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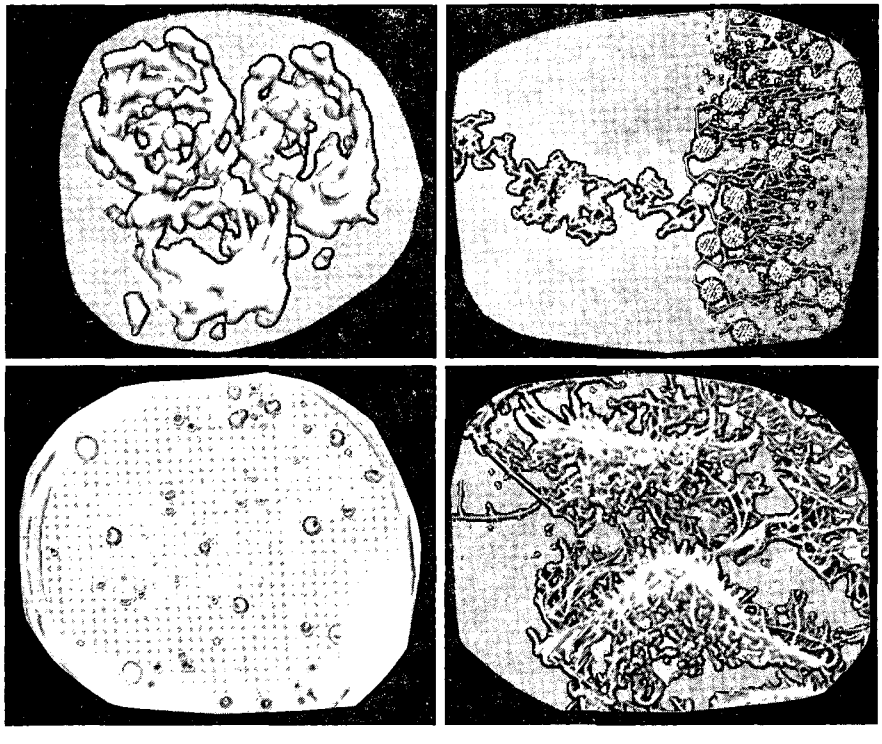

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Trichloroethylene Cancer Risk: Simplified Calculation of PBPK-Based MCLs When Cytotoxic Mechanisms of Action are Presumed

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Abbreviations: ACGIH = American Council of Governmental Industiral Hygienists, AUC = area under the blood-concentration-times-time curve, CF = chloroform, BW = body weight, CH = chloral hydrate, CI = confidence interval, CL = confidence limit, DCA = dichloroacetic acid, EPA = U.S. Environmental Protection Agency, LCL = lower confidence limit, MCL = Maximum Concentration Level, NCI = National Cancer Institute, NTP = National Toxicology Program, PEL = Permissible Exposure Level, PBPK = physiologically based pharmacokinetic, NOAEL = no-observed adverse effect level, SIR = standardized incidence ratio, TBARS = thiobarbituric-acid-reactive substances, TCA = trichloroacetic acid, TCE = trichloroethylene, TWA = time-weighted average.

Abstract

Cancer risk assessments for trichloroethylene (TCE) based on linear extrapolation from bioassay results are questionable in light of new data on TCE's likely mechanism of action involving induced cytotoxicity, for which a threshold-type dose-response model is more appropriate. We show here how algebraic methods can be used to calculate maximum concentration levels (MCLs) for potential TCE-induced cancer in humans from no-effect level (NOAEL) data for corresponding cytotoxic endpoints in experimental animals, assuming: (i) a cytotoxic mechanism of action, (ii) TCE-induced cytotoxicity best correlates with peak metabolite concentration, and (iii) previously developed physiologically based pharmacokinetic (PBPK) models adequately estimate environmental TCE concentrations corresponding to cytotoxicity NOAELs. These methods may be substituted for more tedious, numerical methods typically used to calculate effective dose defined as a peak concentration for PBPK-based risk assessment. Mouse liver and rat kidney were bioassay tumor sites considered positive for this analysis. We first demonstrate reasonable consistency between bioassay tumor/toxicity patterns and the PBPK-predicted cytotoxic-effective doses we calculate for mice and rats using our assumed effective-dose measures. Then, using analytic methods and a standard 1000-fold safety factor, we obtain MCLs for TCE of: 16 ppb (87 $\mu g/m^3$) for TCE in air respired 24 hr/day, 700 ppb (3.8 mg/m^3) for TCE in air respired for relatively brief daily periods (e.g., 0.5 hr while showering/bathing), and 210 μg/liter for TCE in drinking water assuming a daily 2-liter ingestion. Corresponding effective doses obtained for occupational respiratory exposures indicate that current OSHA permissible exposure limits for TCE would produce metabolite concentrations that exceed an *acute* NOAEL for hepatotoxicity in mice, and would not be expected to be protective.

Key Words: Cancer, liver, kidney, mouse, rat, PBPK, threshold, toxicity, trichloroacetic acid.

Introduction

Cancer risk assessments for trichloroethylene (TCE) based on linear extrapolation from bioassay results are questionable in light of TCE's likely cytotoxic mechanism of action (see Appendix). In this paper, we show how simple algebraic methods can be used to calculate maximum concentration levels (MCLs) for TCE-induced cancer in humans from no-effect level (NOAEL) data for corresponding cytotoxic endpoints in experimental animals. In this analysis, we assume a cytotoxic mechanism of action with a threshold-type dose-response, assume that potential TCE-induced cytotoxicity best correlates with peak metabolite concentration, and use a previously developed physiologically based pharmacokinetic (PBPK) model (Allen and Fisher 1993; Fisher and Allen 1993) to estimate environmental concentrations of TCE that correspond to NOAELs for cytotoxicity. The algebraic methods we illustrate may be substituted for more tedious, numerical methods typically used to calculate effective dose defined as a peak concentration for PBPK-based risk assessment. As discussed below, we consider mouse liver and rat kidney to be positive TCE-bioassay tumor sites, and thus to indicate a plausible human cancer risk. Our analysis thus consists of (i) an assessment of the plausibility of our assumed measures of effective dose for these endpoints in light of bioassay toxicity and tumor data, and (ii) calculation of corresponding MCLs for TCE in water and air.

Results of 35 long-term, chronic exposure experiments of TCE are reported in the Carcinogenic Potency Database, including dosing by gavage or inhalation in several strains of rats and mice, and in hamsters (Gold et al. 1984, 1987, 1990, 1993). Results are summarized in Table 1 for organs evaluated as a target site in at least one experiment, along with p-values indicating the statistical significance of each site-specific data set. At each target site, there are individual p-values from several different experiments. With many p-values, some may be small just by chance. Table 1 therefore reports an overall p-value for each site, combining all the data by Fisher's method (Fisher, 1973).

This procedure tests the global null hypothesis (no carcinogenic effect at the site in any of the experiments). The results summarized in Table 1 indicate that:

(1) Target sites differ in rats and mice, and no tumors were induced in hamsters.

(2) For both rats and mice, results are inconsistent across experiments, and at each site some tests are negative.

(3) The strongest evidence for carcinogenicity is for mouse liver tumors, particularly in $B6C3F_1$ mice administered TCE by gavage. Results for administration by inhalation are less consistent across strains, and for two near-replicate tests in male $B6C3F_1$ mice, liver tumors were induced in one test but not in the other. Evidence indicating that some liver cytotoxicity and/or mitogenesis is likely to have occurred at these bioassay doses is discussed in the Appendix.

(4) In rats, there is evidence of carcinogenicity in kidney tubules for TCE given by gavage, but not by inhalation. Nearly all significant results occurred in bioassays evaluated by the National Toxicology Program (NTP) as inadequate due to toxicity and early mortality. In every test where tumors were increased, cytotoxic effects in kidney were observed (see Appendix). In nearly all of the NTP rat experiments with kidney tumors, tumor *decreases* were seen at other sites, with the strongest evidence for adrenal gland. The only significant increase by inhalation (in male Sprague-Dawley rats) was not considered a positive result by Maltoni et al. and was not seen in a near-replicate experiment. When adjusted for the effect of multiple tests, the overall result for malignant tumors of kidney tubules is not statistically significant ($p = 0.11$).

(5) Increases in benign tumors of the testis occurred in some rat strains, but not in others.

(6) Malignant tumors of the mouse lung were significantly increased ($p = 0.017$) in only 1 inhalation experiment out of 12; the only significant result for combined malignant and benign lung tumors was in a different inhalation test ($p = 0.001$). When adjusted for the effect of multiple tests, neither malignant nor combined benign and malignant lung tumors were significantly increased ($p > 0.05$).

