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Trichloroethylene Cancer Risk: Simplified Calculation of PBPK-Based MCLs for Cytotoxic End Points

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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 may be more appropriate. Previous studies have shown that if a genotoxic mechanism for TCE is assumed, algebraic methods can considerably simplify the use of physiologically based pharmacokinetic (PBPK) models to estimate virtually safe environmental concentrations for humans based on rodent cancer-bioassay data. We show here how such methods can be extended to the case in which TCE is assumed to induce cancer via cytotoxicity, to estimate environmentally safe concentrations based on rodent toxicity data. These methods can be substituted for the numerical methods typically used to calculate PBPK-effective doses when these are defined as peak concentrations. We selected liver and kidney as plausible target tissues, based on an analysis of rodent TCE-bioassay data and on a review of related data bearing on mechanism. Tumor patterns in rodent bioassays are shown to be consistent with our estimates of PBPK-based, effective cytotoxic doses to mice and rats used in these studies. When used with a margin of exposure of 1000, our method yielded maximum concentration levels 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. Cytotoxic effective doses were also estimated for occupational respiratory exposures. These estimates indicate that the current OSHA permissible exposure limit for TCE would produce metabolite concentrations that exceed an *acute* no observed adverse effect level for hepatotoxicity in mice. On this basis, the OSHA TCE limit is not expected to be protective. © 1997 Academic Press

INTRODUCTION

Cancer risk assessments for trichloroethylene (TCE)[2] based on linear extrapolation from bioassay results are questionable in light of data indicating likely cytotoxic mechanisms for TCE-induced cancer (summarized in the Appendix). While currently inconclusive, such data are the basis for current regulatory interest in methods to define safe levels of TCE exposure for cancer end points under a cytotoxic mechanism-of-action assumption, even if only for comparison with levels defined by default linear risk extrapolation based on assumed genotoxic mechanisms (EPA, 1996). In this paper, we show how algebraic methods can be used to calculate maximum concentration levels (MCLs) for hypothetical TCE-induced cancer in humans from data on the no observed adverse effect level (NOAEL) for related cytotoxic end points in experimental animals.

Previous PBPK-based risk estimates for TCE have used cumulative or average dose measures, such as average mg TCE metabolized/day/kg body weight, or area under a curve reflecting metabolite concentration vs time (EPA, 1985, 1987; Bogen, 1988; Bogen *et al.,* 1988; Brown *et al.,* 1990; Fisher and Allen, 1993; Clewell *et al.,* 1995). Such dose measures enable the use of simple, algebraic methods to estimate PBPK-based virtually safe environmental concentrations for

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[2] Abbreviations used: ACGIH, American Council of Governmental Industrial Hygienists; BW, body weight; CH, chloral hydrate; CI, confidence interval; DCA, dichloroacetic acid; EPA, U.S. Environmental Protection Agency; MCL, maximum concentration level; NCI, National Cancer Institute; NTP, National Toxicology Program; PEL, permissible exposure limit; PBPK, physiologically based pharmacokinetic; NOAEL, no observed adverse effect level; OSHA, U.S. Occupational Safety and Health Administration; SIR, standardized incidence ratio; TBARS, thiobarbituric acid-reactive substances; TCA, trichloroacetic acid; TCE, trichloroethylene.

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volatile organic compounds like TCE (Bogen and McKone, 1987; Bogen, 1988; Bogen and Hall, 1989). However, these integral measures are appropriate only if the toxic mechanism involves an accumulation of critical (e.g., DNA) damage over time. Rodent-bioassay and related data indicate that TCE-induced liver and kidney cancers most likely depend on cytotoxic mechanisms that correlate better with peak metabolite concentration than with total metabolite yield or average metabolite concentration (Appendix). Therefore, cumulative or average measures of dose are unlikely to be appropriate for these cancer end points.

