2=(VMaxTCVG*CVLiv)/(KMTCVG+CVLiv)
RAMetKid2=(VMaxKidTCVG*CVKid)/(KMKidTCVG+CVKid)

Init AGut=0             Limit AGut>=0
Init AResp=0            Limit AResp>=0
Init AExhResp=0         Limit AExhResp>=0
Init AInhResp=0         Limit AInhResp>=0
Init ALiv=0             Limit ALiv>=0
Init AKid=0             Limit AKid>=0
Init ARap=0             Limit ARap>=0
Init ASlw=0             Limit ASlw>=0
Init AFat=0             Limit AFat>=0
Init ABld=0             Limit ABld>=0

{Respiratory Model Concentrations}
CInhResp=AInhResp/VRespLum    {conc resp lumen during inh, mg/L}
CResp=AResp/VRespEff          {conc resp tract tissue, mg/L}
CExhResp=AExhResp/VRespLum    {conc resp lumen during exh, mg/L}

{Blood Concentrations}
CVGut=(AGut/VGut)*(1/PGut)
CVLiv=(ALiv/VLiv)*(1/PLiv)
CVKid=(AKid/VKid)*(1/PKid)
CVRap=(ARap/VRap)*(1/PRap)
CVSlw=(ASlw/VSlw)*(1/PSlw)
CVFat=(AFat/VFat)*(1/PFat)
CVBld=(ABld/VBld)
CArt=(QC*CVBld+QP*CInhResp)/(QC+(QP/PB))  {arterial blood conc}

{Respiratory Model Mass Balance Equations}
AInhResp'=QM*CInh+DResp*(CResp-CInhResp)-QM*CInhResp
AResp'=DResp*(CInhResp+CExhResp-2*CResp)-RAMetLng
AExhResp'=QM*(CInhResp-CExhResp)+QP*((CArt/PB)-
```

```
CInhResp)+DResp*(CResp-CExhResp)

{Other Mass Balance Equations}
AGut'=QGut*(CArt-CVGut)
ALiv'=(QLiv*CArt)+(QGut*CVgut)-((QLiv+QGut)*CVLiv)-RAMetLiv1-RAMetLiv2
AKid'=QKid*(CArt-CVKid)-RAMetKid1-RAMetKid2
ARap'=Qrap*(CArt-CVRap)
ASlw'=QSlw*(CArt-CVSlw)
AFat'=QFat*(CArt-CVFat)
ABld'=(QFat*CVFat)+((QGut+QLiv)*CVLiv)+(QSlw*CVSlw)+(QRap*CVRap)+(QKid*CVKid)-
(QC*CVBld)

MetTot=(RAMetLng+RAMetLiv1+RAMetKid1+RAMetLiv2+RAMetKid2)*(24/BW)
```

APPENDIX B

Dose Metric Values used in Dose-Response Modeling Obtained from PBPK Inhalation Model

PBPK Estimated Total Metabolized Doses
(mg/kg-day)

JISHA Mouse (Male and female weights: 0.048 and 0.035 kg)		
Exposure Concentration (ppm)	**Male**	**Female**
10	5.10	5.22
50	18.15	18.44
250	72.73	73.94
JISHA Rat (Male and female weights: 0.45 and 0.30 kg)		
Exposure Concentration (ppm)	**Male**	**Female**
50	1.82	1.88
200	6.47	6.67
600	15.32	15.83
NTP Mouse (Male and female weights: 0.037 and 0.025 kg)		
Exposure Concentration (ppm)	**Male**	**Female**
100	32.78	33.38
200	60.25	61.40
NTP Rat (Male and female weights: 0.44 and 0.32 kg,		
Exposure Concentration (ppm)	**Male**	**Female**
200	6.48	6.63
400	11.38	11.66

Responses to Public Comment on the Draft Inhalation Unit Risk Factor for Perchloroethylene

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

(May 2016)

Background

The Office of Environmental Health Hazard Assessment (OEHHA) develops potency values for carcinogenic substances that are candidate Toxic Air Contaminants (TACs) (Health and Safety Code Section 39660) or are listed under the Air Toxics Hot Spots Act (Health and Safety Code Section 44321). These values are used in the Air Resources Board's (ARB) air toxics control programs and also by other State regulatory bodies, to estimate cancer risk in humans.

On February 15, 2016, OEHHA released the draft document, *Perchloroethylene: Inhalation Cancer Unit Risk Factor, Technical Support Document for Cancer Potency Factors, Appendix B, Public Review Draft* (hereinafter referred to as "the document") to solicit public comment. The document presents an updated cancer dose-response analysis for inhalation exposure to perchloroethylene (PCE) and derives a unit risk factor (and a corresponding cancer slope factor) using methodology described in the OEHHA 2009 Air Toxics Hot Spots Technical Support Document for Cancer Potency Factors (hereinafter referred to as the "cancer TSD") and research made available since our last PCE review in 1992. Public comment was received from:

- The Center for Public Environmental Oversight (CPEO)
- The U.S. Department of Defense (DoD)
- The California Chamber of Commerce (CalChamber)
- The Halogenated Solvents Industry Alliance (HSIA)

Responses to these comments are provided below.

1. Responses to Comments Received from the Center for Public Environmental Oversight (CPEO). Comment letter signed by Mr. Lenny Siegel.

CPEO General Comment 1

"I am pleased to see that the Office of Environmental Health Hazard Assessment (OEHHA) is developing an inhalation cancer unit risk factor (URF) for perchloroethylene (tetrachloroethylene). I work with communities across the country where exposure to PCE vapors is all too common. In my own community of Mountain View, California, where I serve on the City Council, we have at least two current development projects on former dry cleaner sites where PCE was released into the subsurface.

"I do not have the expertise to comment on the technical aspects of your study. I am writing simply to say the health and property of a large number of people depend upon you doing your job carefully. Historically, the businesses that produce, use, and release chlorinated compounds have worked incessantly to weaken the exposure standards, and there is no organization with resources on the other side, representing potentially exposed communities, to balance their influence.

"So please be aware of industry's interests and influence as you complete your findings."

Response to CPEO General Comment 1

OEHHA thanks the commenter for providing contextual information regarding current exposures to PCE resulting from historical PCE use and disposal practices. With our currently proposed PCE URF update, OEHHA believes that it has used the best available scientific methodology and toxicity information, and has also taken appropriate steps to offset the remaining analytic uncertainties and data gaps, to provide a health-protective estimate for PCE's carcinogenic potency.

2. Responses to Comments Received from the U.S. Department of Defense (DoD)

DoD General Comment

"The OEHHA Perchloroethylene (PCE) Inhalation Cancer Unit Risk Factor (URF), Public Review Draft does not provide the basis or rationale for the selection of the input values used to calculate the inhalation URF. That is, given the various uncertainties it is unclear whether OEHHA is striving to develop a URF associated with the least amount of uncertainty or a URF associated with the most sensitive endpoints, especially given that the EPA Toxicological Review of Tetrachloroethylene (Perchloroethylene) (EPA 2012) developed an extensive review and analysis of the available data regarding PCE health effects and carcinogenicity, which underwent extensive public and expert peer review by the National Research Council ([NRC] 2010). OEHHA arrived at an inhalation URF approximately 23 times more stringent than the EPA (2012) value and virtually identical to the OEHHA 1992 value. To improve clarity and transparency, OEHHA should clearly state the basis for the selection of input values. In addition the document should include a comprehensive uncertainty analysis and consideration of alternate dose metrics using the OEHHA-adapted Chiu and Ginsberg (2011) model so the impact on the resulting URF associated with the OEHHA decision logic is clear to a reviewer."

Response to DoD General Comment

As noted in the introductory section of the document, OEHHA relies on the 2009 cancer TSD (OEHHA, 2009) for its methodologic basis in developing cancer potency values. The Office strives to determine potency estimates that are appropriate to protect human health. In so doing, we believe it scientifically prudent to consider and make allowances for data gaps and uncertainties in the available toxicologic information. For OEHHA's PCE cancer potency factor derivation, the basis for selection of the input values is discussed throughout the document.

For example, in Section 6, we explain our decision to base the internal dose-metric estimates on the Chiu and Ginsberg PBPK model, given that it is the most up-to-date and comprehensive model for this purpose. In Section 7, a discussion is provided of why rat mononuclear cell leukemia (MCL) was considered to be an appropriate and informative tumor type for use in human cancer risk assessment. Indeed, much of the text is devoted to explaining the various choices of our input assumptions and data. In addition, OEHHA discusses various aspects of uncertainty throughout the document.

Regarding DoD's suggestion that the Office carry out "a comprehensive uncertainty analysis," we do not believe it is necessary or desirable in this instance. In support of our position, we cite the National Academy of Sciences (NAS 2009) opinion on uncertainty analysis in risk assessment:

> "If an uncertainty analysis will not substantially influence outcomes of importance to the decision maker, resources should not be expended on a detailed uncertainty analysis..."

In our review of the current PCE toxicity database, OEHHA has identified several unresolvable uncertainties, a major one of which lies in the toxicokinetic data relating to the level of GST conjugation of PCE in humans. We refer the commenter to Chiu and Ginsberg (2011), who provided a detailed quantitative uncertainty analysis of several aspects of their PBPK model, including a close look at the sources of uncertainty/variability in the human model. In addition, US EPA (2012) explored the range of estimates that would be obtained by utilizing various dose metrics that the Office deemed to be less appropriate for carrying out the dose-response assessment. As noted in the document, OEHHA considered all of this information in its independent analysis.

Based on this comment, however, we have provided additional discussion in the document on several of the more important aspects of uncertainty in the PCE cancer potency factor derivation.

DoD Specific Comment 1

"Page 8, Section 6. The text states the Chiu and Ginsberg (2011) model was adapted by OEHHA, however the basis for this OEHHA adaptation is not provided. For transparency, provide the rationale for the adaptation of the model. Also, the text states the adapted model 'adequately' reproduced the predictions of the original Chiu and Ginsberg (2011) model, however no data or results are provided to support this claim. Recommend a quantitative measure be provided to support the claim that the adapted model is able to reproduce the predictions of the Chiu and Ginsberg (2011) model. Clarify whether the OEHHA adaptation of the model underwent a peer review or validation process."

Response to DoD Specific Comment 1

The development of the inhalation-only version of the Chiu and Ginsberg (2011) model is described in the document and the resulting model code is provided in Appendix A. The document also explains that OEHHA's model uses the relevant inhalation equations

and all of the modeling parameter values from Chiu and Ginsberg (2011), including the maximum likelihood estimates (MLEs) determined via the Markov Chain Monte Carlo calculations. Chiu and Ginsberg's model equations and input parameters were peer-reviewed and extensively validated. This represents "the basis" of the OEHHA-adapted model. Table 2 presents dose-metric estimates that were reported by Chiu and Ginsberg (2011) and were also obtained by OEHHA using its inhalation-only adaptation, at the level of significance presented in the table. Based on the concordance of the estimates, OEHHA deems its adapted model to adequately reproduce the original model results. In order to make this clearer to the reader, the Office has retitled Table 2 and added a clarification in a footnote to the table.

DoD Specific Comment 2

"Pages 9-10, Section 7. The summary of 'selected results' presented in this section does include examples of negative results in genotoxicity tests; however, given the bullet list of positive results, consideration of the uncertainty associated with the genotoxicity of PCE will improve transparency. As EPA (2012) noted, uncertainties with regard to PCE genotoxicity remain. In vivo testing has been equivocal, and although specific PCE metabolites are genotoxic, not all metabolites have been adequately tested to support definitive conclusions regarding their genotoxic potential."

Response to DoD Specific Comment 2

The primary purpose of the document is to revise the dose-response assessment and provide an updated cancer potency value for PCE. Appropriate to this objective, OEHHA streamlined its discussion of PCE as a carcinogenic hazard, including the section on genotoxicity. It is important to note that the new data available since OEHHA's last review have not altered the conclusion that PCE is a potential genotoxic carcinogen via its numerous reactive metabolites. As such, the document section on genotoxicity focuses on the positive results found in the literature. Nonetheless, we do point out several instances where equivocal test results have been obtained.

OEHHA believes that the genotoxicity discussion presented is adequate for this potency-factor update. We do not intend these sections to be comprehensive reviews of the literature. As was noted in the document, detailed genotoxicity reviews have recently been published by US EPA and IARC. Neither of these entities have changed their designations regarding the genotoxic potential of PCE and its metabolites based on the updated information.

DoD Specific Comment 3

"Page 12, Section 7, Paragraph 1. In the subsection 'Primary Studies for Dose-Response Assessment', the text states the JISA 1993 study is of high quality and suitable for the development of an inhalation potency factor, and in comparison to the NTP (1986) study, '...offers the advantage of an additional dose category for each species, as well as the use of several lower exposure concentrations' and had a lower control rate of MCL incidence. However, it appears on Page 22, that despite the advantages associated with the JISA 1993 study, the male mouse liver cancer data from the NTP (1986) study was used to calculate the URF even though the JISA (1993) study included this same endpoint. The basis/justification for including the male mouse liver data from the NTP (1993) [sic] study instead of the results from the JISA (1993) study requires a clear and transparent explanation in the text."

Response to DoD Specific Comment 3

It has been shown that different strains and substrains of rats and mice used in carcinogenicity testing programs display genetic and phenotypic variation as a result of well known mechanisms such as genetic drift. For example, Tiruppathi *et al.* (1990) and Thompson *et al.* (1991) reported that the Japanese and German substrains of the Fischer 344 (F344) rat, but not the US substrain, were deficient in dipeptidyl dipeptidase-4 activity in the kidney and liver. This enzyme has been implicated in the degradation of collagen, blood clotting, immunomodulation, and metabolism of hormonal peptides (Tiruppathi, *et al.*, 1990). While this particular enzymatic variation may not be directly relevant to PCE metabolism, it indicates that F344 rat substrains can display significantly divergent biological traits.

With regard to the mice, the genetic variation issue is accentuated since the two primary PCE rodent studies used different mouse hybrid strains, not substrains.

The two rodent cancer studies for PCE at issue here displayed variability of outcome with respect to types of tumor elevated, as well as the strength of the dose-response relationships for various tumor types. Although it unknown whether these differences resulted from genetic variability, this observation suggests that data from both the JISA (1993) and NTP (1986) provide non-redundant information for the analysis. The document noted that the JISA (1993) study had an advantage of testing animals at several lower doses than the NTP (1986) study. However, OEHHA deemed it important to also use the NTP (1986) data for the reasons listed above. The Office has supplemented the discussion in the appropriate section to make these points clearer to the reader.

DoD Specific Comment 4

"Page 12, Section 7. In the 'Relevance of MCL to Humans' section, OEHHA should supply context for the statement that the NRC expert panel did not reach consensus regarding use of the rat MCL data for human health risk assessment purposes. To improve clarity, the text should indicate the NRC expert panel was comprised of 20 individuals and that the findings of the NRC expert panel were published (169 pages), which allowed transparency regarding recommendations and discussion where the scientific evidence is unclear. The text should also note as stated in the NRC (2010) review, that the '*...majority of the members judged that the uncertainties associated with MCL...were too great to support using the data over that of hepatic or renal cancer for determining quantitative estimates of risk. These members judged that the use of the MCL data could only be justified if it is EPA 's policy to choose the most conservative unit risk when considering a range of options, but that such justification should be distinguished as a policy decision and not a scientific one.*' This recommendation was supported during the subsequent OMB review of the EPA PCE toxicity profile. Recommend including the majority finding of the NRC expert review panel with regards to selection of tumor type for quantitative assessment to improve transparency, rather than only indicating complete consensus between 20 experts regarding complicated biological processes with associated uncertainty was not achieved."

Response to DoD Specific Comment 4

OEHHA generally cites only source materials readily available to the public, to preserve transparency and accountability. In this case we limit our description of the proceedings to the information reported in the panel's summary document. The report summary (NRC 2010, at page 10) indicates the minority position as follows:

> "Other members judged that the MCL data should be used for cancer-risk estimation. Their opinions were based on the observation that reproducible, statistically significant increases in MCL in male and female rats above the background incidence of MCL were found and that MCL was the cancer end point with the highest magnitude of response. They believed that use of the most sensitive response to quantify cancer risk decreases the uncertainty associated with potential differences in metabolism and susceptibility to tetrachloroethylene among exposed populations."

OEHHA generally agrees here with the minority opinion. In addition, in the NRC report section dealing with MCL, the expert panel affirmed US EPA's statement (in justifying the use of MCL) that, "discounting a rodent neoplasm simply because it has no human counterpart is not a scientifically defensible position. Strict site concordance is not a

requirement for relevance in extrapolation of hazard potential." (see NRC, 2010, at page 77). OEHHA agrees with both US EPA and the NRC expert panel on this issue, and has previously stated this methodological principle in our cancer TSD.

Further, as noted by DoD, the NRC panel majority opinion was that US EPA should not use the MCL data "over" (i.e., rather than) the hepatic or renal data. This is because, in the 2008 US EPA draft under review, the agency proposed to use male rat MCL over other tumor types for determining the unit risk value. However, OEHHA's method is to consider the MCL dose-response data *along with* the other tumor data in mice and rats in order to define a potency value that takes account of uncertainty in the data, while also being appropriate to protect human health.

DoD Specific Comment 5

'As the 'reasonable' hypothesis presented is that mononuclear cell leukemia (MCL) is a form of Large Granular Lymphocyte Leukemia (LGLL), which is phenotypically similar to human LGLL, for completeness, the text should also indicate Thomas *et al.* (2007) noted although MCL shares some characteristics with human natural killer-LGLL (NK-LGLL), human NK-LGLL is rare, occurs primarily in the young, and are 'reported mainly from the far-east with strong implications to Epstein-Barr virus as the primary causative agent, which contrasts sharply with the high background incidence in the F344 rat. In addition, Thomas *et al.* (2007) goes on to state '*...more mechanistic information is needed for arriving at scientifically sound conclusions as to its relevance in human cancer risk assessments.*' Also, verify/clarify the finding from the Liao *et al.* (2011) study, which the text states observed similar cellular responses in samples of the two tumor cell types. The Liao *et al.* (2011) study appears to have used a Fischer F344 rat NK-cell leukemia model where RNK-16 cells from in vivo NK-cell leukemic cell line were transplanted intraperitoneally into the rats."

Response to DoD Specific Comment 5

As noted in the Response to DoD Specific Comment 4, OEHHA does not require tumor concordance between rodents and humans in order to use rodent data to estimate human dose-response factors. This was stated in the last paragraph on page 12 of the document. The additional discussion on the possible concordance between rat MCL and human leukemias and lymphomas was provided as supplementary information suggesting the possibility of concordance in this case.

Stating that human NK-LGLL occurs "primarily in the young" is a mischaracterization of Thomas *et al.* (2007). More accurately, Thomas *et al.* (2007) report that when compared

to patients with LGLL of T-cell origin, NK-LGLL "patients are younger, with a median age of 39 years..."

Regarding the Liao *et al.* (2011) paper, it indicates that both human NK-leukemia cells (*in vitro*) and F344 rat MCL tumors (*in vivo*) are susceptible to FTY720 (fingolimod) mediated apoptosis through two specific mechanisms involving suppression of MCl-1, a pro-survival protein, and the alteration of sphingolipid metabolism. This research implies that rat MCL cells and human NK-leukemia cells share fundamental oncologic traits. It is reasonable to consider the possibility these that these traits were produced by similar genetic lesions.

DoD Specific Comment 6

"Page 15, Section 7, Paragraph 2. The basis for the statement that adverse effects on blood and the immune system 'could plausibly give rise to a variety of carcinogenic response,' should be provided, as should the basis for the statement that rat MCL 'may correspond' to other types of human leukemia or lymphoma. Alternatively, the unsupported statements could be removed from the text."

Response to DoD Specific Comment 6

As noted in the response to DoD Specific Comment 5, information on the possible correspondence of rat MCL to human tumor types is provided as supplemental information, and is not the basis for the Office's use of rat MCL in the dose-response assessment. As such, we do not agree with DoD that an extensive discussion is required to support these reasonable toxicologic hypotheses.

DoD Specific Comment 7

"Page 18-19, Section 9. In the last bullet on Page 18, the text indicates the PBPK model for the GST pathway in humans involves large variability or uncertainty. In humans, the range of predicted estimates spans several orders of magnitude. In its review, EPA (2012) noted '...two local maxima were observed for the posterior nodes [sic], each of which the fit to the data was good and substantially similar. However, the model predictions corresponding to each estimate differed by 3,000-fold. It was not clear as to whether this 3,000-fold spread represented uncertainty or variability in the form of a bimodal distribution for human GSH conjugation or both.' OEHHA indicates it is reasonable to assume that some segment of the population could be efficient metabolizers and the larger of the two values is more probable, however no basis is provided for these statements. Provide the basis for these statements, especially since the use of total metabolism as the dose metric incorporates the GST pathway (and

associated uncertainty) in the derivation of each of the tissue-specific URFs used to calculate the proposed inhalation URF."

Response to DoD Specific Comment 7

Regarding the 3000-fold spread in the apparent bi-modal distribution for human GSH conjugation, the document states that Chiu and Ginsberg (2011) were unable to determine how much of the variation was due to uncertainty versus variability, but noted that it could represent variability given the known variation in GST-activity in humans. We refer DoD to Chiu and Ginsberg (2011), pages 224-225, for those authors' evaluation of the human model results. OEHHA has provided additional discussion on uncertainty in the PBPK human GSH conjugation model and its relatively small impact upon the overall results of the dose-response assessment.

DoD also requests a basis for OEHHA's statements in the document that the larger of the two posterior mode estimates for human GSH conjugation is the more probable. We point DoD to Chiu and Ginsberg (2011), page 217, where they state:

> "[T]he parameter optimization procedure revealed two distinct modes in the rate of GSH conjugation — one with 'high' GSH conjugation (the overall posterior mode) and one with 'low' GSH conjugation (a number of the alternative posterior modes). The log-likelihood for the overall posterior mode with high GSH conjugation is 38 units higher than the alternative posterior modes with low GSH conjugation..."

In addition, Table 7 of the same paper reports the post-calibration, posterior mode for the first-order rate constant in the human TCVG pathway as 5.26 L/hr, which is near the high end of the range of posterior modes reported in the same table (0.00194-5.48 L/hr).

DoD Specific Comment 8

"Page 19, Section 9, Paragraph 2. It appears only one metric was chosen for the dose-response analysis, regardless of endpoint. In EPA's assessment, multiple metrics were analyzed after consideration of the most appropriate metric for a particular endpoint. The text is unclear why total metabolism was an appropriate metric for each of the tissue-specific endpoints evaluated in the dose response analysis, especially given the uncertainty (3,000-fold) associated with incorporation of the GST-pathway in the selected PBPK model. Clarify in the text why selection of only one dose metric was considered appropriate for all of the tissue-specific endpoints given no critical analysis of other dose metrics using the OEHHA-adapted model are provided for comparison purposes."

Response to DoD Specific Comment 8

Based on the comment, OEHHA has provided additional discussion in the referenced section of the document to clarify our choice of dose metric.

DoD Specific Comment 9

"Page 21, Section 9, Bullet list. For Bullet 1, it is not clear why the tissue-specific URF or mouse liver tumors from the JISA (1993) study were not used to calculate the inhalation URF. Provide a clear basis/justification for selecting the NTP (1986) mouse liver tumor URF over the JISA (1993) mouse liver tumor URF in the calculation of the inhalation URF. For bullet 4, given the '...URF values for the mouse liver tumors and rat MCL were judged by OEHHA to be more certain in view of the qualitative and quantitative agreement between the two primary studies...' it is unclear why URFs for the brain, testicular, and renal tumors were incorporated into the calculation of the inhalation URF. The basis for including these endpoints in calculation of the inhalation URF should be clearly explained in the text."

Response to DoD Specific Comment 9

To address the comment referring to page 21, bullet 1 of the draft document, we refer the commenter to our "Response to DoD Specific Comment 3."

DoD's comment referring to page 21, bullet 4, requests clarification as to why the Office used dose-response information from the brain, testicular, and renal tumors in the male rat in the NTP (1986) study. The use of this data is based on our cancer TSD, at page 31, which states:

> "...for chemicals that induce tumors at multiple sites, the single-site approach may underestimate the true carcinogenic potential. For example, the overall assessment of cancer risk from cigarette smoking (US DHHS, 1982) or ionizing radiation (NRC, 1990) is not based on risk at one site, such as lung cancer. Instead, total cancer risk is estimated from all the sites at which agent induced tumors are observed (lung, bladder, leukemia, etc), combined."

Further, on page 11 of the document, we identify the three additional tumor types as likely to be elevated above the respective control-group (or historical control) tumor rates and also as suitable for inclusion in the dose-response evaluation.

DoD Specific Comment 10

"The OEHHA document contains no uncertainty analysis. In keeping with standard practices, recommend adding an uncertainty analysis to provide a transparent discussion of the uncertainty associated with the input parameters used to derive the inhalation URF as well as a summary of the justification for selection of the input parameter given the associated level of uncertainty."

Response to DoD Specific Comment 10

OEHHA earlier addressed the DoD General Comment that requested "a comprehensive uncertainty analysis," and in response, has added a short discussion to the document that summarizes the key aspects of uncertainty in the analysis. In response to the present comment, we refer DoD to the revised document section.

DoD Specific Comment 11

"Introduction, Page 1, Paragraph 3. Although this update is said to rely on 'recent toxicological assessments published by the US Environmental Protection Agency (US EPA, 2012a)', OEHHA's methods documentation [OEHHA's current Air Toxics Hot Spots program risk assessment guidelines (OEHHA, 2009)] is out-of-date with regard to EPA's current practice, and apparently current OEHHA practice. Two significant example issues are (1) the use of a linearized multistage model that generates a q1* rather than BMDS that generates a BMDL and (2) use of (body weight)$^{2/3}$ rather than (body weight)$^{3/4}$ for interspecies extrapolation. These differences in procedures can substantially alter the estimated cancer potency. In the next paragraph, however, the document states 'OEHHA used US EPA's Benchmark Dose Software (BMDS)', and on Page 20 (body weight) $^{3/4}$ is used."

Response to DoD Specific Comment 11

DoD mischaracterizes OEHHA's use of the US EPA IRIS toxicity assessment (US EPA 2012). We *drew upon* material from the US EPA assessment, *where appropriate.* Since US EPA's cancer dose-response methods are not entirely the same as OEHHA's, the two methodologies may sometimes diverge, and OEHHA here chooses a more health-protective approach.

DoD claims that OEHHA's guidelines are out of date, and cites two examples related to body-weight scaling and the linearized, multi-stage model. The Office points DoD to the cancer TSD Executive Summary, at page 4, in regard to these examples.

There, we state:

> "OEHHA proposes to use the Benchmark Dose method to compute potency factors rather than the more traditional linearized multistage model (LMS), although the LMS will still be used in some instances.
>
> "OEHHA will use scaling based on body weight to the ¾ power, rather than to the ⅔ power."

As was noted in the draft document, we used the latest version of US EPA's Benchmark Dose software to compute the PCE cancer potency factor, and (¾)-power body weight scaling.

DoD Specific Comment 12

"Summary of Derived Values, Page 1, first partial paragraph. '...the geometric mean of 4 dose-response values was chosen as the best estimate of carcinogenic potency.' This statement is not accurate. At best, the process takes the geometric mean of the estimated cancer potency factors, but as all of the relevant supporting documents for the models state, the cancer potency values derived are not valid within the range of the dose-response data; they are based on extrapolations therefrom. More significantly, both the q1* mentioned in this report and the BMDL used in EPA's current procedures are bounds on the best estimate of the cancer potency. Standard statistical practice when combining data is to combine the best estimates and then re-estimate the desired bound. The difference between these two practices can be quite substantial.

"Although, prior to EPA's 2005 cancer guidelines, EPA sometimes combined q1*s, this is not the best statistical practice. Since (unlike the previous methodology) the best estimate as well as the bound are presented in the IRIS documents and since the method for estimating the bound is provided in the BMD technical guidance, it is not that much more difficult with EPA's current procedures to perform the correct statistical combination of the results. Using the correct statistical procedure has the added advantage of correctly assuming that using more data provides a reduction in the uncertainty and the bound will be closer to the combined best estimate."

Response to DoD Specific Comment 12

The term "best estimate" is not statistical terminology but rather descriptive of OEHHA scientists making a balanced choice of several options for the proposed URF. As noted in the TSD, the proposed URF was obtained by taking a geometric mean of 4 candidate values. This method is supported by the cancer TSD.

Given the various unquantifiable uncertainties that affect each of the URF values, and given that OEHHA judged some of the higher potency estimates to be more uncertain, the Office decided to use a non-statistical, but reproducible rule to choose a mid-range of the available values as a "best estimate" of a URF, "adequate to protect public health."

Calculating the geometric mean of the candidate URFs is equivalent to determining the median of a log-normal distribution defined by these values. The Office does not claim that this protocol is based on any particular statistical method or assumptions. We note that the traditional method of determining "the best" URF value by choosing the most appropriate tumor type, which in many cases is also the most sensitive tumor type, is primarily a qualitative analytical procedure that also eschews more involved statistical methods.

Regarding q1*s, as noted in the response to the previous comment, we used BMDS to calculate the potency factor.

DoD Specific Comment 13

"Multi-Organ Metabolism, Page 5, Third full paragraph. 'The kidney is viewed as the main site for formation of genotoxic metabolites by β-lyase cleavage of TCVC since β-lyase activity is relatively high in this organ.' However, this discussion fails to mention that rats have a much higher rate of production of mutagenic metabolites by this process than humans. To quote one of the authors cited (Rooseboom *et al.* The Journal of Pharmacology and Experimental Therapeutics 294:762-769, 2000, bold added), 'The present study indicated that all tested Se-Cys conjugates (n5 22) indeed underwent b-elimination reactions in human renal cytosol, although the activity was lower than that in rat kidney cytosol. Between 41- and 857- fold lower intrinsic clearances (Vmax/Km) were observed in human kidney cytosol compared with rat kidney cytosol.' Given the quantitative effect of a mutagenic mode of action on cancer potency estimates, this information should be provided to the reader."

Response to DoD Specific Comment 13

The human cytosolic β-lyase activities found for selenium-cysteine (Se-cysteine) conjugates in the Rooseboom *et al.* (2000) paper and referenced in DoD's comment, were based on kidney tissue obtained from three Danish men, all of whom died of cancer, and two of whom were elderly (77 and 78 years). This represents a very narrow sample of the human population and likely underestimates the true variation in human kidney β-lyase activity. Further, this study used the selenium analogues of cysteine conjugates and did not look specifically at TCVC as a cytosolic β-lyase substrate. An

additional caveat on this data is that no information is provided in the paper as to whether these individuals were undergoing chemotherapy or other medical interventions that may have altered their kidney metabolism prior to death.

Green *et al.* (1990) measured kidney cytosolic β-lyase activities for TCVC in rats and 7 human kidney samples indicating a smaller ratio between rat and human intrinsic clearance of about 25. Again, the human sample size is small, and the samples in this study were obtained from cancer patients or individuals suffering from kidney failure, which creates uncertainty regarding data quality.

However, Lash and Parker (2001) noted that: (1) cytosolic protein studies do not provide data on the levels of mitochondrial β-lyase activity, which could play an important part in this metabolic pathway, and (2) renal cytosolic β-lyases are inducible in rat by preexposure to PCE, which indicates that they could be inducible in humans. This could be an important factor increasing β-lyase activity in chronically exposed humans.

OEHHA also points out that potentially genotoxic dichloroketene and TCVC sulfoxides can be formed by alternative pathways involving TCVC oxidation in both kidney and liver (and possibly other tissues as well).

Given these and other uncertainties regarding the full sequence of events in the GST-conjugation pathway, it would be somewhat misleading to present the reader with partial information on the difference between the intrinsic clearance of Se-cysteine conjugates in humans and rats.

DoD Specific Comment 14

"Pharmacokinetic Model, Page 7. 'Table 2 shows a summary of model predictions for several types of dose-metric, as reported by Chiu and Ginsberg (2011).' Since this document did not use the Chiu and Ginsberg model, but rather a simplified version thereof, it would be more useful to see the same information for the model actually used in this analysis. Repeating information that is publically available does not excuse the analyst using a simplified version of the model from presenting the effects of the modifications on the choice of dose metric. The results of the unmodified model are not relevant to this analysis."

Response to DoD Specific Comment 14

Please refer to OEHHA's "Response to DoD Specific Comment 1."

DoD Specific Comment 15

"Pharmacokinetic Model, Page 8, Paragraph 1. 'In spite of the unresolved issues related to PCE's GST metabolism, OEHHA considers the Chiu and Ginsberg model to be the best available methodology for estimating dose metrics in the dose-response assessment.' If the best model produces an up to 3000-fold range for human exposures, it is unclear why OEHHA chose to reanalyze the data with 'a simplified, deterministic version of the model' with a 'pared-down version of the code'. Since the results of the best model are available, they should be used."

Response to DoD Specific Comment 15

Our use of the inhalation-only components of the Chiu and Ginsberg (2011) model, which was clearly described in the document, is not a reanalysis of the data. OEHHA extracted the requisite equations from the full model and utilized Chiu and Ginsberg's maximum likelihood estimates (MLEs) that were calculated by the authors using the Markov Chain Monte Carlo sampling technique. Once these estimates are derived, they can be used to run the model in deterministic mode. The only simplification that OEHHA made was to extract the intact inhalation-only code components from the full model for our more limited objectives of carrying out inhalation dose calculations. Thus OEHHA did use the best model available.

DoD Specific Comment 16

"Genotoxicity and Carcinogenicity, Page 9, Paragraph 1. OEHHA conflates genotoxicity and mutagenicity, and defines neither. As EPA's 2005 supplemental guidance describes procedures for a mutagenic mode of action, it is critical that OEHHA define and differentiate mutagenicity from genotoxicity."

Response to DoD Specific Comment 16

OEHHA methodology as described in the cancer TSD, does not depend upon making a sharp distinction between genotoxicity and mutagenicity.

The cancer TSD, at page 18, states:

> "Genetic damage in exposed organisms includes both gene mutations (point or frameshift), and larger scale effects such as deletions, gene amplification, sister-chromatid exchanges, translocations and loss or duplication of segments or whole chromosomes. These genetic effects of chemical exposures are deleterious in their own right. In addition, <u>since carcinogenesis results from</u>

> somatic mutations and similar genetic alterations, agents that cause genetic damage generally have carcinogenic potential." (emphasis added)

Later sections of the cancer TSD define OEHHA's method of adjusting carcinogenic potency to account for potentially higher sensitivity in early life stages. The cancer TSD, at page 68, explains that OEHHA's methodology for defining Age-Dependent Adjustment Factors (ADAFs) differs from US EPA's method as follows :

> "U.S. EPA is recommending the ADAFs described above only for mutagenic carcinogens, because the data for non-mutagenic carcinogens were considered to be too limited and the modes of action too diverse to use this as a category for which a general default adjustment factor approach can be applied. OEHHA considers this approach to be insufficiently health protective. There is no obvious reason to suppose that the toxicokinetics of non-mutagens would be systematically different from those of mutagens. It would also be inappropriate to assume by default that non-mutagenic carcinogens are assumed to need a toxicodynamic correction factor of 1."

Therefore, contrary to DoD's assertion, it is not critical to our dose-response analysis that we differentiate between genotoxic and mutagenic carcinogens.