Epidemiological studies generally have been evaluated as indicating either negative, inadequate, limited, or quite limited evidence concerning the hypothesis that TCE causes cancer in humans (IARC, 1982,1988,1995; EPA, 1985; Bogen et al., 1988; Fan,

1988; Brown et al., 1990; Weiss, 1996). Several recent studies concluded that environmental and occupational exposures to TCE have not caused cancer in humans (Fagliano et al., 1990; Mallin, 1990; Spirtas et al., 1991; Stewart et al., 1991; Axelson et al., 1994; Koivusalo et al., 1994). The IARC (1995) re-evaluation of epidemiological evidence as "limited" is based on small increases in liver and biliary-tract cancers and non-Hodgkin's lymphoma in two cohort studies of exposed workers (Spirtas et al., 1991; Anttila et al., 1995) and in a third study which was evaluated by its authors as negative (Axelson et al., 1994). None of these three studies controlled for likely confounding factors such as alcohol consumption, diet, and smoking. In the TCE-exposed cohort studied by Anttila et al. (1995), involving 1,698 male and 1,391 female factory workers over a 25-yr period for whom biological monitoring was conducted, only cervical cancer incidence was found to be significantly elevated during the entire study period; however, among workers with 20+ yr since the first exposure measurement, incidence was found to be significantly elevated for all sites, stomach, prostate, lympho-hematopoietic tissues, and for liver in all workers (SIR = 6.07, 95% CI = 1.25-17.7) and in male workers (SIR = 13.0, 95% CI = 2.68-37.9). Liver-cancer incidence was found in this study to be most (albeit not significantly) elevated in workers with the greatest urinary concentrations of the TCE metabolite, trichoroacetic acid (TCA) (Anttila et al., 1995). A recent retrospective cohort study found the incidence of kidney tumors to be significantly increased (SIR = 11.2, 95%CI = 4.49-23.0) among workers in a German cardboard factory who experienced prolonged exposures to TCE dermally and by inhalation of high air concentrations often causing "drowsiness and headaches" (Henschler et al., 1995). In another study, evidence for renal toxicity was not found in workers exposed chronically to ~50 mg/m^3 TCE in air (Seldén et al., 1993).

Based on animal and human evidence, we selected liver and kidney as target sites for deriving MCLs to protect against TCE-induced cancer in environmentally exposed humans. Previous PBPK-based estimates of TCE's human cancer risk have relied on

integral dose measures, such as mg TCE metabolized/day/kg body weight, or area under the blood-concentration-times-time curve (AUC) for TCA (EPA, 1985,1987; Bogen, 1988; Bogen et al., 1988; Brown et al., 1990; Fisher and Allen, 1993; Clewell et al., 1995). Although these measures facilitate convenient algebraic methods to obtain PBPK-based cancer-risk estimates for continuous or time-varying exposures to a volatile, lipophilic parent compound like TCE (Bogen and McKone, 1987; Bogen, 1988; Bogen and Hall, 1989), they are not appropriate unless the mechanism of toxic action is one that integrates the rate of critical (e.g., DNA) damage over time, where damage is instantaneously proportional to toxicant concentration. This does not appear to be the case for TCE-induced liver and kidney cancers in rodent bioassays, which more likely involve cytotoxic mechanisms that correlate better with peak metabolite concentrations (see Methods). Typically, peak concentrations are calculated using numerical methods, if realistically complex PBPK models are used with exposure scenarios involving dynamic (e.g., daily water-ingestion) exposures (Bogen and Hall, 1989; Bogen, 1990). This paper illustrates simpler, algebraic methods to derive PBPK-/NOAEL-based MCLs for human exposure to TCE using the five-compartment PBPK model for TCE/TCA previously developed and applied by Allen and Fisher (1993) and Fisher and Allen (1993).

Using methods described below, we first assess the plausibility of the effective dose measures we used for liver and kidney endpoints by comparing PBPK estimates of these doses to corresponding bioassay data on toxicity and tumor incidence. We then apply traditional safety factors to address uncertainty in extrapolating cytotoxicity NOAELs from animals to humans. Details of PBPK and related analytic methods are presented in Methods. In Results, we assess the plausibility of our measures of effective dose and the MCLs we obtained for liver- and kidney-cancer endpoints that are potentially related to chronic human TCE exposure. The Discussion reflects on the results obtained and their possible regulatory significance.

Methods

NOAEL Identification and Adjustment. We assumed that TCE-induced carcinogenesis in mice is caused indirectly by cytotoxic effects of TCE's primary reactive metabolite, TCA (see Appendix). Peak blood concentrations correlate well with oxidative-damage-mediated hepatotoxicity for chlorinated solvents such as carbon tetrachloride and chloroform (see Bogen, 1990; Larson et al., 1994). Cytotoxic effects involving simultaneous effects on multiple targets (e.g., Ca^{+2} ion pumps destroyed by oxidative stress, or S-phase induction by increased metabolic or repair demands) are likely to have threshold-like (e.g., log-normal) dose-response relationships (Aitchison and Brown, 1957; Bogen, 1990). We therefore assumed that peak TCA concentration in plasma is the effective dose for liver cancer induced by TCE via TCA-mediated cytotoxicity elicited with a threshold-like dose-response. TCA-induced production of reactive substances, indicating oxidative tissue damage in B6C3F1 mice administered single doses of TCA by gavage, was not elevated above control levels at 100 mg/kg body weight (BW) (corresponding to a measured peak concentration, $Max(C_{TCA})$, of TCA in mouse blood plasma of 790 µmol/L or 130 mg/L), but was elevated at 300, 1000 and 2000 mg/kg doses (Larson and Bull, 1992a—see Appendix). The $Max(C_{TCA})$ value of 130 mg/L thus represents an acute NOAEL for TCA-induced liver cytotoxicity in these mice. For kidney, it was assumed that tumors are induced by TCE in rodents or humans only at cytotoxic levels of reactive TCE-conjugation metabolites, which are hypothesized to be formed predominantly or exclusively when oxidative TCE metabolism is relatively saturated (Goeptar, 1995—see Appendix).

By convention, 10-fold safety factors have been applied to an experimental NOAEL to obtain a corresponding MCL (Dourson and Stara, 1983). We used three such factors to reflect: (1) uncertainty in extrapolating an acute NOAEL to chronic concentrations expected to be cytotoxic in rodents, (2) uncertainty in extrapolating biologically effective dose from rodents to humans, and (3) human interindividual variability in

susceptibility to TCA-induced cytotoxicity. In particular, the first factor implies an estimated NOAEL of Max(C_{TCA}) = 13 mg/L for liver cytotoxicity in chronically exposed mice. This value is 7- to 8-fold less than that expected in the B6C3F1 mice recently shown to have significantly increased liver-DNA synthesis after 11 daily doses of 100 mg TCA/kg BW (Dees and Travis, 1994; see Appendix), based on the Larson and Bull (1992a) data cited above and a rate of 1-1.5 day^{-1} for TCA elimination from mouse plasma (Fisher and Allen, 1993).

PBPK Assessment of Cytotoxic Dose Measures. To model effective dose for endpoints of hepatocellular toxicity and liver cancer, we calculated C_{TCA} values for mice and humans for specified TCE or TCA exposure scenarios using the PBPK model described and parameterized by Fisher et al., (1991), Allen and Fisher (1993), and Fisher and Allen (1993). This model comprises four TCE compartments (liver, fat, and richly and poorly perfused viscera, with saturable TCE metabolism assumed to occur in liver) plus one compartment representing TCA in plasma. We first applied the model (implemented in *Mathematica*® 2.2, Wolfram, 1991) numerically to investigate the consistency of our assumed acute mouse NOAEL for liver cytotoxicity with data for liver-cancer incidence in B6C3F1 mice that had been exposed chronically to: TCE 5 day/wk by gavage in corn oil (NCI, 1976; NTP, 1990; male and female mice), TCE 7 hr/day for 5 day/wk by inhalation (Maltoni et al., 1986; male and female mice), and TCA in drinking water (Herren-Freund et al., 1987; male mice). Corresponding C_{TCA} calculations used previously-reported parameter values (Fisher et al., 1991; Fisher and Allen, 1993); mouse respiratory values were additionally based on inverse-variance weighted averages of reported measured fractions of TCE that is metabolized to TCA (i.e., of P_0 values for each sex, from Table 4 of Fisher et al., 1991).