Iterative, numerical methods are typically used to do PBPK calculations of peak concentrations of administered chemicals or metabolites in tissue or blood (Bogen and Hall, 1989; Bogen, 1990). We illustrate below how an alternative, algebraic method that Bogen (1988) applied to TCE, assuming a genotoxic mechanism of action, can be extended to the case in which a cytotoxic mechanism of action (driven by peak metabolite concentrations) is assumed. This method permits simplified estimation of safe concentrations for low-level human exposure scenarios, based on rodent cytotoxicity data. As described below, we first selected plausible target tissues for TCE-induced cancer under an assumed cytotoxic mechanism of action. The merits of this assumption are addressed in the Appendix. We next assessed the plausibility of the measures we used for effective dose in target tissues by comparing PBPK estimates of these doses to corresponding bioassay data on toxicity and tumor incidence. We then applied a margin of exposure of 1000 to address uncertainty in extrapolating acute cytotoxicity NOAELs in animals to safe chronic exposure levels in humans. Cytotoxic effective doses were also estimated for occupational exposure scenarios. Details of methods used for target-tissue selection and for PBPK analysis are presented under Methods. Under Results, we present our basis for target-tissue selection and assess the plausibility of the effective-dose measures and MCLs we obtained for cancer end points potentially related to chronic human TCE exposure. The Discussion and Conclusions reflect on results and regulatory implications.

METHODS

Carcinogenicity data and analysis. 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.,* 1997). These results are summarized in Table 1 for organs evaluated as a target site in at least one experiment. For each target site, the P value reported for each experiment indicates the statistical significance associated with a test of whether the slope of a corresponding fitted one-hit dose–response model is different from 0, taking into account life table data available for bioassays conducted by the National Cancer Institute (NCI) and the National Toxicology Program (NTP) (Peto *et al.,* 1984). With many experiments, some P values may be small just by chance. Therefore, Table 1 also reports an overall P value for each target site, using Fisher's method (Fisher, 1973) to combine all the study-specific results for that site. This procedure tests for each site the global null hypothesis that there is no carcinogenic effect in any of the experiments.

Epidemiological information was also considered for identification of plausible target tissues in humans. Epidemiological studies of TCE generally have been evaluated as indicating either inadequate, negative, or limited/inconsistent evidence of cancer causation (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) reevaluation 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 cohort 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 1698 male and 1391 female factory workers over a 25-year 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+ years since the first exposure measurement, incidence was found to be significantly elevated for several sites, including liver in men and women combined (SIR = 6.07, 95% CI = 1.25–17.7) and in men only (SIR = 13.0, 95% CI = 2.68–37.9). 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).

NOAEL identification and adjustment. In the PBPK analysis described below, TCE-induced cancer in mice is assumed to be caused indirectly by cytotoxic effects of TCE's primary reactive metabolite, TCA (see Appendix for a discussion of data bearing on the merits of this assumption). Peak blood concentrations corre-

TABLE 1
Summary of Bioassay Results for Trichloroethylene by Species, Strain, Route, and Target Site