DoD Specific Comment 17

"Dose-Response Assessment, Pages 18-19. The conclusion at the top of the page 'there are insufficient grounds to evaluate PCE as primarily a non-genotoxic carcinogen using a non-linear model.' has morphed to (by the bottom of the next page), 'Since PCE is considered to be a genotoxic carcinogen'. If OEHHA is following EPA's 2005 cancer guidelines, the appropriate explanation would be that, since the mode of action is not known, the default assumption of low-dose linearity was used. Otherwise, a mode of action analysis, per EPA's 2005 guidelines, should be provided. The only modes of action described in the document are a2u-globulin nephropathy in the male rat, and PPARa activation for mouse liver tumors. No formal mode-of-action analysis is presented for any mode of action, and a 'genotoxic carcinogen' is not described or defined.

"The document should be consistent as to whether PCE is considered to have a mutagenic mode of action, a genotoxic mode of action, or an unknown mode of action. Since the mode of action is used to justify the choice of dose-response model, the observed inconsistency within the document regarding the mode of action must be rectified."

Response to DoD Specific Comment 17

The bottom of page 15 of the document states:

> "PCE's carcinogenic mode of action (MOA) likely involves the genotoxicity of one or more of its oxidative- or GST-pathway metabolites, although the precise mechanisms are unknown."

More accurately, we should have said, "mode(s) of action," and we have corrected this in the document draft. Regarding the issues of genotoxicity, non-genotoxicity, and mutagenicity, and their implications for the dose-response analysis, OEHHA's methodology diverges from that of US EPA (2005) on this point. (Also see the response to the previous comment.) Thus, we emphasize that we are not following US EPA's 2005 cancer guidelines in this respect.

Moreover, the Office notes that while the document identifies various PCE metabolites as potential genotoxicants *and thus as potential carcinogens*, we neither require a finding of mutagenicity nor a "formal mode-of-action analysis" to support our choice of dose metric and our choice of the dose-response model with which to fit the data. OEHHA currently uses the US EPA BMDS implementation of the multi-stage cancer model for dose-response modeling of chemicals identified as potential carcinogens. Further, OEHHA also assumes low-dose linearity of the dose-response function for all potential carcinogens, unless convincing information is available for a carcinogen that would indicate otherwise. This is true for chemicals that OEHHA identifies as genotoxic, or as mutagenic, or even for substances that may act primarily as non-genotoxic carcinogens.

DoD Specific Comment 18

"Dose-Response Assessment, Page 20, 1st Full Paragraph. 'When multiplied by the BMR, the reciprocal of the BMDL gives a unit risk factor that is generally close in value to, and is used in place of (q1*).' This statement requires a citation since it is only true if the lower bound on dose is 'generally close in value to' the upper bound on risk, an assertion that is unlikely. The accurate statement is that the BMR divided by the BMDL provides the slope of the linear extrapolation from the BMDL to the origin. This is rarely, if ever, equivalent to the q1* generated by the 'traditional [linearized] multistage model' cited on the previous page. Problems with the linearized multistage model, e.g., that for some data sets the q1* generated was the upper-bound of the x-axis, are one of the reasons for the use of the benchmark dose approach.

"The linear extrapolations from the linearized multistage model that generates the q1* and the extrapolation from the BMDL from a BMR are not equivalent, as suggested in the quoted sentence. In particular, the BMDS allows the user to choose the BMDR and selection of different BMDRs for the same data generate significantly different estimates (as evidenced by EPA's current draft of RDX with results from various BMRs for the same data). The only equivalence to the BMR divided by the BMDL to the q1* is that both procedures have been used by EPA to estimate cancer potency. The two procedures, however, would not be expected to provide the same estimate of cancer potency."

Response to DoD Specific Comment 18

First, as we noted in the document, OEHHA used the US EPA BMDS implementation of the multi-stage cancer model to fit the dose-response data. With regard to DoD's detailed criticism of OEHHA's comparison of the BMDS multi-stage cancer model (which calculates a BMDL) and the linearized multistage model (which calculates a q1* value), OEHHA is observing the fact that in general values calculated by these two different methods are similar, not arguing about their statistical equivalence. In any event this will not affect the outcome of OEHHA's dose-response calculations or any other substantive aspect of the analysis presented in the TSD. Thus we acknowledge the comment but disagree with it.

DoD Specific Comment 19

"Dose-Response Assessment, Page 20, Third Full Paragraph. '...the combined cancer potency was also estimated for these groups using the multi-site tumor module provided in BMDS.' Based on EPA's 'Technical Background for MS Combo Program', OEHHA may have used this procedure improperly. The background document states that the result of this program, 'are valid only when the tumors are assumed to be independent of one another (conditional on dose level).' OEHHA assumes the same metric is valid for all tumor sites, may be assuming (per comment above, it is not clear) that PCE is a 'genotoxic carcinogen' for all sites, and dismisses organ-specific modes of action for liver and kidney tumors. Thus, it would appear that OEHHA is assuming that the tumors have similar modes of action. If this is an accurate interpretation of the text, the BMDS combo program cannot be used to combine the tumor sites. If OEHHA believes the tumors have different modes of action, the key events that differentiate those modes of action should be clearly stated. Otherwise, the data should be reanalyzed using an appropriate method, e.g., all tumor-bearing animals."

Response to DoD Specific Comment 19

DoD states that the BMDS "MS_combo" program cannot be used to combine tumor site risks unless the tumors are assumed to be independent of one another (conditional on dose level). In using the MS_combo module of BMDS, OEHHA assumes that the various tumor types are largely independent.

OEHHA makes no particular assumptions about the various modes of action operating to create these tumors. In the document, the Office states that PCE's carcinogenicity "likely involves" genotoxicity of its metabolites, but this is only one of many components of a complete mode of action. The expectation that risk of tumorigenesis at different sites contributes independently to the overall cancer risk is a common default assumption (related to the usual assumption of additivity for risks from exposure to different carcinogens), supported by arguments in US EPA and OEHHA cancer risk assessment guidelines and generally supported by experimental observations. There is no implicit assumption that cancers at the different sites are all caused by the same mechanism. In this case there are no specific data indicating that the tumors would be strongly associated statistically or biologically.

DoD Specific Comment 20

Page 22, Table 5. Table 5 is not labeled.

Response to DoD Specific Comment 20

OEHHA has added a label to the table noted in the comment.

3. Responses to Comments Received from the California Chamber of Commerce (CalChamber)

CalChamber General Comment 1

"Overall we question the process being undertaken for the OEHHA PCE potency value update. The U.S. EPA recently (in 2012) updated the Integrated Risk Information System (IRIS) dataset including the full breadth of available science on PCE generating health-protective toxicity information applicable to the entire nation. The outcome of the U.S. EPA's multi-stakeholder, multi-million dollar IRIS update effort (including documented review and responses to interagency reviewers from scientists from other non-EPA federal agencies, White House offices, and independent scientists external to EPA, as well as the public) was publication of a revised PCE inhalation cancer unit risk factor of 2.6E-07 per µg/m^3, backed by a 1,077-page Toxicological Review. The U.S. EPA affirmed (in June 2013) in OSWER Directive 9285.7-86 that IRIS serves as the 'gold standard' for selection of nationwide U.S. EPA Regional Screening Levels (RSL). It seems that instead of departing from current human health risk assessment best practices and unnecessarily revisiting the work only recently performed through a 'gold standard' process, OEHHA could instead adopt the U.S. EPA values without excessive revision and reconsideration, and this approach would both protect human health and thoughtfully steward California’s limited resources."

Response to CalChamber General Comment 1

OEHHA has independent responsibility under California law to develop cancer potency values for protecting the health of people living in California. In May 2009, the Office published its most recent cancer assessment guidance document, "Technical Support Document for Cancer Potency Factors: Methodologies for derivation, listing of available values, and adjustments to allow for early life stage exposures," (the "cancer TSD") pursuant to its responsibilities under California's Air Toxics "Hot Spots" (ATHS) Information and Assessment Act. The dose-response methodology presented in the cancer TSD is based upon the latest scientific knowledge as of the date of its preparation, and was independently reviewed by the Scientific Review Panel on Toxic Air Contaminants (SRP), established by the California Health and Safety Code, Section 39670.

In developing the cancer TSD, OEHHA scientists critically assessed US EPA's cancer risk assessment methods as documented in their 2005 guidelines (US EPA, 2005). OEHHA agrees with various aspects of US EPA's methods, and thus portions of the cancer TSD are consistent with US EPA guidance. However, OEHHA's methodology is

not the same as US EPA's, and where it differs, it tends to be more health-protective. The commenter may refer to the cancer TSD for additional details. It should be noted that federal air pollution law does not preclude California from independently defining health criteria that are more protective (i.e., more restrictive) than those defined by US EPA.

In updating PCE's cancer potency factors, OEHHA relied primarily on our cancer TSD and other previously developed OEHHA guidance, where relevant. Like US EPA, we used "the full breadth of available science on PCE generating health-protective toxicity information..." We have used the best available risk assessment methodology and toxicity data in estimating PCE's cancer potency factors for California, and have provided an adequate level of safety in consideration of the numerous irreducible uncertainties in the available toxicity data.

Finally, the commenter refers to OSWER Directive 9285.7-86, which is a US EPA (2013) document entitled, "Tier 3 Toxicity Value White Paper." In it, US EPA notes that OEHHA's toxicity values are credible because they "rely on best available science," and, "have undergone a high degree of scrutiny and peer review..." We agree with US EPA on this point.

CalChamber General Comment 2

"Similarly, OEHHA appears to be engaged in an exercise of 'raising the bar,' presumably to ensure continually more stringent evaluation of PCE, but fails to cite recent emissions data that show PCE is no longer the health concern for California residents that it may have been in the past. OEHHA should acknowledge that PCE is both less toxic than originally thought, and is hardly detectable in California air as of 2013. With these considerations in mind, a more appropriate focus for scientific consideration and public funding should be pollutants that may pose a greater risk than PCE."

Response to CalChamber General Comment 2

Per California law, OEHHA develops quantitative estimates of cancer potency for chemicals that have been defined as California TACs, and the Office used its cancer TSD and other recent scientific information to do so. OEHHA's methods are not exactly the same as US EPA's, and in some cases utilize more health-protective assumptions in the face of uncertain toxicity information. Thus, the Office may derive higher potency values than would US EPA, even while relying on similar toxicity information and modeling methods.

On CalChamber's second point: The draft PCE cancer potency factor update provides a description of the method by which the Office carried out its dose-response assessment to derive these values. With respect to PCE's carcinogenic effects, our analysis does not indicate that it is less toxic than originally thought. We agree with CalChamber that ambient air concentrations of PCE in California have been diminishing over time, and note that this is a result of successful state regulatory programs designed to achieve this goal. Nonetheless, PCE remains a California TAC, and the Office has statutory responsibility to develop and update the cancer potency factor for PCE as necessary.

CalChamber General Comment 3

"We question the scientific rationale for selection of data from the NTP (1986) study as the basis for calculation of the cancer potency factor in this update, when that same data was rejected previously by both OEHHA (1992) and more recently by the U.S. EPA (2012) as inadequate, of insufficient applicability to humans and, additionally, of insufficient quality for that purpose. The reasons for OEHHA to select the cancer endpoint from the 1986 study in 2016 when that data was rejected in 1992 are not transparent and should be fully explained. The implication of inclusion of the flawed 1986 NTP data mathematically allows OEHHA to calculate a more potent PCE toxicity value, as demonstrated on page 22 of the Public Review Draft. However, this approach is not consistent with best practice in risk assessment. Reliance on NTP (1986) despite previous detailed assessments rejecting same should be revisited, should OEHHA elect to pursue development of a cancer potency factor instead of adopting the IRIS PCE inhalation potency value. At the very least, Peer Review Panel charge questions should include, 'Is a combined cancer site' approach using the 1986 NTP study consistent with sound science? If so, why was this not the approach used by OEHHA in 1992 when it first assessed PCE's cancer potency? Why was this approach not taken by the U.S. EPA in the recent IRIS update for PCE?"

Response to CalChamber General Comment 3

OEHHA's 1992 dose-response analysis did utilize the NTP (1986) study data. The summary section of OEHHA (1992), at page 1-4, states that the data from both rats and mice from the NTP 1986 studies were used to derive the cancer potency estimates. The recommendation section, at page 5-33 of the same document, states that the "carcinogenic risk potency range is taken from the 1986 NTP rat and mouse studies."

However, OEHHA's 1992 analysis, which was based upon pre-2009 guidance, chose a more limited set of tumor data from the NTP (1986) study than was used in the update.

The 1992 evaluation evaluated mouse liver tumors and rat mononuclear cell leukemia, whereas the update included other tumor types for both species.

The inclusion of additional tumor types in the update is consistent with OEHHA's 2009 cancer TSD which uses a fuller range of tumor data and statistical methods of risk summation when increased tumors are observed at multiple sites in the exposed animals. According to the cancer TSD, at page 31:

> "For most carcinogens, the selection of the most sensitive site in the animal studies is recognized as providing a risk estimate which is appropriate to protect human health. However, for chemicals that induce tumors at multiple sites, the single-site approach may underestimate the true carcinogenic potential. [...] Instead, total cancer risk is estimated from all the sites at which agent induced tumors are observed (lung, bladder, leukemia, etc.), combined."

> "For carcinogens that induce tumors at multiple sites and/or with different cell types in a particular species and sex, OEHHA derives the animal cancer potency by probabilistically summing the potencies from the different sites and/or cell types."

Additionally, US EPA did not reject the NTP (1986) data as inadequate. For example, Section 5.3.1 of US EPA's IRIS Toxicological Review for PCE (US EPA, 2012), which describes the choice of data for dose-response calculations, states:

> "[S]everal chronic exposure studies in rats and mice include an oral gavage study in mice and female rats by the National Cancer Institute (NCI, 1977) and two inhalation studies in mice and rats (JISA, 1993; NTP, 1986). These studies established that the administration of tetrachloroethylene, either by ingestion or by inhalation to sexually mature rats and mice, results in increased incidence of tumors. Mouse liver tumors (hepatocellular adenomas and carcinomas) and rat mononuclear cell leukemia (MCL) were reported in both sexes in two lifetime inhalation bioassays employing different rodent strains, and mouse liver tumors were also reported in both sexes in an oral bioassay (NCI, 1977). Tumors reported in a single inhalation bioassay include kidney and testicular interstitial cell tumors in male F344 rats (NTP, 1986), brain gliomas in male and female F344 rats (NTP, 1986), and hemangiomas or hemangiosarcomas in male Crj:BDF1 mice (JISA, 1993)."

> "This analysis considers all three bioassays but focuses primarily on the JISA (1993) study results."

> "The NTP (1986) study was utilized for modeling the increased incidence in renal cancers, brain cancers, and testicular tumors with treatment reported only in this bioassay."

Further, as indicated in Table 5-18 of the IRIS Toxicological Review, US EPA calculated potency values for a variety of mouse and rat tumors using both NTP (1986) and JISA (1993) data sets. Thus, contrary to the commenter's assertion, it appears that US EPA found the NTP (1986) study to be sufficient quality to be considered in its quantitative dose-response evaluation.

Finally, CalChamber refers to the NTP (1986) data as "flawed" but has not provided any justification for this characterization.

CalChamber General Comment 4

"The OEHHA announcement for this review states, 'After the close of the public comment period, the documents will be revised as appropriate by OEHHA, and peer reviewed in 2016 by the State's Scientific Review Panel (SRP) on Toxic Air Contaminants.' If OEHHA elects to pursue independent development of cancer potency factors instead of adoption of the U.S. EPA cancer potency value as suggested, we urge OEHHA to consider an independent third party peer review separately from activities of the State's internal SRP review to increase the breadth and depth of expertise engaged in the process. In addition, release (in advance of any peer review activities) of draft charge questions before the peer review panel work begins would allow public input on the charge questions and encourage a robust review that addresses the issues of OEHHA as well as the broader stakeholder community.

"In summary, recognizing (a) that California faces limited resources, (b) that PCE is both less toxic than previously considered and no longer the air contaminant that it once was, (c) that the U.S. EPA recently thoroughly reviewed PCE science and developed cancer potency factors protective of health across the nation, and (d) that both OEHHA in 1992 and the U.S. EPA in 2012 rejected the data that is the basis for the current proposed draft PCE toxicity value, we encourage OEHHA to reevaluate any benefit to developing this additionally stringent potency value for PCE. Adoption of the IRIS value instead---a value that is founded on a very rigorous scientific process---would allow the State to focus resources and scientific attention on Toxic Air Contaminants of potentially greater concern to citizens of California."

Response to CalChamber General Comment 4

The Scientific Review Panel on Toxic Air Contaminants (SRP) is composed of highly qualified scientists who are professionally active or engaged in the conduct of scientific research, per California Health and Safety Code, Section 39670. The SRP review process thus constitutes the "independent third party peer review" process as called for by the commenter.

Regarding the restatement of comments in the summary paragraph, please refer to the responses to General Comments 1, 2, and 3.

CalChamber Specific Comment 1

"Page 1 of the Public Review Draft notes, 'OEHHA develops potency values for carcinogenic substances that are candidate Toxic Air Contaminants (TACs) or are listed under the Air Toxics Hot Spots Act. These values are used in the Air Resources Board's (ARB's) air toxics control programs and also by other State regulatory bodies, to estimate cancer risk in humans.' The draft fails to note specific examples of where the OEHHA potency value is used, such as in the Multiple Air Toxics Exposure Study (MATES IV) or in California's RWQCB Effects Screening Levels (ESLs). These points are important because the result of OEHHA's proposal to increase the PCE cancer potency value will have ramifications for these programs. Additionally, increasing the PCE cancer potency value as proposed will send a confusing message to the citizens of California because of inconsistency with the message delivered in May 2015 that PCE is no longer the public health concern it once was (e.g., SCAQMD 2015). In addition, cleanups under RWQCB will be evaluated to a more stringent PCE ESL standard and taxpayers will bear the burden of updating that ESL guidance to keep up with OEHHA‟s 'new,' more stringent proposed PCE cancer potency value."

Response to CalChamber Specific Comment 1

The primary purpose of the document is to revise the dose-response assessment and derive updated cancer potency values for PCE based upon new toxicologic information and OEHHA's most recent cancer assessment methodology. The document is not intended to provide a detailed discussion of how cancer potency values are used along with population exposure information to manage health risk in California's various environmental health protection programs and other health risk reduction activities. The commenter appears to confuse the concept of a chemical's cancer potency, which is derived through a risk assessment process, and that chemical's use and prevalence in the environment, which is addressed by regulatory agencies through a risk management process. In addition, we note that the updated PCE cancer potency factor is nearly unchanged from the 1992 value.

CalChamber Specific Comment 2

"Page 2, Section 3 'Major Sources and Uses' cites outdated (2004) facts and figures related to PCE production and demand: the first six lines of Section 3 should be updated to use 2015 figures, or the most recent data available. In addition, the final sentence of Section 3 unnecessarily cites outdated (2010) figures where more current

data are available. OEHHA should acknowledge and cite the MATES IV study conducted in 2012-2013, with a final report published in May 2015 (SCAQMD 2015). That study observed, 'PCE shows a continuing reduction in levels, likely a result of a number of air quality rules leading to the gradual phase-out of its use as an industrial and dry cleaning solvent' in California.

"Specifically, SCAQMD (2015) noted, 'Concentrations of PCE… have become so low such that the typical ambient concentrations are often below the detection limits of the measurements.' The actual measured annual average concentration of PCE in 2012-2013 was 0.03 ppb in the basin studied and SCAQMD found a 37% reduction in PCE between 2009 and 2012. Any update to the public should include the most recently available data so taxpayers are properly informed as to the potential magnitude of a PCE health risk. If OEHHA is not able [to] update Section 3 to use current 2015-2016 figures, the draft should clearly state that 2004 and 2010 data are the most recent available for this effort and thus remain appropriate. Additionally, a summary of MATES IV and findings reported in SCAQMD 2015 (or the most recent statewide report) should be included and explained in context to the PCE update."

Response to CalChamber Specific Comment 2

As noted in the previous response, the primary purpose of the document is to revise the dose-response assessment and derive updated cancer potency values for PCE based upon new toxicologic information and OEHHA's most recent cancer assessment methodology. The document is not intended to provide a detailed PCE emissions inventory analysis, nor is its purpose to carry out an exposure assessment for sites located in the South Coast Air Quality Management District (SCAQMD), such as sites covered in the MATES-IV study.

TAC emissions inventories are developed by the Air Resources Board (ARB) in cooperation with California's local air quality management districts. The PCE emissions data for 2010, provided in Section 3 of the document, represents the most recent statewide estimate published by ARB. The commenter also asserts that Dow Chemical Company's 2008 report of U.S. demand for PCE in 2004 is "outdated," but does not provide an alternative estimate or updated source of information to show that the value is, in fact, outdated. Although the estimate was for 2004 and current demand may be lower, the Dow estimate may still be reasonably representative of current demand, and at a minimum, provides the reader with context on the use of PCE in recent history.

CalChamber Specific Comment 3

"Page 2, Section 5 'National and International Hazard Evaluations' includes only select information resulting in an incomplete representation of available information. A comprehensive discussion of the U.S. EPA (2012) IRIS toxicological profile process (including the NRC 2010 peer review, and other independent reviews) is distinctly missing. As an example of how this selective inclusion of information can be misleading, the Section 5 text might lead the reader to think that specific animal cancers are linked to PCE ingestion. OEHHA wrote, 'The NTP report noted that PCE exposure produced tumors in multiple tissue types of both sexes of mice and rats, by ingestion and/or inhalation. The tumor types cited by NTP were: mononuclear-cell leukemia in rats, tubular-cell kidney tumors in male rats and liver tumors in mice.' However, in actuality, the U.S. EPA (2012) found that only inhalation PCE studies were relevant, as the single ingestion (gavage) study was determined to be 'inconclusive with respect to carcinogenicity due to a high incidence of respiratory disease in all animals and shortened survival in PCE-exposed animals.' U.S. EPA (2012) peer reviewers noted (and the IRIS Toxicological Review reflects) that because the available oral gavage cancer bioassay was inconclusive due to respiratory infection in all groups, and therefore it cannot be concluded based on that ingestion study that PCE caused tubular cell kidney tumors in male rats. In addition, U.S. EPA (2012) summarize rodent studies of PCE addressing renal α2u-globulin accumulation that support the well-known toxicology concept that this type of kidney damage is a phenomenon that occurs only in male rats and that is irrelevant to humans (see Table 4-53 of the IRIS Toxicological Review and related text).

"Text appears to be selectively citing out of context and without relevant technical details. The purpose of this selectivity seems oriented at making the case that PCE causes all manner of cancers, a point central to development of the conclusion on page 22 and which is a necessity to support the approach of taking the geometric mean across cancer endpoints and across studies in multiple species. A more balanced approach is recommended, one that would be more useful to the public in Section 5. OEHHA should select a singular cancer end point and key study upon which to focus (i.e., liver cancer from JISA 1993, or alternate key study and endpoint); data for this endpoint should be summarized with consideration to consensus across national and international assessments so as not to lose the reader in contentious details that combine rats with mice, types of tumors, and multiple studies in a geometric mean method that is not used in other cancer potency derivation methods for human health risk assessment."

Response to CalChamber Specific Comment 3

The commenter asks for a "comprehensive discussion of the US EPA (2012) IRIS toxicological profile process (including the NRC 2010 peer review, and other independent reviews)." The purpose and scope of the document is clearly described in the document introduction. A comprehensive discussion of the U.S. EPA (2012) toxicological profile is not part of the purpose or scope of the document.

With regard to the document statement that, "The NTP report noted that PCE exposure produced tumors in multiple tissue types of both sexes of mice and rats, by ingestion and/or inhalation. The tumor types cited by NTP were: mononuclear-cell leukemia in rats, tubular-cell kidney tumors in male rats and liver tumors in mice," we agree that the sentences at issue should be edited to convey the NTP determinations more accurately and have made the appropriate edits to the document.

The commenter also notes that US EPA determined that the data for rats in the 1977 National Cancer Institute (NCI, 1977) oral gavage study was not useful for use in its inhalation dose-response analysis due to problems with respiratory disease and shortened survival of the exposed animals. OEHHA does not rely on the NCI (1977) oral study for its quantitative dose-response analysis, but uses appropriate information from the study as qualitative supplemental information. OEHHA's analysis of kidney tumors in rats is based on the NTP (1986) inhalation study.

CalChamber also notes that the US EPA (2012) toxicological review includes a discussion of renal α2u-globulin accumulation. OEHHA's document for PCE also includes a discussion of this issue and why it is unlikely to be relevant in the case of rat kidney tumors induced by PCE exposure.

CalChamber also disagrees with OEHHA's use of tumor data from multiple studies, species, and multiple tissue types to inform the choice of a cancer potency value for PCE. CalChamber states that 1) OEHHA should not use a geometric mean of multiple dose-response estimates and 2) OEHHA should select a single cancer end point from a single study. OEHHA refers the commenter to our responses to CalChamber Specific Comments 6, 7, and 8, which are concerned with the same issue.

CalChamber Specific Comment 4

"On page 22, OEHHA quantitatively relies upon the NTP (1986) rat leukemia data, ignoring the Science Advisory Board of the U.S. EPA's observation that although leukemias were observed in the PCE-exposed rats in the NTP (1986) inhalation bioassay, control rats in another 1986 NTP bioassay (for methylene chloride, captured

in the IRIS Toxicological Review for that substance) showed the same incidence of leukemias as the PCE-exposed animals. The NTP (1986) rat leukemia data were specifically deemed questionable, and after extensive peer review detailed in the 2010 NRC report, were ultimately not incorporated into the U.S. EPA (2012) IRIS potency factor for PCE. OEHHA should acknowledge that exclusion of this data from use in development of a potency factor is both credible and appropriate considering the rate of spontaneous leukemias in the 1986 NTP studies and in recognition of the robust consideration already given the data. If OEHHA does not adopt the U.S. EPA PCE potency value and pursues an independent revision to the PCE potency factor, then additionally, OEHHA should add discussion of this weakness in the data to text on page 15, delete the NTP (1986) rat leukemia data from Tables 4, 5, and 6, and delete the weighting for this endpoint from any quantitative 'combined site' modeling. This will impact the NTP male rat 'multiple tumor' row on the page 22 table, and will change the largest (most conservative) URF that OEHHA has calculated for the NTP 'combined site' input to the geometric mean of 6.06E-06 per $\mu g/m^3$. Inappropriately including the NTP (1986) leukemia data results in miscalculation of the PCE potency factor resulting in an excessively conservative number which, consequently, generates a value inconsistent with what the U.S. EPA (2012) established to be reliable and appropriate after extensive public comment and multi-stakeholder, external peer review."

Response to CalChamber Specific Comment 4

We refer the commenter to the detailed discussion in the document supporting the use of rat MCL in the dose-response assessment. In addition, also see OEHHA's responses to DoD Specific Comments 4 and 5, which provide additional discussion of the rat MCL issue.

CalChamber mischaracterizes OEHHA's technical approach when it says that the Office "relies upon the NTP (1986) rat leukemia data [...]" Instead, OEHHA's method is to consider the MCL dose-response data from both the JISA (1993) and NTP (1986) studies, *along with* the other tumor data in mice and rats in order to define a potency value that uses a range of the high-quality dose-response information, takes account of uncertainty in the data, and is "appropriate to protect human health," per our cancer TSD.

Finally, regarding use of the NTP (1986) rat MCL data: Please see the OEHHA response to DoD Specific Comment 3. Additional discussion on this topic is provided in the response to CCC Specific Comment 11.

CalChamber Specific Comment 5

"In Tables 5-7 and mathematically in the potency factor calculated for PCE on page 22, OEHHA includes another irrelevant and poor quality cancer endpoint from the NTP (1986) data set. As noted previously in Specific Comment 3, U.S. EPA (2012) explained the poor human relevance of the kidney tumors in male rats found in the NTP (1986) bioassay. CDC/NIOSH and OSHA agreed with the U.S. EPA, the EPA Science Advisory Board and NRC (2010) that these tumors may not be good predictors of human risk; no other modern U.S. risk assessment uses the NTP (1986) rat kidney tumor data. If OEHHA does not adopt the U.S. EPA PCE potency value and pursues an independent revision to the PCE potency factor, then at a minimum, OEHHA should strike male rat 'renal adenoma or carcinoma' data from all tables and text, including removing it from Table 4, Table 5, and the Table 6 row for individual modeling endpoints, as well as from the 'combined site' dataset, and then remodel the results. Use of the rat kidney tumor data, as with the NTP (1986) rat leukemias, is in stark contrast to the position of every other regulatory body that has had expert peer review and critical toxicology input on the topic of relying quantitatively on this endpoint from the NTP (1986) study, which OEHHA did not rely upon in 1992. A Peer Review Panel charge question should be, 'Should OEHHA include renal α2u-globulin nephropathy-influenced kidney cancer endpoints in the quantitative potency factor, even though OSHA, CDC/NIOSH and U.S. EPA all acknowledge this male rat phenomenon is not relevant to humans?' "

Response to CalChamber Specific Comment 5

OEHHA restates part of its response to CalChamber General Comment 3, as follows:

CalChamber mischaracterizes US EPA's assessment and use of the NTP (1986) study data. US EPA did not reject the NTP (1986) data as inadequate. For example, Section 5.3.1 of US EPA's IRIS Toxicological Review for PCE (US EPA, 2012), which describes the choice of data for dose-response calculations, states:

> "Several chronic exposure studies in rats and mice include an oral gavage study in mice and female rats by the National Cancer Institute (NCI, 1977) and two inhalation studies in mice and rats (JISA, 1993; NTP, 1986). These studies established that the administration of tetrachloroethylene, either by ingestion or by inhalation to sexually mature rats and mice, results in increased incidence of tumors. Mouse liver tumors (hepatocellular adenomas and carcinomas) and rat mononuclear cell leukemia (MCL) were reported in both sexes in two lifetime inhalation bioassays employing different rodent strains, and mouse liver tumors were also reported in both sexes in an oral bioassay (NCI, 1977). Tumors

> reported in a single inhalation bioassay include kidney and testicular interstitial cell tumors in male F344 rats (NTP, 1986), brain gliomas in male and female F344 rats (NTP, 1986), and hemangiomas or hemangiosarcomas in male Crj:BDF1 mice (JISA, 1993)."

> "The NTP (1986) study was utilized for modeling the increased incidence in renal cancers, brain cancers, and testicular tumors with treatment reported only in this bioassay."

Further, as indicated in Table 5-18 of the IRIS Toxicological Review, US EPA calculated potency values for a variety of mouse and rat tumors using both NTP (1986) and JISA (1993) data sets. Thus, contrary to the commenter's assertion, it appears that US EPA found the NTP (1986) study to be of sufficient quality to be considered in its quantitative dose-response evaluation.

CalChamber mischaracterizes the NRC (2010) and US EPA positions on the use of NTP (1986) rat kidney tumor data, stating incorrectly that, "Use of the rat kidney tumor data [...] is in stark contrast to the position of every other regulatory body that has had expert peer review and critical toxicology input on the topic [...]"

On the contrary, the NRC peer review report, at page 71, stated:

> "Renal-tubular adenoma and carcinoma were observed in male rats in the NTP (1986) bioassay and to a lesser extent in the Japan Industrial Safety Association (JISA 1993) studies.
>
> "There is a very low spontaneous incidence of renal tumors in Fischer 344 rats (Haseman *et al.* 1998). Induction of renal tumors in rats by tetrachloroethylene is therefore easily observed against a low background. In addition, the controls had only benign tumors, not malignant tumors, whereas the high-dose group had two malignant tumors. In the draft IRIS assessment, EPA calculates the chance that two animals will have a rare tumor to be less than 0.001, giving biological relevance to the finding.
>
> "Overall, the dose-dependent induction of renal tumors in one experiment against the low background incidence of renal tumors in rats exposed to tetrachloroethylene indicates that tetrachloroethylene can induce renal tumors in rats. After integrating the results of the studies, the committee concluded that tetrachloroethylene induces renal tumors in rats. EPA considers the renal tumors to be suggestive of an effect and notes that it is similar to the effects of other chlorinated ethanes and ethylenes. The committee agrees with EPA's assessment."

At page 73 of the NRC report, the expert panel states:

> "The draft IRIS assessment concludes that a mutagenic mode of action cannot be ruled out. The committee agrees with this assessment. A mutagenic mode of action is supported by the findings after exposure to the structurally similar trichloroethylene.

> "While the mode of action of tetrachloroethylene tumorigenesis is not understood, the α2μ-globulin nephropathy and peroxisome proliferator modes of action are not consistent with experimental results. A mutagenic mode of action cannot be ruled out."

Finally, regarding the question of renal α2u-globulin nephropathy, the OEHHA document included a detailed discussion of this issue and why it is unlikely to be relevant in the case of rat kidney tumors induced by PCE exposure.

CalChamber Specific Comments 6, 7, and 8

"Comment 6: On page 22, OEHHA departs from standard toxicology practice, as well as that of U.S. EPA and the recommendation of the NRC (2010) in its peer review of PCE, in combining multiple tumor types. In fact, once rat kidney and leukemia data are properly removed from Table 4, none of the NTP (1986) rat endpoints are statistically significant (see Table 4 footnote 'c') and OEHHA can properly focus on the JISA (1993) data set. If OEHHA declines to adopt the U.S. EPA approach or incorporate these revisions, an independent Peer Review Panel should be asked to address the question, 'Is it scientifically valid and appropriate to include, and combine, all the NTP endpoints to derive the 'multiple tumor' URFs shown in OEHHA"s table on page 22 for use in a human potency factor?' This combination implies all the endpoints are equally relevant to humans, and that all endpoints are equally robust, which is scientifically not the case as detailed in specific comments above.

"Comment 7: On page 22, OEHHA departs from standard toxicology practice and also crosses over studies to derive a 'geometric mean' for a human health cancer potency factor. While this approach is sometimes used for ecological risk assessment, this is uncommon practice in modern toxicology methods for human risk assessment. An appropriately qualified independent peer review panel should thus be charged with the question, 'Is it appropriate for OEHHA to estimate human PCE health risks using a geometric mean for multiple studies and cancer endpoints combined, or should OEHHA select a key, relevant cancer endpoint and estimate cancer risks for that specific (and appropriately sensitive) cancer endpoint?' If the approach is retained, the reason for OEHHA"s departure from selecting a key study (as U.S. EPA guidance and the NRC 2010 peer review report recommended) to prefer a nonstandard method (adopting a geometric mean approach) should be clarified.

"Comment 8: The method OEHHA uses to arrive at the PCE inhalation potency factor on page 22 is inconsistent with the U.S. EPA guidance for human health toxicity value development, and also inconsistent with other state and international (Canada, France, etc.) human toxicology and health risk assessment guidance on the topic. No authoritative health body recommends against selecting a key study in favor of generating a 'geometric mean' across studies in different species. U.S. EPA (2005) Guidelines for Carcinogen Risk Assessment also do not infer concordance across species. We recommend OEHHA follow a validated, authoritative method that includes key study selection and is consistent with other U.S. methods for human health risk assessment."

Response to CalChamber Specific Comments 6, 7, and 8

CalChamber takes issue with OEHHA's use of dose-response data from more than a single key study, as well as using a geometric mean value to derive the proposed cancer potency value. CalChamber appears to believe that there is only one acceptable and accepted way to choose the primary data set for dose-response analysis and only one acceptable and accepted method of choosing the best potency factor from a set of possible candidates. OEHHA disagrees with CalChamber's assertions.

The cancer TSD suggests, as a default option, identifying a single study that represents the best estimate of potency, but does not prohibit using alternative methods (e.g. geometric mean) for deriving potency factors. In the case of PCE, OEHHA judged that both the JISA (1993) and the NTP (1986) studies provided acceptable and non-redundant dose-response information suitable for a quantitative estimate of cancer potency. However, as we noted in several sections of the document, the NTP (1986) study, while important to include in the quantitative determination, was also considered to be more uncertain for reasons stated in the document.