In female mice dosed daily by gavage or inhalation, the calculated weekly C_{TCA} patterns exhibited five daily peaks, followed by virtual emptying of all compartments by the end of the (nondosed) weekend, as noted by Fisher and Allen (1993). Such

complete emptying was not predicted, however, for male mice (Fig. 1), so care was taken to run all calculations for 2 to 3 wk to ensure attainment of virtual dynamic equilibrium in estimated peak C_{TCA} values. At dynamic equilibrium (after 6-7 wk of simulated exposure), bioassay and human effective liver dose, Max(C_{TCA}), was defined as the average of peak C_{TCA} values occurring on the last three days of each week, because, unlike 5-day weekly averages, these 3-day averages all had coefficients of variation ≤ ~5%. Max(C_{TCA}) values for 30-g mice exposed to 5 g/L (~1000 mg/kg-BW/day) TCA in drinking water were calculated using the above-referenced 1st-order model of TCA elimination from plasma in male mice. This calculation assumed 10 drinks of 100 mg/kg BW per day, the first of which was assumed to yield Max(C_{TCA}) = 130 mg/L (Larson and Bull, 1992a); Eq. (3) below was then used to obtain the corresponding peak value at dynamic equilibrium.

Malignant liver-tumor incidence data for bioassays of TCE- and TCA-exposed B6C3F1 mice (Table 1) were used to calculate increased tumor probability, P, assuming background independence. Each value P, based on a given bioassay duration t, was adjusted to the value P^* expected for a standard lifetime bioassay of duration t^* = 104-wk, using TD_{50} assumptions (Peto et al., 1984); i.e., $P^* = 1-(1-P)^{(t^*/t)^2}$. Data from these bioassays were then assigned to groups involving (1) all respiratory TCE doses and TCA dose in drinking water, (2) all gavage TCE doses to male mice, and (3) all gavage TCE doses to female mice. To facilitate comparison of these groups, a lognormal dose-response model, $P^* = \Phi\{\log_{10}[\mathrm{Max}(C_{TCA}) - \mu]/\sigma\}$ was fit to data in group 1. Similar fits of the parameter μ were then obtained for data groups 2 and 3, using the same value of σ obtained with group 1 for both groups 2 and 3 (due to the small number of points in the latter two groups).

To model effective dose for endpoints of nephrotoxicity and kidney cancer in rats and humans, PBPK models described and parameterized by Fisher et al. (1991) and Fisher and Allen (1993) were used to calculate weekly maxima for daily peak values of

the ratio Max(B)/K_m (defined in Table 2), which when >>1 indicates saturation of oxidative TCE metabolism. As for mice, care was taken to use only rat and human values obtained after virtual dynamic equilibrium was attained (1 wk for rats, 6-7 wk for humans). The plausibility of this ratio as a measure of effective kidney-cytotoxic dose was assessed by comparing calculated values to the degree of kidney toxicity observed in male Sprague-Dawley rats exposed to TCE by inhalation (7 hr/day, 5 day/wk) (Maltoni et al., 1986) and in male F344 rats exposed to TCE by gavage in corn oil (5 day/wk) (NTP, 1990). These are the only two studies of TCE-exposed rats that indicate statistically significant ($p < 0.05$) induction of malignant tubular-cell kidney tumors (see Table 1). The Maltoni et al. (1986) inhalation study also has the best available NOAEL for kidney toxicity in male rats.

Simplified Approach to Obtain Dynamic MCLs. As an alternative to using numerical PBPK calculations, we used a "steady-state" analytic approach to PBPK analysis (Bogen and McKone, 1987; Bogen, 1988; Bogen and Hall, 1989), applied to the Fisher-Allen PBPK model cited above. This approach was used to calculate PBPK-based MCLs for both static exposure to TCE in air, and dynamic exposure to TCE in drinking/household water. These MCLs for air and water correspond to cytotoxicity-related measures of effective dose at steady state and at dynamic equilibrium, respectively. Specifically, the steady-state metabolized fraction, f_{mr}, of respired TCE was estimated as

$$f_{mr} = \left[1 + \frac{Q_a}{P_b}\left(\frac{K_m}{V_{max}} + Q^{-1}\right)\right]^{-1} , \tag{1}$$

in which the parameters Q_a, Q, P_b, V_{max}, and K_m are described and corresponding parameter values used for humans are listed in Table 2. The corresponding steady-state metabolized fraction of an ingested dose was estimated as

$$f_{mo} = \left[1 + \frac{K_m}{V_{max}}\left(\frac{P_b}{Q_a} + Q^{-1}\right)^{-1}\right]^{-1} . \tag{2}$$

An analytic approach was also taken for exposure scenarios involving repeated, relatively brief daily infusions into a first-order system, resulting in multiple dosing kinetics characterized by a "sawtooth" approach to dynamic-equilibrium oscillation between a maximum and relative minimum values (see, e.g., Wiegand et al., 1963). Thus, after a sufficiently lengthy regime of such multiple dosing, peak concentrations at virtual dynamic-equilibrium all equal a fraction

$$f_{\text{deq}} = \frac{1-e^{-kt}}{1-e^{-kt_p}} \tag{3}$$

of the steady-state concentration that would be achieved if system input were continuous, where t is the assumed daily infusion duration, t_p is the exposure/non-exposure cycle period (t_p = 24 hr), and k is the first-order rate constant governing loss (in this case, of TCA from plasma).

Results

Consistency of Dose Metrics with Data on Liver and Kidney Endpoints. Figure 2 plots normalized, increased probability of hepatocellular carcinoma incidence as a function of estimated effective dose, Max(C_{TCA}), for seven B6C3F1 mouse bioassays. All values of significantly increased liver-tumor incidence correspond to Max(C_{TCA}) values >130 mg/L, the NOAEL identified by Larson and Bull (1992a) for *acute* liver cytotoxicity. The lowest such value, 153 mg/L, is for dosed males in the NTP (1990) gavage study. Figure 2 also shows lognormal fits obtained (see Methods) to data from groups involving (1) all respiratory TCE and drinking-water TCA doses (square- and diamond points), (2) all oil-gavage TCE doses to male mice (open round and triangular points), and (3) all oil-gavage TCE doses to female mice (solid round and triangular points). Parameter estimates obtained for group 1 (not gavage) were μ = 490 mg/L and σ = 0.078; μ-estimates obtained for groups 2 and 3 (oil-gavage) were substantially lower (240 and 160 mg/L, respectively, conditional on σ = 0.078).

Table 3 lists PBPK-based estimates of toxicity-related effective dose calculated (as described in Methods) for kidney endpoints in male rats exposed chronically to TCE by inhalation (Maltoni et al., 1986) and by gavage (NTP, 1990). The values of the TCE-metabolism saturation index, Max(B)/K_m, shown for rats in Table 3 are substantially >1 for all exposed groups, and kidney toxicity was observed in all but one of these groups (the rats exposed to the lowest TCE concentration by inhalation). The corresponding values calculated for liver-effective dose shown for rats in this table are all substantially below 130 mg/L, the NOAEL for acute liver toxicity in B6C3F1 mice discussed above.

Prediction of Occupational Hazard. Table 3 also refers to different human occupational (8-hr/day, 5-day/wk) scenarios involving time-weighted average (TWA) respiratory exposures to ≤100 ppm TCE, which is the OSHA (1993) permissible exposure level (PEL). All the scenarios correspond to a calculated effective kidney dose ≤ ~1, indicating that TCE metabolism is not expected to be saturated in humans exposed to these levels. In contrast, the corresponding values shown for effective liver dose, Max(C_{TCA}), are all greater than the 130-mg/L *acute* NOAEL in mice (i.e., without safety factors), except that for the smallest TCE concentration considered (20 ppm—the mean exposure of 1,670 TCE-exposed workers reported by Axelson et al. [1994] not to have had increased cancer rates, but considered by IARC [1995] to have had increased liver/biliary-tract cancer incidence).

Simplified MCL Calculation. The PBPK model used implies that the blood concentration, $C_{TCA}(t)$, of TCA in humans at time t changes at the rate

$$\frac{dC_{TCA}(t)}{dt} = \frac{P_0}{V_d}\left(\frac{B(t)V_{max}}{B(t)+K_m}\right)\frac{MW_{TCA}}{MW_{TCE}} - k_e C_{TCA}(t) \quad , \tag{4}$$

in which $B(t)$ is the concentration of TCE in venous blood exiting liver, and the other variates and their corresponding values used by Allen and Fisher (1993) are listed in Table 2. Substituting the appropriate values, Eq. (4) may be rewritten

$$\frac{dC_{TCA}(t)}{dt} = 0.0578\,L^{-1}\left(\frac{B(t)V_{max}}{B(t)+K_m}\right) - 0.00783\,hr^{-1}C_{TCA}(t) \quad , \tag{5}$$

here the parenthesized quantity in Eqs. (4)-(5) is simply the rate of TCE metabolism at time t conditional on $B(t)$. After continuous respiratory exposure to a given TCE concentration (C_{in}) in air, $B(t)$ eventually attains a steady-state value $B(\infty)$ that corresponds to a rate of TCE metabolism equal to $Q_a C_{in} f_{mr}$, where $f_{mr} = 0.671$ (using Eq. (1) in Methods and the reference Q_a value listed in Table 2). The steady-state solution to Eq. (5) for small C_{in} (such that $B(\infty) << K_m$) is therefore

$$\begin{aligned} C_{TCA}(\infty) &= 7.38\,hr\,L^{-1}Q_a C_{in} f_{mr} \\ &= 1450\,C_{in} \quad . \end{aligned} \tag{6}$$

We obtained a corresponding MCL for cytotoxicity-induced liver cancer by substituting the NOAEL TCA concentration of 130 mg/L for $C_{TCA}(\infty) = \mathrm{Max}(C_{TCA})$ in Eq. (6), and dividing this concentration by a 1000-fold safety factor, The MCL thus obtained is ~0.090 μg/L (16 ppb) for TCE in continuously respired air. At this air concentration, it is easily shown analytically (Bogen, 1988) that in this case $B(\infty) = 6.0\ 10^{-5}\ K_m$, which satisfies the $B(\infty) << K_m$ constraint.