Species and route[a]	Tumor type and strain	Sex	Exposure (weeks)	Experiment (weeks)	Nonzero doses[a] $\times 10^{-2}$	Tumor incidences[a]					Malignancy P value	Mix P value	Study[a]
						Control	Malignant incidence			Benign + malignant (numerators)			
Mouse	Liver												
Inh (ppm)	B6C3F1	F	78	154	1, 3, 6	3/88	4/89	4/88	9/85		0.05		1
	B6C3F1	M	78	154	1, 3, 6	1/59	1/31	3/38	6/37		0.005		1
	B6C3F1	M	78	135	1, 3, 6	17/77	19/47	27/67	21/63		0.244		1
	Han:NMRI	F	78	129	1, 5	0/29	0/30	0/28		(0, 0, 0)	1	1	2
	Han:NMRI	M	78	129	1, 5	1/30	0/29	0/30			1	1	2
	IC	F	104	107	0.5, 1.5, 4.5	0/49	0/50	0/50	0/46	(0, 0, 0, 1)	1	0.146	3
	Swiss	F	78	145	1, 3, 6	0/84	0/89	0/86	1/86		0.185		1
	Swiss	M	78	145	1, 3, 6	4/66	2/53	8/59	13/61		0.002		1
Gav (mg/kg/day)	B6C3F1	F	78	90	9, 18[a]	0/20	4/50	11/50		(0, 4, 11)	0.003	0.003	4
	B6C3F1	M	78	90	12, 24[a]	1/20	26/50	30/50		(1, 26, 30)	<0.0005	<0.0005	4
	B6C3F1	F	103	105	10	2/50	13/50			(6, 22)	<0.0005	<0.0005	5
	B6C3F1	M	103	105	10	8/50	31/50			(14, 39)	<0.0005	<0.0005	5
											$<10^{-9}$[b]	$<10^{-7}$[b]	
Mouse	Lung												
Inh (ppm)	B6C3F1	F	78	154	1, 3, 6	0/90	1/90	0/89	0/87	(2, 6, 7, 14)	1	0.001	1
	B6C3F1	M	78	154	1, 3, 6	0/85	0/86	0/88	0/88	(1, 2, 2, 1)	1	1	1
	B6C3F1	M	78	135	1, 3, 6	0/90	0/89	0/90	1/90	(12, 6, 7, 7)	0.186	1	1
	Han:NMRI	F	78	129	1, 5	1/29	3/30	0/28			1	1	2
	Han:NMRI	M	78	129	1, 5	5/30	3/29	1/30			1	≥0.948[c]	2
	IC	F	104	107	0.5, 1.5, 4.5	1/49	3/50	8/50	7/46	(6, 5, 13, 11)	0.017	0.062	3
	Swiss	F	78	145	1, 3, 6	2/88	0/89	0/88	2/88	(15, 12, 12, 16)	0.733	0.679	1
	Swiss	M	78	145	1, 3, 6	0/78	0/73	0/78	1/74	(10, 5, 12, 12)	0.183	0.229	1
Gav (mg/kg/day)	B6C3F1	F	78	90	9, 18[a]	0/20	2/50	2/50		(1, 4, 7)	0.291	0.193	4
	B6C3F1	M	78	90	12, 24[a]	0/20	0/50	1/50		(0, 5, 2)	0.319	0.622	4
	B6C3F1	F	103	105	10	1/50	0/50			(1, 4)	1	0.090	5
	B6C3F1	M	103	105	10	3/50	1/50			(7, 6)	1	0.455	5
											0.65[b]	0.089[b]	
Rat	Kidney, tubular cell carcinoma												
Inh (ppm)	Sprague–Dawley	F	104	159	1, 3, 6	0/101	0/89	0/88	1/88	(0, 1, 0, 1)	0.176	0.514	1
	Sprague–Dawley	M	104	159	1, 3, 6	0/87	0/86	0/80	3/85		0.021		1
	Sprague–Dawley	F	104	159	1, 3, 6	0/38	0/39	0/39	0/39	(0, 0, 0, 0)	1	1	1
	Sprague–Dawley	M	104	159	1, 3, 6	0/33	0/32	0/36	1/37	(0, 0, 0, 1)	0.197	0.197	1
	Sprague–Dawley	F	104	107	0.5, 1.5, 4.5	0/50	0/50	0/47	1/51[d]	(0, 0, 0, 1)	0.155	0.155	3
	Han:WIST	F	78	156	1, 5	0/28	0/30	0/30		(0, 0, 1)	1	0.179	2
	Han:WIST	M	78	156	1, 5	0/29	0/30	1/30			0.176		2
Gav (mg/kg/day)	ACI	F	103	104	5, 10	0/50	1/50	1/50		(0, 3, 1)	0.126	0.066	6
	ACI	M	103	104	5, 10	0/50	1/50	0/50		(0, 1, 0)	0.360	0.360	6
	Aug	F	103	104	5, 10	0/50	2/50	0/50		(1, 4, 0)	1	0.197	6
	Aug	M	103	104	5, 10	0/50	1/50	0/50		(0, 2, 1)	0.646	0.139	6
	Fischer 344	F	103	103	5, 10	0/50	0/50	1/50		(0, 0, 1)	0.213	0.213	5
	Fischer 344	M	103	103	5, 10	0/50	0/50	3/50		(0, 2, 3)	0.014	0.008	5
	Marshall	F	103	104	5, 10	0/50	1/50	1/50		(1, 2, 1)	0.071	0.283	6
	Marshall	M	103	104	5, 10	0/50	0/50	1/50		(0, 1, 1)	0.120	0.097	6