Given that the candidate potency values are each impacted by uncertainty, and given that OEHHA considered some of the higher potency estimates to be more uncertain, the Office decided to use the first of the four selection options presented in the California Department of Health Services cancer risk assessment guidelines (CDHS, 1985). OEHHA chose a mid-range potency from the available values (i.e., the geometric mean) as a "best estimate" for PCE's cancer potency, a value that the Office also judged to be "adequate to protect public health."

Calculating the geometric mean of the potencies is equivalent to determining the median of a log-normal distribution defined by these values. US EPA has used this method occasionally (e.g., see the current US EPA IRIS slope factor for DDT) (US EPA,

2016). An example in which OEHHA has used a geometric mean value is the Public Health Goal for methyl-t-butyl ether (MTBE) (OEHHA, 1999).

Finally, we also provide the following discussion on this topic from a recent textbook on health risk assessment (Theodore and Dupont, 2012, at page 216):

> "In deriving slope factors, the available information about a chemical is evaluated and an appropriate data set is selected. [...] If animal data are used, the species that responds most similarly to humans (with respect to factors such as metabolism, physiology, and pharmacokinetics) is preferred. When no clear choice is possible, the most sensitive species is given the greatest emphasis. <u>Occasionally, in situations where no single study is judged most appropriate, yet several studies collectively support the estimate, the geometric mean of estimates from all studies may be adopted as the slope factor. This practice ensures the inclusion of all relevant data</u>." (emphasis added)

CalChamber Specific Comment 9

"Despite contentious scientific issues related to the Chiu and Ginsberg (2011) model for PCE metabolism, OEHHA considers the Chiu and Ginsberg model to be the best available methodology for estimating dose metrics in the dose-response assessment. This is a departure from U.S. EPA (2012) recommendations on what Chiu and Ginsberg (2011) modeling can (and cannot) confirm, based on the variability of up to 3,000 in relation to one pathway that occurs in humans. For a model (revised by OEHHA in Appendix A) to be used in the PCE update, it should be validated and independently verified by third party (peer review) PBPK modeler(s) prior to acceptance. Then, it should be confirmed to be 'fit for purpose' as the U.S. EPA had done with its interpretation of the 2011 model. Neither the general public nor California lawmakers should be expected to understand Appendix A and its implications on the OEHHA PCE cancer potency value. The independent peer review panel should be asked whether this model is appropriately used and should be able to confirm or refine its use in the PCE potency value."

Response to CalChamber Specific Comment 9

OEHHA refers the commenter to the responses to DoD Specific Comments 1 and 15 which address the issues raised by this comment.

CalChamber Specific Comment 10

"California citizens will be told their PCE cancer risk is 23 times higher than U.S. EPA would calculate (at the same PCE air concentration) for citizens of Arizona or Nevada,

complicating human health risk assessment of shared PCE impacts from point sources whose emissions might cross state boundaries. The California public will not have an 'apples to apples' comparison of their own health improvements over time as compared to the health of other basins or states. This implication, particularly on neighboring states within U.S. EPA Region 9, highlights a significant disconnect between OEHHA and the remainder of the U.S., warranting complete and transparent independent peer review."

Response to CalChamber Specific Comment 10

The CalChamber comment refers to potential regulatory complexities that are beyond the scope of the document analysis and OEHHA's responsibility, which in this case, is to develop a cancer potency value for PCE based on up-to-date scientific information and our cancer TSD (OEHHA 2009). CalChamber's implication that OEHHA should consider PCE impacts in neighboring states as a factor in conducting its own PCE risk assessment would actually undermine OEHHA's statutory responsibility to conduct its risk assessments based purely on scientific criteria. And, as previously stated, the upcoming SRP review constitutes the statutorily mandated peer review of the draft PCE assessment.

CalChamber Specific Comment 11

"In Appendix B on page 42, contrasting details of the JISA (1993) and NTP (1986) studies emphasizes another area where OEHHA departs from best practices: In modern standard toxicology, scientists preferentially rely quantitatively upon studies that are as close to the anticipated inhalation concentration in the 'real world' exposure as possible. This [is] among the many reasons why U.S. EPA and its NRC (2010) peer reviewers downgraded the NTP (1986) study in favor of the JISA (1993) study when selecting the key PCE inhalation cancer study. While neither PCE inhalation cancer study included exposures truly reflective of current California air concentrations, the JISA dose levels are far closer than those used in the NTP study. Specifically, the 1993 JISA data was for PCE exposures at three dose levels (10 ppm, 50 ppm, and 250 ppm for mice). While even the lowest JISA dose level of 10,000 ppb (10 ppm) PCE is still 333,333-times higher than the 2013 PCE air concentration of 0.03 ppb measured in some California air (SCAQMD 2015), it is inconsistent with current scientific practice for OEHHA to use the far higher NTP (1986) concentrations with the more appropriate JISA data available.

"California's health will be protected by use of the more relevant JISA (1993) inhalation study data which are closer to the actual/anticipated PCE exposures that could be encountered in 2016 and beyond. U.S. EPA and its peer review panel did not use the

NTP (1986) study because it failed to have a sufficient number of doses (e.g., two in the NTP study, versus three used in the JISA dataset) to have an acceptable dose-response curve. In addition, the JISA requires less extrapolation to relevant concentrations among the purview of regulatory agencies (i.e., the lowest JISA dose was far closer to the air concentrations being assessed than the NTP 1986 dose ranges). If the NTP (1986) data is retained for quantitative use in the PCE potency factor update, OEHHA should transparently explain the rationale for citing a lesser quality, two- dose, high-dose study over the more recent, better quality, three-dose, lower-dose data set. Further, OEHHA should explain why it is has elected to disregard the U.S. EPA (2005) Guidelines for Carcinogen Risk Assessment regarding preference for low-dose studies designed to avoid excessive extrapolation for use in human health risk assessment. The Peer Review Panel charge questions should include, 'Is the dose range in the key study/studies that form a quantitative basis for the OEHHA potency factor update relevant to current and future California exposures being assessed?' "

Response to CalChamber Specific Comment 11

OEHHA disagrees with the commenter that its use of both the JISA (1993) and the NTP (1986) rodent study data "departs from best practices." The document noted, in this case agreeing with US EPA, that the JISA (1993) study had an advantage of testing animals at several lower doses than the NTP (1986) study. However, OEHHA deemed it important to also use the NTP (1986) data. We also note that US EPA (2012) used the NTP (1986) study data in developing a list of candidate dose-response values, and considered these values in its final deliberations. OEHHA also refers CalChamber to our response to DoD Specific Comment 3, that provides further support for use of both the JISA (1993) and NTP (1986) data.

Regarding US EPA (2005) guidelines and a preference for using lower-dose studies, we agree with US EPA on this issue, but point out to CalChamber that the preference is conditional on other aspects of the studies being equal. In the present case, aside from differing dose levels the studies are not equivalent in terms of animal models tested, as described above.

CalChamber Specific Comment 12

"According to the U.S. EPA Risk Characterization Handbook (2000) guidelines on transparency, as well as the NRC (2010) reminder on best practices, it is expected that any human health risk assessment will clearly and transparently convey to the public the certainty with which a cancer potency value is developed, and avoid inference of a 'false sense of certainty.' In contrast to this expectation, the OEHHA PCE potency value

Public Review Draft contains no uncertainty analysis, which may lead to the presumption the toxicity value put forth is a scientific fact, as opposed to a calculated value that departs from mainstream validated procedures used elsewhere in the U.S. and the world. The Draft should be revised to include an uncertainty analysis with clear and transparent discussion of the uncertainty related to the cancer potency value including all deviations from generally recognized good practice and potential implications of the approach taken."

Response to CalChamber Specific Comment 12

We disagree with CalChamber that the document "contains no uncertainty analysis." In developing the cancer potency factor for PCE, OEHHA discussed various aspects of uncertainty throughout the document. OEHHA refers the commenter to its response to DoD's General Comment for further discussion of this issue. Based on the comment, however, we have provided additional discussion in the document on several of the more important aspects of uncertainty.

CalChamber Specific Comment 13

"The reports and/or guidance detailed below (or their underlying technical methods) are likely to be impacted by the OEHHA draft PCE potency value change. It is unclear whether the wide-ranging impacts across multiple California programs were appropriately considered, as these efforts are not cited in the OEHHA draft PCE development. Because impacts of the OEHHA draft on these and other California initiatives could be substantial, a regulatory impact analysis (including consideration of the financial burden of updating all methodologies that involve PCE health risk assessment in the state of California, relative to the benefit of increasing the PCE cancer potency value) should be conducted:

- South Coast Air Quality Management District (SCAQMD). 2015. Multiple Air Toxics Exposure Study in the South Coast Air Basin (MATES). May. http://www.aqmd.gov/docs/default-source/air-quality/air-toxic- studies/mates-iv/mates-iv-final-draft-report-4-1-15.pdf?sfvrsn=7.
- California Regional Water Quality Control Board (RWQCB). 2007. http://www.waterboards.ca.gov/rwqcb2/water_issues/available_documents/esl.pdf"

Response to CalChamber Specific Comment 13

The Office develops potency values for carcinogenic substances that are candidate Toxic Air Contaminants (TACs) (Health and Safety Code Section 39660) or are listed

under the Air Toxics Hot Spots Act (Health and Safety Code Section 44321). These values are used in the Air Resources Board's (ARB's) air toxics control programs and also by other State regulatory bodies, to estimate cancer risk in humans.

OEHHA is not statutorily mandated to carry out the analyses requested by the commenter.

CalChamber Specific Comment 14

"The assessments and guidelines detailed below do not appear to have been adequately considered, cited or incorporated sufficiently in the OEHHA draft PCE development document:

- While NRC (2010) is cited in the OEHHA assessment, OEHHA did not implement the NRC (2010) findings (as detailed in specific comments above). The Peer Review Panel should require that deviations from the NRC (2010) recommendations are identified clearly and discussed transparently to assist the public in understanding why OEHHA concludes it must differ from the NRC.
- In 2007, the Environmental Council of States advocated for 'assessments which have been externally and independently peer reviewed, where reviewers and affiliations are identified. Other things being equal, there should also be a preference for assessments with more extensive peer review. Panel peer reviews are considered preferable to letter peer reviews.' As noted in General Comment 4, such a review would improve the quality of OEHHA"s PCE draft and provide a medium for OEHHA to justify decisions on deviating from findings in the U.S. EPA IRIS assessment of PCE and its many transparent peer reviews including NRC (2010) and public comment. When set side-by-side with the U.S. EPA IRIS assessment of PCE and its many transparent peer reviews including NRC (2010) and public comment, it is obvious that the less extensive 'peer review in 2016 by the State’s Scientific Review Panel on Toxic Air Contaminants' does not fully meet externally and independent peer review criteria.
- As a final point, we encourage OEHHA to consider U.S. EPA's Science and Technology Policy Council's Peer Review Handbook, 4th edition, October 2015 as a resource for guidance on best practice in peer review. If OEHHA elects not to follow recommendations on peer review set forth in the U.S. EPA 2015 Handbook, the rationale for not undertaking an independent external peer review of the PCE draft potency value should be clearly stated in the document.

"The approaches outlined above have been relied upon nationwide by the U.S. Environmental Protection Agency and other states, and, indeed, in other nations.

OEHHA is encouraged to apply these same balanced and scientifically sound approaches."

Response to CalChamber Specific Comment 14

OEHHA has independent responsibility under California law to develop cancer potency values for protecting the health of people living in California. In updating PCE's cancer potency factors, OEHHA relied primarily on our cancer TSD and other previously developed OEHHA guidance, where relevant.

In developing the cancer TSD, OEHHA scientists critically assessed US EPA's cancer risk assessment methods as documented in their 2005 guidelines (US EPA, 2005). OEHHA agrees with various aspects of US EPA's methods, and thus portions of the cancer TSD are consistent with US EPA guidance. However, OEHHA's methodology is not the same as US EPA's (and where it differs, it tends to be more health-protective). The commenter may refer to the cancer TSD for additional details.

Before finalization, draft cancer potency documents developed by OEHHA are independently reviewed by the Scientific Review Panel on Toxic Air Contaminants (SRP), established by the California Health and Safety Code, Section 39670. We refer CalChamber to this California law for more information on the scientific qualifications of the SRP members. OEHHA may, on a case-by-case basis, utilize information developed by the NRC working groups, but is not bound by federal peer-review processes.

OEHHA has used the best available risk assessment methodology and toxicity data in estimating PCE's cancer potency factors for California. The potency value provides an adequate level of safety in consideration of the numerous irreducible uncertainties in the available toxicity data. It should be noted that federal air pollution law does not preclude California from independently defining health criteria that are more protective (i.e., more restrictive) than those defined by US EPA.

4. Responses to Comments Received from the Halogenated Solvents Industry Alliance (HSIA)

HSIA Comment 1

"MCL lacks relevance for humans."

"In its review of the draft IRIS assessment in 2010, the majority of the NRC panel recommended against the use of MCL data from F344 rats to calculate a cancer slope factor for regulatory use."

"HSIA urges OEHHA to reconsider recommending a cancer potency value based on the MCL data. Its propensity to develop spontaneous MCL shows that the F344 strain does not reflect either the general human population or any significant sensitive sub-population."

"As noted in the NRC review of the draft IRIS assessment, 'NTP has decided to stop using its F344/N rat colony in its bioassays for reasons that include the high background rate of MCL'..."

"The NRC panel judged that "the use of the MCL data could be justified only if it is EPA's policy to choose the most conservative unit risk when considering options but that such justification should be distinguished as a policy decision, not a scientific one."

" [T]he weight of the evidence does not justify use of the F344 rat MCL data for risk assessment."

Response to HSIA Comment 1

The document provides a detailed discussion to support OEHHA's use of rat MCL in the dose-response assessment, to which we refer the commenter. OEHHA also refers the commenter to our responses to DoD Specific Comments 4 and 5, which provide further support for the use of rat MCL in our analysis.

In addition, regarding the NRC panel statement noted in the comment, that using rat MCL represents a "policy decision, not a scientific one," OEHHA strives to determine potency estimates that are "appropriate to protect human health," a policy stated in our cancer TSD. In so doing, we believe it scientifically prudent to consider and make allowances for data gaps and uncertainties in the available toxicologic information. We also note that the potency value obtained from the rat MCL data is not the highest ("most conservative") estimate that could be obtained. In OEHHA's analysis, the highest estimate was obtained by calculating the combined risk of four tumor types found in the NTP rat study.

HSIA Comment 2

"OEHHA inappropriately minimizes the uncertainty associated with the glutathione conjugation pathway in metabolism of PCE."

"...EPA developed a 'harmonized' PBPK model that included consideration of the GSH pathway, but conceded that 'the GSH conjugation pathway in humans remains highly uncertain and/or variable, and that additional data are needed to better quantify that pathway in humans.' "

"An important consideration in evaluating the role of the GSH pathway in PCE toxicity is the exposure dose. Mice have been shown to metabolize PCE to trichloroacetic acid (TCA) to a greater extent than rats; human activity is reported to be even lower than that in rats. In both rats and humans, saturation of this CYP-dependent oxidation of PCE is reported to occur at exposure concentrations of 100 ppm or greater, raising the potential of exposure-dependent metabolite patterns. It should be noted that the F344 rats were exposed to PCE concentrations of 200 and 400 ppm in the NTP (1986) bioassay and 50, 200, or 600 ppm in the JISA (1993) study. In addition to potential impacts on metabolite patterns at/around CYP saturation, consideration must be given to the relevance of bioassay exposure levels to most human exposures.

"As with oxidative metabolism, the primary pathway for metabolism of PCE, *in vitro* studies of GSH conjugation in mice, rats, and humans have shown considerable intra- and interspecies variability. Reported conjugation rates also differ by several orders of magnitude between laboratories. However, in order for GSH conjugation to be relevant in humans, there must be a significant capacity to form glutathione conjugates. In our view, this has not been demonstrated."

Response to HSIA Comment 2

In this comment, HSIA quotes US EPA (2012) that, "the GSH conjugation pathway in humans remains highly uncertain and/or variable..." OEHHA agrees with US EPA (2012) that the PBPK modeling analysis for the GSH conjugation pathway in humans contains a relatively large amount of uncertainty and/or variability, and that additional data are needed to better quantify that pathway in humans. The Office points out, however, that the GSH conjugation pathway could be strongly determined by variability (i.e., actual biological variation within the population) as opposed to uncertainty. Moreover, there is still a large amount of uncertainty regarding the importance of the CYP 450 and/or FMO-3 oxidation branch of the GSH conjugation pathway, which produces additional genotoxic metabolites, and may be more important in humans than in rodents (e.g., see Irving and Elfarra, 2012). Population variability and toxicokinetic uncertainty should properly be addressed by making appropriate health-protective assumptions in the quantitative cancer potency assessment.

The main problem from a regulatory perspective is that the harmonized PBPK model of Chiu and Ginsberg (2011) was not able to decipher how much of the large spread in the model predictions for the GSH conjugation pathway was due to variability and how much was due to uncertainty. In using total metabolism as the preferred dose metric, OEHHA considered the uncertainty in the available scientific information and, in contrast to US EPA, has chosen a modeling approach that will produce a more health-protective potency estimate. This is consistent with the cancer TSD, where OEHHA states its policy of developing cancer potency factors that are "adequate to protect public health."

Based on this comment, however, OEHHA has included additional discussion on the uncertainty/variation in the human PBPK model. Our analysis indicates that this uncertainty has much less impact upon the overall dose-response calculation when using total metabolized dose as the dose metric than might be assumed based on the 3000-fold spread in the PBPK model estimates for human glutathione conjugation. OEHHA finds that the level of "conservatism" added to the analysis by including the GST-pathway in the total dose-metric, is a factor of 10, approximately.

Related to this, HSIA also comments that:

> "The notion of a high proportion of PCE being metabolized via the glutathione conjugation pathway is based largely upon the trichloroethylene (TCE) work of Lash and co-workers utilizing a questionable analytical technique. The technique is based on an indirect method developed by Reed and involves liquid chromatographic (LC) separation followed by derivatization and UV detection."

OEHHA points out that the analysis method used by Lash and coworkers has been a standard and widely used method in glutathione metabolism research and that Lash *et al.* (1999), for example, looked into whether their TCE results could be in error and have repeatedly confirmed the accuracy of their results. In the above-cited article, they write:

> "Rates of GSH conjugation of [TCE] in human liver and kidney subcellular fractions reported in the present study are up to an order of magnitude greater than those reported by Green *et al.* (1997) [...] The controversy between our present and previous results and those of Green *et al.* (1997) has not been resolved. Differences in analytical methods (radiolabeled substrate with HPLC separation versus derivatization and HPLC separation) may contribute to the discrepancies in measured rates. Beyond this, no other explanations have been found to explain the discrepancies. Corrections were made in the present studies for nonenzymatic DCVG formation and our analytical method involved measurement of the N-dinitrophenyl derivative of DCVG and quantitation with respect to authentic standard. Hence, we were certain of the identity of the measured compound."

Regarding "potential impacts on metabolite patterns at/around CYP saturation," data from PCE metabolism studies and the Chiu and Ginsberg (2011) PBPK model indicate that saturation of the oxidative pathway tends to increase the rate of glutathione conjugation (e.g., by less than a factor of 2 in mice), possibly due to reduced competition for substrate between the two enzymatic pathways. However, this does not indicate that the GSH pathway would not be operative at lower environmental exposures.

HSIA Comment 3

"OEHHA's choice of total PCE metabolism as the dose metric in PBPK modeling is inappropriate."

"HSIA agrees with EPA that the currently available data on the role of GSH conjugation in PCE toxicity do not support using total metabolism as the dose metric for dose-response analysis. We urge OEHHA to reconsider its selection. In addition, given the lack of any defined mechanism linking PCE and MCL in F344 rats, there is no justification for OEHHA's decision to select total PCE metabolism as the dose metric for development of a potency factor based on that endpoint."

Response to HSIA Comment 3

The comment appears to be largely a restatement of HSIA Comments 1 and 2. Therefore OEHHA refers the commenter to our responses to Comments 1 and 2. In addition, in Comment 3, HSIA submits that:

> "[T]here are no data to support a role for metabolites of PCE in the generation of MCL in Fisher F344 rats and that, as affirmed by EPA and the NRC, a role for GSH-derived metabolites in renal or hepatocellular tumors is still controversial and associated with a high degree of uncertainty and variability."

The document provides a detailed discussion of research indicating that the metabolism of PCE in rodents produces a variety of reactive and potentially genotoxic (and therefore potentially tumorigenic) metabolites through both the CYP450 oxidation and GST conjugation pathways. Several of these metabolites are stable enough to circulate widely throughout the organism and thus impact tissues other than the liver or kidney. In addition, the document notes that tissues other than the liver or kidney can also independently metabolize PCE to potentially genotoxic metabolites (e.g., the document points out that lymphocytes have been found to contain a variety of CYP isoforms that could oxidize PCE or one of its metabolites).

However, the document also notes that the precise mechanisms by which these potentially genotoxic substances cause increased tumor formation are unknown. Nonetheless, OEHHA's cancer TSD does not require complete knowledge of a chemical's mode of action, nor does it require evidence of tumor concordance between the animal model and humans in order to use the dose-response data for estimation of the cancer potency.

Regarding the involvement of GSH-derived metabolites in rat liver tumors, the document discusses the multiple branch points that occur in the GST conjugation pathway, one of which involves CYP450 or Flavin monoxygenase-3 (FMO-3) oxidation of TCVC to potentially genotoxic α,β-unsaturated sulfoxides. This pathway could occur substantially in the liver, given its relative abundance of CYP450 and FMO-3 enzymes. Regarding rat kidney tumors, the NRC (2010) peer review report, at page 73, states (references omitted):

> "The draft IRIS assessment concludes that a mutagenic mode of action cannot be ruled out. The committee agrees with this assessment. A mutagenic mode of action is supported by the findings after exposure to the structurally similar trichloroethylene. Some metabolites derived from S-(1,2,2-trichlorovinyl) glutathione (TCVG), the glutathione conjugate of tetrachloroethylene, have been shown to be mutagenic in bacterial systems or to cause unscheduled DNA synthesis. Others react with DNA in vitro. S-(1,2,2-Trichlorovinyl)-L-cysteine (TCVC) causes a greater response than dichlorovinyl cysteine in mutagenicity tests using Salmonella and in renal toxicity."

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Public Health Goal for
TETRACHLOROETHYLENE
In Drinking Water

NOT A REGULATORY STANDARD

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PREFACE

Drinking Water Public Health Goals

Pesticide and Environmental Toxicology Section

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency

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 (amended Health and Safety Code, Section 116365), amended 1999, 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. Section 116365 specifies that the PHG is to be based exclusively on public health considerations without regard to cost impacts. 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 which can cause chronic disease shall be based upon currently available data and shall be set at levels which 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 the existence of groups in the population that are more susceptible to adverse effects of the contaminants than a normal healthy adult.
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. In cases of insufficient data to determine a level of no anticipated risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
7. 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.
8. The PHG may be set at zero if necessary to satisfy the requirements listed above.
9. OEHHA shall consider exposure to contaminants in media other than drinking water, including food and air and the resulting body burden.
10. PHGs adopted by OEHHA shall be reviewed every five years and revised as necessary based on the availability of new scientific data.

PHGs published by OEHHA are for use by the California Department of Health Services (DHS) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations, drinking water standards adopted by DHS are to consider economic factors and technical feasibility. Each standard adopted shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By federal law, MCLs established by DHS must be at least as stringent as the federal MCL if one exists.

PHG documents are used to provide technical assistance to DHS, and they are also informative reference materials for federal, state and local public health officials and the public. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not to be utilized as target levels for the contamination of other environmental media.

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

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PUBLIC HEALTH GOAL FOR TETRACHLOROETHYLENE IN DRINKING WATER

SUMMARY

A public health goal (PHG) of 0.06 µg/L is established for tetrachloroethylene (PCE, also known as perchloroethylene) in drinking water. The PHG is based on carcinogenic effects observed in experimental animals. Exposure to PCE is carcinogenic for rodents, inducing liver cancer in mice by inhalation (NTP, 1986) or ingestion (NCI, 1977), and leukemia in rats by inhalation (NTP, 1986). Statistically significant increases in the incidence of tumors at several sites have also been observed in certain studies of workers in the dry-cleaning industry (Blair et al., 1990; Ruder et al., 1994).

For the calculation of the PHG, cancer potency estimates were made based on the recommended practices of the 1996 United States Environmental Protection Agency (U.S. EPA, 1996) proposed guidelines for carcinogenic risk assessment. According to these methods, a polynomial model is fit to the experimental data in order to establish the lower 95 percent confidence bound on the dose associated with a 10 percent increased risk of cancer (LED_{10}). The PHG was calculated assuming a *de minimis* theoretical excess individual cancer risk level of 10^{-6} from exposure to PCE. Cancer potency estimates were derived, using time-dependent models, from the observed incidences of hepatocellular carcinoma in male and female mice exposed orally to PCE. For water-derived inhalation exposures, estimates were derived from the incidences of hepatocellular adenoma or carcinoma in mice, and mononuclear cell leukemia in rats of both sexes exposed by inhalation to PCE. Based on these considerations, Office of Environmental Health Hazard Assessment (OEHHA) has established a PHG of 0.06 µg/L for PCE in drinking water.

An estimate of the concentration of PCE in drinking water protective against chronic toxicity other than cancer was derived based on neurobehavioral endpoints (related to delayed reaction times) observed in epidemiological studies of humans with occupational or environmental exposures to inhaled PCE. Uncertainty factors, allowing for extrapolation from LOAELs (0.29, 4.15 and 8.48 mg/kg-d) to NOAELs and for interindividual variation in the human population, ranged from 30 to 100. The geometric mean of three such estimates was used to derive an estimated health protective concentration in drinking water of 11 ppb (11 µg/L).

The U.S. EPA has established a maximum contaminant level goal (MCLG) of zero mg/L PCE. A maximum contaminant level (MCL) of 0.005 mg/L PCE has also been established (U.S. EPA, 1989). The California Department of Health Services currently lists an MCL of 0.005 mg/L (5 ppb). The OEHHA PHG incorporates several differences from the earlier U.S. EPA evaluation, including: 1) interspecies scaling according to the ¾ power of body weight, 2) a time-to-tumor analysis of the NCI (1977) oral mouse bioassay data, and 3) a more sophisticated human pharmacokinetic model for low-dose oral and inhalation exposures. It also is based exclusively on public health considerations, whereas the MCLs may include considerations of economic and technical feasibility.

INTRODUCTION

The California Safe Drinking Water Act of 1996 requires protection of the public from drinking water contaminants. This document is a revision and reassessment of a 1992 Office of Environmental Health Hazard Assessment (OEHHA) document entitled *Health Effects of Perchloroethylene* (DHS, 1992). The purpose of this document is to establish a PHG for perchloroethylene (tetrachloroethylene or PCE) in drinking water. In our evaluation, an extensive analysis of the available scientific literature was made and prudent assumptions were made as necessary to assure absence of adverse health effects.

PCE is a perchlorinated two-carbon olefin. Total production of PCE in the United States has been decreasing over the last decade. In 1983, production of PCE was 547 million pounds while the production decreased to 271 million pounds in 1993 (ATSDR, 1997). The primary uses of PCE are as a chemical intermediate for the production of chlorofluorocarbons and as a solvent used in cleaning operations (metal cleaning, vapor degreasing, and dry cleaning). In addition, numerous household products contain some level of PCE. Due to widespread use, PCE is a common environmental contaminant.

Every effort was made to incorporate all aspects of potential exposure. Various sources such as air (environmental and occupational), soil, and water were considered. Typical exposures to PCE from drinking water are not expected to result in any acute health effects, due to the low levels involved. Exposure from drinking water can be in the form of household airborne exposures from showering, flushing of toilets, and other contact with water.

PCE is readily absorbed through the lungs and gastrointestinal tract, and to a lesser extent, it can be absorbed through the skin. Acute and chronic neurological changes, and liver and kidney toxicity, have been reported in humans and animals exposed to PCE. However, these effects were observed with higher exposures than typical background levels in the general indoor or outdoor environment.

As in the previous assessment of PCE, the findings observed in the rodent cancer bioassays were used for the calculation of the PHG. These assays have shown that oral administration of PCE leads to an increased incidence of hepatocellular carcinoma in B6C3F1 male and female mice (National Cancer Institute, 1977). The tumor incidence was also increased in both rats (mononuclear cell leukemia) and mice (hepatocellular carcinoma) exposed to PCE by inhalation (National Toxicology Program, 1986). The California Department of Health Services (DHS) currently lists an MCL of 0.005 mg/L (5 ppb). An MCL of 0.005 mg/L PCE has also been established by the U.S. EPA (U.S. EPA, 1989).

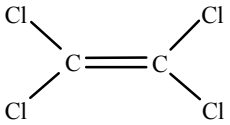
Based on considerations discussed in this document, OEHHA establishes a PHG of 0.06 ppb for PCE in water. Some of the considerations used in this calculation were interspecies scaling according to ¾ power of body weight, a time-to-tumor analysis of the NCI (1977) oral mouse bioassay data, and a more sophisticated human pharmacokinetic model for low-level oral and inhalation exposures.

CHEMICAL PROFILE

Chemical Identity

Tetrachloroethylene is a perchlorinated two-carbon olefin. The chemical formula, structure, synonyms and identification numbers are listed in Table 1 and are adopted from the International Research Agency for Research on Cancer (IARC) (IARC, 1995b).

Table 1. Chemical Identity of Tetrachloroethylene (IARC, 1995b)

Chemical name	Tetrachloroethylene
Synonyms	Perchloroethylene, Tetrachloroethene, PCE, Carbon Bichloride, Ethylene Tetrachloride
Trade names	Ankilostin, Antisol 1, Didakene, Dilatin PT, Fedal-Un, Nema, Perchlor, Perclene, PerSec, Tetlen, Tetracap, Tetraleno, Tetroguer, Tetropil
Chemical formula	C_2Cl_4
Chemical structure	
Identification numbers	
Chemical Abstracts Service (CAS) Registry number:	127-18-4
NIOSH Registry of Toxic Effects of Chemical Substances (RTECS)® number:	KX3850000
U.S. EPA Hazardous Waste number:	K043; U210; D039
Oil and Hazardous Materials/Technical Assistance Data System (OHM/TADS) number:	7216847
Hazardous Substances Data Bank (HSDB) number:	49 403 55
National Cancer Institute (NCI) number:	NCI-C04580

Physical and Chemical Properties

Important physical and chemical properties of tetrachloroethylene are given in Table 2 (adopted from IARC, 1995b). Tetrachloroethylene is slightly soluble in water and is readily volatile.

Table 2. Physical and Chemical Properties of Tetrachloroethylene (IARC, 1995b, except as noted)

Property	Value
Molecular weight	165.83
Color	Colorless
Physical state	Liquid
Odor	Ether-like
Odor threshold	50 ppm (NIOSH, 1978)
	1.0 ppm in air (ATSDR, 1993)
	0.3 ppm in water (ATSDR, 1997)
Taste threshold	0.3 mg/L water (U.S. EPA, 1998)
Melting point	-19°C
Boiling point	121°C (at 760 mm Hg)
Flash point, *etc.*	Non-flammable
Solubility	
Water	0.15 g/L (at 25°C)
Organic solvents	Soluble in ethanol, diethyl ether, benzene
Biological fluids	Unknown
Density	1.6227 g/cm^3
Octanol-water partition coefficient (log K_{ow})	3.4
Vapor pressure	9.975 mm Hg (at 13.8°C)
Henry's law constant	1.8 x 10^{-2} atm-m^3/mol (ATSDR, 1997)
Conversion factor[1]	1 ppm = 6.782 mg/m^3

[1]Calculated assuming the ideal gas law, PV= *n*RT, where the partial molar volume at 25°C is 24.45 L/mol: then ppm = mg/m^3 x MW / 24.45.

Production and Uses

The primary uses of PCE are as a chemical intermediate, primarily in the production of chlorofluorocarbons, and as a solvent, primarily for cleaning operations. The breakdown of the use of PCE is approximately 55 percent as a chemical intermediate, 25 percent in metal cleaning and vapor degreasing, 15 percent in dry-cleaning operations, and 5 percent for miscellaneous other uses (ATSDR, 1997). Numerous household products contain some level of PCE. PCE's use in the production of chlorofluorocarbons (CFCs) has declined in recent years due to reduced production of CFCs stemming from efforts to protect the stratospheric ozone layer from depletion. Solvent recovery operations and recycling have also reduced demand for PCE production.

Dry-cleaning grade PCE is supplemented with stabilizers including amines or epoxide/ester mixtures, which reduce hydrolytic decomposition.

Total production of PCE in the U.S. has been estimated at 271 million pounds in 1993, a decrease from 547 million pounds in 1983 (ATSDR, 1997). According to the U.S. EPA's 1992 Toxic Release Inventory, 68 facilities in California manufacture or process PCE, with total maximum on-site amounts ranging from 0 to 10 million pounds (ATSDR, 1997).

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Air

Environmental Exposures

The high volatility of PCE leads to considerable potential for release into the air from the various uses of the compound, particularly in cleaning operations, although releases at sites of chemical production are also possible. PCE is nearly ubiquitously present in air, with significant levels detected in areas ranging from remote to urban. Significantly higher levels have been reported in areas in close proximity to its use, such as at dry-cleaning facilities.

The California Air Resources Board (ARB) reported a statewide mean air concentration of 0.134 ppb for 1996, which represented the lowest value in a decreasing trend for reporting beginning in 1990 (highest reported mean: 0.277 ppb PCE in 1990) (ARB, 1998).

Mean U.S. ambient air concentrations compiled from over 2,500 monitoring points have shown a range from 0.16 ppb PCE in rural/remote areas to 0.79 ppb PCE in urban/suburban areas to 1.3 ppb PCE near emission sources (ATSDR, 1997, citing Brodzinsky and Singh, 1982).

In its Toxic Chemical Release Inventory (TRI), U.S. EPA reported air emissions of approximately one million pounds of PCE in 1994 (U.S. EPA, 1994a). Reported emissions to air have declined yearly from those reported in 1987 when over 5 million pounds of PCE were released. This is likely to be a considerable under-estimation of PCE releases because of limits to the required reporting to the TRI.

The half-life of PCE in air has been estimated at 96 days (ATSDR, 1997).

Occupational Exposures

As noted above, various industrial activities including dry cleaning involve the use of PCE, and significant exposures of workers may result, in addition to the release of PCE to the general environment.

As part of an industry-wide study (Ludwig et al., 1983) to assess the health effects of long-term, low-level exposures to PCE, the National Institute for Occupational Safety and Health (NIOSH) conducted industrial hygiene surveys at 44 commercial dry cleaning facilities in five states. Time-weighted average (TWA) and peak exposures to PCE were determined. Among the various jobs in a dry cleaning facility, Ludwig et al. (1983) found that the machine operators (dry cleaners) had the greatest PCE exposure. TWA exposures of machine operators ranged from 4.0 to 149.0 ppm PCE (geometric mean = 22 ppm). The geometric mean PCE exposure of the

pressers, seamstresses, and front counter workers were 3.3 ppm, 3.0 ppm and 3.1 ppm, respectively. The geometric mean 5-minute peak PCE exposure during textile transfer was 44 ppm while the mean 15-minute exposure was 33 ppm.

Investigations of PCE exposures in the workplace have also been undertaken as part of epidemiological studies of health effects; a number of such studies are described in the later sections of this report covering human health effects.