For TCE-contaminated air that is inhaled for some relatively brief period t, the right-hand side of Eq. (6) must be multiplied by the fraction f_{deq} defined in Eq. (3) (see Methods and Table 1). For example, t = 0.50 hr might be assumed to reflect daily shower/bathroom use, in which case the corresponding value, $f_{deq} = 0.0228$, and a 1000-fold safety factor implies a 44-fold larger respiratory MCL (~700 ppb) for that daily exposure period, assuming other household air concentrations are negligible.

Similarly, Eq. (5) implies that small daily bolus TCE ingestions, each resulting in an approximately constant infusion of M (mg) over time t (hr), corresponds to a steady-state TCA concentration in blood of

$$\mathrm{Max}[C_{TCA}(\infty)] = 7.38\ hr\ L^{-1}\left(\frac{M f_{mo}}{t}\right) f_{deq} \quad , \tag{7}$$

in which f_{mo} (= 0.908) and f_{deq} were defined in Eqs (1-2) (Methods). Fisher and Allen (1993) assumed that human gastrointestinal TCE absorption was a first-order process with a rate constant of 5.5 hr^{-1}, which corresponds to a gastrointestinal uptake half-time of 7.56 min. However, for $t \leq 2$ hr, the fraction (f_{deq}/t) in Eq. (7) is relatively insensitive to different values of t, remaining approximately 0.0455 hr^{-1}. Therefore, Eq. (7) implies that ~427 g TCE must be ingested per day to achieve a peak TCA concentration in blood—again using a 1000-fold safety factor—equal to 0.130 mg/L at dynamic equilibrium, assuming ~100% absorption within 2 hr. This intake corresponds to an MCL of approximately 210 ppb for TCE in drinking water ingested at 2 L/day. Again, it is easily shown analytically that $B(\infty) = 1.1\ 10^{-3}\ K_m$ in this case (see Bogen, 1988), which satisfies the $B(\infty) << K_m$ constraint.

The MCLs obtained for TCE in water and air address liver cancer assuming a cytotoxic mechanism of action. Because these MCLs both satisfy a $B(\infty) << K_m$ constraint (by a factor of ~1000 or more), the corresponding values of the ratio $\mathrm{Max}(B)/K_m$ are also <<1 by a factor of ~1000 or more. The MCLs therefore also apply to kidney cancer under the assumption used that this endpoint results from cytotoxicity caused by reactive metabolites produced when TCE metabolism is relatively saturated.

Discussion

The comparison of calculated effective kidney doses vs. kidney toxicity incidence in rats (Table 3) shows that the measure of effective kidney dose used, $\mathrm{Max}(B)/K_m$, is consistent with relevant rat toxicity data. Effective liver doses we calculated are likewise consistent with data on increased liver-cancer rates observed in bioassay mice (Fig. 2), insofar as our predicted NOAEL of $\mathrm{Max}(C_{TCA})$ = 13 mg /L for chronic hepatocellular toxicity in mice falls well below all calculated effective liver doses at which significantly increased incidence has been observed for liver cancer (as well as induced DNA synthesis indicative of mitogenesis—see Appendix). However, Fig. 2 shows a clear difference between data from the NCI (1976) and NTP (1990) studies of

mice exposed to TCE by oil-gavage vs. data from both the Maltoni et al. (1986) study of mice exposed to TCE by inhalation and the Herren-Freund et al. (1987) study of mice exposed to TCA via drinking water. The inhalation/drinking-water data (squares, diamonds) lie on a gradually inclining continuum of tumor probability vs. Max(C_{TCA}), which departs substantially from background only where Max(C_{TCA}) > 250 mg/L. In contrast, the oil-gavage data (circles, triangles) reflect substantially elevated risks at Max(C_{TCA}) values between 100 and 200 mg/L.

The relatively greater risk per unit effective dose among oil-gavage B6C3F1 mice is consistent with the observed enhancement of liver toxicity and carcinogenicity of chloroform when administered by oil-gavage rather than via drinking water (Jorgenson et al., 1985; Larson et al., 1994). An increased potency of Max(C_{TCA}) arising from TCE administered in oil might be expected from the fact that the PBPK model we have used assumes that TCE is transferred to liver and kidney by blood perfusion, without consideration of higher TCE concentration in blood-borne oil droplets delivered to metabolizing and/or target cells (e.g., fat:blood partition coefficient of ~30-40 was used in the model for TCE in mice). Whatever mechanism is involved, the oil-gavage (but not the inhalation or drinking-water) data indicate enhanced liver cancer in mice is likely at Max(C_{TCA}) levels somewhat less than 130 mg/L. This highlights the importance of additional chronic toxicity data for TCE- and TCA-exposed mice with and without coadministration of oil. Liver cytotoxicity was not observed to be elevated in mice given an acute dose of 100 dichloroacetic acid (DCA) per kg BW (Larson and Bull, 1992a), whereas hepatocellular toxicity + increased liver cancer was observed in male B6C3F1 mice exposed for 104 wk via drinking water to ~93 mg DCA/kg-day (Daniel et al., 1992). Thus, chronic DCA exposure is apparently more hepatotoxic than an acute DCA dose. Such a difference might also be expected for TCA, although TCA is somewhat less hepatotoxic than DCA (see Appendix).

Our study has shown that PBPK-based MCLs can be calculated for cytotoxic endpoints, involving non-integral measures of effective dose, using analytic instead of more cumbersome numerical methods. By this method we obtained MCLs of 16 ppb for TCE in continuously respired air, 700 ppb in 30-min peak-daily air concentration (e.g., in the shower), and 210 ppb in ingested water (at 2 L/day). These results may have important implications concerning how best to manage risk posed by environmental TCE exposures. If our assumption of cytotoxic mechanisms for TCE's potential carcinogenic action in humans is correct, then, for example, the U.S. EPA and California MCLs of 5 ppb for TCE in water (C.F.R. 141.61(a)(5); CDHS, 1994) may be unnecessarily conservative by a factor of ~40. This conclusion pertains not only to purely ingestive exposure, but also to realistic multiroute exposure to TCE from household water, because TCE concentrations in shower and bathroom air are only ~2% and ~0.4%, respectively, of those in household water (McKone, 1987; Andelman, 1990). In contrast, cytotoxic effective doses predicted for occupational respiratory exposure scenarios (Table 3) indicate that the current OSHA (1994) PEL for TCE corresponds to a TCA concentration that exceeds an *acute* NOAEL for TCA-induced hepatotoxicity in mice. This analysis supports the earlier conclusion of Gold et al. (1994), based on direct comparison of administered doses (in mg/kg/day) of rodent carcinogens in bioassays vs. total respired occupational doses, that OSHA PELs may fail to protect workers from risks posed by carcinogenic chemicals in the workplace.

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APPENDIX:
Cytotoxicity and TCE-Induced Cancer

TCE is relatively unreactive, but is metabolized to a reactive epoxide which decomposes in the microsomal environment to chloral hydrate (CH), and in a relatively aqueous cytosolic environment to dichloroacetic acid (DCA), *N*-(hydroxyacetyl)-aminoethanol, glyoxylic acid, formic acid and/or carbon monoxide; CH in turn is oxidatively metabolized in rodents and humans principally to the metabolites trichloroacetic acid (TCA) and trichloroethanol, of which TCA has the longest half-life in circulated blood (Dekant et al., 1984,1986a; EPA, 1985; Rouisse and Chakrabarti, 1986; Davidson and Beliles, 1991; Larson and Bull; 1992a; Templin et al., 1993). The metabolites are then subject to further spontaneous degradation, enzyme-mediated oxidative and/or reductive metabolism, and/or glutathione- or glucuranide-conjugation (Davidson and Beliles, 1991; Larson and Bull; 1992a; Templin et al., 1993). In rodents and humans, TCA partitions to extracellular water and is very tightly and extensively bound to plasma protein. Roughly 50-80% of metabolized TCE is excreted as urinary TCA and free plus glucuranide-conjugated urinary trichloroethanol (Bogen et al., 1988; Davidson and Beliles, 1991). In both rats and mice, TCA has been shown to be metabolized (probably oxidatively) to DCA (Larson and Bull; 1992a), which appears as a relatively small percentage (1-2%) of urinary metabolites (Hathaway, 1980; Dekant et al., 1984; Green and Prout, 1985; Larson and Bull, 1992a); in TCE-exposed humans, DCA has not been measured as a urinary or other metabolite (Hathaway, 1980; Dekant et al., 1984; Green and Prout, 1985); only ~5% of TCE-epoxide formed is degraded to either DCA or other non-CH-metabolites (Allen and Fisher, 1993).