	Osborne–Mendel	F	78	110	5, 10[a]	0/20	0/50	0/50		(0, 0, 0)	1	1	4
	Osborne–Mendel	M	78	110	5, 10[a]	0/20	1/50	0/50		(0, 1, 0)	0.822	0.822	4
	Osborne–Mendel	F	103	104	5, 10	0/50	0/50	0/50		(0, 0, 1)	1	0.195	6
	Osborne–Mendel	M	103	104	5, 10	0/50	0/50	1/50		(0, 6, 2)	0.196	0.002	6
	Sprague–Dawley	F	52	140	0.5, 2.5	0/30	0/29	0/26		(0, 0, 0)	1	1	1
	Sprague–Dawley	M	52	140	0.5, 2.5	0/22	0/24	0/21		(0, 0, 0)	1	1	1
											0.11[b]	0.0065[b]	
Rat	Testis tumour					All benign (for testis only)							
Inh (ppm)	Sprague–Dawley	M	104	159	1, 3, 6	5/81	11/73	24/71	22/76			<0.0005	1
	Sprague–Dawley	M	104	159	1, 3, 6	1/33	5/32	6/36	9/37[e]			0.017	1
	Han:WIST	M	78	156	1, 5	4/29	0/30	3/30				1	2
Gav (mg/kg/day)	ACI	M	103	104	5, 10	36/50	23/50	17/50				0.116	6
	Aug	M	103	104	5, 10	34/50	30/50	26/50				0.631	6
	Fischer 344	M	103	103	5, 10	46/50	47/50	32/50				0.064	5
	Marshall	M	103	104	5, 10	17/50	21/50	32/50[e]				0.0005	6
	Osborne–Mendel	M	78	110	5, 10[a]	0/20	0/50	0/50				1	4
	Osborne–Mendel	M	103	104	5, 10	0/50	0/50	1/50				0.199	6
	Sprague–Dawley	M	52	140	0.5, 2.5	0/30	0/30	1/30				0.174	1
												0.00002[b]	
Hamster	All tumor-bearing animals												
Inh (ppm)	Syrian Golden	F	78	129	1, 5	2/30	3/29	3/30[f]			0.739		2
	Syrian Golden	M	78	129	1, 5	6/30	2/30	4/30			1		2
Hamster	Hepatocellular carcinoma												
Inh (ppm)	Syrian Golden	F	78	129	1, 5	1/30	0/29	0/30[f]		(1, 1, 0)	1	1	2
	Syrian Golden	M	78	129	1, 5	1/30	0/30	0/30		(1, 0, 0)	1	1	2

[a] Abbreviations: (Routes) Inh, ppm in air by inhalation 7 hr/day for 5 day/week; Gav, mg/kg/day by gavage in oil 5 days/week. Studies: 1, Maltoni et al. (1986); 2, Henschler et al. (1980); 3, Fukuda et al. (1983); 4, NCI (1976); 5, NTP (1990); 6, NTP (1988). Administered daily exposures are listed; NCI (1976) values listed for mice were applied for 66 weeks (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 weeks (means were 549 and 1097 mg/kg for low- and high-dose groups of either sex). All study-specific P values test the null hypothesis that the dose–response slope is 0; tests for NCI and NTP studies are based on a life-table analysis, and those for other studies are based on 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 value. Note: all NTP (1988) studies were reported as "inadequate" for determining carcinogenicity due to reduced survival and other factors.

[b] Fisher's χ^2 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 $\geqslant$ the values shown.

[d] Clear cell carcinoma.

[e] One malignant tumor in high-dose group.

[f] TCE carcinogenicity is also indicated by data on female B6C3F$_1$ mice exposed to 0, 2, 6.67, and 20 mmol/liter TCA in drinking water for 82 weeks, after which liver carcinoma incidence was 0/40, 0/40, 0/19, and 5/20, respectively ($P < 0.05$) (Pereira, 1996). Similar results were obtained in a 1-year mouse bioassays (Herren-Freund et al., 1987; Pereira and Phelps, 1996).

late 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 used peak TCA concentration in plasma as a biological effective-dose surrogate for TCE-induced liver cancer, which we assumed to be elicited by TCA-mediated cytotoxicity produced with a threshold-like dose response.

In B6C3F$_1$ mice administered single doses of TCA by gavage, production of reactive substances indicating oxidative tissue damage was not elevated above control levels at 100 mg/kg body weight (BW), but was elevated at higher doses (Larson and Bull, 1992a—see Appendix). The 100 mg/kg dose produced a measured peak concentration, Max(C_{TCA}), of TCA in mouse blood plasma equal to 130 mg/liter (790 μmol/liter) (Larson and Bull, 1992a). A Max(C_{TCA}) value of 130 mg/liter thus represents an acute NOAEL for TCA-induced liver cytotoxicity in these mice. For kidney, we 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 have 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. Note that the first factor implies an estimated NOAEL of Max(C_{TCA}) = 13 mg/liter for liver cytotoxicity in chronically exposed mice. This value is 10- to 40-fold less than the value of Max(C_{TCA}) expected in B6C3F$_1$ mice in which liver DNA synthesis was increased after 11 daily doses of 100 mg TCA/kg BW (Dees and Travis, 1994; see Appendix), based on the TCA pharmacokinetics data of Larson and Bull (1992a).