Indoor Exposures

Indoor air levels of some volatile organic compounds (VOCs) have been found to be higher than corresponding outdoor concentrations. Pellizzari et al. (1986) measured corresponding matched pairs of indoor and outdoor concentrations of several VOCs for different highly urban-industrialized residential U.S. communities. Over all the geographical areas evaluated, the outdoor air quality for the reported chemicals was better than in the residences. PCE was one chemical reported to have higher indoor medians and maximum levels than the corresponding outdoor concentrations. In some cases, the median ratios (indoor to outdoor) were greater than ten. Source or explanations for these observations were not identified.

Increases of indoor PCE air concentrations may arise due to some of the same sources responsible for the environmental and occupational exposures. These sources could include the proximity to a dry-cleaning establishment, emission of PCE from dry-cleaned clothes, or emissions of PCE for workers clothing. In addition, increased indoor air levels of PCE as a result of exhaled breath from workers have been implicated as an additional source (Wallace et al., 1991a; Thompson and Evans, 1993). Wallace et al. (1991a) found levels of PCE in indoor air on the order of 100 $\mu g/m^3$ for these homes while the average indoor level of PCE was found to be 5 to 10 $\mu g/m^3$ in non-PCE worker homes (Wallace et al. 1991b). Both Wallace et al (1991a) and Pellizzari et al. (1986) found a ten-fold increase in indoor PCE levels. These studies indicate that indoor air exposures could lead to a greater exposure to PCE than outdoor air exposures.

Soil

Contamination of soil with PCE may result from the disposal of sludge and wastewater containing PCE, particularly from recycling operations.

Water

The numerous industrial uses and its presence in consumer products may lead to the release of PCE into water. Cleaning processes have the potential to create PCE contaminated wastewater. PCE contamination of water arises primarily from the disposal of spent sludge and the storage and disposal of solvents.

PCE has been detected in most water supplies including drinking water, ground water, surface waters, and rainwater. U.S. EPA has estimated that 5.3 percent of the U.S. population (11.4 million people in 1985) may be exposed to PCE at levels in excess of 0.5 $\mu g/l$, and 0.4 percent of the population (0.87 million) may be exposed at levels in excess of 5 $\mu g/l$ (U.S. EPA, 1985, as described in IARC, 1995b).

The use of vinyl-lined asbestos-cement water pipes resulted in the contamination of the municipal water supply in Cape Cod, Massachusetts, in the late 1970s (IARC, 1995b). PCE concentrations

of 1.5 to 7750 µg/l were reported at different points of approximately 650 miles of piping used, depending on the level of water use.

Trace amounts of PCE may also be formed during the disinfection of water through chlorination (NAS, 1977).

Geographically, patterns of PCE contamination in California are usually in concert with use patterns, with the highest levels of PCE contamination occurring in the historically most heavily urbanized areas. In a state-wide sampling survey of large water systems under the Assembly Bill 1803 program, approximately 70 percent of the PCE contaminated wells were found in Los Angeles County (DHS/DIR, 1986).

The U.S. EPA TRI reported less than 12 pounds of PCE released to water for each of the years from 1987 to 1994 (U.S. EPA, 1994b).

Extent of Contribution of Tap Water to Total PCE Exposure

The National Academy of Sciences (NAS, 1982) and the U.S. EPA (U.S. EPA, 1985) have assumed that 20 percent of total exposure to organic chemicals may be attributed to drinking water. This assumption is applied when suggesting the 'no adverse response level(s)' (SNARL) for chronic effects, or when calculating U.S. EPA recommended maximum contaminant levels (RMCLs) for drinking water. The literature on PCE occurrence has been reviewed and found that tap water contaminated with PCE contributed much less than 20 percent to an individual's total exposure to PCE (Letkiewicz et al., 1982). The figures of Letkiewicz et al. (1982) for percent contribution at contamination levels of 0.5 µg/L and DHS calculations using their occurrence data and assuming 2 µg/L PCE in tap water are provided in Table 3. These figures provide an example of water contribution to total PCE exposure of an individual. They ignore the contributions from PCE found in indoor air attributable to non-tap water sources such as that arising from recently dry-cleaned clothing and from dermal and inhalation exposures which are attributable to tap water sources (e.g., exposure received while showering). PCE in indoor air was not found to be correlated with PCE tap water concentrations, because the major contributions were from environmental sources other than water (e.g., dry-cleaned clothes) (the exposure to PCE during showering and other activities of high water use was not measured) (Wallace et al., 1986). The ratio of median PCE concentrations in indoor to outdoor air has been reported to range from 2 to 27 (Hartwell et al., 1984a; Hartwell et al., 1984b).

DHS (1987) reviewed studies by Wallace et al. (1986), who measured concentrations of PCE in the air to which study participants were exposed in Los Angeles and Contra Costa counties. Personal monitors sampled over 24-hour periods, but were not placed in the bathroom while subjects showered and bathed. DHS (1987) concluded that when PCE levels in the water supply are relatively high (>10 ppb), tap water may be a predominant source of PCE exposure. However, for water sources where PCE concentrations are 0.5 ppb or less, exposures from other sources, particularly indoor air, may be more important.

Table 3. Percent Contribution of Tap Water to Total Exposure to PCE

Water concentration of PCE	Urban areas	Rural areas	Area of maximum air pollution
0.5 μg/L[a]	2.9%	4.3%	0.04%
2.0 μg/L[b]	10.7%	14.9%	0.02%

[a]Data reproduced from Letkiewicz et al. (1982); ignores exposures from dermal absorption and inhalation while bathing; assumes 70 kg adult ingesting 1 L/day.
[b]Derived by DHS (1987) using the assumptions of Letkiewicz et al. (1982) regarding exposures.

Infants

Exposure calculations typically do not consider differences in exposure for different age groups. Because virtually all of an infant's fluid and nutritional requirements are met by breast milk and/or formula, and because, on a bodyweight basis infants consume approximately ten times the fluid volume of adults, there is the potential for greater exposure of infants to PCE through contaminated drinking water. Exposures can occur directly in formula feeding and indirectly *via* breast milk. A case of enlarged liver and obstructive jaundice was reported in an infant exposed to PCE via breast milk (Bagnell and Ellenberger, 1977). The mother of the infant regularly visited a dry-cleaning establishment to lunch with her husband who worked there as a leather and suede cleaner. One hour after visiting the husband, the mother's breast milk was found to contain 10 mg/L PCE that decreased to 3 mg/L after 24 hours. The infant's symptoms disappeared one week after cessation of breast-feeding. There are no definitive data at present to determine the amount of PCE received through breast-feeding attributable to PCE in tap water. That PCE exposure can occur via breast milk has also been demonstrated by Erickson et al. (1980) who found PCE present in all 42 samples of human milk collected in Bayonne and Jersey City (Anonymous , 1996), Pittsburgh (PA), Baton Rouge (LA) and Charleston (WV) (Erikson et al., 1980). Concentrations ranged from 0.1 to 43 μg/L with a median value of 1.25 μg/L. The mothers resided in areas with relatively high water and air concentrations of PCE.

A survey on U.S. infant dietary patterns has been conducted (Martinez and Dodd, 1983). At one month of age, approximately 50 percent of infants receive formula, 44 percent breast milk, and 6 percent breast milk plus formula. Taking into account the volatilization of PCE from tap water through boiling and the different types of infant formula used, Letkiewicz et al. (1982) estimate that 22 percent of formula-fed infants receive fluids contaminated at PCE levels found in the water supply. These data indicate that approximately 11 percent (=0.5 x 0.22) of the infant population could receive up to ten times the exposure, on a mg/kg basis, of adults to PCE through drinking contaminated tap water.

Food

In its review of PCE content in food, IARC (1995b) listed the detection of PCE in numerous items including milk, meat, fish, mollusks, margarine, oils, coffee, tea, fruits and vegetables, cereals, pork and beans, baby foods, milk chocolate, baked goods, peanut butter, and nuts. In products where PCE has been detected, reported concentrations ranged from 1 to 124 μg/kg. IARC estimated daily PCE intake to be 160 μg/day (Zimmerli et al., 1982). Another estimate of

total PCE intake from all sources (including air and water as well as food) was reported to be 113-144 μg/day (IARC, 1995b; citing Bauer, 1981; von Düszeln et al., 1982).

Other Sources

PCE has been reported to be produced naturally in several algae of temperate, subtropical and tropical origin and in a red microalga (IARC, 1995b). IARC also reports that PCE is present in consumer products including cosmetics and cough medicine.

METABOLISM AND PHARMACOKINETICS

PCE is readily absorbed through the lungs and gastrointestinal tract and, to a lesser extent, can be absorbed through the skin. Between 50 and 70 percent of inhaled PCE is reported to be absorbed by humans (Bolanowska and Golacka, 1972; Ohtsuki et al., 1983; Imbriani et al., 1988). After oral administration to rodents, PCE is absorbed rapidly, and the percentage of dose recovered approaches 100 percent (Pegg et al., 1979). Once in the body, PCE disperses into all tissues. Steady-state tissue concentrations are a function of the absorbed dose, partitioning factors (which reflect the lipophilic nature of PCE), and rates of metabolic conversion and elimination.

A substantial proportion of PCE absorbed systemically is exhaled unchanged. This is pronounced at high doses since the metabolism of PCE appears to be saturable. Studies in which radiolabeled PCE was administered to animals have found that trichloroacetic acid is the major urinary metabolite; carbon dioxide is a commonly produced exhaled metabolite. Other urinary metabolites that have been detected in rodent studies include trichloroethanol, chloride, dichloroacetic acid, oxalic acid, ethylene glycol, S-(1,2,2 trichlorovinyl)glutathione, N-acetyl-S-(trichlorovinyl)-L-cysteine (N-Ac-TCVC), N-trichloroacetyl-aminoethanol, and N-oxalyl-aminoethanol. Several of these metabolites, including trichloroacetic acid, trichloroethanol, and N-Ac-TCVC have been observed in the urine of humans exposed to PCE (Yllner, 1961; Daniel, 1963; Dimitrieva, 1967; Ikeda and Ohtsuji, 1972; Ikeda et al., 1972; Moslen et al., 1977; Costa and Ivanetich, 1980; Monster et al., 1983; Dekant et al., 1985; Koppel et al., 1985; Green et al., 1990).

Two pathways of PCE metabolism have been identified. Which metabolites are responsible for PCE's toxic effects, including carcinogenicity, is not known and therefore the relative importance of these pathways remains unclear. Cytochrome P_{450} oxidation is the first step of the most quantitatively significant metabolic pathway for PCE. An epoxide intermediate has been postulated, although this compound has not been isolated *in vivo*. More recently it has been suggested that the epoxide, if formed at all, may not be released from the cytochrome P_{450} catalytic site, and that the first free product is trichloroacetyl chloride, a rearrangement product which is itself highly reactive. Trichloroacetic acid is the stable product of this pathway, and is produced by hydrolysis. The second metabolic pathway for PCE involves conjugation with glutathione. Subsequent degradation of the resulting mercapturic acids by several routes, including one catalyzed by the β-lyase enzyme in the kidney, produce further metabolites from this pathway some of which are potentially reactive.

Published studies on the absorption, distribution, metabolism, and elimination of PCE have been extensively reviewed in a previous California risk assessment document (OEHHA, 1992), and by IARC (IARC, 1995b). A recently updated Toxicological Profile (ATSDR, 1997) also reviewed the pharmacokinetics of PCE in humans and experimental animals. Since there have not been substantial changes in our understanding of these phenomena since those reviews were

undertaken, the reader is referred to these authorities for a complete description. This report will confine itself to noting some more recent findings, which confirm or extend the earlier accounts.

Metabolism

The primary route of PCE metabolism in humans and rodents occurs via oxidation. Cytochrome P_{450} 2B proteins and activities were induced after exposure of male Wistar rats to 500 to 2000 mg/kg PCE p.o. in corn oil for five days, suggesting that these enzymes may be involved in PCE metabolism (Hanioka et al., 1995a; Hanioka et al., 1995b). Several Phase II enzyme activities (DT-diaphorase, glutathione-S-transferase and UDP-glucuronyl transferase) were also induced. The induction of cytochrome P_{450} 2B1 and 2B2 proteins was at a level similar to that seen after phenobarbital treatment. Oxidation of PCE results in formation of trichloroacetyl chloride, possibly through an epoxide intermediate. Trichloracetyl chloride can react with amino groups of cellular proteins or undergo hydrolysis to produce trichloroacetic acid. Trichloroacetic acid is the primary urinary metabolite of PCE in rodents and humans. This compound has been the basis for biological monitoring of workers exposed to PCE. The intermediate product, trichloroacetyl chloride, is a potentially reactive compound. N^{ε}-(trichloroacetyl)-L-lysine was detected in the liver of rats exposed to PCE, consistent with the formation of protein adducts by trichloroacetyl chloride (Birner et al., 1994).

Glutathione conjugation in the liver is a second metabolic pathway for PCE. In some earlier discussions of the glutathione pathway (reviewed by OEHHA, 1992) it had been asserted that this pathway was entirely absent in humans. However, recent reports have identified a derivative of this pathway, N-acetyl-S-(trichlorovinyl)-L-cysteine (N-Ac-TCVC), in the urine of dry-cleaning workers (Birner et al., 1996) and experimentally exposed volunteers (Volkel et al., 1998). N-Ac-TCVC is thought to be formed by cleavage and subsequent acetylation of the conjugate. A comparative study of the production of N-Ac-TCVC in rats and humans was recently published by this group (Volkel et al., 1998). Six volunteers were exposed to 10, 20, and 40 ppm PCE for six hours; urine was collected for the following 72 hours (Volkel et al., 1998). Wistar rats were exposed to 10, 20, 40, and 200 ppm and urine collected and analyzed in a similar manner. In humans, the cumulative quantity of TCA excreted was 100-fold higher than the quantity of N-Ac-TCVC. On a μmol/kg basis rats excreted more of both metabolites than humans, however the ratio of TCA to N-Ac-TCVC was somewhat higher in rat urine. In addition, dichloroacetic acid was detected in rat but not human urine. The authors hypothesize that dichloroacetic acid is derived from beta-lyase cleavage of TCVC in the kidney, via a reactive dichlorothioketene intermediate. In support of this hypothesis, exposure to rats resulted in formation of a covalent protein adduct, N^{ε}-(dichloroacetyl)-L-lysine in kidney cells (Birner et al., 1994). The formation of sulfoxides from PCE glutathione conjugates of sulfoxides from PCE glutathione conjugates reactive electrophiles, and are cytotoxic, raising the possibility that the glutathione pathway may also play a role in the cytotoxicity and carcinogenicity of PCE at other sites in addition to the kidney.

Pharmacokinetic Models

Although the metabolic and mechanistic studies noted above have increased the understanding of PCE metabolism in both rodents and humans significantly, they have not resolved issues basic to the use of more sophisticated pharmacokinetic modeling approaches, such as whether the metabolites responsible for the observed carcinogenic effects arise via the cytochrome P_{450} mediated oxidative pathway or the glutathione conjugation pathway, or both. Some information is available on human pharmacokinetics of inhaled PCE, but the consistency of these data among

themselves, and the extent to which they allow for uncertainty and variability in exposed human populations, has been subject to extensive and complex discussion (Bois et al., 1990; Hattis et al., 1993; Bois et al., 1996). The results of several pharmacokinetic models that have been used to estimate the fraction of PCE metabolized by the oxidative pathway in animals and humans are summarized below. Emphasis is placed on those models which have been used to generate dose estimates for the animal carcinogenicity bioassays, and on a recent model for human metabolism. The models discussed consider only the oxidative pathway for metabolic transformation of PCE. As discussed above, this is the major pathway in both humans and rodents, and appears to be qualitatively similar across species.

Animal Models

Bogen et al. (1987) used a steady-state metabolism model, with empirical data from metabolism studies in rodents, to predict metabolized doses of PCE for the animals in the cancer bioassays (1987). The metabolism of PCE in rats and mice was assumed to follow Michaelis-Menten kinetics. This assumption is consistent with the cytochrome P_{450} enzymes mediating the major pathway of metabolism. Urinary metabolite data reported by Buben and O'Flaherty (1985) for PCE-exposed mice were used for the calculations of metabolized dose excreted in the urine (M_u) after oral exposures, using a Michaelis-Menten type equation:

$$M_u = (D\ V_{max})/(D + K_m)$$

where D = applied oral dose (from 20 to 2000 mg/kg-d), V_{max} = apparent maximum rate of metabolism and K_m = apparent Michaelis constant (all in units of mg/kg-d). In addition, the fraction of total metabolism that is excreted in the urine was estimated from mass-balance results obtained by Schumann et al. (1980). Based on these experimental data,

$$M_u/M = 0.8$$

where M = total metabolites formed and M_u = metabolites observed in urine (both in mg/kg-d). The following values of V_{max} and K_m, corrected for fraction of metabolites observed in urine, were consistent with the Buben and O'Flaherty data for mice:

$$V_{max} = 170 \text{ mg/kg-d} \qquad K_m = 660 \text{ mg/kg-d.}$$

Using these parameter values in the model, the data from the Schumann et al. (1980) study are predicted reasonably well, but the predictions of the data from another study (Mitoma et al., 1985) were not as accurate. Due to less reliable collection and quantitation of metabolites, Bogen et al. elected not to use the Mitoma data for metabolite estimates.

Bogen et al. (1987) also applied their method to predict doses for mice and rats exposed by the inhalation route. A correction for the different body weights of the experimental animals and those to be simulated was included in view of the dependence of metabolic capability and respiration rate (expressed as a volume per unit time) on body weight to the $^2/_3$ power. Thus:

$$M = \frac{D\ V_{max}\ (w_1/w_2)^{1/3}}{D + K_m\ (w_2/w_1)^{1/3}}$$

where D = inhaled concentration in ppm, M = total metabolites formed, V_{max} = maximum rate of metabolism and K_m = apparent Michaelis constant (all in mg/kg-d). The parameter w_1 is the body weight of the animals used in the calibration experiment and w_2 is that of the animals to be simulated (e.g. those in the bioassay). Data on total metabolites formed in Sprague-Dawley rats exposed by inhalation, obtained by Pegg et al. (1979), were used to derive metabolic parameters.

Values used were V_{max} = 52.982 mg/kg-d, K_m = 273.32 ppm, w_1 = 0.25 kg [the mean body weight of the rats in the Pegg et al. (1979) study], and w_2 = 0.44 kg for the male rats, and 0.32 kg for the female rats, in the (NTP, 1986) bioassay.

For mice, the inhalation data obtained by Schumann et al. (1980) were insufficient to calculate the required parameters, because only one inhalation exposure level was tested. Instead, the V_{max} value obtained from the oral studies discussed above (Buben and O'Flaherty, 1985) was assumed to be applicable to the inhalation studies, and the Schumann et al. data were used for validation. The value of K_m for inhalation was then extrapolated by means of the body-weight correction noted above from the rat value of Pegg et al. (1979). Values used were V_{max} = 170 mg/kg-d, K_m = 126 ppm, w_1 = 0.0245 kg [the mean body weight of the mice in the Schumann et al. study], and w_2 = 0.037 kg for the male mice, and 0.032 kg for the female mice, in the NTP bioassay. The metabolized doses for the cancer bioassay studies calculated using the methodology developed by Bogen et al (1987) are shown in Table 4. This is the same animal dosimetry that was used in a previous California risk assessment of inhaled PCE (OEHHA, 1992).

Table 4: Metabolized Dose of PCE in NCI (1977) and NTP (1986) Bioassays

	Applied dose	Metabolized Dose/day[a]	Corrected for 5 days/week	Corrected for weeks dosing	Correction for study length[b]
NCI Oral Study					
Male mice					
	536	76.19	54.42	47.16	30.57
	1072	105.22	75.16	65.14	42.21
Female mice					
	386	62.73	44.81	38.84	25.17
	772	91.65	65.46	56.73	36.77
NTP Inhalation Study					
Male mice, bw = 0.037 kg					
	100	60.59	43.28	43.28	43.28
	200	86.01	61.43	61.43	61.43
Female mice, bw = 0.032 kg					
	100	65.42	46.73	46.73	46.73
	200	92.10	65.78	65.78	65.78
Male rats, bw = 0.44 kg					
	200	16.56	11.83	11.83	11.83
	400	24.04	17.17	17.17	17.17
Female rats, bw = 0.32 kg					
	200	19.65	14.03	14.03	14.03
	400	28.01	20.01	20.01	20.01

[a]Metabolized doses in mg/kg-d
[b] Calculated as Applied Dose X (lifetime/study duration)^3. The figures in this column are those tabulated by Bogen et al. (1987) and OEHHA (1992).

Other physiologically-based pharmacokinetic (PBPK) models for animals, and dosimetry for the bioassays derived from them, are also available. Chen and Blancato (1987) used metabolite

excretion data from rodents to fit a PBPK model based upon that of Ramsey and Andersen (1984). The experimental data selected were those reported by Pegg et al. (1979) and Schumann et al. (1980) for rats and mice exposed to radiolabeled PCE orally and by inhalation. Estimates of V_{max} and K_m were generated by optimizing the fit of the model to the experimental data. The resulting estimates of V_{max} and K_m, were 0.003 mg/min and 1.472 mg/liter for mice and 0.0059 mg/min and 2.938 mg/L for rats, respectively (note that these values are not directly comparable to those derived by Bogen et al. (1987), because of different units). Based on these metabolic parameters, the PBPK model of Chen and Blancato was used to estimate metabolized doses for the carcinogenesis bioassays (Table 5). The doses predicted are quite similar to those generated by Bogen et al. (1987), but are somewhat higher in all cases. Both approaches result in a higher percentage of PCE metabolized at lower applied doses and thus a less than proportional increase in dose of metabolites for a doubling of exposure level as administered in the bioassays.

A PBPK model of inhalation exposure to PCE was also developed by Bois et al. (1990). Parameter estimates for rat, mice, and humans were drawn from the literature, except for the metabolic parameters, which were obtained by fitting. The results of four experimental studies in mice and four in rats were used in the fitting process. The metabolized doses predicted by the model for the NTP rat bioassay are quite similar to those reported by Bogen et al. and Chen and Blancato, but the doses derived for mice diverge somewhat (Table 5).

As shown in Table 5, the three independent efforts produced dose estimates for the bioassay exposures that are in good agreement. Because the approach of Bogen et al. (1987) is the only source which provides estimates for both the NTP and NCI studies, in both rats and mice, these estimates will be used for analysis and route-to-route comparisons of the carcinogenesis bioassay data in rodents in the dose response assessment section of this report.

Dallas and colleagues obtained additional data on uptake and distribution of PCE in rats after exposure by inhalation and oral routes, and intraarterial infusion, and used this to refine their PBPK model of PCE distribution (Dallas et al., 1994a; Dallas et al., 1994b; Dallas et al., 1994c; Dallas et al., 1995). This model explicitly included estimates of exposure of organ systems, such as the brain, which are sites of toxicity endpoints of interest to these authors. They also demonstrated the feasibility in principle of route-to-route extrapolation using the PBPK modeling approach. This new approach is important since it improves the validation of the model by incorporating new experimental data on specific organs in animals. The Dallas et al. studies did not assess metabolite production.

Table 5: **Metabolized Doses in NCI and NTP bioassays predicted by three different models**

Study	Species/ Sex	Administered Dose or Exposure Concentration	Predicted Dose[1]				
			Bogen et al. (1987)	Chen and Blancato (1987)	Chen and Blancato (adjusted)[2]	Bois et al. (1990[3])	Bois et al. (adjusted)[4]
NCI (1977)	*Mouse*						
	male	536 mg/kg-d	30.6	56.3	36.5		
		1,072 mg/kg-d	42.2	75.3	48.8		
	female	386 mg/kg-d	25.2	40.6	26.3		
		772 mg/kg-d	36.8	57.5	37.3		
NTP (1986)	*Mouse*						
	male	100 ppm	43.3	47.2		7.3	22.1
		200 ppm	61.4	66.3		12.7	38.4
	female	100 ppm	46.7	55.0		7.3	22.1
		200 ppm	65.8	76.8		12.7	38.4
	Rat						
	male	200 ppm	11.8			9.8	13.5
		400 ppm	17.2			13.9	19.1
	female	200 ppm	14.0			9.8	13.5
		400 ppm	20.0			13.9	19.1

[1]Dose units are mg metabolized/kg-d, lifetime TWA
[2]Adjusted for study length with conversion factor: $(90\text{weeks}/104\text{weeks})^3$ (Bogen et al., 1987)
[3]Bois et al. did not report separate estimates for males and females
[4]Bois et al. predictions were adjusted from the published units of mg/d-$kg^{2/3}$ to mg/kg-d

Pharmacokinetic Models of PCE Exposure in Humans

A PBPK model of PCE uptake, metabolism and elimination in humans was developed by Bois and colleagues (Bois et al., 1996). To account for interindividual variation, a population level statistical model was linked to the PBPK model during the fitting process. Prior estimates of the population parameter distributions were based on literature values. Empirical data from the human exposure studies of Monster et al. (Monster et al., 1979) were used for calibration simulations, carried out using a Markov chain Monte Carlo simulation approach with Bayesian updating. The fitted posterior distributions of model parameters were then used to make predictions of metabolized doses for specific exposure scenarios. At higher exposure concentrations (50 ppm in air), the average fraction metabolized predicted by the fitted parameters was low (mean 1.7, 95 percent confidence limits 0.52 - 4.1 percent). This is in line with what has been observed in several empirical studies of occupationally exposed workers (OEHHA, 1992; ATSDR, 1997) as well as human exposure data that were not used in model fitting (e.g. Volkel et al., 1998) and the results of several other PBPK model predictions (Hattis et al., 1990 and 1993). However at a low exposure level (1 ppb in air), such as might be

encountered in environmental inhalation exposures to PCE, the predicted proportion of PCE metabolized was much higher (mean 36, 95 percent confidence limits 15 – 58 percent). Saturation of metabolism in continuously exposed humans was predicted to begin at exposure concentrations between 1 and 10 ppm. Unfortunately, measured human data on metabolite excretion at low exposure levels are not available.

Relatively little has been done to explore the kinetics of PCE in humans following oral exposure; the published models have been primarily concerned with inhalation exposures. However, the parameter values derived from fitting Bois and colleagues' model to inhalation data have also been used to predict the fraction of PCE metabolized under conditions of oral exposure (F.Y. Bois, personal communication). A compartment for uptake from the gastrointestinal tract to the liver was added to the published model for this purpose. As was done for inhalation exposure, 5,000 simulations were performed using random draws from the fitted distributions for population parameter values. Under simulated conditions of exposure to 1μg/L in drinking water assuming intake of 2 L/day and complete absorption, the fraction metabolized was 54 percent (95 percent confidence interval 28-78 percent). This is higher than the corresponding estimate for exposure to low concentrations by inhalation. The difference between routes is biologically plausible, since absorption is more efficient by the oral route and PCE absorbed through the gut is subjected directly to metabolic processes in the liver before entering systemic circulation (and thus enabling exhalatory clearance).

An alternative approach to full pharmacokinetic modeling for determining the fraction of PCE metabolized at low exposure levels in humans was presented by McKone and Bogen (Bogen and McKone, 1988; McKone and Bogen, 1992). Assuming that at low exposure levels metabolism can be described by a linear function (the V_{max}/K_m ratio), these investigators derived a formula from the Ramsey-Anderson PBPK model at steady state:

$$\text{Fraction metabolized, oral} = \left[1 + \frac{1}{K}\left(\frac{P_b}{Q_a} + \frac{1}{Q_l} \right)^{-1} \right]^{-1}$$

K is the V_{max}/K_m ratio, P_b is the blood/air partition coefficient, Q_a is alveolar flow, and Q_l is blood flow to the liver. Using their estimated parameter values with this approach, McKone and Bogen reported that 34 percent of an oral dose of PCE that is below metabolic saturation is predicted to be metabolized (range 5 – 63 percent) (McKone and Bogen, 1992). Alternatively, if the point estimates of parameter values from the fitted human model of Bois et al. (1996) are substituted (except following McKone and Bogen for the alveolar ventilation rate of 354 L/hr), the above formula produces an estimate of 87 percent metabolized. This estimate is somewhat higher than the upper 95 percent confidence limit resulting from simulations in the Bois et al. PBPK model (78 percent), but shows reasonable agreement.

A similar formula was derived for the maximal fraction metabolized at steady state during inhalation exposure. This formula is also an appropriate approximation for dermal exposure, since a dermally absorbed dose is delivered to systemic circulation, as is an inhaled dose, and is not subject to first pass effects:

$$\text{Fraction metabolized, inhalation} = \left[1 + \frac{Q_a}{P_b}\left(\frac{1}{K} + \frac{1}{Q_l}\right)\right]^{-1}$$

Using this equation, and taking into account a range of possible V_{max} and K_m values, McKone and Bogen (1992) estimated that the average fraction of inhaled PCE metabolized in humans could range from 0.04 to 0.46 (geometric mean 0.25). Using the fitted parameter estimates of Bois et al. (1996) results in a revised estimate of 0.59, very close to the upper confidence limit of 0.58 that resulted from simulations of inhalation exposure in the Bois et al. PBPK model.

Bogen and McKone also theorized that as the inhaled or ingested concentration approaches zero, metabolism of the dose becomes very rapid, such that V_{max} can be considered to approach infinity. Under this assumption, they argued that metabolism of respired or dermal doses would be subject to a theoretical physiological upper limit of 73 percent metabolized (Bogen and McKone, 1988; McKone and Bogen, 1992). Substituting the parameter estimates derived from the fitted human PBPK model of Bois et al. into the formula of Bogen and McKone, this theoretical maximum becomes 68 percent. For ingested PCE, the assumption of infinite metabolism as exposure level approaches zero results in a theoretical upper limit of 100 percent metabolized fraction.

For the purposes of determining a PHG, the 95 percent upper confidence limits on the fractions predicted at low dose by the human PBPK model will be used to determine human doses. However, the agreement between these figures and those derived from the approach of Bogen and colleagues provides added confidence in their relevance.

Bois et al. (1996) noted a 30-fold variation in the fraction metabolized in a sample of 25,000 simulations, and a two-fold difference in the maximal metabolic rate within the study population of six males. Findings of substantial interindividual variability have been noted in several other studies. For example, Jang and colleagues reported that TCA in urine was significantly lower in a sample of six Asians in comparison to a sample of six Caucasians (Jang et al., 1997a), indicating potential ethnic differences. Another study reported a two-fold spread in PCE blood concentrations in a small study group (Opdam, 1989). Metabolism of other compounds by cytochrome P_{450} 2B enzymes has also been found to vary. The use of upper confidence limits on the fraction metabolized in humans is reasonable considering the extensive interindividual variability in pharmacokinetics expected in the diverse California population.

Issues in PCE Pharmacokinetics and Other Studies

Hattis and colleagues reviewed several modeling efforts for PCE with particular reference to uncertainties in predictions of low-dose metabolism rates (Hattis et al., 1990). Bois et al. also examined the issue of uncertainty, and investigated sensitivity of pharmacokinetic and cancer risk predictions to key pharmacokinetic parameters (Bois et al., 1990). They established that the most important parameters are K_m and V_{max} for the metabolism of PCE, and the partition coefficients for distribution of PCE between blood, air, and various tissue compartments. Hattis et al. (1993) reported that the value for one of these parameters, the coefficient for partitioning of

PCE between blood and air, used by most investigators is likely to be too low; a value between 15 and 18 for humans was recommended. The fitted estimate of the partition coefficient in the Bois

et al. human model, 16, falls within this range. Bois et al. (1990) also showed that the precise details of model structure with regard to grouping of tissues did not have a major effect on model predictions; in fact a model based on single body compartment with Michaelis-Menten metabolism and reversible pulmonary exchange was adequate to fit the data they examined. Subsequently, it was argued that parameter uncertainty was not an important source of variation in model output (Gearhart et al., 1993). This conclusion was disputed by Bois et al. (1996), who pointed out that although the variability and/or uncertainty attributable to a single parameter might not be extremely large, the overall uncertainty and variability of the result was considerable due to the combined effect of uncertainty and variability in all the parameters of the model.

Hattis and colleagues examined a large number of different PBPK models and found anomalies in the model fits to human PCE exposure data which they suggested were due to either heterogeneity of the fat compartment, or diffusion of PCE between fat and muscle compartments (Hattis et al., 1993). However it does not appear, based on the work by Bois et al. (1990), that they make a large difference to the prediction of the extent of metabolism of inhaled PCE by humans exposed at very low doses.

In addition to the model developed by Bogen and colleagues (1987) and those evaluated by Hattis et al. (1993), Gearhart et al. (1993) and Bois et al. (1990; 1996), several other PBPK models for PCE have been proposed. These include a model developed by Rao and Brown (1993) specifically to address uptake of PCE as a result of showering and bathing in contaminated water. Byczkowski and colleagues described a computerized simulation of their experimental findings on the transfer in milk of PCE from exposed lactating rats to their pups (Byczkowski et al., 1994; Byczkowski and Fisher, 1995). Jang and Droz applied a PBPK model to their data from experimental exposures of six Asian and six Caucasian volunteers (Jang and Droz, 1997b), but the results are not directly comparable to other human PBPK models, since metabolism was assumed to follow linear kinetics.

Reitz and colleagues reported experimental studies on intraarterial and inhalation exposure of rats and mice *in vivo*, and also various measurements *in vitro* with rodent and human tissues and extracts, in order to determine parameters for incorporation into a refined PBPK model (Reitz et al., 1996). The model was used to fit experimental data in rodents, and measured results from human occupational exposure studies, and to predict tissue concentrations at various times during and after inhalation exposure. The authors concluded that their enhanced model with improved experimental and fitted parameter estimates was successful in fitting the experimental data available in rodents exposed to PCE. They also attempted to refine parameter estimates for humans based on the data sets available to earlier authors. This latter attempt is somewhat frustrated by the inconsistencies between different results noted previously (Hattis et al., 1993; OEHHA, 1992). However, two overall conclusions were reached by Reitz et al. (1996) which are important for the purpose of this report:

1. Their various model predictions, and the available experimental data, are consistent with the fraction of oral dose metabolized in experimental animals as originally estimated by Chen and Blancato (1987) and therefore with the Bogen et al. (1987) approach used in this document for dose-response modeling of the animal cancer data.
2. The estimates of fraction metabolized by humans exposed by inhalation at high and low doses are still somewhat uncertain, but not inconsistent with earlier estimates as reported by Hattis (1990). Although Reitz et al. (1996) do not cite the work of Bois et al. (1996), it appears that the range of values proposed is consistent with Bois' estimates of the mean values and confidence bounds at high and low doses.

TOXICOLOGY

Estimates of human health risks resulting from exposure to a toxic substance may be based on evidence of effects in humans exposed to the chemical, when data are available. Frequently, however, specific human data are absent or inadequate for this purpose. In this case, estimates of human health risks are based on an assessment of animal dose-response data. The toxicity of PCE was reviewed previously by OEHHA (1992), and prior to that evaluation by IARC (1979), Reichert (1983), WHO (1984), and the U.S. EPA (1980, 1982, 1984, 1985a, 1985b). Also, ATSDR (1993) compiled a Toxicological Profile for PCE, which was recently updated (ATSDR, 1997). This section provides a further update to these reviews, with emphasis on the endpoints and specific studies used in the later sections of this report to determine a recommended PHG level.

Toxicological Effects in Animals

In this section, animal PCE toxicity studies are reviewed, including data from bioassays conducted to evaluate the carcinogenicity of PCE. Bioassay results are also used as the basis of the quantitative assessment of carcinogenic potency. The bioassay results are therefore described individually in this report. However not all the non-neoplastic toxicity endpoints are of significance for the determination of the PHG (as noted in the following section on Calculation of the PHG). Therefore, except where relevant for calculation of the PHG, or for recent reports not covered by the reviewers noted above, these non-neoplastic effects will be described in general terms only. The reader is referred to these reviews, and particularly those by OEHHA (1992) and Isacson et al. (1985), for further details.