Liver Cancer. In mice, acute and chronic cytotoxicity induced by TCE occurs primarily in liver, the principal site of TCE metabolism. Liver toxicity in mice correlates well with the total amount of TCE metabolized, indicating that major forms of TCE-induced chronic cytotoxicity are almost certainly caused by TCE metabolism or

metabolites rather than by TCE itself (Buben and O'Flaherty, 1985; Prout et al., 1985; EPA, 1985,1987; Rouisse and Chakrabarti, 1986; Davidson and Beliles, 1991). Several studies have shown that liver and kidney weights were significantly increased, and liver-related serum enzyme levels were significantly perturbed, in different strains of mice (including B6C3F1) exposed chronically to TCE by inhalation or by corn-oil gavage (Kjellstrand et al., 1982,1983a-b; Buben and O'Flaherty, 1985). These toxic effects were observed at doses lower than those administered in positive cancer bioassays (Table 1). However, in male Swiss-Cox mice dosed with TCE in corn oil 5 day/wk for 6 wk by gavage, elevated serum SGPT levels were observed at daily doses of 1600-3200, but not at 200-800 mg TCE/kg BW, which was considered evidence of a threshold for induction of liver cytotoxicity (Buben and O'Flaherty, 1985; Bruckner et al., 1989). All male and female B6C3F1 mice given 100, 250, 500 or 1000 mg TCE/kg BW in corn oil by gavage for 10 days were observed to be clinically ill and show some liver histopathology (Dees and Travis, 1993). While reports of TCE-induced liver cancer in bioassays (NCI, 1976; NTP, 1988,1990; Maltoni et al., 1986) do not implicate frank hepatocellular toxicity (Table 1), these studies included neither blood-chemistry data nor detailed examinations of mild or subtle cytotoxicity; furthermore, if a tumor was present, toxic lesions were often not recorded (J. Ward, U.S. National Toxicology Program, pers. commun.).

Major TCE metabolites (chloral hydrate, TCA and DCA) all induce liver cancer in mice when administered in buffered drinking water (Herren-Freund et al., 1987; Bull et al., 1990; DeAngelo and Daniel, 1990; DeAngelo et al., 1991; Daniel et al., 1992). TCE is hepatocarcinogenic in mice but not rats, which is consistent with the higher peak-plasma concentrations of TCA and DCA measured for B6C3F1 mice administered TCE in drinking water than those measured for similarly exposed F344 rats (Larson and Bull, 1992b). In mice, strain differences in metabolism are also noteworthy. TCA accounts for 7-12% of an oral TCE dose in B6C3F1 and Swiss mice, but is only a trace urinary

metabolite in NMRI mice (Dekant, 1986b). Liver and lung tumors were induced in TCE-exposed B6C3F1 and Swiss mice, but not in NMRI mice (Table 1). TCA and DCA are clearly hepatotoxic in mice at chronic tumorigenic oral doses, and somewhat less hepatotoxic in rats when administered in drinking water (Bull et al., 1990; Mather et al., 1990). After single oral doses of NaOH-buffered TCA or DCA (or CCl_4, as a positive control) in water, elevated TBARS (thiobarbituric-acid-reactive substances indicative of lipoperoxidative-stress-induced cell killing in liver, similar to that induced by CCl_4) was observed in B6C3F1 mice given 300, 1000 and 2000 (but not 100) mg TCA/kg BW, and in mice given 300 and 1000 (but not 100) mg DCA/kg (Larson and Bull, 1992a). Results for F344 rats were similar to those for mice, except that 300 mg TCA/kg failed to elevate TBARS in rats (Larson and Bull, 1992a).

DCA is a quite minor urinary TCE metabolite in rats (in which TCE is not hepatocarcinogenic) and mice (in which TCE is hepatocarcinogenic). In male B6C3F1 mice administered approximately 2000 mg (15 mmol) TCE per kg BW in 1-2% aqueous Tween 80, the measured area under the blood-concentration-times-time curve (AUC) for DCA was approximately 30-fold smaller than that for TCA (Larson and Bull, 1992b; Templin et al., 1993). Templin et al. (1993) concluded that DCA and TCA production kinetics were consistent with the hypothesis that TCE-induced mouse-liver cancers may be caused by either DCA or TCA or both. However, substantially increased DCA levels did not arise from TCE doses increasing from 100 to 2000 mg/kg in mice (Templin et al., 1993), in contrast to the increase in mouse liver cancer observed over this dose range (Table 1, Fig. 2). Thus, DCA does not easily explain a substantial fraction of mouse-liver cancers induced by TCE, and TCA is a more likely explanation.

Several mechanisms have been suggested to explain why TCE-induced cytotoxic effects in liver, such as cell division increased by peroxisome proliferation, may be fundamentally related to pharmacodynamics of TCE hepatocarcinogenicity in mice. Substantial peroxisome proliferation is observed in mice, but the effect is weaker in rats,

after administration of TCE by gavage in corn oil for 10 days; this has been considered evidence that differential peroxisome proliferation may explain differences in TCE-induced hepatocarcinogenicity in mice vs. rats (Elcombe, 1985; Elcombe et al., 1985; McClain, 1994). These observations also have been used to support the hypothesis that TCE is unlikely to induce liver cancer in humans, because monkey and human hepatocytes are much less susceptible to induction of peroxisome proliferation than rodent hepatocytes (Elcombe, 1985; Eacho et al., 1986; McClain, 1994).

Multistage theories of carcinogenesis imply that dose-related changes in cell-proliferation kinetics may alter tumor risk, without direct involvement of genotoxicity (Armitage and Doll, 1957; Moolgavkar and Knudson, 1981; Moolgavkar, 1983; Moolgavkar et al., 1988; Bogen, 1989; Ames and Gold, 1990; Cohen and Ellwein, 1990,1991; Preston-Martin, 1990; Monticello and Morgan, 1994). For some chemicals (e.g., chlorinated solvents that injure liver cells through oxidative stress), dose-response data for tumors and related cytotoxicity are substantially nonlinear or threshold-like, in accordance with cell-kinetic multistage predictions that presume a cytotoxic mechanism of action (Bogen, 1990; Larson et al., 1994; Smith et al., 1995). Acute and subchronic TCE administration in corn oil by gavage has been shown to significantly increase S-phase-DNA synthesis and proliferation in mouse hepatocytes (Mirsalis et al., 1985,1989; Dees and Travis, 1993,1994). Dees and Travis (1993,1994) assessed liver toxicity and hepatocellular proliferation in male and female B6C3F1 mice given 0, 100, 250, 500 and 1000 mg TCE/kg BW in corn oil by gavage for 10 days, and 100 μCi/kg [^{3}H]thymidine 6 hr prior to sacrifice. Not only did all treated mice appear clinically ill and show some liver histopathology (as noted above), but radiolabeled hepatocytes were also reported to be significantly increased in all treated groups. In a parallel study using TCA rather than TCE, all treated groups of mice appeared healthy, but all dose groups (100-1000 mg TCA/kg BW in corn oil by gavage for 11 days) had significant increases both in liver weights and in levels of hepatocellular [^{3}H]thymidine indicating DNA (Dees and

Travis, 1994). More recently, Ferreira-Gonzalez et al. (1995) showed that hepatocellular carcinomas induced by chronic lifetime exposure to DCA (at 1 or 3.5 g/L) or TCA (at 4.5 g/L) in drinking water have *ras* oncogene mutations with an overall incidence—and in the case of TCA a mutagenic spectrum—approximately equal to that seen in spontaneous tumors. This finding supports the hypothesis that TCE metabolites accelerate the growth of spontaneous tumors via cytotoxicity-mediated cell kinetic effects, rather than create new malignant cells via direct genotoxic effects.