Acute Toxicity

Acute lethality, particularly after inhalation exposures to high concentrations of PCE, typically results either from the CNS depression or from cardiac sensitization to adrenaline resulting in arrhythmia. These effects are seen with many other volatile halogenated compounds, but PCE is apparently one of the more potent of the common solvents. (In the series of halogenated C_1-C_3 alkanes and alkenes, cardiac sensitization and anesthetic potency are determined by various physical parameters related to molecular weight.) There is no evidence of substantial interspecies variations in susceptibility, other than variations in the effects of different exposure concentrations and time due to interspecies pharmacokinetic variations.

Liver and kidney damage after PCE exposures (by various routes) has been described by various authors. Some experiments have shown such effects after single inhalation, oral or intraperitoneal doses. The nature of the effects is similar to (although more limited than) those seen in subacute or subchronic repeated dose experiments, described below.

Significant exposure levels for acute exposures (ATSDR, 1997) are shown in Table 6.

Subacute and Chronic Toxicity

Liver toxicity has been observed after treatment with PCE by various routes. This has been revealed by increases in liver-specific transaminase levels in serum, by observation of various histological effects (swelling, vacuolation, centrilobular necrosis, hyperplasia) and by biochemical changes (decrease in the adenosine triphosphate (ATP) content, increase in the content of lipids and triglycerides) indicative of damage to hepatocytes. Mice are generally

substantially more susceptible than rats to this effect. After exposure to PCE, increased numbers of peroxisomes and increased peroxisomal cyanide-insensitive palmitoyl CoA oxidation were reported in mouse liver, but not in rat liver. In mice, liver weight increases and induction of cytochrome P_{450} after repeated PCE treatment have been reported.

In the bioassay of tetrachloroethylene by gavage (NCI, 1977) described in the section on carcinogenicity, there was no reported increase in any non-carcinogenic hepatic lesions in either mice or female rats. However, in the inhalation bioassay (NTP, 1986), male and female mice of both exposure groups showed dose related liver degeneration and necrosis. Under the conditions of this study, rats did not develop hepatic lesions in response to exposure to PCE.

Kidney damage after oral, intraperitoneal or inhalation PCE exposure has been reported in rodents (especially male rats) and dogs (ATSDR, 1997). With shorter treatment regimens and lower doses this may be limited to functional impairments, increases in kidney weight and modest histological alterations. However, more extensive histological changes are seen after extended exposures at high doses, including desquamation of tubular epithelium and intratubular casts. In male rats, hyaline droplet formation has been reported. This was described as an α2u globulin nephropathy by the original authors (Green et al., 1990; Bergamaschi et al., 1992) and by ATSDR (1997). However, there appears to be some doubt as to whether the findings in male rat are fully compatible with this explanation. In any case, there must be other mechanisms by which PCE produces renal toxicity, since nephropathy (which may be severe or fatal after chronic exposures at high doses) is observed in female rats and both sexes of mice, none of which are susceptible to α2u globulin nephropathy. The observations by Birner et al. (1994) of a glutathione-based metabolic route (which may be active in the kidney) to a reactive intermediate are described in the section on Metabolism. This may indicate an additional possible mechanism for renal toxicity of PCE.

The NCI cancer bioassay of PCE by gavage (NCI, 1977) documented a high incidence of toxic nephropathy in mice and rats for all dose groups. Toxic nephropathy was defined as degenerative changes in the proximal convoluted tubule, fatty degeneration, and necrosis of the tubular epithelium. In the inhalation bioassay (NTP, 1986), kidney casts, nephrosis, and tubular cell karyomegaly were reported in mice. In rats, a dose-related increase of renal tubular cell karyomegaly was reported in both sexes. Male rats exhibited a dose-related increase in renal tubular cell hyperplasia; one high-dose female was also affected.

Table 6: Significant Exposure Levels for Acute Exposure to Perchloroethylene

Species, Strain, Sex	Route	Level, Type	Effect
Rat			
Sprague-Dawley, M	Oral (single dose)	3005 mg/kg, LD_{50}	Lethality
Sprague-Dawley, F	Oral (single dose)	3835 mg/kg, LD_{50}	Lethality
Fischer 344, M/F	Inhalation (4 h)	3768 ppm, LC_{50}	Lethality
Fischer 344, F	Oral (single dose)	500 mg/kg, NOAEL 1500 mg/kg, LOAEL	Lacrimation, gait disturbance, decreased motor activity
Wistar, M	Oral (5 days)	1000 mg/kg, NOAEL	Decreased body weight gain
Fischer 344, F	Inhalation (6 h/d, 5d/wk, 2 wk)	875 ppm, NOAEL 1750 ppm, LOAEL	Decreased body weight gain
Various, M/F	Oral (5-10 days)	500 mg/kg, NOAEL 1000 mg/kg, LOAEL	Increased liver weight, liver enzyme induction
Mouse			
Swiss-Webster, M	Oral (single dose)	8139 mg/kg, LD_{50}	Lethality
?, M/F	Inhalation (4 h)	5200 ppm, LC_{50}	Lethality
?, F	Inhalation (4 h)	200 ppm, LOAEL	Liver fatty degeneration
$B6C3F_1$, M	Oral (10 days)	1000 mg/kg, LOAEL	Hepatic and renal peroxisome proliferation
$B6C3F_1$, M/F	Oral (11 days)	100 mg/kg, LOAEL	Hepatocellular swelling
$B6C3F_1$, M/F	Inhalation (6 h/d, 5d/wk, 2 wk)	400 ppm, LOAEL	Liver fatty changes, peroxisome proliferation

Source: ATSDR (1977), citing Isacson et al. (1985)

Developmental and Reproductive Toxicity

Developmental and reproductive effects of PCE were reviewed by OEHHA (1992), IARC (1985a,b) and ATSDR (1997). The teratogenic activity of PCE has been studied in rats (Schwetz et al., 1974; Schwetz et al., 1975; Beliles et al., 1980; Nelson et al., 1980), mice (Schwetz et al., 1975) and rabbits (Beliles et al., 1980). Maternal exposure levels ranged from 100 to 1800 ppm PCE. Tinston (1995, in an unpublished report cited by ATSDR, 1997) undertook a multigeneration study in rats exposed to PCE. At 1000 ppm there were some decreases in litter size and survival during lactation, but these were not seen at 300 ppm.

Although some minor effects have been seen in the progeny in these studies, PCE is not generally considered a rodent teratogen. Developmental toxicity, in the form of morphological abnormalities, was also observed in an *in vitro* study in which ten-day old embryos from Sprague-Dawley rats were removed to culture medium containing PCE (Saillenfait et al., 1995). There are some possible effects of PCE on the developing nervous system following exposure to PCE *in utero* (Nelson et al., 1980) or neonatally (Fredriksson et al., 1993). In general, however,

investigations of developmental effects of PCE have shown evidence of maternal toxicity, rather than specific adverse effects on the progeny.

Immunotoxicity

Kroneld and associates conducted *in vitro* experiments using isolated human peripheral blood lymphocytes (Kroneld, 1987). Lymphocytes were exposed to PCE at concentrations of 0.2, 1.6, 16, 160 µg/L. Significant decreases in ^{3}H-thymidine intake were noted at concentrations of 1.6 µg/L and greater.

Groups of 140 female CD-1 mice were exposed to 0, 25 or 50 ppm PCE for three hours (Aranyi et al., 1986). Challenge by inhaled *Streptococcus zooepidemicus* resulted in significantly increased mortality in animals exposed to 50 ppm PCE relative to controls. Pulmonary bactericidal activity against *Klebsiella pneumoniae* was significantly decreased in mice exposed to 50 ppm PCE. However, the significance of the effects observed is unclear because the mortality observed in the control animals in the 25 ppm experiment was greater than that of PCE-exposed animals in the 50 ppm experiment. Furthermore, exposure to 25 ppm PCE for three hours per day for five days did not result in changes in *Streptococcus* mortality or pulmonary bactericidal activity.

Miyano and associates (1987) reported that PCE caused decreased cell viability in mouse spleen cell cultures (Miyano and Nakano, 1987). Lipopolysaccharide-stimulated B-lymphocyte transformation and Con A-stimulated T-lymphocyte transformation were also inhibited by PCE.

Neurotoxicity

Acute high exposure to PCE typically induces CNS depression. Initial depression can progress to loss of consciousness, anesthesia, and respiratory failure with prolonged or massive exposure. Chronic exposures at lower levels have been reported to result in alterations in biochemical parameters in the central nervous system. These effects were reviewed by ATSDR (1997) and OEHHA (1992). The following account describes a small number of recent studies of PCE neurotoxicity in animals, which were not cited by Alewife et al. (1992) or in the recent review by ATSDR (1997).

Wang et al. (1993) exposed rats to PCE (300 – 600 ppm) by inhalation for 4–12 weeks. Total brain weight increase was inhibited at 600 ppm for 4 or 12 weeks, and tissue weight, total protein and DNA were reduced in specific regions after 12 weeks at this concentration. Studies of specific biochemical markers indicated reductions in numbers of brain cells, probably glial cells rather than neurons, and interference with the metabolism of cytoskeletal proteins in both types of cell.

Umezu et al. (1997) examined the effects of intraperitoneal injections of PCE on the behavior of mice. Loss of righting reflex was noted, with an ED_{50} of 4209 mg PCE/kg. Inhibition of operant behavior was noted at 2000 mg PCE/kg. Negative effects were noted on conflict behavior in two tests at 250-500 mg/kg. The study also examined the effects of trichloroethylene, which were similar to those of PCE.

Mattson et al. (1998) examined various neurotoxicological end-points in rats during and following inhalation of 50 – 800 ppm PCE for 6 hours/day, 5 days/week for 13 weeks. Long--latency flash evoked potentials (FEPs) in the visual cortex were found to have greater amplitude in the group exposed to 800 ppm PCE. The authors described the toxicological significance of this finding as "unknown." Other neurotoxicological parameters (FEPs in the cerebellum;

auditory, somatosensory, or caudal nerve evoked potentials; clinical or histopathological observations) showed no dose-related effects. The NOEL for amplitude changes in visual cortex FEPs was 200 ppm.

Genetic Toxicity

A number of investigators have reported data on the genetic toxicity of PCE, using microorganisms, mammalian cells in culture, and a variety of genetic assays *in vivo* in mammals and in *Drosophila.* The genetic toxicity of PCE has been extensively reviewed by OEHHA (1992), by IARC (1995b) and by ATSDR (1997), and the reader is referred to these sources for details of the studies. The reviewers generally concluded that the evidence for genotoxicity of PCE is negative, or at most, equivocal. However, this may be related to the difficulty in designing a test system with appropriate sensitivity and metabolic activation, rather than necessarily indicating the absence of a role for genotoxicity in the overall toxic and carcinogenic effects of PCE.

The results in the majority of cases for the assays measuring forward or reverse mutations in prokaryotes (*Salmonella typhimurium, Escherichia coli*) *in vitro* have been negative. A few positive results were obtained which were apparently associated with the presence of mutagenic impurities or stabilizers in the PCE, or were of doubtful significance. Some positive or equivocal results were obtained with eukaryotic systems *in vitro*, including mitotic recombination in *Saccharomyces* and induction of unscheduled DNA synthesis in cultured mammalian cells, but most results in these types of test systems were also negative. Some reports of effects *in vivo* (DNA single strand breakage in liver, micronucleus induction and altered sperm morphology, in rodents) have appeared. However, these results are often hard to interpret, and are not considered to provide clear evidence of direct genotoxicity of PCE or its metabolites.

A recent study examined the effect of oral PCE exposure (800 mg/kg-d, five days per week for up to 76 weeks) on proto-oncogenes in the livers of mice (Anna et al., 1994). Oncogene mutations were examined in the spontaneously occurring tumors (hepatocellular adenomas and carcinomas) found in control mice, and in those from exposed mice. The frequency of mutations at codon 61 of the H-*ras* gene was significantly reduced in exposed mice, and a possible shift in the spectrum of mutations at this codon was noted. The frequencies of other mutations in the H-*ras* gene, and the K-*ras* gene were very low in concurrent or historical controls. On the other hand, in tumors from exposed mice these mutations contributed 4 and 13 percent respectively of the total observed *ras* mutations.

Several metabolites of PCE are known to be mutagenic. Tetrachloroethylene oxide (PCE oxide) is believed to be the first intermediate formed by microsomal oxidation of PCE. PCE oxide was found to be mutagenic to *Salmonella typhimurium* TA1535 (without metabolic activation) but not to *E. coli* WP2 uvrA. PCE oxide gave positive results in a differential growth inhibition test using the DNA-polymerase-deficient strain, pol A 1 of *E. coli* (Kline et al., 1982).

Trichloroacetic acid (TCA) is excreted in the urine of rodents and humans exposed to PCE. In an Ames test conducted with metabolic activation, TCA (0.45 mg/plate) and trichloroethanol (7.5 mg/plate) were not mutagenic to *S. typhimurium* strains TA98 and TA100. There is some indication, however, that trichloroethanol can induce sister-chromatid exchange in cultured human lymphocytes (Gu et al., 1981), although this potential metabolite has not been consistently detected in humans. TCA may also contribute to "non-genotoxic" mechanisms of liver carcinogenesis in rodents, since it is known to be carcinogenic in mice (Herren-Freund et al., 1987) and to induce peroxisome proliferation (Odum et al., 1988).

The proposed metabolism of PCE to a glutathione conjugate by the mercapturic acid pathway and beta-lyase, resulting in formation of a genotoxic metabolite, is discussed further in the section in this report on Metabolism. The PCE glutathione conjugate S-(1,2,2-trichlorovinyl)glutathione (TCVG) was found to be mutagenic in *S. typhimurium* TA 100 when a suitable metabolic activation system was included in the assay (Vamvakas et al., 1989). TCVG incubated with rat kidney particulate fraction containing high concentrations of gamma-glutamyl transpeptidase (GGT) and dipeptidases was highly mutagenic. PCE was not mutagenic in the absence of metabolic activation, or under conditions supporting oxidative metabolism. PCE was, however, mutagenic in the Ames assay upon incubation purified with rat liver glutathione (GSH) S-transferases, GSH and rat kidney fractions. Under these conditions, it was expected that TCVG would be formed in the mixture. TCVG was formed from PCE in isolated perfused rat liver and excreted in bile, and this bile was mutagenic in the presence of kidney particulate fractions. Serine borate, an inhibitor of GGT, or aminooxyacetic acid, an inhibitor of β-lyase, reduced mutagenicity.

Carcinogenicity

The National Toxicology Program has conducted two lifetime bioassays on PCE (NCI, 1977; NTP, 1986). Additionally, three other studies have addressed the question of PCE carcinogenicity (Theiss et al., 1977; Rampy et al., 1978; VanDuuren et al., 1979).

Gavage studies

Mouse (NCI, 1977)

The National Cancer Institute conducted a long-term study (NCI, 1977) in which $B6C3F_1$ mice were administered PCE in corn oil by gavage, 5 days/week (78 weeks + an additional 12 week observation period). Mice were 25 days old at initial treatment. The time-weighted average daily doses of PCE were 536 and 1,072 mg/kg for male mice, 386 and 722 mg/kg for female mice, 471 and 941 mg/kg for male rats, and 474 and 949 mg/kg for female rats. A statistically significant increase ($P<0.001$, Fisher Exact test) in hepatocellular carcinoma was observed in both males (controls 2/17; 536 mg/kg-d 32/49; 1072 mg/kg-d 27/48) and females (controls 0/20; 386 mg/kg--d 19/48; 772 mg/kg-d 19/48). Incidences of hepatocellular carcinoma in concurrent groups of untreated mice were 2/17 (males) and 2/20 (females). The NCI concluded that under the conditions of this study, PCE was a liver carcinogen to $B6C3F_1$ mice of both sexes.

Questions have been raised about the purity of PCE used in the NCI mouse and rat gavage bioassays. The PCE was produced by Aldrich Chemical Co. and had a purity of 99 percent. However, epichlorohydrin (ECH) was apparently used as a stabilizer. It has been suggested that the presence of this contaminant may have directly contributed to tumor induction. ECH is a direct-acting alkylating agent and is mutagenic (Kucerova et al., 1997; Bridges, 1978). VanDuuren and associates demonstrated that ECH was carcinogenic in mice when injected subcutaneously (VanDuuren et al., 1974). A subsequent study by Laskin and colleagues showed that ECH induced neoplastic lesions of the nasal cavity of rats (Laskin et al., 1980). Most of these tumors were carcinomas of the squamous epithelium. A study by Konishi and co-workers and Kawabata also showed that ECH fed discontinuously to rats in drinking water at a concentration of 1500 ppm (and at a lifetime TWA dose of approximately 40.2 mg/kg-d) induced a significantly increased incidence of papillomas and squamous cell carcinomas of the forestomach above that of control animals (Konishi et al., 1980; Kawabata, 1981).

The exact quantity of ECH present in the PCE used in the NCI study is not known, but it has been estimated that high-dose male mice received 0.42 mg/kg-d (U.S. EPA, 1985a). This represents one percent of the dose that elicited squamous cell carcinomas in rats. Furthermore, ECH appears to initiate tumors by a localized tumorigenic reaction at sites
where it is in direct contact with tissue, such as nasal or forestomach squamous-cell epithelium (U.S. EPA, 1984). No animal in the NCI bioassay developed tumors at these sites. ECH is among the weakest of the more than 50 suspect carcinogens evaluated by the EPA Carcinogen Assessment Group, having an estimated upper-bound carcinogenic potency, or effect per unit dose at low doses, to humans of 9.9×10^{-3} $(mg/kg\text{-}d)^{-1}$, based on increased nasal cavity tumor incidence in rats exposed to ECH via drinking water (U.S. EPA, 1984). Using the methodology of the U.S. EPA, the equivalent potency to mice would be 9.9×10^{-3} x (fm/fh), where fm and fh are the fractions of body weight consumed as water by mice and humans, respectively (U.S. EPA, 1984). The potency for ECH to mice is therefore estimated to be 0.058 $(mg/kg\text{-}d)^{-1}$. Using this potency estimate, the highest dosed animals (high-dose male mice) in the NCI bioassays would be expected to incur an increased cancer risk of (0.42) x (0.058) - 0.024, or less than 2.5 percent (NCI, 1977). Therefore, it is unlikely that ECH contributed significantly to the observed increased tumor incidence in PCE-exposed mice in the NCI bioassay (NCI, 1977).

Rat (NCI, 1977)

Male and female Osborne-Mendel rats received 471 mg/kg-d or 941 mg/kg-d, and 474 mg/kg-d or 949 mg/kg-d PCE, respectively, by gavage in corn oil (78 weeks + an additional 32 week observation period). Early mortality occurred in all groups of rats dosed with PCE. Half of the high-dose males had died by week 44 and half of the high-dose females died by week 66. The survival time of control animals ranged from 88 to 102 weeks. The NCI determined that there was a statistically significant association ($p<0.001$) between increased dosage of PCE and increased mortality. The early mortality observed in rats and its statistical association with dose of PCE indicate that the doses given to rats in this bioassay were inappropriately high. Because the optimum dosages were not used and because significant early mortality occurred, these results preclude making conclusions regarding the carcinogenicity of PCE in rats.

Inhalation studies

Mouse (NTP, 1986)

A study of the carcinogenic potential of PCE by inhalation in mice was conducted by Battelle Pacific Northwest laboratories for the National Toxicology Program (NTP, 1986). In this experiment, B6C3Fl mice were exposed to 99.9 percent pure PCE by inhalation, 6 hours/day, 5 days/week for 103 weeks at concentrations of 0, 100, or 200 ppm. Hepatocellular adenoma and hepatocellular carcinoma in males, and hepatocellular carcinoma in females were observed. The incidences of hepatocellular carcinoma relative to controls were significant ($P<0.01$, Fisher Exact test) for mid- and high-dose males (controls 7/49; 100 ppm 25/49; 200 ppm 26/50) and females (controls 1/48; 100 ppm 13/50; 200 ppm 36/50). The NTP determined that, under the conditions of this study, there was "clear evidence of carcinogenicity" of PCE for both sexes of $B6C3F_1$ mice.

Rat (NTP, 1986)

In parallel to the mouse study, a PCE inhalation study in rats was conducted (NTP, 1986). In this experiment, F344/N rats were exposed to 99.9 percent pure PCE by inhalation, 6 hours/day,

5 days/week for 103 weeks at concentrations of 0, 200, or 400 ppm. Treated male rats had lower survival rates than control animals (controls, 23/50 animals; 200 ppm, 20/50; 400 ppm, 12/50). Survival rates among female rats showed little variation (controls, 23/50 animals; 200 ppm, 21/50; 400 ppm, 24/50). A statistically significant increase in mononuclear cell leukemia was observed in mid- and high-dose males (controls 28/50; 200 ppm 37/50; 400 ppm 37/50) and females (controls 18/50; 200 ppm 30/50; 400 29/50). In males, increases in renal tubular cell adenomas (controls 1/49; 200 ppm 3/49; 400 ppm 2/50) and tubular cell adenocarcinomas (controls 0/49; 200 ppm 0/49; 400 ppm 2/50) were observed. Although these increases were not statistically significant according to NTP (1986), subsequent commentators and investigators have generally concluded that the results have toxicological significance in view of the low historical incidence of such tumors in F344 rats (Green et al., 1990; Birner et al., 1994). The combined incidence of tubular cell adenoma or adenocarcinoma was: controls, 1/49; 200 ppm, 3/49; 400 ppm, 4/50. The NTP determined that, under the conditions of this study, there was "clear evidence of carcinogenicity" of PCE for male F344/N rats, and "some evidence of carcinogenicity" of PCE for female F344/N rats.

Rat (Rampy et al., 1978)

Male and female Sprague-Dawley rats were exposed to 300 or 600 ppm PCE by inhalation for 6 hours/day, 5 days/week for 12 months. Animals were then observed for an 18-month follow-up period. High-dose males had slightly greater mortality than did controls. No treatment-related tumors were observed in either male or female rats. Interpretation of this study is limited by the short duration of the exposure and by the fact that it was reported only as an abstract.

Other Routes

Mouse i.p. study (Theiss et al., 1977)

Six to eight weeks old male A/St mice were given 80, 200, or 400 mg/kg of PCE in tricaprylin by intraperitoneal injection three times per week. Each group received 14, 24, or 48 injections. Animals were sacrificed 24 weeks after the first injection and examined histologically for the presence of pulmonary tumors. No treatment-related tumors were observed.

Mouse dermal studies (VanDuuren et al., 1979)

ICR/Ha Swiss mice were treated with 163 mg PCE applied once to surface skin. Fourteen days after treatment, phorbol myristate acetate, a promoter, was applied to the same area three times a week for 428 to 576 days. A second group received 54 mg PCE by topical application three times a week for 440 to 594 days. No treatment-related tumors were observed in either male or female mice.

Summary of Evidence of Carcinogenicity in Animals

The NCI bioassay of PCE found that administration of PCE by gavage was associated with a statistically significant increased incidence ($p<0.001$) of hepatocellular carcinoma (NCI, 1977). This increase was documented in low and high-dose PCE-treated $B6C3F_1$ mice of both sexes. A decrease in the time to first tumor development was also observed in treated mice of both sexes and both dose groups. Early mortality prevented an analysis of PCE's carcinogenic potential in

rats. The NCI concluded that under the conditions of this study, PCE was a liver carcinogen to $B6C3F_1$ mice of both sexes (NCI, 1977).

The final report of the NTP inhalation bioassay on PCE was released in 1986 (NTP, 1986). The NTP determined that, under the conditions of this study, there was "clear evidence of carcinogenicity" of PCE for male F344/N rats, "some evidence of carcinogenicity" of PCE for female F344/N rats, and "clear evidence of carcinogenicity" of PCE for both sexes of $B6C3F_1$ mice. In rats, these conclusions were based on an increased incidence of mononuclear cell leukemia in males and females. Male rats also developed renal tubular cell neoplasms (a rare type of tumor). The evaluation of carcinogenicity in mice was based on an increased incidence of hepatocellular adenoma and hepatocellular carcinoma in males, and an increased incidence of hepatocellular carcinoma in females.

In 1979, IARC reviewed the NCI study on PCE as well as the animal carcinogenicity studies of Rampy and associates (Rampy et al., 1978) and Theiss and colleagues (1977). Only two short-term assays were evaluated (Greim et al., 1975; Cerna and Kypenova, 1977). At this time, IARC determined that there was "limited evidence" that PCE was carcinogenic in mice (IARC, 1979). Recently, IARC re-evaluated the evidence of carcinogenicity of PCE, including the NTP (1986) data. The working group concluded that there was “sufficient evidence” that PCE is carcinogenic to animals (IARC, 1995b) .

Toxicological Effects in Humans

Acute and Subacute Toxicity

Numerous case reports of short-term exposure to PCE, studies of occupationally exposed workers and experimental studies of human volunteers have demonstrated acute toxicity of PCE to humans (reviewed in Isacson et al. (1985); OEHHA (1992); OEHHA (1999)). The reader is referred to these sources for additional details. It does not appear likely that the acute toxicity described would be important at the exposure levels anticipated as a result of exposures to PCE contaminated drinking water. The effects described in humans resemble the acute toxicity of PCE in animals. Symptoms associated with effects on the central nervous system predominate: nausea, speech difficulty, lightheadedness, anesthesia, and eye and throat irritation have been observed. In most cases, effect levels are at or above 100 ppm. Hepatic necrosis and hepatitis have also been reported occasionally, particularly following substantial oral doses (*ca.* 0.12 mL/kg).

Neurotoxicity

Human and animal studies of acute and chronic duration have shown that, like many other solvents, the nervous system is a critical target organ for the effects of PCE. Recent reviews by the ATSDR (1997) and OEHHA, (1992) have surveyed many of the available studies. Selected findings of these documents are noted below; the discussion is supplemented with more recent data not available at the time of the earlier reviews.

Acute exposure has been studied in short-term experimental studies in humans. At very high concentrations, anaesthetic effects occur; these effects are generally thought to be reversible (ATSDR, 1997). At lower exposure concentrations (ranging from 50-600 ppm) for acute exposure periods (10 minutes to 1 week) a variety of CNS symptoms such as dizziness, ataxia, loss of coordination, balance, and increased latency of visual evoked potentials have been

reported (ATSDR, 1997). Effects on visual evoked potentials, coordination, and vigilance were noted in 15 male volunteers exposed to 50 ppm for 4 hr/day, 4 days, in comparison to a similar group exposed to 10 ppm (Altmann et al., 1992). Based on this study, ATSDR considered 10 ppm to be an acute NOAEL for PCE.

Subchronic exposure to volunteers (0, 25, or 100 ppm for 5.5 hours/day for 11 weeks) resulted in significant decreases in an index of coordination at 100 ppm. Exposure to 100 ppm for 4 weeks caused alterations in EEG patterns, suggesting a state of drowsiness similar to the first stages of anesthesia (Stewart et al., 1977, 1981, as cited in ATSDR, 1997).

Chronic effects of PCE exposure on the nervous system have been studied in occupationally exposed workers, mostly dry-cleaners, and in one study of environmental exposure to residents in the vicinity of dry-cleaning shops. A wide variety of tests for neurotoxicity have been performed. The most important effects appear to be neurobehavioral and cognitive effects such as longer response times and decreased memory functions. Visual effects including dyschromatopsia (acquired color vision loss) are also potentially important neurotoxic endpoints in humans. Effects on the peripheral nervous system, such as would be detected in tests of motor functions, appear to be less frequently affected by PCE exposure than central nervous system endpoints.

Delayed reaction times to different stimuli types have been observed in a number of tests conducted in people with exposure to PCE. In a study of 60 female dry-cleaning workers with an average employment duration of 10.1 years, Ferroni and colleagues found statistically significantly longer simple reaction times and longer reaction time in tests of shape comparison (Ferroni et al., 1992). Exposure was determined by 4-hour air samples taken in each workplace at two different times of year (median 15 ppm), and by blood concentration of PCE (median 145 mg/L). Delayed reactions were also reported in an earlier study of German workers exposed to an average of 30 ppm PCE (Seeber, 1989). While the effects were statistically significant in low exposure (average exposure level 12 ppm for 141 months) and high exposure (54 ppm, 127 months) groups, there were no significant differences between the two exposure groups for any of the test results reported. A recent paper reported that laundry operators exposed to PCE had significantly delayed vocal reaction time to words displayed on a computer screen compared to unexposed controls (Spinatonda et al., 1997). The duration of the response was also shorter, particularly for meaningless words. Average exposure was 8 ppm; years of employment were not given. Altmann and colleagues tested a number of cognitive and psychomotor functions in a group of 14 people with at least one year of residence neighboring a dry-cleaning shop (Altmann et al., 1995). The median air concentration in the home was 0.2 ppm, average residence duration was 10.6 years, and blood concentrations averaged 17.8 μg/L. Significant differences from controls in simple reaction time, visual memory for shapes and a vigilance were observed. The findings remained significant after adjustment for age, education, and gender. This study is limited by the small sample size, but appears to identify the lowest exposure level at which chronic effects of PCE exposure have been reported. In contrast to these findings, Lauwerys and colleagues (1983) did not find effects on reaction time in two tests administered to workers exposed to an average of 21 ppm for 6.4 years.

Adverse effects on memory, particularly visual memory, have been reported in PCE-exposed people. Echevarria and colleagues followed up on clinical findings in four patients examined for PCE-induced encephalopathy with a field study in workers (Echeverria et al., 1995). The clinical findings on the four patients included decrements in memory, motor, attention, and visuospatial functions; these findings were used to design a field study of 65 dry-cleaning workers in PCE-only shops. Exposure was determined by PCE measurements in breath and air. Two exposure levels were noted: low (11 ppm) and high (41 ppm). Both current exposure and lifetime cumulative exposure (accounting for other occupational and hobby exposure) were used

as explanatory variables in the analysis of results from a variety of neurobehavioral tests. Visually-mediated functions, particularly those associated with short-term memory for visual designs, were significantly associated with cumulative exposure, but not current exposure. These effects were also associated with alcohol intake, emphasizing the importance of controlling for alcohol in studies of this kind. In the aforementioned study of environmentally exposed subjects (Altmann et al., 1995), a test for visual memory showed differences between exposed subjects and unexposed controls.

Other outcome measures that have been assessed in neurotoxicity studies of chronic PCE exposure include tests for attention, cognitive function, verbal skills, and motor function. Lauwerys and colleagues (1983) did not find any differences between their exposed workers and controls in a test of attention, whereas Seeber et al. (1989) reported a significant difference in an attention task. Echevarria and colleagues concluded that there were few effects on verbal and cognitive functions, and proposed that PCE may target parts of the limbic and frontal systems dealing with visual response and memory (Echevarria et al., 1995).

Mood changes and other subjective symptoms were reported with increased prevalence in exposed groups in two studies (Lauwerys et al., 1983; Cai et al., 1991). However, findings of similar symptoms were not significant in the paper of Lauwerys et al. (1983) and effects on emotional liability were restricted to the low exposure group in a third study (Seeber, 1989). Motor functions also appear to be relatively unaffected in chronic studies. One positive result for a finger tapping test was reported (Ferroni et al., 1992). Another study appears to have been negative, although only selected results were reported (Seeber, 1989). Altmann et al. (1995) reported no effect in a finger-tapping test in subjects with low environmental exposure.

Acquired dyschromatopsia has been observed in several studies of occupational exposure to organic solvents at relatively low levels, as well as in alcoholics, and may be a sensitive indicator of PCE neurotoxicity. The mechanism of acquired dyschromatopsia is not known, but could involve direct effects on cone cells in the retina or axonopathy of the optic nerve. Mergler found evidence that solvent-induced loss of color vision is due to neural rather than direct ocular damage (Mergler et al., 1987).

In 1992, Nakatsuka and colleagues reported on a study of color vision in groups of workers exposed to toluene, PCE alone or PCE in combination with trichloroethylene (Nakatsuka et al., 1992). Color vision was somewhat worse in control subjects as compared to any of the exposed groups. However, other authors have pointed out that this study failed to control for alcohol intake, an important factor for color vision outcomes (Valic et al., 1997). Further, there is some question about whether the test administered was sufficiently sensitive (Gobba et al., 1998). Color vision was tested in a group of dry-cleaning workers, using a desaturated color test and a sensitive measurement scale (Cavalleri et al., 1994). Subjects were selected from workers with low alcohol intake. Exposure assessed by personal sampling for a full shift. A statistically significant increase in color confusion was observed in exposed subjects, mostly affecting blue-yellow discernment. When the data for ironers with lower exposure (4.8 ppm, range 0.5-11.3) were analyzed separately from data for dry-cleaners (7.3 ppm, range 0.4-31.2), the dry-cleaning workers were found to have a significant increase in dyschromatopsia that was not observed in ironers. The same group of workers was followed up two years later (Gobba et al., 1998). Exposure in one group had been 1.7 ppm and increased to 4.4 ppm, whereas in the other group, exposure had been 3.0 ppm and had decreased to 0.7 ppm. Color confusion index had worsened in the subgroup that had experienced an increase in exposure level, and no improvement was observed in the subgroup with decreased exposure level. Thus, PCE effects on color vision appear to be cumulative and persistent.

A third study considered potential interactive effects of alcohol and solvent exposure (Valic et al., 1997). Exposure to PCE and/or trichloroethylene was determined by urinary TCA levels; most of the 31 PCE/TCE subjects were classified as having "mild exposure". Neither cumulative exposure to solvents (toluene, xylene, TCE, PCE) nor TCA in urine were correlated with color confusion in Lanthony's d-15 test. However, there was a significant increase in color confusion in workers co-exposed to solvents and high alcohol intake, with the TCE/PCE group showing the most pronounced effect.

In conclusion, neurobehavioral and cognitive effects, including effects on visual perception, are sensitive to relatively low PCE exposures; changes in function have been measured in workers at exposure levels below current occupational standards.

Reproductive and Developmental Toxicity

Adverse effects on fertility and pregnancy outcomes have been described in several studies of men and women occupationally exposed to PCE. Since earlier reviews by ATSDR (1997) and IARC (1995 a, b, c) were published, one further study on pregnancy outcomes was identified that is pertinent to the current review (Doyle et al., 1997). The reader is referred to the previous assessments for more complete review of studies published before 1997.

Serum prolactin levels were significantly increased relative to controls in a group of women with occupational exposure to PCE (Ferroni et al., 1992). In another study, it was noted that women with potential exposure to PCE in laundry/dry-cleaning reported menstrual disorders, including PMS, menorrhagia, and dysmenorrhea more frequently than women in the same work who were considered unexposed (Zielhuis et al., 1989). Two studies have reported a possible association between dry-cleaning work and reduced fertility, although the small number of study subjects in both cases limits the usefulness of the results. In a study comparing infertile to fertile couples, the women in infertile couples were more likely to have exposure to dry-cleaning chemicals; specific exposures were not assessed (Rachootin and Olsen, 1983). In a second study, couples in which the men were employed in dry-cleaning were compared to couples in which the men were employed in laundries (Eskenazi et al., 1991a). Men who worked as dry-cleaners and their partners experienced a longer time to conception and were more likely to have sought treatment for infertility than laundry workers. Minor differences in sperm morphology and motility were also found, and were related to the concentration of PCE in expired air (Eskenazi et al., 1991b).

Spontaneous abortion among women with employment in laundry or dry-cleaning was investigated in studies in four Nordic countries, reported together by Olsen and colleagues (Olsen et al., 1990). Spontaneous abortion was increased slightly in workers classified as having low exposure to PCE and further increased in the high exposure group (OR 2.9, 95 percent CI 0.98-8.4). The association in the overall cohort was largely due to an increased risk of spontaneous abortion in Finnish women (Kyyrönen et al., 1989). In that study, an odds ratio of 3.4 (CI 1.0-11.2) was observed. A survey of occupational exposures among cases of spontaneous abortion found a significant association with employment in laundry/dry-cleaning (Lindbohm et al., 1984). A much smaller study of 67 Italian dry-cleaning workers noted a marginally significant increased risk of spontaneous abortion in comparison to women who worked in the home (Bosco et al., 1987). A Canadian study did not find an association, except for a weak effect on past pregnancies (McDonald et al., 1986). A significant effect of exposure to PCE during the first 20 weeks of pregnancy was reported in a study of spontaneous abortion in women exposed to PCE and other solvents in California (OR 4.7, 95 percent CI 1.1-21.1) (Windham et al., 1991). However, the results are based upon only seven cases with PCE exposure, four of which were also exposed to trichloroethylene. A recent retrospective study reported reproductive outcomes in 7305 women with employment history that included dry-cleaning or laundry in the

UK (Doyle et al., 1997). An odds ratio for spontaneous abortion of 1.67 (95 percent CI 1.17-2.36), adjusted for age, previous pregnancies and year, was observed for women who worked as dry-cleaner operators versus those who did not report any work in dry-cleaning or laundry during pregnancy or the three months preceding.

Spontaneous abortion has also been studied with paternal exposure as the descriptor of interest. A case control study nested in a cohort of male workers biomonitored for solvent exposure in Finland did not find an association between PCE exposure in men and spontaneous abortion in their wives (Taskinen et al., 1989).

Several of the above-cited studies have also assessed the rates of stillbirth, congenital malformation, and low birth weight in children born to women with potential exposure to PCE. No significant associations have been observed. Thus there is evidence from several studies that employment in occupations that potentially involve PCE exposure can have adverse effects on pregnancy; the most marked effect reported to date is increased spontaneous abortion. It is likely that these effects are most pronounced when exposure coincides with the early stages of pregnancy; only one study focussed on exposure during this period. Fertility may also be impacted in exposed men and women, although the available data are quite limited.

Genetic Toxicity

The genetic toxicity of PCE exposure in humans has not been adequately evaluated. A small study of factory workers exposed to high (92 ppm PCE [geometric mean]) or low (10-40 ppm) found no evidence of cytogenetic damage to lymphocytes or altered cell cycle kinetics (Ikeda et al., 1980). Air concentrations of PCE were also reflected in trichloro-compound content from urinalysis. No differences between either group and concurrent controls were observed with respect to lymphocyte damage. No increase in sister chromatid exchanges in lymphocytes was found in a study of 27 subjects exposed to PCE compared with control subjects matched by age, sex, smoking habits, and place of residence (Seiji et al., 1990).

Immunotoxicity

Groups of "exposed" (n=21) and "unexposed" (n=16) workers in a dry-cleaning plant were examined for differences in numerous immunological parameters (Andrýs et al., 1997). Both groups were also compared with long-term laboratory reference values. Exposure was measured at time-weighted average concentrations of 11-752 mg/m^3. While immunological parameters of metabolic activity did not tend to fall outside the normal range for either group, the exposed group did have statistically significantly different levels for several metabolic activity parameters relative to the in-plant control group (phagocytes, α_2-macroglobulin, C3 and C4 complement component, salivary secretory IgA, blast transformation test). Both plant groups also showed some departure from the long-term reference values (α_2-macroglobulin, C3, percent lymphocytes).

Chronic Toxicity (Other Endpoints)

The neurotoxic and reproductive effects of PCE have been discussed above. Other effects of chronic exposure include indicators of renal and hepatic toxicity. The literature previous to 1997 was adequately summarized by ATSDR (1997).

As discussed in the section on animal toxicology, above, the kidney is a target organ for cancer and other toxic effects in rodents exposed to PCE. While effects were often weak, changes in at

least one renal-effect parameter were observed in six of eight studies of nephrotoxicity associated with PCE exposure (ATSDR, 1997). An additional study, recently published, reported a nearly two-fold increase in the mean urinary concentration of retino-binding protein in a group of exposed workers as compared to an unexposed group (Verplanke et al., 1999). No significant differences were seen in the concentration of albumin or the activities of alanine aminopeptidase, beta-galactosidase, or N-acetyl-glucosaminidase. Exposure was assessed by concentration of PCE in exhaled air and a work history that included hours spent in particular tasks. The exposed and control groups were similar in sex age, mass smoking, and alcohol intake. The geometric mean of daily exposure to PCE was estimated to be 1.2 ppm, with employment ranging from 0.1 to 32 years, lower exposure than that estimated in other studies of nephrotoxicity. This confirms that pre-clinical toxic effects on the kidney may occur at relatively low exposure to PCE.

Hepatic effects, as indicated by alterations in serum levels of liver enzymes, have been assessed in several studies of workers, with mostly negative results (ATSDR, 1997). One study used ultrasound analysis to assess morphological changes in liver of dry-cleaning workers (Brodkin et al., 1995). Significant differences in exposed and unexposed groups were found by ultrasound, yet no changes in blood clinical chemistry markers were noted. No new studies of chronic liver toxicity were located that have not been previously reviewed.

Carcinogenicity

There are a number of reviews of cancer epidemiology for humans exposed to PCE (ATSDR, 1997; IARC, 1995a,b; OEHHA, 1992; U.S. EPA, 1985). The epidemiological studies are primarily concerned with inhalation exposure in occupational settings, particularly in the dry-cleaning workers. A recent working group convened by the International Agency for Research on Cancer evaluated the carcinogenicity of PCE and of dry-cleaning occupations and concluded that the evidence for carcinogenicity is limited for both (IARC, 1995a, b). While the available studies of human carcinogenicity do not provide an adequate basis for quantitative analysis of PCE risk, the limited findings in humans lend support to the sufficient evidence of cancer in animals, resulting in an overall evaluation of PCE as a probable human carcinogen, group 2A (IARC, 1995b). Detailed reviews of available epidemiological studies can be found in the documents cited above; a brief summary of findings follows. References to work published since the IARC document was prepared are included in the findings below.

A number of occupational cohort studies have examined the potential relationship between inhalation exposure to PCE and various cancer types. The most significant tumor endpoints that have emerged from the epidemiological studies are esophageal, lymphohematopoietic, and female genital cancers. Cancer of the bladder, liver, kidney, lung, and breast have also been associated with PCE exposure in at least one study. The majority of occupational studies with relevance to PCE exposure concern the dry-cleaning industry in the U.S. (most recent updates in Blair et al., 1990 and Ruder et al., 1994) or laundry and dry-cleaning occupations in Nordic countries (Lynge and Thygesen, 1990). A cohort of chemical industry workers (Olsen et al., 1989), air force base employees (Spirtas et al., 1991) and a study of Finnish workers in a variety of occupations have also provided important information (Anttila et al., 1995). This report emphasizes the results of cohort studies, but results of descriptive mortality and case-control studies are included where pertinent. Interpretation of the majority of the epidemiology studies of PCE is hampered by concomitant exposure to other solvents in the studied industries, limited treatment of risks of smoking and alcohol intake, and/or poor definition of occupational history in death certificate-based studies. Other issues with these studies include low exposure levels (e.g. Anttila et al.,

1995) making detection of an effect unlikely, and numbers of exposed cases too low for quantitative assessment.

A statistically significant excess of mortality from cancer of the esophagus (SMR 2.1) was observed in two U.S. cohorts of dry-cleaning workers (Blair et al., 1990; Ruder et al., 1994). In the Blair study, significant excess was found only in black men. Ruder and colleagues defined a sub-cohort for whom PCE was the primary solvent exposure; in this group an SMR of 2.6 for esophageal cancer was observed, but the excess was not statistically significant. Three other cohort studies of PCE-exposed workers did not provide data on esophageal cancer. One population-based study of esophageal cancer cases did not identify any cases with history of employment in laundry or dry-cleaning; however, only 99 cases were examined (Siemiatycki, 1991, as cited by IARC, 1995). Another population-based case control study of esophageal cancer reported an elevated, non-significant odds ratio (3.6; 95 percent CI: 0.5-27.0) for ever having worked in dry cleaning (Vaughan et al., 1997). Excesses of esophageal cancer have been identified in two mortality studies that included dry cleaners (Walker et al., 1997; Office of Population Censuses and Surveys, 1986, as cited by IARC, 1995a). One of these, a recent mortality study based on the NIOSH national occupational mortality surveillance database, found a statistically-significant excess of esophageal cancer in black men aged 15-65 employed in laundry or dry-cleaning (Walker et al., 1997). Non-significant excesses were observed in other race/sex categories. The inclusion of laundry workers, presumably without PCE exposure, in the study would be expected to reduce the likelihood of observing an association. This study was not yet available when IARC reviewed the evidence for PCE carcinogenicity. While the association between occupational exposure to PCE and esophageal cancer has been perhaps the most consistent cancer finding, potential confounding by combined smoking and alcohol consumption, a risk factor for this cancer, has not been fully accounted for (Weiss, 1995). Further, there is not yet an explanation for the greater susceptibility in black men suggested in two studies.

No consistent pattern has been observed for the association of PCE with lymphohematopoietic cancers in humans, but several reports have noted increased risks. This class of cancers is of particular interest in light of the increased leukemia rate in rats exposed to PCE. Olsen and colleagues (1989) reported an elevated SMR for leukemia/aleukemia in a cohort of chemical workers; the SMR was statistically significant when local cancer rates were used for comparison. However, there were only 11 total cancer deaths in the cohort of 2610 subjects, and exposure was not limited to PCE. A drinking water study also reported increased relative risk of leukemia in the group with the highest exposure to PCE (90th percentile of exposure); however the result was based on only two cases (Aschengrau et al., 1993). Elevated risks of non-Hodgkin's lymphoma (NHL) have been reported in three cohort studies (Blair et al., 1990; Anttila et al., 1995; Spirtas et al., 1991). In contrast, a case control study and a recent mortality study did not find any association between PCE and NHL (Siemiantycki et al., 1991; Walker et al., 1997). A second case-control study of NHL found an increased OR of 2.0 for employment in laundry or dry cleaning, after adjustment for smoking, although the association was not statistically significant. The New Jersey Department of Health conducted a study of 18 organic chemicals in drinking water in 75 towns (Cohn et al., 1994). Incidence rates of high-grade lymphoma were elevated in females from the towns with the highest concentrations of PCE in drinking water. After stratifying towns into 0, 0.1-5.0 and >5.0 ppb exposure levels, the relative risk of high-grade NHL was significantly higher in the highest exposure stratum compared to controls. However, PCE levels in drinking water were also highly correlated with trichloroethylene levels, so whether the lymphoma risk was due to PCE is not clear.

Three cohort studies have indicated that occupational exposure to PCE may be associated with cervical or other female genital cancers (Blair et al., 1990; Anttila et al., 1995; Ruder et al., 1994). However, in an earlier study of mortality in female laundry and dry-cleaning workers (which

solvents were used was not known), the increased risk of cervical cancer observed in the first analysis was no longer apparent when workers in other low-wage jobs were used as a reference group (Katz and Jowett, 1981). This has raised the question of whether significant confounding by socio-economic status could have occurred in the analysis of the cohort studies.

Both of the major U.S. dry cleaner cohorts (Ruder et al, 1994; Blair et al., 1990) reported an increased risk of bladder cancer. One study defined a subcohort of workers whose exposures were primarily to PCE; the bladder cancer risk was not elevated in the subcohort. Two Nordic laundry/dry-cleaning cohorts did not experience excess bladder cancer (Lynge and Thygesen, 1990; Malker and Weiner, 1984). Two proportionate mortality studies of laundry and dry cleaning workers reported some increases in bladder cancer risk (Katz and Jowett, 1981; Office of Population Censuses and Surveys, 1986, as cited by IARC, 1995a), as did a study of a population in Massachusetts exposed via drinking water (Aschengrau et al., 1993). A significant increased risk of bladder cancer was reported in non-whites employed 6 months or longer in dry-cleaning (Silverman et al., 1989). A recent occupational surveillance study in Canada concluded that the elevated odds ratio for bladder cancer observed in laundry/dry-cleaning workers (after adjustment for smoking, coffee drinking, and other possible risk factors) merited further surveillance follow-up (Teschke et al., 1997). Whether smoking and/or exposure to other solvents could play a key role in bladder cancer observed in these studies is not clear (Weiss, 1995). IARC concluded that the risk for cancer of the bladder "may be increased by employment in dry-cleaning", but also that "little or no information was available" about the specific relation to PCE from the majority of bladder cancer studies (IARC, 1995b).

Liver and kidney have been identified as target tissues for tumor formation in studies of animals exposed to PCE. However, these sites have not been consistently reported in epidemiological studies. Among 8567 female laundry and dry-cleaning workers identified from an occupational cancer registry, liver cancer incidence was significantly higher than the comparison population (standardized incidence ratio 3.4, 95 percent CI 1.4-7.0) (Lynge and Thygesen, 1990). An update of the study, with ten additional cases, reported an SIR of 2.7 (95 percent CI 1.5-4.5) (Lynge, 1994). However, a nested case control study indicated that the excess risk occurred in laundry, not dry-cleaning workers (Lynge et al., 1995). Kidney cancer was elevated in some studies of dry-cleaning workers, but the findings were not consistent across studies. A recent review of the evidence for renal cell cancer, in which the results of at least 13 studies were considered, concluded that there is "little evidence of an increased risk of renal-cell cancer" with exposure to PCE (McLaughlin and Blot, 1997).

A recent article has found an association between PCE exposure and lung cancer among individuals residentially exposed to high cumulative levels of PCE-contaminated drinking water. Paulu et al. (1999) reported moderately elevated adjusted odds ratios (3.7, 3.3, 6.2, and 19.3) for lung cancer among those individuals whose exposure level was above the 90th percentile without a latent period or with 5, 7, and 9 years of latency, respectively. The controlled variables for this analysis included age of diagnosis or index year, vital status at interview, sex, occupational exposure to PCE, benzene, and other solvents, usual number of cigarettes smoked, history of cigar or pipe use, living with a smoker, and occupational history associated with lung cancer.

Most studies that assessed breast cancer reported no effects or reduced risks in women with occupational exposure to PCE. Aschengrau and colleagues recently reported preliminary results of a breast cancer case-control study in women exposed to PCE in drinking water (Aschengrau et al., 1998). While there was no overall association between PCE exposure and breast cancer, significantly elevated odds ratios were observed for the most highly exposed women when seven or nine years latency was assumed. A larger study is underway. A recent survey of mortality based on NIOSH records identified elevated risks of breast cancer in men formerly employed in laundry/dry-cleaning work (Walker et al., 1997). The risks were based on only two cases in black

men and four in white men, but these are very rare cancers and the proportional mortality ratios were statistically significant.

In conclusion, epidemiological studies provide evidence that PCE is possibly carcinogenic in humans. Especially important tumor sites are the esophagus and lymphatic system, but available data are quite limited in terms of the ability to quantify these cancer risks. This is particularly the case for risks associated with oral exposure to PCE, since the majority of the human data, and the most quantitatively informative studies, concern occupational exposure by the inhalation route. Most of the evidence for human carcinogenicity is based on solvent exposures in the dry-cleaning industry, of which PCE is a major but not exclusive component. Studies of solvent exposures in other industries also implicate PCE as a possible risk factor for human cancer, but typically involve exposure to multiple solvents and other potentially carcinogenic agents.

The epidemiological studies performed have relatively low power to detect an effect, and uncertain dose quantitation in most cases. Because of this, both the potential of PCE to induce human cancers, as well as quantitative estimates of the human risk associated with particular levels of exposure, must be inferred from animal data. In Appendix 1 of this report, the predictions made using the animal data are compared to the observations in the some of the more quantitatively reliable epidemiology studies. In general, the upper bound predictions of excess human cancers due to PCE exposure do not exceed the increased cancer incidences observed to be associated with PCE exposure in the studies. However, any such conclusions must be regarded as tentative in view of the difficulties in interpreting the human data.

DOSE-RESPONSE ASSESSMENT

Non-Cancer Effects

Studies of non-cancer toxicity in humans associated with chronic exposure to PCE derive primarily from the occupational health literature, and involve the inhalation exposure route. No studies addressing chronic effects of ingested PCE in humans were identified. Several studies in workers exposed by inhalation have identified neurotoxic effects associated with relatively low chronic exposures in humans (see Neurotoxicity section); these appear to be the most sensitive endpoints for chronic non-cancer toxicity in humans. One study of renal toxicity indicators found significant effects in a group of workers calculated exposure levels in the low ppm range (Verplanke et al., 1999). This study is more difficult to use for risk assessment, however, because the exposure estimates were calculated based on pre-shift exhaled air concentrations of PCE; no measurements of workplace air were available for validation of the computed exposure levels.

Three recent studies have reported statistically significant increased reaction times in neurobehavioral tests in people with PCE exposure compared to control subjects (Altmann et al., 1995; Ferroni et al., 1992; Spinatonda et al., 1997). None of these studies identify a no-observed-adverse-effect-level (NAOEL) for chronic neurotoxicity in humans.

An alternative endpoint for non-cancer risk assessment is reduction in color vision, which has been investigated in two groups of exposed workers, and followed up in one of these (see neurotoxicity section, above). An advantage of using the color vision data is that one study reported results for two subgroups with different exposure levels, thus providing some dose-response information (Cavalleri et al., 1994). A statistically significant effect was seen in the higher exposure group (mean 7.3 ppm, SD 8.2) and not in the 16 workers exposed to the lower concentration (mean 4.8 ppm, SD 3.5), thus suggesting a possible NOAEL. However, the number of subjects is too low to clearly define a NOAEL. Also, the exposure ranges of the group

with no effect and the group with a significant effect overlap, making the difference in results between the groups somewhat less convincing. In addition, the effect of PCE on color vision has not been confirmed in other studies.

Therefore, the selected endpoint of concern for determining a health protective drinking water level for non-cancer toxicity is delayed reaction time. The average daily dose associated with the exposure level and duration in each of the three studies cited above is estimated below. These doses provide estimates of the human LOAEL in each study. Since PCE itself is considered to be the relevant compound for neurotoxicity, metabolic models are not needed and the dose calculations are based on PCE concentrations. Exposures reported in ppm are converted to mg/m^3 (see Table 2 for conversion factor). Default breathing rates of 20 m^3/day and 10 m^3/workshift are assumed, and an inhalation absorption factor of 0.7 is used below to account for incomplete uptake of PCE by this route. Body weights are assumed to be 60 kg for females and 70 for males, and working time to be 5 days per week (non-working periods, e.g. vacations, are unspecified, but are assumed not to affect the critical dose rate for development of chronic toxicity).

Altmann et al. 1995:

Fourteen subjects with environmental exposure to PCE (median indoor air concentration 1.36 mg/m^3; average duration 10.6 yr.) had decreases in measures of simple reaction time, vigilance and visual memory for shapes compared to 23 age and gender matched controls.

$1.36\ mg/m^3 \times 20\ m^3/d \times 0.7 = 19\ mg/d$

Using 65 kg body weight for mixed gender study subjects = 0.29 mg/kg-d

Spinatonda et al, 1997:

Vocal reaction time (to visual stimuli) was increased in 35 workers with median exposure to 53.9 mg/m^3 PCE in comparison to 39 controls matched for age and education.

$53.9\ mg/m^3 \times 10\ m^3/d \times 5/7 \times 0.7 = 270\ mg/d$

Gender not specified, assume 65 kg average body weight = 4.15 mg/kg-d

Ferroni et al, 1992:

60 female dry-cleaning workers with median exposure to 15ppm PCE for an average duration of 10.1 years had significant differences in simple reaction time, vigilance, and finger tapping compared to 30 controls comparable in age, gender, and vocabulary test scores.

$15\ ppm \times 6.78\ mg/m^3 \times 10\ m^3/d \times 5/7 \times 0.7 = 509\ mg/d$

Using average body weight of 60 kg = 8.48 mg/kg-d

It appears that no single study is sufficiently reliable to be used as the primary basis for a health protective standard for chronic non-cancer effects. The Altmann et al. (1995) study was based on an environmentally rather than occupationally exposed population, and appears to define the lowest lowest-observed-adverse-effect-level (LOAEL). It might therefore be selected as the most health protective basis for risk assessment. However, the results are based on only 14 exposed subjects. The study by Spinatonda et al. (1997) is somewhat larger (35 subjects), although this is still quite small, and the population studied was occupationally exposed, which may be less

predictive of the general population due to the "healthy worker" effect. The sample size in the Ferroni et al. (1992) occupational study is larger, however the reporting of the study results is rather sparse on details. To account for the different strengths and weaknesses of the three studies, a geometric mean of the estimates of a human health protective concentration (after application of uncertainty factors appropriate to each study) will be used below in the risk assessment calculations.

Carcinogenic Effects

Selection of Data for Estimation of PCE Carcinogenic Potency

The bioassay used as the basis of an oral potency estimate for this report is the National Cancer Institute (NCI) study of mice exposed to PCE by gavage (NCI, 1977). The EPA used this gavage study as the basis of a carcinogenic potency assessment in 1985 (U.S. EPA, 1985), and used studies of mice and rats exposed to PCE by inhalation by National Toxicology Program (NTP, 1986) to update this assessment in 1986 (U.S. EPA , 1986). Both the NCI and NTP studies were used in the risk assessment prepared for the California Air Resources Board (OEHHA, 1992). In each of the reported bioassays PCE caused a significantly increased tumor incidence. Since human epidemiological data suitable for dose-response assessment are not available, these two bioassay data sets represent the only long-term animal exposure studies with well-defined, exposure-response data that indicate a positive carcinogenic response for PCE in animals. The current objective of calculating a public health goal for drinking water exposure to PCE is best addressed by using the gavage study. Since the human exposure is also by the oral route, the uncertainties of route-to route extrapolation are thus avoided. However, the findings of the more recent NTP inhalation study provide important supporting data. Because of this, the analysis of the inhalation study as performed by OEHHA (1992) is included for comparison.

In the mouse gavage study (NCI, 1977), incidence of hepatocellular carcinomas increased with dose for both males and females. Table 7 gives the tumor incidence data (see U.S. EPA, 1985a). In contrast to the overall tumor incidence data given earlier (in the description of the animal carcinogenicity studies), the incidence of tumors in Table 7 is reported as that in animals which survived at least until the appearance of the first carcinoma in each study. This appeared at week 24 for female mice and at week 41 for male mice in the NCI study. The mortality-adjusted incidence data are used here in preference to unadjusted incidence data to partially correct for the influence of competing mortality risks.

Also shown in Table 7are tumor incidence data from the inhalation studies (NTP, 1986) in the rat and mouse. Mononuclear-cell leukemia was the only tumor type observed to be significantly increased in rats at either the low or high dose levels. Again, in order to partially correct for competing mortality, the incidence values appearing in the table refer to animals surviving at least until the appearance of the first mononuclear-cell leukemia, at week 53 for males and at week 60 for females.

The tumor types observed to be significantly increased in the mice exposed by inhalation are hepatocellular carcinoma and hepatocellular adenoma. Since these two tumor types are of the same cellular origin, it is conventional to combine them for purposes of potency estimation. The incidence rates (carcinoma only, and adenoma or carcinoma) in the table are reported for animals surviving at least until the appearance of the first hepatocellular carcinoma, which was at week 60 for males and week 67 for females.

Estimation of Carcinogenic Potency

Dose Adjustments

Adjustments to the experimental exposures are required to calculate the lifetime daily exposure levels. Thus, for inhalation exposures, the reported dose must be multiplied by:

H/24: where H is the hours of exposure per day. This converts the exposure period to a time weighted average for 24 hours daily continuous exposure.

D/7: where D is the number of days exposed per week. This converts the dosing schedule to a time weighted average for a continuous (7 days/week) exposure.

L_e/L: where L_e is the length of the experimental exposure and L is the lifespan of the animal (the longer of L or 24 months). This converts the experimental protocol to a continuous lifetime exposure.

Respiration rate is observed to be proportional to body surface area (or, approximately, to body weight to the two-thirds power) (Guyton, 1947; Adolph, 1949; U.S. EPA, 1980; Anderson et al., 1983; Calabrese, 1983). Therefore, assuming 1 ppm PCE = 6.78 mg/m^3, and standard values (Anderson et al., 1983) for mouse and rat respired volume per day at the "average" body weights for the experimental animals (see Table 7) :

Total respired dose (averaged over time):

(for mice) = Applied dose x (6/24) x (5/7) x 0.0345 x (body weight of mouse/0.025)$^{2/3}$ x 6.78 / body weight

(for rats) = Applied dose x (6/24) x (5/7) x 0.105 x (body weight of rat/0.113)$^{2/3}$ x 6.78/body weight

In the NCI study, PCE in corn oil was administered by gastric intubation to male and female B6C3F$_1$ mice for 5 days/week for 78-weeks, beginning at about 5 weeks of age, followed by 12 additional weeks of pre-sacrifice observation (NCI, 1977). In the NTP inhalation rat study, groups of fifty 8- to 9-week-old male and female F344/N rats were exposed to PCE by inhalation at 0, 200, or 400 ppm for 6 hours/day, 5 days/week over 2 years (NTP, 1986). In the NTP inhalation mouse study, groups of fifty 8- to 9-week-old male and female B6C3F$_1$ mice were exposed to PCE by inhalation at 0, 100, or 200 ppm for 6 hours/day, 5 days/week over 2 years (NTP, 1986). Table 7 shows the average administered daily doses (in units of mg/kg), for the male and female vehicle control, low- and high-dose groups in the NCI oral study, and the exposure concentrations in the mouse and rat inhalation studies, alongside the tumor incidences data for each group. The dose corrections noted above were used to convert the experimental dose values to time-weighted average doses for the oral study, and total respired doses (averaged over time) for the inhalation studies.

Table 7: Dose-Response Data For Selected Cancer Bioassays of PCE

Study, species (strain)	Sex and weight	Administered dose or conc.	TWA Applied dose (mg/kg-d)	Tumor Type	Tumor Incidence[a]
NCI, 1977	Male	0 (mg/kg-d)	0	hepatocellular	2/20

Mice	0.030	536[b]	215.0[d]	carcinoma	32/48
($B6C3F_1$)		1072[b]	430.1[d]		27/45
	Female	0	0	hepatocellular	0/20
	0.025	386[b]	154.9[d]	carcinoma	19/48
		772[b]	309.7[d]		19/45
NTP, 1986	Male	0 (ppm)	0	hepatocellular	7/49
Mice	0.037	100[c]	146.6[e]	carcinoma	25/47
($B6C3F_1$)		200[c]	293.2[e]		26/50
	Female	0	0	hepatocellular	1/44
	0.032	100[c]	153.9[e]	carcinoma	13/42
		200[c]	307.8[e]		36/47
	Male	0	0	hepatocellular	16/49
	0.037	100[c]	146.6[e]	adenoma or	31/47
		200[c]	293.2[e]	carcinoma	40/50
		0	0	hepatocellular	4/44
	Female	100[c]	153.9[e]	adenoma or	17/42
	0.032	200[c]	307.8[e]	carcinoma	38/47
NTP, 1986	Male	0	0	mononuclear-	28/50
Rats	0.44	200[c]	143.0[e]	cell leukemia	37/48
(F344/N)		400[c]	286.0[e]		37/50
	Female	0	0	mononuclear-	18/49
		200[c]	159.0[e]	cell leukemia	30/50
	0.32	400[c]	318.1[e]		29/50

[a]Tumor-incidence denominator excludes animals dying before the first corresponding tumor type observed in each study.
[b]Average daily gavage dose in mg/kg-d, for a 5 d/wk exposure over 78 wk of a 90-wk bioassay.
[c]Average administered inhalation exposure in ppm 6 h/d, 5 d/wk over 2-years.
[d]Time-weighted average (TWA) dose (following Anderson et al., 1983).
[e]Total respired dose averaged over time (following Anderson et al., 1983).

Selection of pharmacokinetic models

Pharmacokinetic analyses have been used to estimate the "effective" dose for use in risk assessment calculations. In the case of PCE, the relevant quantity is considered to be the amount of PCE metabolized. Ideally, a pharmacokinetic model could be used to extrapolate dose measures to low-level exposures of humans. Although some more recent publications have expressed disagreements or uncertainties over the pharmacokinetic modeling of human exposures, the calculation of effective dose in the animal studies has not been seriously challenged. In the earlier section on metabolism and pharmacokinetics, available models for use in determining effective dose in the two bioassay studies were discussed. Dose estimates resulting from three different approaches (Bogen et al., 1987; Bois et al., 1990; Chen and Blancato, 1987) were compared; all three are in reasonably good agreement. The methodology of Bogen et al. (1987) was retained, since this is the only model that provided dose estimates for mice and rats exposed by both inhalation and orally. The calculations were repeated to separate out the adjustments for dose frequency, dose duration and study duration, which are dealt with separately by the program used for potency calculations.

Selection of extrapolation model

According to the proposed guidelines for carcinogen risk assessment (U.S. EPA, 1996), the type of extrapolation employed for a given chemical depends on the existence of data supporting linearity or non-linearity, or a biologically-based or case-specific model. When data are not sufficient to construct a case-specific model, and are not inconsistent with linearity, the default is to use a linear extrapolation. In the case of PCE, although there are significant uncertainties as to the actual mode of action, several lines of evidence suggest that a linear low-dose extrapolation assumption is reasonable. Although only limited genotoxicity has been reported for pure PCE, this may be because of the well-known difficulties in testing highly volatile materials, and the failure to include appropriate metabolic systems in many experiments. At least two classes of identified metabolites (tetrachloroethylene epoxide, and the halothioketenes produced by β-lyase action on the mercapturic acids) are considered to have mutagenic activity. On the other hand, metabolites such as trichloroacetic acid are known rodent carcinogens, which are often argued to operate by a mechanism other than direct genotoxicity via covalent modification of DNA. Various indirect mechanisms involving cytotoxicity or modulation of genetic control systems are also argued as important in carcinogenesis by halocarbons, especially when the target tissue is mouse liver. However, these counter-proposals are hypothetical, and attempts to identify positively what an alternative mechanism might be have been unsuccessful. On balance therefore, it is assumed for the purposes of risk assessment that PCE is acting as a directly genotoxic carcinogen. In this case a linear low-dose extrapolation model is appropriate, and a constrained polynomial ("multistage") model is suitable when fitting tumor incidence data in the observed range.

Earlier guidelines for cancer risk assessment, including those formerly used by OEHHA (DHS, 1985) have required the use of the linearized multistage (LMS) model to estimate an upper bound on the low-dose potency (q_1^*). This was used, for example, in the previous PCE risk assessment prepared by California (OEHHA, 1992). However, more recent OEHHA methodologies, and the proposed U.S. EPA (1996) guidelines for carcinogen risk assessment, recommend a linear extrapolation approach based on the 95 percent lower bound on the dose which produces a 10 percent tumor incidence (LED_{10}). A multistage polynomial is used to fit data in the observable range, unless some other dose-response curve is specifically indicated by available data. As noted above, for PCE this curve-fitting approach is appropriate.

Interspecies extrapolation

A numerical adjustment must be made to convert the q_1* calculated from the animal data to a q_1* relevant to humans. Cross-species scaling of carcinogen doses by the 3/4 power of body weight is adopted as proposed by the U.S. EPA (1996), instead of the previous use of the 2/3 power (OEHHA, 1994). Therefore, to convert the animal potency value to an estimated human potency, a scaling factor equal to the ratio of human to animal body weight is raised to the ¼ power is applied:

$$q_1^* \text{ (human)} = q_1^* \text{ (animal)} \times (\text{human body weight/animal body weight})^{1/4}$$

That is, for example, $(70 \text{ kg}/0.034 \text{ kg})^{1/4}$, or 6.74 for mice. The scaling factor is used to account for interspecies differences in dose rate and response rate. Thus, even if a pharmacokinetic model is used to account for dose differences between species, scaling may still be necessary to account for potential interspecies differences in sensitivity to the resulting internal doses.

For inhalation exposures, U.S. EPA has at times in the past assumed equivalence between different species for exposures to a given atmospheric concentration. This provides roughly

similar dose scaling in effect, due to the way that breathing rate and related parameters affecting uptake scale with body weight. More recently, physiologically based pharmacokinetic modeling has been seen as a preferable approach to both dose estimation and inter-species scaling of inhalation exposures, where data are available to support this.

The potency calculations selected for use in setting a Public Health Goal, below, are based on a dose metric defined as the fraction of PCE metabolized in the bioassay animals. Scaling by the 3/4 power of body weight method discussed above is used to convert the risk estimates from animal to human. The resulting oral and inhalation potencies in Tables 10-12 are expressed in units of (mg PCE metabolized/kg-d)$^{-1}$. For those potencies, application to human risk estimation requires conversion of the fraction metabolized back to external exposure units for humans. This latter computation is subsumed in the computations of exposure factors, as explained below, but has the same effect on the PHG as correcting the q_1* values.

Potency estimates

In all cases the Tox_Risk (v. 3.1, KS Crump Division, Clement International Corp., Ruston, LA) program was used to fit the multistage model to the quantal data sets. The q_1* cancer potencies or the 95 percent upper bound on the linear slope at low dose (LMS) were calculated directly by the program. Carcinogen slope factors (CSFs) are based on the LED_{10} (the 95 percent lower bound on the dose that is predicted to give a 10 percent tumor incidence). The carcinogen slope factor (CSF) is $0.1/LED_{10,}$ in units of (mg/kg/day)$^{-1}$. For the curve fitting to estimate the LED_{10}, we have employed a $p \geq 0.05$ criterion for the χ^2 goodness of fit statistic of the optimized polynomial.

Quantal analysis with applied dose metric

In Table 8 are summarized the cancer potency values derived by both the LED_{10} method (and the LMS model, for comparison with earlier results) from the data sets in the NCI (1977) oral study, using the time-weighted average applied dose as the dose metric. The potency estimates for hepatocellular carcinoma in male and female mice are quite similar whether based on the q_1* or the CSF. However, the fit of the time-independent version of the polynomial model to the data is not good in either case. The χ^2 goodness of fit criterion is barely met in the case of the female mouse data, and failed for the male mouse data.

Table 8: PCE Mouse Oral Study - Administered Dose as Dose Metric

Study	Sex	Tumor site and type	q_1* (mg/kg-d)$^{-1}$	LED_{10} mg/kg-d	CSF (mg/kg-d)$^{-1}$
NCI, 1977	male	Hepatocellular Carcinoma	0.024	4.38	0.023
	female	Hepatocellular Carcinoma	0.022	4.86	0.021

The q_1* values quoted here differ significantly from earlier estimates (e.g. by OEHHA, 1992) due to different methodology, in particular the use of the ¾ rather than $^2/_3$ power of body weight for interspecies scaling. When this difference is allowed for, the present calculations agree with the

values reported (as q_{mouse} values) by Bogen et al. (1987) and (OEHHA, 1992) for the female mice Table 9. For the male mice OEHHA (1992) followed Bogen et al. (1987) in ignoring the high dose group data in their calculation because of the poor fit.

Table 9: q_1* Values in the NCI Oral Mouse Study of PCE

Methodology	q_1*_{mouse} (mg/kg-d)$^{-1}$ Males	Females	Scaling index	q_1*_{human} (mg/kg-d)$^{-1}$ Males	Females
This report	0.0035	0.0030	2/3	0.046	0.042
			3/4	0.024	0.022
Bogen et al. (1987)	0.0064	0.0030	2/3	0.085	0.042

Quantal analysis with metabolized dose metric

Use of the metabolized dose (determined as described in the section on pharmacokinetic models, and shown in Table 6) as the dose metric allows comparison of potencies derived from oral and inhalation studies. Accordingly, in Table 10 the cancer potency values derived by the LED_{10} method and the LMS model using metabolized dose are shown for both the NCI (1977) oral study in mice, and the NTP (1986) inhalation study in mice and rats. As in the case of the applied dose analysis, the figures for q_1* are in agreement with those reported by OEHHA (1992) after allowing for the use of a different interspecies scaling index, apart from the male mice in the NCI (1997) gavage experiment. In addition, the differences between the LMS model results and the CSFs derived from the LED_{10} approach are not large. Note that the units are in mg-metabolized, and so they are not directly comparable to the potencies derived from the applied dose metric, above.