In light of such pharmacodynamic considerations, current risk assessments for TCE based on linear-type risk extrapolations from bioassay liver-tumor data are increasingly considered implausible (Abelson, 1993; Steinberg and DeSesso, 1993). Steinberg (1993) recently made a similar point regarding CH. As noted above, CH is a primary TCE metabolite that is also carcinogenic and also metabolized to TCA and DCA. Noting that CH is a widely used sedative in both adults and children, Steinberg (1993) argued that a threshold model is appropriate for evaluation of cancer risks posed by medical uses of CH in humans because the rodent-cancer bioassay dose-response relationships for CH and its breakdown products TCA and DCA are "nonlinear", and because these bioassays all involved high, necrogenic doses which appear to be necessary for tumor induction.

Kidney Cancer

Different molecular mechanisms have been hypothesized to explain a possible link between chronic TCE exposure and kidney cancer in male rats (Table 1; NCI, 1976; NTP 1988,1990) and for an observed cluster of renal-cell tumors in highly exposed German factory workers (Henschler et al., 1995). In rats, renal cytotoxicity is a central element of one set of hypotheses. Most male and female rats chronically exposed to TCE in NCI and NTP bioassays involving F344 and other rat strains developed toxic nephrosis and ≥ 90% of rats (and mice) developed cytomegaly, which generally was most pronounced

in male rats; however, kidney tumors were positive/increased only in some male and no female rats (Table 1; NCI, 1976; NTP 1988,1990).

The *S*-dichlorovinyl-*L*-cysteine (DCVC) TCE conjugates, *S*-(1,2-dichlorovinyl)-*L*-cysteine (1,2-DCVC) and *S*-(2,2-dichlorovinyl)-*L*-cysteine, the corresponding mercapturic acids (DCV-Nac) formed by DCVC *N*-acetylation, *N*-acetyl-*S*-(1,2-dichlorovinyl)-*L*-cysteine and *N*-acetyl-*S*-(2,2-dichlorovinyl)-*L*-cysteine, and the glutathione TCE conjugate, *S*-(1,2-dichlorovinyl)glutathione (transformed to DCVC by γ-glutamyl-transferase as well as peptidase enzymes), are all rodent (and possible human) TCE metabolites that can produce proximal tubular necrosis and other (e.g., proliferative) nephrotoxic lesions in rat (and possibly human) kidney after conversion to reactive, mutagenic intermediates by (primarily cytosolic) cysteine conjugate β-lyase (Elfarra et al., 1984,1986a-b; Dekant et al., 1986b-c, 1989; Vamvakas et al., 1988; Commandeur et al., 1991; Wallin et al., 1992; Goeptar et al., 1995). In the absence of β-lyase activity, rat kidney cells are almost completely protected against 1,2-DCVC-induced cytotoxicity (Elfarra et al., 1986a; Stephens et al., 1986). Exposure of cultured rat kidney cells to 1,2-DCVC has also been shown to induce *c-fos* and *c-myc* protooncogene expression to levels that are also induced by growth factor (fetal calf serum) or by the tumor promoter, 12-*O*-tetradecanoylphorbolacetate (Vamvakas and Köster, 1993). This indicates the possibility that this TCE metabolite may be directly mitogenic to kidney cells, and thus promote kidney neoplasia. It is not currently known which toxic β-lyase metabolites are most responsible for induced cytotoxicity, gene expression, and likely mutagenicity in kidney cells exposed to TCE-conjugates (Goeptar et al., 1995). It is known that TCE-conjugation metabolites, such as DCV-Nac via the mercapturic acid pathway, are trace TCE metabolites formed in rodents (Commandeur and Vermeulen, 1990; Dekant et al., 1986a-b,1990) and possibly also in humans (Birner et al., 1993). Approximately 3-4 mmol/L DCV-Nac was measured in urine obtained 16 hr post exposure from workers exposed to "varying amounts" of TCE "during an 8-hr

work shift when cleaning metal parts" in a TCE bath, which concentrations were roughly 40% of those found in rat, and ~30-100% of those found in mouse, urine 22 hr after oral gavage exposure to 50 mg/kg TCE (Birner et al., 1993). However, occurrence of TCE-induced rat-kidney tumors is considered unlikely in the absence of severe chronic kidney damage, like that produced when amounts of TCE conjugation with cysteine and glutathione become significant upon saturation of the oxidative cytochrome-P_{450} pathway for TCE metabolism —as occurs in rats, but not mice, that are highly exposed to TCE (Goeptar et al., 1995).

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Table 1. Summary of bioassay results for trichloroethylene by species, strain, route and target site

Species / Route[a]	Tumor type / Strain[a]	Sex	Exposure (wk)	Experiment (wk)	Nonzero doses[a] $\times 10^{-2}$	Control	Tumor incidences[a]: Malignant Incidence			Benign + Malignant (Numerators)	Malig. p val	Mix p val	Paper[a]
Mouse	**Liver**												
Inh	B6C	F	78	154	1, 3, 6	3/88	4/89	4/88	9/85		.05		Mal86
(ppm)	B6C	M	78	154	1, 3, 6	1/59	1/31	3/38	6/37		.005		Mal86
	B6C	M	78	135	1, 3, 6	17/77	19/47	27/67	21/63		.244		Mal86
	NMR	F	78	129	1, 5	0/29	0/30	0/28		(0,0,0)	1	1	Hen80
	NMR	M	78	129	1, 5	1/30	0/29	0/30			1	1	Hen80
	IC	F	104	107	.5, 1.5, 4.5	0/49	0/50	0/50	0/46	(0,0,0,1)	1	.146	Fuk83
	Swi	F	78	145	1, 3, 6	0/84	0/89	0/86	1/86		.185		Mal86
	Swi	M	78	145	1, 3, 6	4/66	2/53	8/59	13/61		.002		Mal86
Gav	B6C	F	78	90	9, 18[a]	0/20	4/50	11/50		(0,4,11)	.003	.003	NCI76
(mg/kg/d)	B6C	M	78	90	12, 24[a]	1/20	26/50	30/50		(1,26,30)	<.0005	<.0005	NCI76
	B6C	F	103	105	10	2/50	13/50			(6,22)	<.0005	<.0005	NTP90
	B6C	M	103	105	10	8/50	31/50			(14,39)	<.0005	<.0005	NTP90
											$< 10^{-9}$ [b]	$<10^{-7}$ [b]	
Mouse	**Lung**												
Inh	B6C	F	78	154	1, 3, 6	0/90	1/90	0/89	0/87	(2,6,7,14)	1	.001	Mal86
(ppm)	B6C	M	78	154	1, 3, 6	0/85	0/86	0/88	0/88	(1,2,2,1)	1	1	Mal86
	B6C	M	78	135	1, 3, 6	0/90	0/89	0/90	1/90	(12,6,7,7)	.186	1	Mal86
	NMR	F	78	129	1, 5	1/29	3/30	0/28			1	1	Hen80
	NMR	M	78	129	1, 5	5/30	3/29	1/30			1	≥.948 [c]	Hen80
	IC	F	104	107	.5, 1.5, 4.5	1/49	3/50	8/50	7/46	(6,5,13,11)	.017	.062	Fuk83
	Swi	F	78	145	1, 3, 6	2/88	0/89	0/88	2/88	(15,12,12,16)	.733	.679	Mal86
	Swi	M	78	145	1, 3, 6	0/78	0/73	0/78	1/74	(10,5,12,12)	.183	.229	Mal86
Gav	B6C	F	78	90	9, 18[a]	0/20	2/50	2/50		(1,4 7)	.291	.193	NCI76
(mg/kg/d)	B6C	M	78	90	12, 24[a]	0/20	0/50	1/50		(0,5 2)	.319	.622	NCI76
	B6C	F	103	105	10	1/50	0/50			(1,4)	1	.090	NTP90
	B6C	M	103	105	10	3/50	1/50			(7,6)	1	.455	NTP90
											.65 [b]	.089 [b]	