Table 10: Rat and Mouse Studies of PCE - Metabolized Dose as Dose Metric

Study	Species, Sex	Tumor site and type	q_1* (mg-metab/ kg-d)$^{-1}$	LED_{10} mg-metab/ kg-d	CSF (mg-metab/ kg-d)$^{-1}$
NCI, 1977 (oral)	Mouse, male	Hepatocellular Carcinoma	0.22	0.48	0.21
	Mouse, female	Hepatocellular Carcinoma	0.16	0.65	0.15
NTP, 1986 (inhalation)	Mouse, male	Hepatocellular Adenoma or Carcinoma	0.16	0.66	0.15
	Mouse, female	Hepatocellular Adenoma or Carcinoma	0.071	1.32	0.076
NTP, 1986 (inhalation)	Rat, male	Mononuclear Cell Leukemia	0.23	0.46	0.22
	Rat, female	Mononuclear Cell Leukemia	0.15	0.69	0.15

Time-to Tumor analysis

The impact of mortality in the bioassays is significant, especially in the NCI (1977) oral mouse study. The use of the pharmacokinetic correction in the quantal analysis reported above results in an improved fit of the model to the data points, within acceptable limits for both sexes. However, the fit is still not good. Evidently, the approximate correction for mortality based on time to first appearance of the tumor is insufficiently accurate in this case. Interpretation of this study is further complicated by the fact that the dose given to both high and low dose groups was increased after 11 weeks. Both the mortality and the variation in dose rate are accommodated by a time-to-tumor model, so this analysis was used for all data sets. The time-to-tumor analysis had been previously used, and preferred, by OEHHA (1992) for the NTP (1986) study. However the individual animal data are not provided in the publicly available technical report of the oral study (NCI, 1977). These latter data were obtained specially for this analysis (Dr. L. Gold, personal communication to Dr. A.G. Salmon, 1998).

In order to determine the metabolized dose at different stages of the experiment, the pharmacokinetic model developed by Bogen et al. (1987) was applied to the dose rates for the individual experiment segments as shown in Table 11.

Table 11: Metabolized Dose in Individual Segments of the NCI (1977) PCE Bioassay

	Applied dose (mg/kg-d)	Metabolized Dose (mg-metab/kg-d)	Corrected for 5 d/week
Males: Low dose			
Weeks 1-11 (11 weeks)	450	68.92	49.23
Weeks 12-78 (67 weeks)	550	77.27	55.19
Males: High dose			
Weeks 1-11 (11 weeks)	900	98.08	70.05
Weeks 12-78 (67 weeks)	1100	106.25	75.89
Females: Low dose			
Weeks 1-11 (11 weeks)	300	53.13	37.95
Weeks 12-78 (67 weeks)	400	64.15	45.82
Females: High dose			
Weeks 1-11 (11 weeks)	600	80.95	57.82
Weeks 12-78 (67 weeks)	800	93.15	66.54

The calculations were made using the model and source data described earlier. These dose rates and the individual tumor and mortality data were fit to the multistage in dose, Weibull in time model provided by Tox_Risk v. 3.5. This program is designed to provide an estimate of q_1* according to the previously standard linearized multistage dose extrapolation model. It was also used to provide an estimate of the end-of-life LED_{10} at 104 weeks, a value comparable to the LED_{10} estimate obtained with the quantal polynomial model after adjustment for non-standard experimental durations. Results of these calculations are shown in Table 12. Again, the resulting dose and risk figures are based on mg-metabolized.

Tox_Risk does not report quality of fit measures for this model comparable to the χ^2 goodness of fit criterion reported for the quantal models. However, the fit of the time dependent models to the data appears to be adequate by inspection of the graphical output and other informal criteria. Potency values reported here for the NTP inhalation studies in rats and mice are similar to those reported by OEHHA (1992), allowing for the incorporation of the ¾ power of bodyweight factor for interspecies scaling[1]. Use of the time-to-tumor model, the explicit inclusion of the varied dose rates, and the pharmacokinetic model result in potency estimates between two and five times higher than the quantal analyses of the same data sets. This reflects the better allowance for the substantial intercurrent mortality in this study when using the time-dependent model. The fits to

[1] However, it should be noted that Bogen et al. (1987) used the Weibull 82 program for their time-dependent calculations [which were the source of the numbers reported by OEHHA (1992)]. There are differences in methodology between this earlier program and Tox_Risk v 3.5. The earlier authors do not report time-dependent calculations for the NCI oral study. The program used by Bogen et al. does not allow for the simultaneous application of a multistage time-to tumor model and allowance for varying dose rates during the study. However this option is available with the more recent software (and the more powerful hardware now used to run it).

the data also appear to be more satisfactory. Because of these considerations, the full time-dependent analysis used here is the preferred approach for obtaining a potency estimate from the NCI (1977) oral data sets.

Table 12: Rat and Mouse Studies - Metabolized Dose of PCE, Time Dependent Model

Study	Species, Sex	Tumor site and type	q_1* (mg-metab/ kg-d)$^{-1}$	LED_{10} (mg-metab /kg-d)	CSF (mg-metab/ kg-d)$^{-1}$
NCI, 1977 (oral)	Mouse, male	Hepatocellular Carcinoma	0.42	0.25	0.40
	Mouse, female	Hepatocellular Carcinoma	0.78	0.13	0.74
NTP, 1986 (inhalation)	Mouse, male	Hepatocellular Adenoma or Carcinoma	0.20	0.53	0.19
	Mouse, female	Hepatocellular Adenoma or Carcinoma	0.062	1.4	0.071
NTP, 1986 (inhalation)	Rat, male	Mononuclear Cell Leukemia	0.26	0.40	0.25
	Rat, female	Mononuclear Cell Leukemia	0.18	0.58	0.17

The estimates obtained using the time-dependent methodology and the metabolized dose metric are consistent within one order of magnitude, regardless of route or species. Apart from the low estimate obtained in the female mice in the NTP (1986) inhalation study, they are all consistent within a factor of five. In view of the overall uncertainties involved in the estimation methodology, it is reasonable to regard all these estimates as consistent with one another.

Carcinogen risk assessment guidelines used by OEHHA normally recommend selection of human cancer potency estimates based on the most sensitive study, site, and species. This applies unless there is evidence to indicate that the most sensitive site(s) are not relevant to human cancer induction, or represent data sets with unusually wide error bounds. However, the selection of a potency value may take into account the appropriateness of the route of exposure in the various studies, and a geometric mean of several estimates may be chosen where several similar values are available. In this case, the values from the NCI oral (gavage) study are preferred both because of appropriateness of the route to a public health goal for drinking water, and by the most sensitive study/site/species criterion. The values for male and female mice are not regarded as significantly different, so the geometric mean of the two values may be chosen. The preferred value for the oral cancer potency is therefore a CSF of 0.54 (mg-metabolized/kg-d)$^{-1}$ for liver carcinomas in male and female mice in the gavage studies by NCI (1977).

The oral cancer potency is the result of primary interest from the standpoint of drinking water consumption. However, as described below, some inhalation and dermal exposure is expected to result from contamination of a public water supply with PCE. In addition to the estimate of oral carcinogenic potency, estimates based on inhalation studies were produced. Although these

estimates are not so different as to be clearly inconsistent with the oral estimate, it is appropriate to make use of this route-specific information, since it appears to be of at least as high quality as the estimate for the oral route. As in the oral case, the estimates calculated using the metabolized dose basis do not differ greatly (by a factor of 3.5 between the highest and lowest values). The geometric mean of these four values was chosen, giving a preferred value for the inhalation CSF of 0.15 (mg-metabolized/kg-d)$^{-1}$. This is based on liver carcinomas or adenomas in male and female mice, and mononuclear cell leukemia male and female rats, in the NTP inhalation studies.

There is no direct evidence as to the appropriate potency estimate to be used for dermal exposures. This route has been considered analogous to inhalation exposure in that the toxicant enters the general circulation directly, rather than via the portal circulation and the liver as for oral exposures. So by default the inhalation value will be used for this route also.

The oral and inhalation potency estimates are expressed as risk per mg of PCE metabolized/kg-d. To apply these potencies to human exposures, a conversion from metabolized dose back to external exposure is needed. As noted earlier in the section on Pharmacokinetics, the most recent and reliable estimates of the percentage of ingested and inhaled doses (and the associated inter-individual variability) that are metabolized by humans in low exposure conditions were derived by Bois et al. (1996, and in a personal communication from Dr. Bois). Based on that model, at low exposure levels such as could occur in ambient media (1 ppb in air or water), the predicted mean proportion of PCE metabolized was 54 percent (95 percent confidence limits 28 – 79 percent) for the oral route, and 36 percent (95 percent confidence limits 15–58 percent) for inhaled PCE. In order to protect sensitive sub-populations and individuals adequately, it is appropriate to use the 95 percent upper confidence limit of these values in determining the public health goal for PCE.

Conversion between metabolized dose and external doses is carried out by correcting the exposure factors, below, rather than correcting the CSF values. Both approaches result in the same adjustment of the PHG estimates.

CALCULATION OF PHG

Calculations of concentrations of chemical contaminants in drinking water associated with negligible risks for carcinogens or non-carcinogens must take into account the toxicity of the chemical itself, as well as the potential exposure of individuals using the water. Tap water is used directly as drinking water and for preparing foods and beverages. It is also used for bathing or showering, and in washing, flushing toilets, and other household uses that may result in dermal and inhalation exposures.

Exposure Factors

Bogen et al. (1987) showed that significant inhalation and dermal absorption of PCE from tap water occurs during bathing and other periods of high domestic water use. The amount of PCE absorbed from water in this way is uncertain due to the variability of use patterns, and uncertainties in physical and biological models. The ratio of the amount inhaled to the amount ingested was estimated to range from one to six (Cothern et al., 1984; Andelman, 1985; Foster and Chrostowski, 1986; McKone, 1987; Bogen et al., 1987). Data on dermal absorption are more limited, although it has been suggested that dermal exposure may contribute a dose up to that equivalent to drinking two liters per day of water or more (Brown et al., 1984). Various other sources, not related to the drinking water supply, may also contribute substantially to human environmental exposure to PCE, as noted in the earlier section on exposure.

The CalTOX exposure model[2] was used to examine the likely exposures to PCE from drinking water contamination by all routes, including inhalation and dermal absorption as well as ingestion. This uses a range of calculated, measured or assumed parameters to describe the movement of a contaminant between different environmental media, and a standardized series of exposure assumptions to estimate the exposure impact from these media. Data specific to PCE are included with the program in the "Datacal" module. For particular individuals, the physiological parameters such as body size and area, volume of water consumed as tap water, or other fluids, and volume of air inhaled per day are obviously highly variable depending on climate and level of exercise. Exposures as a result of bathing, showering and other indirect routes related to water contamination also may vary considerably, depending on building type, occupancy periods and behavior. CalTOX uses assumed values and distributions for these inputs, which are considered representative of average California residents, and provides uncertainty estimates on the results. In order to estimate relative route and media contributions for PCE the model was specified for "Average Residential Exposure in California." Relative contributions from inhalation, dermal absorption and ingestion were unchanged for initial applied concentrations (to root and vadose zones) of 1 – 100 ppm PCE. These model inputs resulted in contamination levels of water and other media covering ranges considered typical of low-level environmental contamination. Volume of drinking water consumed was referred to the "standard" value of 2.0 L/day used in other PHG calculations, rather than to a model-specific consumption rate estimate. The resulting predictions of route-specific exposure, expressed in terms of liter equivalents per day, are shown in the second column of Table 13. Alternative physiological or behavioral parameters input to the model resulted in some variation in the result, but this remained broadly similar for most plausible inputs. Inputs of the body size, consumption and breathing parameters for a 70 kg male used by Bogen et al. (1987) resulted in a 6 percent lower contribution from inhalation, due mainly to the assumption of a lower breathing rate during exercise. However, this difference was not considered significant in view of the overall uncertainties in the result.

Table 13 also converts the exposure volumes to human dose of metabolites, using the fraction of PCE metabolized predicted by the pharmacokinetic model of Bois et al. (1996). A discussion of the pharmacokinetic model, the findings, and rationale for its use was presented in the earlier section of this document on pharmacokinetics. The fraction metabolized for PCE taken up by the dermal route was considered to follow the same kinetics as respired doses, since both are delivered to the systemic circulation and are not subject to first-pass effects in the liver. It is important to note that the metabolism of PCE in humans is not predicted to be linear with exposure. The conversion between exposure and metabolized dose used in Table 13 was developed for continuous exposure to 1 ppb by inhalation and intake of drinking water containing 1 μg/L. It should be noted that, because of the non-linear relationship between exposure and metabolism in humans (Bois et al, 1996), use of the same adjustment factors for high exposure scenarios would not be appropriate.

Table 13: Exposure to PCE in Water for an Average California Resident

Route of PCE Exposure from	Avg. Dose (mg/kg-d)	Exposure: Vol. Equiv.	Fraction Metabolized	Metabolized: Vol. equiv.	% of Total

[2] CalTOX™ 2.3 (beta): Eight-Compartment Multimedia Exposure Model. Copyright (c) 1996 Regents of the University of California and California Department of Toxic Substances Control.

Water		(L/day)	(UCL)	(L/day)	Dose
INHALATION	1.27×10^{-6}	3.54	0.58	2.05	*50.0%*
INGESTION:					
Water	7.16×10^{-7}	2.00	0.79	1.58	38.5%
Produce, meat, etc.	2.48×10^{-8}	0.07			
Total ingestion	7.41×10^{-7}	2.07	0.79	1.64	*40.0%*
DERMAL UPTAKE	2.51×10^{-7}	0.70	0.58	0.41	*10.0%*
DOSE SUM	2.26×10^{-6}	6.31		4.1	100.0%

Bogen and colleagues (1987) calculated "best estimates" of 2.2, 2.8 and 2.2 liter equivalents (for the 70 kg 'reference' adult) associated with exposures by the oral, inhalation and dermal routes, respectively (before adjustment for percentages metabolized). Their estimate of the relative input by inhalation is similar to that generated by the CalTOX model, and the estimate of dermal uptake is higher by a factor of 3. This latter difference clearly represents a difference in assumptions either about the likely exposure scenario, or about the extent of dermal uptake of PCE. CalTOX includes a more extensive range of information about exposures to California residents than was available to Bogen et al. at the time of their assessment. It also may benefit from physical parameters and process models of dermal uptake which have been validated for a number of different organic solvents, particularly since (as noted previously) the data on dermal uptake of PCE are relatively sparse. The CalTOX results will therefore be used, yielding an equivalent uptake of 2.07 L/day orally and 4.24 L/day by dermal or inhalation routes. It should however be noted that there is a substantial range of both variability and uncertainty for these values, which should be taken into account in evaluating specific exposure situations. Where more specific, measured data are available it would be preferable to use them.

Noncarcinogenic Effects

The estimated daily doses at which three different studies identified delayed response time in neurobehavioral tests will form the basis of the calculation below. Because a NOAEL was not defined (only one exposure group was reported in these studies) an uncertainty factor is required in each case; a factor of 10 is appropriate for extrapolation to a presumed no-effect level for the cognitive and visual effects noted in study subjects. Exposure durations in all studies averaged eight years or greater, and therefore no uncertainty factor is applied below to correct for less than lifetime exposure. It should be noted that there may have been subjects with considerably less than the average exposure time, however. An additional uncertainty factor of ten is needed to account for the interindividual variability in the diverse California population, compared to the adult and relatively healthy workers in the occupational study cohorts. In the Altmann et al. (1995) study the exposed group was environmentally rather than occupationally exposed. Although this group may therefore be less selected than an occupational cohort, it is likely that due to its small size, and other selective influences in the study design, it still does not reflect the true diversity of the total California population. An uncertainty factor of 3 is therefore included in this case.

Calculation of a public health-protective concentration (C, in mg/L) for PCE in drinking water for non-carcinogenic endpoints follows the equation:

$$C = \frac{\text{LOAEL (mg/kg-d)} \times 70\text{ kg} \times \text{RSC}}{\text{UF} \times \text{L/day}} = \text{mg/L}$$

where,

LOAEL	=	lowest-observed-adverse-effect-level,
RSC	=	relative source contribution (3 percent, based on data cited in Table 3 for urban areas with 0.5 µg/L PCE in drinking water),
UF	=	uncertainty factors (10 to account for use of a LOAEL rather than NOAEL, and 10 or 3 for potentially sensitive human subpopulations, as described above),
L/day	=	adult daily water consumption rate. (As described in the section on exposure factors, 6.31 L/d is the estimated intake by all routes of PCE present in tap water).

Using the LOAEL for each study, and substituting the values for the RSC, UF, and intake rate into the above equation, estimates of the concentration of PCE in drinking water protective against the chronic neurotoxic endpoints observed in the cited studies are derived:

Altmann et al. (1995):

$$C = \frac{0.29\text{ mg/kg-d} \times 70\text{ kg} \times 0.03}{30 \times 6.31\text{ L/day}} = 3.22 \times 10^{-3}\text{ mg/L}$$

Spinatonda et al. (1997)

$$C = \frac{4.15\text{ mg/kg-d} \times 70\text{ kg} \times 0.03}{100 \times 6.31\text{ L/day}} = 1.38 \times 10^{-2}\text{ mg/L}$$

Ferroni et al. (1992)

$$C = \frac{8.48\text{ mg/kg-d} \times 70\text{ kg} \times 0.03}{100 \times 6.31\text{ L/day}} = 2.82 \times 10^{-2}\text{ mg/L}$$

The geometric mean of these estimates is 1.1×10^{-2} mg/L, or 11 ppb. The protective concentration derived below for cancer is significantly lower, and therefore the drinking water concentration proposed below to protect against carcinogenic effects is also protective against non-cancer chronic toxicity. A reference dose (RfD) of 0.01 mg/kg-d was previously established by the U.S. EPA (IRIS, 1988). The LOAELs reported here yield an estimated safe dose level, as the geometric mean of the three results, of 0.032 mg/kg-d, after application of the previously chosen uncertainty factors. This is higher than the U.S. EPA RfD, but OEHHA considers the human data to be preferable for risk assessment purposes. The RfD was based on a NOAEL for hepatotoxicity in mice exposed to PCE by oral gavage for only 6 weeks. While there is significant uncertainty concerning the actual exposure levels in the epidemiological studies, a NOAEL is not available, and inter-route extrapolation is required to use the human data, it is important to eliminate the additional uncertainty that comes from interspecies extrapolation.

Carcinogenic Effects

For carcinogens, the following general equation can be used to calculate the public health-protective concentration (C) for PCE in drinking water (in mg/L):

$$C = \frac{BW \times R}{\sum_{(\text{all routes})} (q_1^* \text{ or CSF} \times \text{L/day})} = \text{mg/L}$$

where,

BW	=	adult body weight (a default of 70 kg),
R	=	*de minimis* level for lifetime excess individual cancer risk (a default of 10^{-6}),
q_1* or CSF	=	cancer slope factor, q_1* is the upper 95 percent confidence limit on the cancer potency slope calculated by the LMS model, and CSF is a potency derived from the lower 95 percent confidence limit on the 10 percent tumor dose (LED_{10}). CSF = 10 percent/ LED_{10}. Both potency estimates are converted to human equivalent [in $(\text{mg-metab/kg-d})^{-1}$] using $BW^{3/4}$ scaling,
L/day	=	daily volume of water consumed by an adult. A default of 2 L/day is used for direct oral consumption, but this may be modified to allow for indirect oral exposures from drinking water and percentage metabolized. Other routes are represented by equivalent daily water consumption volumes, again corrected for the metabolized fraction, and appropriate CSF or q_1* values.

The potency estimates for carcinogens are calculated by both methods because a substantial part of our current experience-base is with the LMS model. The new methodology, which is based on the LED_{10} and is similar to that proposed by U.S. EPA (1996) in its proposed guidelines for carcinogen risk assessment, has only been in regular use for the past three years. It may therefore present problems of interpretation, particularly when comparisons with earlier risk estimates are necessary. The LMS model focuses on the linear low dose extrapolation, and analysts (e.g., U.S. EPA) have often accepted relatively poor fits to the observed tumor incidence data. The new 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, 1996). In the specific case of PCE considered here the two methods show no major divergences in results, reinforcing the confidence in use of the LED_{10} methodology, which is considered generally preferable from a theoretical viewpoint.

For the carcinogenic endpoint and the linear approach based on the oral and inhalation CSFs of 0.54 and 0.15 $(\text{mg/kg-d})^{-1}$ the water concentration equivalent to a negligible lifetime theoretical cancer risk of 10^{-6} can be calculated as follows:

$$C = \frac{1 \times 10^{-6} \times 70 \text{ kg}}{(0.54 \times 1.64 \text{ L/d}) + (0.15 \times 2.46 \text{ Leq/d})} = 5.6 \times 10^{-5} \text{ mg/L} = 0.06 \text{ ppb (rounded)}$$

where 1.64 L/d is the estimate of water and other oral ingestion (corrected for percentage metabolized), and 2.46 Leq/d is the estimate of inhalation and dermal exposure equivalents from showering, bathing, flushing toilets and other household activities using PCE contaminated water derived above, using CalTOX, and corrected for percent metabolized. The PHG is therefore 0.06 ppb), based on the induction of hepatocellular carcinoma in male and female mice exposed orally to PCE (and, for water-derived inhalation exposures, on the induction of hepatocellular adenoma or carcinoma in mice, and mononuclear cell leukemia in rats, exposed by inhalation to PCE).

RISK CHARACTERIZATION

PCE is used as a chemical intermediate, and as a solvent, primarily for cleaning operations (metal cleaning and vapor degreasing, dry-cleaning). Many household products contain some PCE. This results in opportunities for airborne exposures, particularly near the site of PCE-using industrial or dry-cleaning activities, and when handling recently-dry-cleaned clothing. Drinking water exposures may occur as a result of environmental releases of PCE from leaking storage tanks, from industrial wastes, and through spills on site or during transportation. The public health risks of exposure to PCE can be characterized as follows:

Acute and Chronic Health Effects

Typical exposures to PCE in drinking water are not expected to result in any acute health effects, due to the low levels involved. This includes household airborne exposures from showering, flushing toilets, etc. Various health complaints, including neurological changes, have been reported as a result of exposures to inhaled PCE in a domestic context. However, these involved exposures to higher levels than expected in a typical household situation. These levels resulted from, for example, proximity to commercial dry-cleaning operations, or improper handling and storage of recently dry-cleaned clothing or furnishings. Acute and chronic neurological changes, and liver and kidney toxicity, have also been reported in humans and animals exposed to PCE. However, the effective levels for these changes do not indicate that humans exposed to typical background levels in the general indoor or outdoor environment are at significant risk of experiencing such effects.

Carcinogenic Effects

Some studies have suggested a positive relationship between working in the dry-cleaning industry and cancer. Although interpretation of these results is complicated, due (among other issues) to concurrent exposures to other potentially carcinogenic agents, they are considered to provide limited evidence of a carcinogenic effect of PCE in humans. In animal studies, inhalation exposure to PCE produced increased incidences of mononuclear cell leukemia in male and female rats, kidney tumors in male rats, and liver tumors in mice. Oral administration of PCE produced liver tumors in mice. A summary of our evaluation is given below.

- OEHHA considers PCE to be an animal carcinogen and a possible human carcinogen.
- Three separate cancer bioassays (two in mice, one in rats) have shown PCE induced tumors at several sites, in two species, in both sexes, by oral and inhalation routes of exposure.
- A fourth bioassay (in rats, with oral exposure) was non-positive as a result of severe non-neoplastic toxic effects, and therefore neither confirms nor conflicts with the other data.
- Another small inhalation experiment in male rats was also non-positive, but due to the study design appears to have had limited power to detect any carcinogenic effect. Two other studies (one using intraperitoneal injection, and the other skin exposure) also found no dose-related tumors. However, due to the route differences and restriction of the sites examined (which did not include sites found positive in other studies) these also neither confirm nor conflict with the results of the positive studies.
- The cancer study results in mice show consistency, in that liver tumors were induced by PCE exposures via both oral and inhalation routes.

- The oral studies in male and female mice were considered adequate for risk assessment of drinking water exposures to PCE. This is despite the early mortality in the studies, which was accommodated by using time-to-tumor data and a time-dependent dose response model.
- The inhalation studies in rats and mice were also considered adequate for risk assessment of exposures by the inhalation route.
- There is some evidence of cancer in humans as a result of PCE exposures, but the equivocal nature of this evidence (especially its confounding by concurrent exposure to other potentially carcinogenic chemical exposures), and its unsuitability for quantitative risk assessment, are significant limitations. There is also uncertainty as to the relevance to human cancer causation of the tumors induced by PCE in rodents. However, the occurrence of tumors in animals at several sites adds considerably to the weight of evidence supporting the conclusion that PCE should be considered a possible human carcinogen.
- Site concordance between the various species in which positive or suggestive results have been obtained is not observed, but this is not necessarily expected except in cases where certain special site-specific mechanisms have been demonstrated.
- PCE genotoxicity data is mostly negative or equivocal. On the other hand, a minor metabolic pathway involving glutathione conjugation, leading to a highly mutagenic intermediate, has been identified. The standard mutagenicity assays usually fail to demonstrate this effect. It has been hypothesized that this route is important in the production of the kidney toxicity and tumors observed in rats. The significance of these observations for the observed carcinogenic effects of PCE at other sites, and in other species including humans, is unclear. It is possible, although unproven, that entirely different mechanisms, including non-genotoxic effects such as protein-deposition nephropathies, or interactions of PCE or its metabolites with growth-regulating receptors, may also contribute to the effects observed in the rat kidney.
- The mechanisms by which exposure to PCE leads to the observed liver tumors in mice, and leukemia in rats, are unknown. In the case of the liver tumors it is possible that non--genotoxic mechanisms (such as those involving interactions with a peroxisome proliferator activated receptor) contribute to the effects observed, but this is unproven. Directly genotoxic mechanisms involving activation of PCE to reactive metabolites have also been proposed, but the nature of these metabolites has not been clearly established. Such mechanisms might involve either oxidative metabolism or glutathione conjugation, but neither of these possibilities has been definitively shown to contribute to toxic effects in the liver or hematopoietic system.
- It appears probable that metabolism of PCE is required for carcinogenicity. The evidence is not however sufficient to demonstrate the relevance of the hypothetical specific modes of action, whether genotoxic or otherwise, at the sites where carcinogenesis is observed (in either animals or humans). In the absence of such evidence, dose metrics based on the overall extent of metabolism of parent compound, PCE, were chosen for the dose-response assessment.
- Lack of knowledge of the mode(s) of action of PCE or its metabolites in causing cancer in rats and mice, and the implication of these processes for human disease, is a limitation of this risk assessment.
- Cancer potency estimates derived from different studies, species, sites, and routes of administration are similar, although some differences between the oral and inhalation routes are observed.
- The PHG of 0.06 ppb is based primarily on an average of the quantitatively similar CSFs for liver tumors in male and female mice exposed to PCE by the oral route. A slightly different (but still broadly consistent) CSF was derived for the inhaled component of exposures

derived from drinking water contamination with PCE. This latter value was based on the average of CSFs for liver tumors in male and female mice, and leukemias in male and female rats, exposed to PCE by the inhalation route. If the PHG value were based on individual tumor sites (specific to the oral and inhalation routes) instead of an average, the values would range from 0.04 to 0.09 ppb.

- The CSFs are upper-bound estimates defined by the 95 percent confidence limit on the ED_{10}. It is theoretically possible that the true value of the cancer potency of PCE in humans could exceed these values, but that is considered unlikely. It is plausible that the true value of the human cancer potency for PCE has a lower bound of zero, based on statistical and biological uncertainties including interspecies extrapolation and mode of action.
- The estimate of multi-route exposure employed in the PHG calculation was 6.31 Leq/day (of which 4.1 Leq/day is metabolized). Use of an alternative exposure model (in which the total exposure is 7.2 Leq/day) employed in earlier risk assessments would yield a health protective level of 0.051 ppb, using the same metabolized dose conversion and slope factors as the basis of the estimate. Estimates of the health protective levels based on other data and assumptions (including median rather than upper bound estimates of the percentage of inhaled or ingested PCE that is metabolized) might be as high as approximately 0.1 ppb. However, these estimates are not recommended since they fail to make appropriate allowance for the expected inter-individual variation in the human population.
- The estimate of additional exposure via the inhalation route is 3.54 Leq/day (of which 2.05 Leq/day are metabolized). This is higher than the default value of two Leq/day suggested by U.S. EPA (based on average estimated showering exposures of a number of typical VOCs). This estimate reflects the greater volatility and lower water solubility of PCE, compared to some other VOCs commonly found in drinking water, and is based on reasonable extrapolations from the known physical properties of PCE.

According to this analysis, the health protective concentration of PCE in water associated with a negligible theoretical extra lifetime cancer risk is 0.06 ppb. This includes an estimate of inhalation exposure from showering in PCE contaminated water, flushing toilets, and other household activities involving tap water. The primary sources of uncertainty in the development of the PHG for PCE in drinking water are also the general issues of uncertainty in any risk assessment, particularly inter- and intra-species extrapolation, extrapolation from high to low doses, and issues relating to possible human exposure. A more formal analysis of the uncertainties used in our approach is possible, but would not affect the PHG and has therefore not been included.

The PHG of 0.06 ppb was calculated based on the carcinogenic potency of PCE. In calculating the PHG, a *de minimis* theoretical excess individual cancer risk level of 10^{-6} was assumed. The corresponding levels for cancer risk levels of 10^{-5} or 10^{-4} are 0.6 and 6 ppb, respectively.

For PHGs, OEHHA's use of the relative source contribution (RSC) has followed U.S. EPA drinking water risk assessment methodology, with a few exceptions. U.S. EPA has treated carcinogens differently from noncarcinogens with respect to the use of RSCs. For noncarcinogens, RfDs (in mg/kg-d), DWELs (in mg/L) and MCLGs (in mg/L) are calculated using UFs, body weights and DWC (in Leq/day) and RSC, respectively. The typical RSC range is 20 to 80 percent (0.2 to 0.8), depending on the scientific evidence. In this case, evidence indicated an RSC below this range could be justified based on exposure to PCE from multiple sources. However, this was not used for development of the PHG, since the PHG was based on carcinogenicity.

U.S. EPA follows a general procedure in promulgating MCLGs:

- For Groups A and B (strong evidence of carcinogenicity), MCLGs are set to zero;
- For Group C (limited evidence of carcinogenicity), **either** an RfD approach is used (as with a noncarcinogen) but an additional UF of one to ten (usually 10) is applied to account for the limited evidence of carcinogenicity, **or** a quantitative method (calculation of potency and low-dose extrapolation) is used and the MCLG is set in the 10^{-5} to 10^{-6} cancer risk range;
- For Group D (inadequate or no animal evidence of carcinogenicity), an RfD approach is used to promulgate the MCLG.

For approaches that use low-dose extrapolation based on quantitative risk assessment, U.S. EPA does not factor in a 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 assumed 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 a cancer potency value based on low-dose extrapolation. (U.S. EPA has categorized PCE as a probable/possible Group B/C) carcinogen, and OEHHA has indicated in this risk assessment that it is appropriate to calculate a cancer potency by low-dose extrapolation.) This is an area of uncertainty and scientific discussion, and it is unclear how this assumption impacts the overall health risk assessment.

OTHER REGULATORY STANDARDS

The U.S. EPA has established a Maximum Contaminant Level Goal (MCLG) of zero mg/L PCE. A Maximum Contaminant Level (MCL) of 0.005 mg/L PCE has also been established which U.S. EPA believes would protect against the potential health problems identified in its report and is "the lowest level to which water systems can reasonably be required to remove this contaminant should it occur in drinking water" (U.S. EPA, 1989).

The California Department of Health Services currently lists a Maximum Contaminant Level of 0.005 mg/L (5 ppb). This is based in part on the earlier Proposed Maximum Contaminant Level document (DHS, 1987), which proposed a Maximum Contaminant Level of 2 ppb PCE. This concentration in drinking water was calculated to be associated with exposure producing a *de minimis* excess cancer risk value of one case in one million persons. Both the DHS (1987) assessment and the health risk assessments by U.S. EPA (1984, 1986) used similar data sets to those examined in the present analysis. The *de minimis* level (0.7 ppb) indicated by the U.S. EPA analysis is approximately 10-fold higher than that presented here. (However, the cancer risk assessment of PCE is no longer presented in U.S. EPA's IRIS database.) The difference results primarily from the use in this document of 1) interspecies scaling according to ¾ power of body weight, 2) a time-to-tumor analysis of the NCI (1977) oral mouse bioassay data, and 3) a more sophisticated human pharmacokinetic model for low-level oral and inhalation exposures. The DHS (1987) document also did not use the time dependent analysis of the oral study. In addition, the final value presented was an average of estimates obtained using a wide range of different assumptions about exposure, uptake, and interspecies scaling, some of which were not included for consideration by either U.S. EPA (1984, 1986) or the present authors. The DHS and U.S. EPA MCL standards reflect risk management choices in addition to the health protective levels.

The California Air Resources Board has identified PCE as a Toxic Air Contaminant, with an upper bound estimate of the human lifetime risk of carcinogenicity of between 2 and 72×10^{-6} per ppb exposure (best value 54×10^{-6}/ppb). Various other regulatory standards for occupational and environmental exposures to PCE exist, but these are primarily designed to

address the hazards of short-term exposures to PCE rather than long-term environmental exposures such as those from polluted air or drinking water. The current U.S. EPA and California standards (RfD of 0.01 mg/kg-d; the California Hot Spots program inhalation chronic REL is based on this number) for non-cancer health effects are based on extrapolation from liver toxicity in mice. The protective non-cancer level established in this document is derived using human data and is approximately three times higher (equivalent to 0.032 mg/kg-d).

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APPENDIX 1:
COMPARISON OF EXCESS CANCERS PREDICTED BY ANIMAL-DERIVED POTENCY FOR PCE TO COHORT DATA

The data from two cohort studies (Ruder et al, 1994; Blair et al, 1990) are considered. Both are mortality studies of members of dry-cleaning unions in U.S. cities. A number of limitations of both studies have been noted in IARC, 1995b, the main part of this document, and other analyses. Nonetheless, these two studies comprise the best available human data on carcinogenicity of PCE.

Assumptions

Exposure levels

No measurements of exposure levels are available for either of the cohort studies. A study conducted by the National Institute of Occupational and Industrial Health (NIOSH) in 1977-1979 measured exposure to workers performing four task categories in 44 U.S. dry-cleaning operations (Ludwig et al, 1983). Assuming exposure conditions were similar, the results of the NIOSH survey can be used to provide surrogate data for the dry cleaners in the Ruder and Blair studies. This also assumes that the distribution of dry vs. wet transfer shops in the cohorts was similar to that in the NIOSH study. Taking the geometric means (GM) for each of the four job categories described by Ludwig et al. (1983), time-weighted average (TWA) exposure to PCE for various dry-cleaning jobs is:

Operators	22.0 ppm
Pressers	3.3 ppm
Seamstress	3.0 ppm
Counter	3.1 ppm
GM:	5.1 ppm (35 mg/m^3)

The GM over all four job categories (35 mg/m^3) is used in this analysis to represent typical exposure in the cohorts. The GM is not likely to significantly underestimate exposure, because most workers in dry-cleaning operations perform the lower exposure tasks. Ludwig et al. (1983) reported that a "typical workforce" consisted of one operator, and six pressers, seamstresses, and counter clerks. Further, the Blair study reported similar standard mortality ratios (SMRs) in both the high and mid exposure groups, indicating that excess risks are not likely to be restricted to the minority of workers with the highest TWA exposure levels. In that study, the highest SMRs were found in the largest group, those exposed to intermediate levels of PCE.