Species / Route[a]	Tumor type / Strain[a]	Sex	Exposure (wk)	Experiment (wk)	Nonzero doses[a] × 10^{-2}	Control	Tumor incidences[a] Malignant Incidence			Benign + Malignant (Numerators)	Malig. p val	Mix p val	Paper[a]
Rat	Kidney, tubular cell carcinoma												
Inh (ppm)	SD	F	104	159	1, 3, 6	0/101	0/89	0/88	1/88	(0,1,0,1)	.176	.514	Mal86
	SD	M	104	159	1, 3, 6	0/87	0/86	0/80	3/85		.021		Mal86
	SD	F	104	159	1, 3, 6	0/38	0/39	0/39	0/39	(0,0,0,0)	1	1	Mal86
	SD	M	104	159	1, 3, 6	0/33	0/32	0/36	1/37	(0,0,0,1)	.197	.197	Mal86
	SD	F	104	107	.5, 1.5, 4.5	0/50	0/50	0/47	1/51[d]	(0,0,0,1)	.155	.155	Fuk83
	Wis	F	78	156	1, 5	0/28	0/30	0/30		(0,0,1)	1	.179	Hen80
	Wis	M	78	156	1, 5	0/29	0/30	1/30			.176		Hen80
Gav (mg/kg/d)	ACI	F	103	104	5, 10	0/50	1/50	1/50		(0,3,1)	.126	.066	NTP88
	ACI	M	103	104	5, 10	0/50	1/50	0/50		(0,1,0)	.360	.360	NTP88
	Aug	F	103	104	5, 10	0/50	2/50	0/50		(1,4,0)	1	.197	NTP88
	Aug	M	103	104	5, 10	0/50	1/50	0/50		(0,2,1)	.646	.139	NTP88
	F34	F	103	103	5, 10	0/50	0/50	1/50		(0,0,1)	.213	.213	NTP90
	F34	M	103	103	5, 10	0/50	0/50	3/50		(0,2,3)	.014	.008	NTP90
	Mar	F	103	104	5, 10	0/50	1/50	1/50		(1,2,1)	.071	.283	NTP88
	Mar	M	103	104	5, 10	0/50	0/50	1/50		(0,1,1)	.120	.097	NTP88
	OM	F	78	110	5, 10[a]	0/20	0/50	0/50		(0,0,0)	1	1	NCI76
	OM	M	78	110	5, 10[a]	0/20	1/50	0/50		(0,1,0)	.822	.822	NCI76
	OM	F	103	104	5, 10	0/50	0/50	0/50		(0,0,1)	1	.195	NTP88
	OM	M	103	104	5, 10	0/50	0/50	1/50		(0,6,2)	.196	.002	NTP88
	SD	F	52	140	.5, 2.5	0/30	0/29	0/26		(0,0,0)	1	1	Mal86
	SD	M	52	140	.5, 2.5	0/22	0/24	0/21		(0,0,0)	1	1	Mal86
											.11[b]	.0065[b]	
Rat	Testis tumor					All benign							
Inh (ppm)	SD	M	104	159	1, 3, 6	5/81	11/73	24/71	22/76			<.0005	Mal86
	SD	M	104	159	1, 3, 6	1/33	5/32	6/36	9/37[e]			.017	Mal86
	Wis	M	78	156	1, 5	4/29	0/30	3/30				1	Hen80
Gav (mg/kg/d)	ACI	M	103	104	5, 10	36/50	23/50	17/50				.116	NTP88
	Aug	M	103	104	5, 10	34/50	30/50	26/50				.631	NTP88
	F34	M	103	103	5, 10	46/50	47/50	32/50				.064	NTP90
	Mar	M	103	104	5, 10	17/50	21/50	32/50[e]				.0005	NTP88
	OM	M	78	110	5, 10[a]	0/20	0/50	0/50				1	NCI76
	OM	M	103	104	5, 10	0/50	0/50	1/50				.199	NTP88
	SD	M	52	140	.5, 2.5	0/30	0/30	1/30				.174	Mal86
												.00002[b]	

Species Route[a]	Tumor type Strain[a]	Sex	Exposure (wk)	Experiment (wk)	Nonzero doses[a] $\times 10^{-2}$	Control	Tumor incidences[a,f] Malignant Incidence		Benign + Malignant (Numerators)	Malig. p val	Mix p val	Paper[a]
Hamster	All tumor-bearing animals											
Inh (ppm)	Syr	F	78	129	1, 5	2/30	3/29	3/30		.739		Hen80
	Syr	M	78	129	1, 5	6/30	2/30	4/30		1		Hen80
Hamster	Hepatocellular carincoma											
Inh (ppm)	Syr	F	78	129	1, 5	1/30	0/29	0/30	(1,1,0)	1	1	Hen80
	Syr	M	78	129	1, 5	1/30	0/30	0/30	(1,0,0)	1	1	Hen80

[a] Abbreviations: Inh = inhalation 7 hr/day for 5 day/wk, Gav = gavage in oil 5 day/wk; B6C = B6C3F1, NMR = Han:NMRI, Swi = Swiss, SD = Sprague-Dawley, Wis = Han:WIST, Aug = August, F34 = Fischer 344, Mar = Marshall, OM = Osborne-Mendel, Syr = Syrian golden; Fuk83 = Fukuda et. al. (1983), Hen80 = Henschler et. al. (1980), Mal86 = Maltoni et. al. (1986), NCI76 = NCI (1976), NTP88 = NTP (1988), NTP90 = NTP (1990). Administered daily exposures are listed; NCI (1976) values listed for mice were applied for 66 wk (means were 1169 and 2339 mg/kg for low- and high-dose males and 869 and 1739 mg/kg for low- and high-dose females), and for rats were applied for 62 wk (means were 549 and 1097 mg/kg for low- and high-dose groups of either sex). All p-values for NCI and NTP studies are based on a life-table analysis; p-values for other studies are based on a test involving the summary incidence data shown (Gold et al., 1984). For benign+malignant tumors, numerators are listed in parentheses and corresponding p-values are indicated under the heading "mix p val". Note: all NTP (1988) studies were reported as "inadequate" for determining carcinogenicity due to reduced survival and other factors.

[b] Fisher's chi-square test was used to test the nonrandomness of sets of p-values corresponding to multiple studies within each of the indicated sets of species-and-site-specific studies (Fisher, 1973). The p-value of each summary test is given below each corresponding list of single-study p-values.

[c] Tumor incidences could not be combined for benign and malignant tumors; however, the p-values for all possible combinations were all ≥ the values shown.

[d] Clear cell carcinoma.

[e] One malignant tumor in high dose group.

[f] Also indicative of TCE carcinogenicity are results of a bioassay of male B6C3F1 mice exposed to 2 g/liter TCA in drinking water for 61 wk, at which time they were sacrificed (Herren-Freund et al., 1987). Hepatocellular carcinoma (carcinoma+adenoma) incidence was 0/22 (2/22) in control and 7/22 (8/22) in dosed animals; p = 0.001 (p = 0.027).

Table 2. Parameters in PBPK Model of TCE and TCA in Humans[a]

Parameter	Description		Value	Unit
C_{in}	Respired concentration of TCE in air		–	mg/L
M	Ingested TCE mass		–	mg
V_d	Volume of fluid distribution for TCA		7.1	L
$C_{TCA}(t)$	Concentration of TCA in V_d at time t		–	mg/L
$B(t)$	Concentration of TCA in venous blood exiting liver at time t		–	mg/L
Q_a	Alveolar ventilation rate,	reference	292.2	L/hr
		occupational	416.6	L/hr
Q	Blood perfusion rate to liver	reference	89.9	L/hr
		occupational	108.3	L/hr
P_b	Blood/air partition coefficient for TCE		9.2	–
P_0	Fraction of all metabolized TCE converted to TCA		0.33	–
V_{max}	Maximum rate of TCE metabolism		345.6	mg/hr
K_m	Michaelis constant (= $B(t)$ | [TCE metabolism rate = $V_{max}/2$])		1.5	mg/L
k_e	TCA clearance rate		0.00783	hr^{-1}
MW	Molecular weight,	TCE	131.4	g/mol
		TCA	163.4	g/mol

[a]PBPK model parameters and corresponding reference values are those reported by Fisher and Allen (1993) pertaining to trichloroethylene (TCE) and trichloroacetic acid (TCA) in a reference 70-kg adult. Higher occupational values for Q_a (10 m^3/day) and Q ($0.26Q_a$) were assumed for the present study.

Table 3. Toxicity-Related Estimates of TCE Metabolism in Bioassay Rats and Occupationally Exposed Humans[a]

Species (Strain)	Weight (kg)	Reference	TCE exposure scenario[b]	Metabolic measure[c] Max(C_{TCA}) (mg/L)	Metabolic measure[c] Max(B)/K_m (unitless)	Toxicity[d] liver (%)	Toxicity[d] kidney (%)	Cancer[d] liver (%)	Cancer[d] kidney (%)
Rat (SD)	.575	Maltoni et al. (1986)	Inhalation, 0 ppm	0	0	–	0	–	0
			Inhalation, 100 ppm	11	59	–	0	–	0
			Inhalation, 300 ppm	11	59	–	22	–	0
			Inhalation, 600 ppm	12	190	–	82	–	3.5
Rat (F344)	.350	NTP (1990)	Gavage, 0 mg/kg	0	0	–	0	–	0
			Gavage, 500 mg/kg	32	14	–	98	–	0
			Gavage, 1000 mg/kg	32	14	–	98	–	6.0
Human	70	Axelson et al. (1994)	Inhalation, 20 ppm	55	0.079	–	–	–(+)[e]	–
		ACGIH TLV	Inhalation, 50 ppm	136	0.21	–	–	–	–
		OSHA PEL	Inhalation, 100 ppm	264	0.51	–	–	–	–
		OSHA PEL	Inhalation, 0/200/300 ppm	260	1.1	–	–	–	–

[a]PBPK models and parameter values (see Table 2) are those reported by Fisher et al. (1991) for the rat (scaled as in that study for the weights indicated) and by Fisher and Allen (1993).

[b]Exposure scenarios refer to lifetime bioassays by inhalation (7 hr/day, 5 day/wk) and by gavage in corn oil (5 day/wk), and the following occupational (8-hr/day, 5-day/wk) scenarios: 20 ppm = mean exposure of 1,670 TCE-exposed workers studied by Axelson et al. (1994); 50 ppm = ACGIH's "TLV" allowed time-weighted average (TWA) exposure (ACGIH, 1993); 100 ppm = OSHA's allowed TWA exposure, and 4×{0 ppm for 62.5 min, 200 ppm for 52.5 min, 300 ppm for 5 min} is an OSHA-allowed daily pattern of maximal exposure pulses (OSHA, 1993).

[c]The weekly maxima, Max(C_{TCA}) (peak TCA concentration in plasma) and Max(B)/K_m (indicating, when >>1, saturated oxidative TCE metabolism), reflect assumed effective doses for cytotoxic endpoints in liver and kidney, respectively (see Methods).

[d]Listed values refer to animals with tubular cytomegaly/megalonucleocytosis (toxicity) and malignant tumors (cancer); – = not applicable or increased incidence not observed or reported.

[e]IARC (1995) interprets the Axelson et al. (1994) data on liver/biliary-tract tumors as suggestive, in contrast to the study's authors, who concluded there was no association between TCE exposure and cancer.

Figure Legends

Figure 1. Peak plasma concentration of trichloroacetic acid (TCA) in B6C3F1 mice administered trichloroethylene (TCE) 5 day/wk for one week, estimated by the physiologically based pharmacokinetic (PBPK) model of Fisher et al., (1991) and Allen and Fisher (1993), after virtual dynamic equilibrium is established according to this model (after 2-3 wk of such exposure). **(a)** Upper and lower curves correspond to high- and low-dose males, respectively, exposed by gavage (NCI, 1976; see Table 1); **(b)** upper, middle and lower curves correspond to positive studies of high-, middle- and low-dose males, respectively, exposed by inhalation (Maltoni et al., 1986; see Table 1).

Figure 2. Increased hepatocellular carcinoma probability vs. predicted weekly peak plasma concentrations of trichloroacetic acid (TCA) in female (solid points) and male (open points) B6C3F1 mice administered trichloroethylene (TCE) or TCA. These mice were exposed to TCE in oil 5 day/wk by gavage in studies by NCI (1976) (circles) and NTP (1990) (triangles); to TCE by inhalation 7 hr/day and 5 day/wk in positive studies by Maltoni et al. (1986) (squares); and to TCA in drinking water in the study by Herren-Freund et al. (1987) (diamond). Tumor probabilities were normalized for a standard lifetime bioassay duration (see Methods). Error bars denote ± 1 SD assuming ~binomial sampling error in estimated risks. Lognormal fits (dashed curves—see Methods) are shown for data groups involving (1) all respiratory TCE doses (squares) and drinking-water TCA doses (diamonds), (2) all gavage TCE doses to male mice (open circles and triangles), and (3) all gavage TCE doses to female mice (solid circles and triangles).

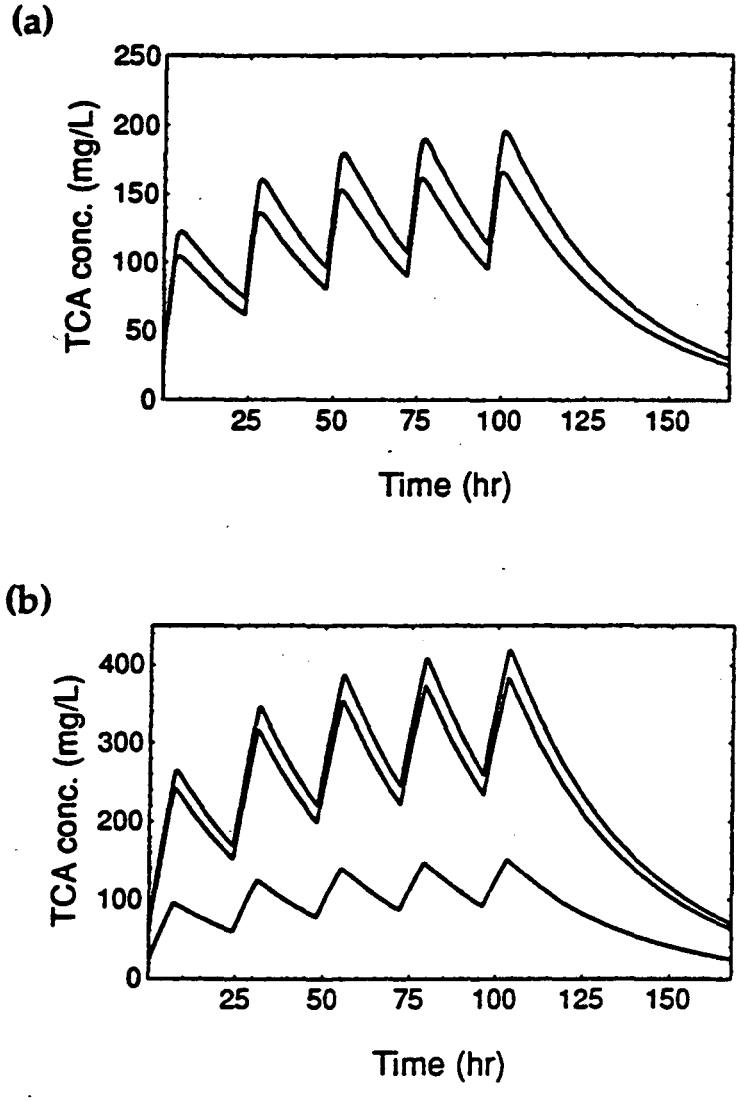

Figure 1

(Bogen and Gold, "Pharmacokinetic Extrapolation ..."; ↑ = up)

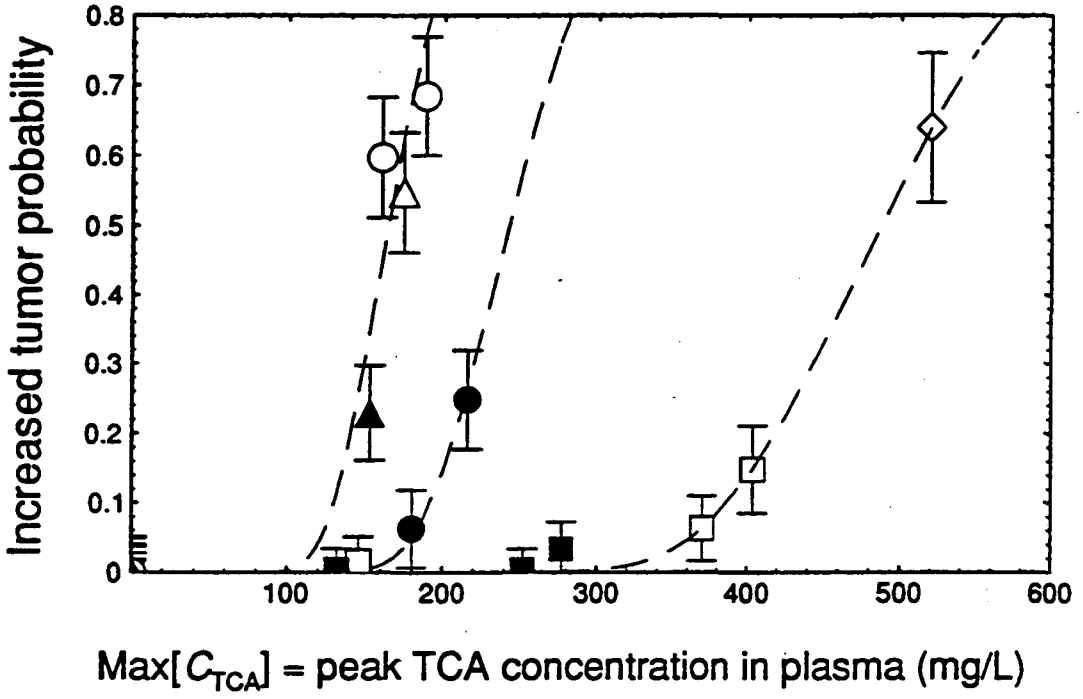

Figure 2

(Bogen and Gold, "Pharmacokinetic Extrapolation ..."; ↑ = up)

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