Exposure duration

The duration of exposure is estimated by the years of union membership, following the assumptions made in the analyses of the epidemiologic cohorts performed by the original authors.

Fraction metabolized

This analysis assumes that it is the metabolites of PCE that are likely to be associated with human cancers in exposed workers. Therefore, the exposures above were converted to metabolized doses, using the results of the human pharmacokinetic model of Bois et al. (1996). Based on visual estimation from the graph presented by Bois et al (1996), the mean fraction of inhaled PCE metabolized at 5 ppm is 12 percent of inhaled dose (95 percent CI: 5 - 30 percent). Both the mean and upper 95 percent confidence bound on the metabolized fraction are used below.

Body weight The Blair cohort was 75 percent women and the Ruder cohort was 65 percent women. A bodyweight of 65 kg (average of male and female default bodyweights) is assumed in dose estimation for both cohorts.

Dose estimation

Application of the above assumptions results in an estimated average daily dose for workers in both cohorts of 0.46 mg-metabolites/kg-d:

$$35\ mg/m^3 \times 10\ m^3/d \times 5\ d/7\ d \times 0.12 \times 1/65\ kg = 0.46\ mg\text{-}metab/kg\text{-}d$$

Or, using the upper bound on metabolite production, the daily dose becomes:

$$35\ mg/m^3 \times 10\ m^3/d \times 5\ d/7\ d \times 0.3 \times 1/65\ kg = 0.15\ mg\text{-}metab/kg\text{-}d$$

Potency

The lifetime cancer risk due to a daily dose from inhalation exposure was derived from animal data, as described in the documentation for the PHG. The upper bound potency estimate for exposure by inhalation was 0.15 $(mg\text{-}metab/kg\text{-}d)^{-1}$. Using this value, the cancer risk for one year is assumed to be:

$$\text{Risk per year at 1 mg-metab/kg-d} = 0.15/70\ yr = 0.002$$

To make a prediction of the expected number of excess cancers due to PCE exposure in the epidemiological cohorts, the total person-years of exposure to 1 mg-metab/kg-d is multiplied by this annualized risk estimate.

Predictions for the Ruder et al. (1994) data set:

1701 workers at risk

Mean duration of exposure = 9.6 years

Total "Dose-Years" = 1701 workers x 9.6 years x 0.46 mg-metab/kg-d = 7,512. (Using the mean estimate of the fraction of inhaled PCE converted to metabolites.)

Predicted excess cancers due to PCE exposure, derived from animal potency estimates:

7,512 x 0.002 = **15 excess cancers.**

If the upper bound on metabolite production is used in place of the average, "dose years" increase to 18,780 and **38 excess cancers** are predicted.

To compare what the animal-derived potency estimates predict for the cohort to the reported results of the cohort analysis, we select the SMR for total cancer, because the inhalation potency for the rodent model is taken to be indicative of any human cancer. In

the Ruder cohort, the SMR was 1.23, based on 209 deaths observed, 170 expected. This means **39 excess cancers** occurred in the study cohort during the period of observation. Thus, the cancer potency based on rodents shows a slight under-prediction, but reasonable agreement with the human data in this very rough analysis.

It should be noted that the predictions for the cohort made using the animal potency assume a lifetime of follow-up; the actual years of observation of these cohorts was considerably less. Given the same exposures, but follow-up increased to lifetime, more excess cancers might be expected from in the cohort data, somewhat increasing the tendency of the animal-derived potency to underestimate.

Predictions for the Blair et al. (1990) data set:

5365 workers at risk.

Mean duration of exposure = 6.6 years

Total "Dose-Years" = 5365 workers x 6.6 years x 0.46 mg-metab/kg-d = 16,288
(Based upon the mean estimate of the fraction of inhaled PCE converted to metabolites.)

Predicted excess cancers due to PCE exposure, using animal potency:

16,288 TDY x 0.002 = **33 excess cancers**

Using the upper bound on the human metabolized dose, the "dose years" for this cohort are 40,720 and **81 excess cancers** are predicted.

For comparison, the SMR for total cancers reported in the study was 1.16, based on 294 observed, 254 expected, indicating that **40 excess cancers** occurred in the study cohort. For this cohort, the animal potency agrees well with the observed SMR for cancer.

Conclusions

The number of cancers due to PCE exposure that is predicted by the animal-derived inhalation potency shows reasonable agreement with the number of excess cancers actually observed for both the PCE-exposed cohorts. While the analysis is necessarily quite crude, and is based on rough average estimates for a considerable number of variables, it may provide some confidence that the potency derived from rodent bioassay data used to set the PHG is not overly conservative. It was concluded in the PHG that the epidemiological data are not adequate to produce a quantitative estimate of the risk of PCE, and the same limitations apply here.

Responses to Major Comments on Technical Support Document

Public Health Goal
For
TETRACHLOROETHYLENE
In Drinking Water

Prepared by

Pesticide and Environmental Toxicology Section
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

August 2001

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INTRODUCTION

The following are responses to major comments received by the Office of Environmental Health Hazard Assessment (OEHHA) on the proposed public health goal (PHG) technical support document for tetrachloroethylene as discussed at the PHG workshop held on November 5, 1999, or as revised following the workshop. Some commenters provided comments on both the first and second drafts. For the sake of brevity, we have selected the more important or representative comments for responses. Comments appear in quotation marks where they are directly quoted from the submission; paraphrased comments are in italics.

These comments and responses are provided in the spirit of the open dialogue among scientists that is part of the process under Health and Safety Code Section 57003. For further information about the PHG process or to obtain copies of PHG documents, visit the OEHHA Web site at www.oehha.ca.gov. OEHHA may also be contacted at:

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RESPONSES TO MAJOR COMMENTS RECEIVED ON THE PUBLIC HEALTH GOAL FOR TETRACHLOROETHYLENE

United States Environmental Protection Agency, National Center for Environmental Assessment

Comment 1. "The unique features of this assessment, as compared to other recent assessments (State of New York, 1997; ATSDR, 1997; Canada, 1993) are the use of the pharmacokinetic models described on pages 10 to 18 to estimate the metabolized dose in both animals and humans and the use of the CalTOX exposure model described on pages 47 and 48 to include inhalation and dermal exposure from drinking water sources as separate routes of exposure attributable to the presence of perchloroethylene in tap water. Since not enough information was given about these models, we are unable to endorse them and the conclusions drawn from them, although the piecing together of the various models into a coherent assessment seems logical."

Response 1. We agree that this complicated risk assessment would require some additional details and collaboration to reproduce. The CalTOX model has been previously described and used for various assessments; it is available free of charge on the Web site of the Human and Ecological Risk Division of the California Department of Toxic Substances Control (www.cwo/~herd1/ctox_dwn.htm) for those who might desire a better understanding of the methods.

Comment 2. "The section on environmental occurrence would be a better place to describe the CalTOX exposure model than having it buried on page 47 and 48. It is a significant part of the document and fits logically in this section."

Response 2. The addition of the CalTOX exposure model to the environmental occurrence section would not be appropriate. In this section, sources of potential exposure to tetrachloroethylene (PCE) are described. The CalTOX exposure model was used in the document to assess the exposure to PCE and does not describe a source of exposure.

Comment 3. "There is an apparent inconsistency between the text on page 6 describing Table 3 and the footnote a of Table 3 on page 7. The text says that inhalation exposures while bathing have been considered in Table 3, whereas the footnote says that inhalation while bathing has been ignored."

Response 3. The corresponding text has been revised to remove the inconsistency.

Comment 4. "The study length correction as given [in Table 4] was applied as a divisor, while a cursory reading of the footnote suggests it was a multiplier."

Response 4. The correction for study length is obtained by first dividing the lifetime of animals by study duration and taking the result to the 3rd power. This value is used as a multiplier in the correction for study length. Footnote (b) in Table 4 was revised to more accurately reflect the calculation performed.

Comment 5. "In reading the description of the calculations leading to Table 7, page 39, an unanswered question arises: namely, does the assessment account for the fraction of the inhaled dose in animals that is not absorbed? Although this question is not relevant to Table 7 because that table deals with administered dose, the reader would be helped by an indication here about where in the document that issue is dealt with. We could not find such a discussion."

Response 5. Every effort was made to incorporate all aspects of potential exposure. Although a specific section for the unabsorbed dose was not identified in the document, the topic is indirectly touched upon in the Pharmacokinetic Models section. The fraction of the inhaled dose that is not absorbed is excreted from the body. As discussed in the Metabolism and Pharmacokinetics section, 50 percent to 70 percent of the inhaled dose is absorbed. Thus, 50 percent to 30 percent of the inhaled dose is then not absorbed.

Comment 6. A discrepancy was observed in the reported incidence of mononuclear cell leukemia in the low and high dose groups between the values reported in Table 7 and the values reported in NTP Technical Report #311.

Response 6. The data represented in Table 7 are correct.

Comment 7. "One has a hard time seeing how the administered dose metric in Table 8 is converted to the metabolized dose metric in Table 10. The only indication of how this is done is in Table 4 on page 12, where the model of Bogen et al. (1987) is excerpted. But it would be of general interest as well as specific interest in going from Table 8 to Table 10 to see, either through an equation or preferably a graph, the non-linear function of metabolized vs. administered dose that Bogen et al. (1987) used to generate the data quoted in Table 4."

Response 7. Bogen et al. (1987) used a series of equations to convert administered metric dose to metabolized dose. Because of the complexity involved, the reader was directed to the article by Bogen et al. (1987) for a more thorough explanation of the process the researchers used in their conversion. No simple graphic representation was available for this lengthy calculation. We feel that this approach is more appropriate than providing the details on for the purposes of the PHG document.

Comment 8. "For the curious reader, some data on the "improve fit" of the time to tumor model would be of interest. [Page 43, line3] This would include not only the "fit" statistics, but also a graph of the data and the two models."

Response 8. See response to comment # 7.

Comment 9. "The title should refer to NCI (1977) PCE gavage bioassay and the applied dose should be in units of mg/kg-day." [Table 11]

Response 9. Text was revised.

Comment 10. "The sentence starting with "However, it should…" and ending with "…used to run it)." could be a footnote for those who have to reconcile the 1992 OEHHA document with this document. [Page 44, lines 6-13] However, for readers of this document it is a confusing comment because in this document you did not use Bogen's analysis of the tumor incidence."

Response 10. Text was revised in order to clarify this paragraph.

Comment 11. "Although from metabolic considerations inhalation and dermal exposure both bypass the liver and avoid the first pass effect, the obvious difference between the routes is how the perchloroethylene is transferred from air to blood. [Page 46, 1st paragraph] This paragraph needs to say how that transfer was accounted for before one can accept using an inhalation potency for a dermal route of exposure."

Response 11. In this paragraph, we have indicated that there is no direct evidence as to the appropriate potency estimate to be used for dermal exposures. So by default, the inhalation value was used for this route. In terms of differences between these two routes, there are other differences than just the transfer of PCE from air to blood, such as rates of metabolism and other dispositional parameters that could impact the potency estimate. Further discussion on this subject can be found in the calculation of the public health goal (PHG) section of the document.

Comment 12. "Since the CalTOX model considers the variation of input parameters, some indication of the variability of the output parameters in Table 13 could be given and summarized in the Risk Characterization section. This would give some basis for stating the uncertainty of the final water concentration calculated on page 49."

Response 12. Variability in the CalTOX module represents only a small part of the total variability and uncertainty in the analysis. Good variability (sensitivity analysis) tools are not available for the other calculations. Conducting the full variability analysis in CalTOX therefore did not seem very productive.

Comment 13. "This [Table 13] is a bit confusing, because it is not completely clear that the inhalation and dermal doses are all part of the water dose (which as we may recall from earlier in the analysis only makes up around 3%-11% of one's total exposure). The title makes it clear that this table refers to water exposure, but the wording of the column and row headings reintroduce ambiguities. One idea would be to change the column heading for the last column from "% of total dose" to "% of total dose from water."

Response 13. Table was modified to remove ambiguity.

Comment 14. "The calculation of the water concentration corresponding to a cancer risk of 10^{-6} turns out to be on the order of magnitude of 10^{-2} μg/L, but the values for metabolized dose in Table 13 used in this calculation assumed concentrations of 1.0 μg/L (see page 47, bottom), which is on the order of 100 times higher than the result. The question is whether the non-linear relationship between metabolized dose and administered dose should be explicitly considered in this calculation, since this relationship could change considerably over a concentration range of a factor of 100. The only information given in the document on this point is in Table 4, page 12, which shows noticeable non-linearities at (comparatively) very high doses, but says nothing about lower concentrations."

Response 14. As the commenter notes, the calculation assumes a linear relationship at low concentrations. This is expected for any regular mathematical function of this type. Both 1.0 and 0.01 μg/L are in the low, presumed-linear range. The available data showing non-linear factors involves much higher concentrations.

Comment 15. "The risk characterization section fails to discuss the implications of the relative source contribution (RSC) first introduced on page 49. If tap water constitutes only 3% of the total exposure to tetrachloroethylene, the entire document is apparently devoted to only a minor source of exposure. This fact is certainly important in making regulatory decisions, and needs to be mentioned in any credible characterization of the risk of tap water contamination. This is true for both non-cancer risks, where the RSC is an explicit factor, and in cancer risk estimation, where it is not explicitly considered."

Response 15. Tap water should be only a small contributor to exposures to PCE on average, but certain individuals may receive (and have received in the past) much greater exposures by this route. The RSC in this case is calculated at an equivalent drinking water level ten times the PHG value and is based on actual water contamination data. Although RSC is acknowledged as an estimated value in our PHG calculations, it is defensible. The Office of Environmental Health Hazard Assessment (OEHHA) defines the RSC as appropriate for use in non-cancer risk assessments only; this approach explicitly directs that the RSC is not used in cancer calculations.

Other routes of exposure are already addressed by other California regulatory programs, notably the Toxic Air Contaminants regulations which use a similar risk assessment methodology for inhalation exposures to that presented for the PHG program.

Lawrence Livermore National Laboratory, University of California

Comment 1. "[T]here is very little consideration given to the fact that exposure to PCE occupationally as a chemical intermediate occurs in combination with exposure to many other solvents. Environmental exposure to PCE likewise occurs in combination with many other exposures including airborne benzene, PAHs, smog, etc. No consideration is given to chemical interactions and should be to set exposure limits. Most solvents are acted on by similar detoxification/bioactivation processes (CYP2E1, for example) which can lead to potentiation of effects. This should be considered."

Response 1. OEHHA and other regulatory agencies are currently attempting to develop methods for evaluating environmental exposures to a combination of compounds. At present, we can only acknowledge this as one of the uncertainties.

Comment 2. "Non-quantitative statements about dose or effect are frequently made throughout the document that should be quantified." *The document* "could be made more informative by including some quantal indication."

Response 2. Public Health Goals provide estimates of the levels of chemical contaminant in water that would pose no significant risk to individuals, including the most sensitive subpopulations, consuming the water daily, over an entire lifetime. In our extensive review of the best available toxicological data, we focused the quantitative analysis on the critical data that posed the most significant risk to individuals in order to calculate the PHG. As for the remaining data which is adequate for the purpose and it would not be critical for the development of the numerical goal, more descriptive information (non-quantitative statements) was provided not to distract from the critical information were used. The references cited in the document provide more quantitative evaluations of the data, for those who would wish to have a more in-depth study of the subject.

Comment 3. "PCE and some of its metabolites have been used medicinally and its surprising that such information [on molecular mechanisms of PCE toxicity] is lacking. Has this literature been examined to make sure all relevant information on PCE metabolism in humans has been considered?"

Response 3. A discussion of medicinal use or molecular mechanisms of PCE toxicity has not been incorporated into this document because the mechanism or mechanisms of PCE carcinogenicity is/are not well characterized. Such a discussion would be outside the scope of a PHG document at this time, since it cannot currently contribute to setting a PHG.

Comment 4. Very little information is provided on the DNA and protein binding of PCE. Some information is in the literature on this topic but seems ignored under the carcinogenesis sections. This is information that adds to the weight of data supporting a role for PCE in carcinogenesis and other mechanisms of toxicity.

Response 4. The topic of DNA and protein binding was briefly discussed in the sections on metabolism, genotoxicity, and cancer potency estimation. . We agree that this information adds to the weight of data supporting a genotoxic potential for PCE, and it was assumed for the purposes of risk assessment that PCE is acting as a directly genotoxic carcinogen partly on the basis of these data. However, a more extensive discussion of the binding experiments and adduct formation would not be relevant to the derivation of the PHG value, therefore no further discussion on this topic is provided.

Lawrence Berkeley Laboratory, University of California

Comment 1. "[T]here are some instances where the references used are out of date, and more recent information could and should be cited."

Response 1. The indicated references were obtained and reviewed. The appropriate data were incorporated into the document.

Comment 2. "The data evaluation and interpretation are consistent with the state of the art in this field and well described and defended. The OEHHA staff is to commended for making use of many alternate and innovative approaches—metabolized dose as a basis for estimating cancer, time-to-tumor models, linear extrapolation models, multipathway exposure assessments, etc. However, as noted below there is a need for a more formal analysis of the uncertainties in these approaches and some discussion of how sensitive the PHG is to the assumptions and models used."

Response 2. An overall sensitivity analysis would add even more complexity to this already difficult and complicated analysis. Software tools are available for a semi-automated sensitivity analysis only on certain parts of the calculation, such as CalTOX; a consistent approach could not have been extended through the chain of calculations in a reasonable period of time. We acknowledge this as a limitation to the quantitative evaluation and description of our approach, and have noted this in the Risk Characterization section.

Comment 3. "The one area that was left out is a discussion of all potential sources of PCE exposure. There is no mention of exposure to indoor sources of PCE other than tap water and this should be added. There should be some discussion of indoor levels of PCE and indoor exposures. PCE was one of the chemicals considered in the EPA Total Exposure Assessment Methodology (TEAM) studies. The PHG report should provide some perspective on the relative contribution of indoor sources to PCE exposure." *Two references were cited for inclusion.*

Response 3. An additional section titled "Indoor Exposures" was added to the document.

Comment 4. "The report is lacking discussion of uncertainty and variability in the overall characterization of risk. There should be some discussion of uncertainty with dose/response function—in particular how likely is PCE to be a human carcinogen, how reliable and accurate

are the dose response models, and how sensitive is the value of the PHG to models and assumptions selected to characterize the dose/response function? There should also be discussion of the uncertainty and variability of the exposure factors selected—in particular, does the 2 L/day assumption of tap water consumption represent a mean or an extreme value, how representative is 3.54 L/day equivalent inhalation from household water uses, and how much to these assumptions impact the magnitude of the PHG value?"

Response 4. Discussion of uncertainty and variability is included in the PHG document for both the uncertainty with dose/response function and variability of the exposure factors selected.

Comment 5. "Summary. This is actually an Executive Summary and should be referred to as such. The summary discussion should include some discussion of why the PHG value of 0.056 ppb differs so much from the EPA MCL of 5 ppb."

Response 5. A discussion of the reasons for the difference in values for the PHG and the U.S. Environmental Protection Agency (EPA) has been incorporated into the summary. We see no reason to retitle it "Executive Summary" and have left its title unchanged.

Comment 6: "The summary should provide a brief discussion of how feasible it is to make routine measurements of PCE concentrations of 0.06 ppb in water supplies. Is this below the level of detection by most common methods?"

Response 6. The PHG is determined based only on public health considerations, and does not consider detection limits or cost-benefit analyses. These factors are intentionally excluded from discussion in the PHG document.

Comment 7. Introduction. The document has no introduction. Why? Most risk assessment documents provide some type of introduction providing an overview of what is presented in various sections of the report as well as some background on motivations for the study. It would be useful to include that in this document.

Response 7. An introduction was added to the PHG document.

Comment 8. "Table 2 on page 4 and page 47. Are the chemical properties reported here the values that were used for the CalTOX calculations reported on page 47 or were the values in the CalTOX data set used."

Response 8. Table 2 contains the physical and chemical properties of PCE as described by IARC and ATSDR. These parameters were not the ones used in the CalTOX calculations, from the standard CalTOX data set. The values used in the CalTOX calculations were values that were obtained following a survey of the literature. The average value calculated from this search was incorporated into the CalTOX data set. Some slight differences exist in the values presented in Table 2 compared to those obtained from the survey and used in CalTOX.

Comment 9. "Why is there no consideration of Pharmacodynamics? Metabolism serves as a loss mechanism in a PBPK model. Pharmacodynamics provides a somewhat broader assessment of the rate of and products of a various metabolism processes. I suggest that this section be renamed "Pharmacokinetics and Pharmacodynamics" and include more information on the behavior of metabolism products. In particular there should be some discussion of metabolism pathways."

Response 9. Discussion of the metabolic pathways and pharmacokinetics can be found in the Metabolism and Pharmacokinetic Section of the document. A general discussion regarding the absorption, metabolism, distribution, and excretion is presented. In addition, metabolism and the behavior of the metabolic products were briefly considered in this section. The addition of more detailed information on pharmacodynamics could be of general interest, but would not appear to be directly relevant to the calculation of the PHG value. In the document, previous reviews (OEHHA, 1992; IARC, 1995; ASTDR, 1997) on this topic are cited for readers who would like to obtain more detailed information.

Comment 10. "There is no information provided here on tissue distribution and its importance for assessing the ratio of metabolism to intake. Why not?"

Response 10. A discussion of tissue distribution is found within the Metabolism section. A more detailed discussion is not provided in the document because the distribution of PCE among tissues is extensive once the material is absorbed; no particular sensitivities related to differential distribution are noted.

Comment 11. "There is no discussion here on the use of biomarkers in human studies of PCE and cancer. There should be some discussion of the feasibility of biomarkers and if possible some discussion of who has attempted this or on why no one has yet attempted biomarker-based studies."

Response 11. A discussion of biomarkers in human studies of PCE and cancer has not been incorporated into this document because the mechanism(s) of PCE carcinogenicity is/are not well characterized. Further elaboration of the feasibility of biomarkers or as to why no one has attempted biomarker-marker studies is outside the scope of a PHG document at this time, since it cannot currently contribute to setting a PHG.

Comment 12. The last paragraph of page 40, "provides a good discussion and illustration of the use of the linear extrapolation approach with the LED_{10}. However, use of this new methodology raises some questions and concerns. First, how sensitive are the results of the analysis to the benchmark selected. That is what happens for example if the approach is applied to the LED_5 instead of the LED_{10}? Also, the assumption is made here that the EPA will follow through with the proposed 1996 guidelines. The guidelines have not be[en] finalized or issued. What happens if there are major revisions to these guidelines?"

Response 12. Yes, the PHG values are sensitive to the assumptions used, but formal sensitivity analyses are not conducted. LED_{10} was selected as a point of departure for risk assessment

because it is generally the lowest (round number) point within the observable range. This makes it a good stable point for low-dose extrapolation. Extrapolations based on a LED_5 might be half the value that they would be if based on an LED_{10}, but that depends on both the slope of the dose-response curve and any decision as to whether the same uncertainty factors should be applied from the lower effect level. As required by the Safe Drinking Water Act, we will review and revise as necessary any public health protective concentration when new scientific data are available or significant regulatory changes occur. OEHHA utilizes the methodology described in U.S. EPA's 1996 draft guidelines where appropriate and will review any subsequent versions and incorporate them as appropriate.

Comment 13. "In using the CalTOX model, the authors of this study missed an opportunity to carry out a quantitative uncertainty analysis on the absolute and relative exposure by different pathways. The CalTOX model was designed to provide easy access to uncertainty/variability assessments. The Datacal.xls data set that is referred to includes both a mean value and coefficient of variation of exposure factors such as drinking water intake. At a minimum these values should be reported and discussed."

Response 13. An uncertainty analysis only of the exposure aspects appeared to us to be of limited utility, whereas a comparable evaluation of the entire analysis really was not feasible. Only the CalTOX model includes uncertainty analysis as a built-in functionality, and CalTOX has been used in only a few of the PHGs to date. For all these reasons, sensitivity analysis is not a standard part of the PHG development process.

Comment 14. "Why report the PHG as 0.056 ppb? Given the overall uncertainties in the parameters used and the reliability of the calculations provided, there is no justification for reporting more than 1 significant figure for the PHG, that is write it as 0.06 ppb."

Response 14. We agree that the PHG values have no more than one significant figure. Our use of two digits in some cases may serve to emphasize the value as the result of a simple calculation. However, in this case we have decided to go ahead and round off the PHG to 0.06 ppb. .

Comment 15. "[O]ne area that is left out is the "para-occupational" exposure. That is, the exposure to non-workers as a result of chemicals brought home from work by a worker residing in their household. PCE has been proposed and evaluated as an example of a significant para-occupational exposure…" (*reference provided*).

Response 15. The appropriate data were included in the PHG document under the "Indoor Exposures" subsection.

Halogenated Solvents Industry Alliance, Inc.

Comment 1. "Risk assessments that accumulate worst case assumptions and that predict risks that are patently too high at low dose are not in the best interests of the public. Such overly conservative risk assessments draw attention to situations that are, in reality, of negligible risk whilst circumstances of true concern are overlooked."

Response 1. The Safe Drinking Water Act of 1996 requires protection of the public from drinking water contaminants, using prudent assumptions as necessary to assure absence of adverse health effects. Lack of explicit knowledge about human health effects requires incorporation of some assumptions about toxic mechanisms and cross-species extrapolations. Our evaluation involved an extensive analysis of the available scientific literature and acknowledgement of several types of uncertainty; none of the steps involves a worst-case scenario. When new scientific data are available to reduce the uncertainty, the conclusions can be reevaluated.

Comment 2**.** *Regarding animal tumor types:*

"Mouse Liver – Passing mention is made of the mechanisms of induction of rodent liver tumors in association with peroxisome proliferation. European regulators, the US Food and Drug Administration, and many scientists now acknowledge that rodent liver tumors associated with peroxisome proliferation have no relevance for man (and are therefore not a basis for calculating human risk). If regulatory conservatism prevents this conclusion despite substantial evidence regarding perchloroethylene (PCE) and its principle metabolite trichloroacetic acid (TCA), the relationship between mouse liver tumors and peroxisome proliferation should, at least, be reviewed. This is critical in the light of the decisions regarding linear versus non-linear dose response relationships analyzed below."

"Rat mononuclear cell leukemia (MNCL)– The incidence of MNCL in control rats in the NTP inhalation study on PCE was 56 % in males and 37 % in females. The background incidence in the F344 rat is known to be "very high and variable." The human analogue of rat MNCL is extremely rare and there are no types of spontaneous tumor in man that approach the levels of incidence of this rat tumor type. Since it is likely that the natural propensity for developing MNCL can be triggered by mechanisms not normally associated with tumorigenesis, it is doubtful whether an increased incidence should be regarded as an indicator of carcinogenicity. However, even if considered of relevance on a qualitative basis, there is no justification for using the increased incidence in a quantitative calculation of risk because of the special susceptibility of the F344 rat strain."

Response 2**.** Cancer risk assessments do not assume concordance between species in particular tumor types, because of species- and strain-specific differences, as rightly pointed out above. The important point is that a particular chemical interacts with cells to produce tumors. We agree that the F344 strain may be unusually sensitive to some carcinogenic mechanisms, compared to other rodent strains. Its relative sensitivity for chemical-induced carcinogenesis, compared to humans, is unknown. Because of the multiple tumor types resulting from administration of PCE and its metabolite TCA, the argument that tumors secondary to

peroxisomal proliferation are not relevant to humans is of limited significance; the overall pattern of carcinogenicity is more compelling.

Comment 3**.** "Mention is made in several places of the possibility of the formation of PCE oxide, a metabolite, that if generated as a free molecule, could be expected to be genotoxic. There is no evidence that this metabolite is formed as a free molecule. Moreover, the negative results of various types of in vivo or in vitro genetic toxicity tests, where active metabolism occurred, show that PCE oxide is unlikely to be of any significance. It is likely that PCE oxide, if it exists at all, never leaves the site of formation on the enzyme before further chemical alteration occurs."

Response 3. Several investigators consider PCE oxide to be a credible potential genotoxin in the PCE metabolic pathway. We acknowledge the diversity of scientific opinion on this point in the Metabolism and Pharmacokinetic and Genetic Toxicity sections, but feel that this is a relevant postulate regarding carcinogenic mechanisms.

Comment 4. *The amount of dose assumed to be metabolized is too high. The work of Reitz et al. (1996) should have been used for the calculation.*

Response 4. The work by Reitz et al. (1996) was reviewed and considered in the preparation of this document. The various model predictions, available experimental data, and the fraction of dose metabolized used by Reitz et al. (1996) were found to be consistent with results of Chen and Blancato (1987), Bois et al. (1996), and the Bogen et al. (1987) approach used in this document for dose-response modeling of the animal cancer data.

Comment 5. "The evidence indicates that exposure to PCE is not associated with substantial risk but is not sufficiently robust to show that PCE is not carcinogenic to man. However, it can be said that there is no clear evidence of carcinogenicity – this is a more accurate conclusion than "possibly carcinogenic" (p. 35). Of the tumor sites identified, the incidence of esophageal cancer cannot be evaluated without the confounders of smoking and drinking being taken into account. It should be noted that both factors individually are associated with elevations in esophageal cancer but together display a very large degree of synergy. It is not clear which part of the "lymphatic system" is identified. There is no indication that PCE exposure is associated with leukemia and the result for non-Hodgkin's lymphoma was only reported in a proportion of the studies (suggesting "unremarkable" findings when not reported) and statistically significant elevations were not observed."

Response 5. We think the varied evidence justifies a positive statement (possibly carcinogenic) rather than a disclaimer (no clear evidence of carcinogenicity). We are aware of the significant role confounders can play. As discussed in Appendix 1 of the document, predictions made using the animal data support our conclusion of "possibly carcinogenic." In addition, the IARC has judged PCE as a probable human carcinogen while the U.S. EPA has listed PCE as a probable/possible human carcinogen based on support of the limited findings in humans to the sufficient evidence of cancer in animals.

Comment 6. "The existing human neurobehavioral studies are not a suitable basis for setting a PHG. The authors of the PHG document have identified some of the problems with these studies but there are many other concerns. For example, it is very difficult to avoid bias, the selection of appropriate control subjects is critical, and multi-endpoint studies are prone to showing occasional statistically significant differences that have arisen by chance."

Response 6. OEHHA considers that there are sufficient data available to support the use of the human neurobehavioral effects as the non-cancer effects observed with PCE exposure. In order to account for the different strengths and weaknesses of the three studies cited in the document, a geometric mean of the estimates of a human health protective concentration was used in the risk assessment calculations.

Comment 7. "The study by Verplanke et al. (1999) reported no changes in those parameters normally used as clinical indicators of kidney damage. The one possible change reported, increased urinary retinol binding protein, was considered to represent possible minor alterations only in kidney tubules."

Response 7. OEHHA considers the findings by Verplanke et al. (1999) to be important in supporting the possibility of an alternative mechanism for renal toxicity by PCE. The nearly two-fold increase in mean urinary concentration of retino-binding protein reported by Verplanke et al. (1999) for one group of exposed workers confirms the changes in at least one renal parameter observed in six of eight rodent studies, where nephropathy has been observed in female rats and both sexes of mice.

Comment 8. The effects that can be considered to be associated with exposure to PCE are the onset of anesthesia (human information) and liver effects (rodent). Since rodents may be more sensitive to liver effects than man, the quantitative interpretation may be difficult. The effects on rat kidney would also require careful quantitative interpretation, if they are relevant to man at all, given the information in Volkel et al. (1998).

Response 8. These points are noted and considered.

Comment 9. "The selection of an extrapolation model for a PCE should be discussed on an end point basis. Thus "halothioketenes" relate to kidney responses and should not be used to justify a linear treatment of mouse liver tumors. In fact, the evidence that PCE does not induce mouse liver tumors by a genotoxic mechanism is extensive and fits with what is known for a wide range of rodent liver carcinogens associated with peroxisome proliferation. If a risk assessment is based on mouse liver tumors (despite the probable irrelevance to man), the extrapolation model should not be linear. An example of the type of analysis that is appropriate has been published for diethylhexylphthalate (Doull, J et al., Reg Tox Pharmacol **29**:327-357). For any kind of credible risk assessment for PCE, there has to be a full discussion of peroxisome proliferation (and associated phenomena such as cell proliferation, apoptosis and oxidative stress) and the role of trichloroacetic acid (TCA)."

Response 9. The evidence that liver tumors caused by PCE are related to peroxisomal proliferation is rather tenuous, and we have not assumed so in our evaluation. In that regard, the amount of detail provided on peroxisomal proliferation in the PHG document seems appropriate for our purpose. The calculation method we utilized is appropriate for the assumed genotoxic mechanism.

Comment 10. "The assumption that a "bodyweight to the power ¾" conversion should be applied despite the use of metabolized dose in both rodents and man is unjustifiable. The suggestion that it is needed to adjust for "sensitivity" means that it is being used as an uncertainty or safety factor that has no basis in biology. Every piece of mechanistic information relevant to PCE (and TCA) and mouse liver tumors leads to the conclusion that man, if he or she responds at all, is less sensitive than the mouse to a given metabolized dose. The absence of an induction of rat tumors provides support for this view. The power conversion should only apply when the metabolism in two species cannot be related by a PBPK treatment and, even then, the scientific justification for its use is very weak."

Response 10. OEHHA does not share the commenter's optimistic interpretation of the nature and direction of uncertainties in the interspecies comparison for PCE carcinogenic effects. Indeed, as noted in an appendix to the PHG document, the limited evidence from epidemiological studies is broadly consistent with the estimate proposed based on the mouse data and using the default interspecies extrapolation procedure applied in other PHG risk assessments. In the absence of more definitive evidence, OEHHA elected to use this standard default approach which is both consistent with the available data and protective of public health. OEHHA has previously emphasized that interspecies extrapolation needs to allow for toxicodynamic as well as toxicokinetic differences between species, and that variation and uncertainty from both these causes is accommodated by the default interspecies extrapolation procedure, in the absence of better information. The power conversion used in this document also follows the current proposed U.S. EPA guidelines.

Comment 11. "EPA does <u>not</u> classify PCE as a Group B carcinogen (page 54). The EPA Science Advisory Board rejected that classification and the IRIS database currently shows no official classification."

Response 11. EPA considers PCE as an intermediate between a probable and possible human carcinogen (Group B/C); this is now correctly noted in the document. Other groups, such as the IARC, have classified PCE as a probable human carcinogen (Class 2A).

Comment 12. "When the imperfections of the epidemiology data and the uncertainties of the animal-based calculation of risk are considered, this comparison should not appear in any public document. OEHHA recognized many of the difficulties in interpreting the epidemiology data for PCE and admit that it is inappropriate for calculating risk. The epidemiology studies used are an insecure basis for calculations for a number of reasons (including exposures to other solvents, potential uncorrected confounders of drinking and smoking, no correction for socioeconomic status). It should also be noted that the "PCE-only" sub-cohort does not show an overall

increase in cancer (and remains true for a very recent update of this cohort). As discussed above, the calculation of risk based on animal tumors has a number of flaws. The comparison is thus being made on the weakest possible sets of data and certainly should not be taken as supporting the calculations of the PHG."

Response 12. OEHHA believes that the animal data on PCE carcinogenicity are adequate for deriving a carcinogenic risk factor, and that the human data provide supporting evidence.

Association of California Water Agencies

Comment 1. The suggestion was made to include two recent publications associated with PCE exposure and percutaneous absorption (references provided).

Response 1. The identified articles were reviewed. The PHG document was revised to include some of the findings reported by Paulu et al. (1999).