PBPK assessment of cytotoxic dose measures. To model effective dose for liver toxicity and 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 hepatocellular carcinoma incidence in B6C3F$_1$ mice that had been exposed chronically to TCE 5 day/week by gavage in corn oil (NCI, 1976; NTP, 1990; male and female mice), TCE 7 hr/day for 5 day/week by inhalation (Maltoni *et al.,* 1986; male and female mice), and TCA in drinking water (Pereira, 1996; female mice). Our C_{TCA} calculations used PBPK parameter values that were reported previously (Fisher *et al.,* 1991; Fisher and Allen, 1993); mouse respiratory values were additionally based on inverse-variance-weighted averages of the measured fraction, P_0, of TCE metabolized to TCA, using the sex-specific P_0 values reported by Fisher *et al.* (1991).

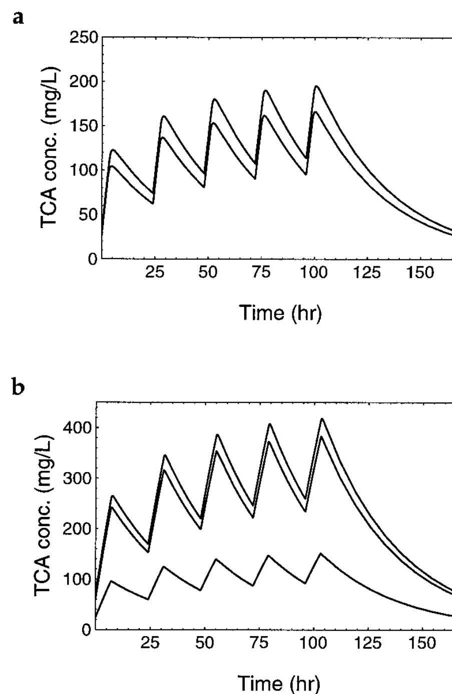

FIG. 1. Plasma concentration of TCA in B6C3F$_1$ mice administered TCE 5 day/week for 1 week, estimated by the PBPK model of Fisher *et al.* (1991) and Allen and Fisher (1993), after virtual dynamic equilibrium is established according to this model (after 2–3 weeks of such exposure). (a) Top and bottom curves correspond to high- and low-dose males, respectively, exposed by gavage (NCI, 1976; see Table 1); (b) top, middle, and bottom curves correspond to positive studies of high-, middle-, and low-dose males, respectively, exposed by inhalation (Maltoni *et al.,* 1986; see Table 1).

For female mice dosed daily by gavage or inhalation, predicted 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). The same pattern was predicted for rats. Such complete emptying was not predicted for male mice (Fig. 1), however, so care was

taken to run all calculations for 2–3 weeks to ensure attainment of dynamic equilibrium in estimated peak C_{TCA} values. For humans, 6–7 weeks of simulated exposure were required to attain virtual dynamic equilibrium. For all exposure scenarios considered, the daily peak C_{TCA} values occurring on the last 3 days of each simulated week at dynamic equilibrium were always found to have coefficients of variation $\leqslant \sim 5\%$. Effective liver dose, Max(C_{TCA}), was therefore defined for all species as this 3-day average value at dynamic equilibrium.

Max(C_{TCA}) values in female B6C3F$_1$ mice exposed by Pereira (1996) to TCA in drinking water (see Table 1, footnote *f*) were calculated based on data of Larson and Bull (1992a) indicating that 20 and 100 mg TCA/kg BW administered in water by gavage yields Max(C_{TCA}) values of 230 and 790 mmol/liter in B6C3F$_1$ mice, respectively, with first-order TCA elimination from plasma at rates $\geqslant 0.12$ hr^{-1}. The mice exposed chronically to TCA in drinking water initially drank ~20% of their BW per day (Pereira, 1996), which decreased to ~10% of BW by the middle of the study (M. A. Pereira, personal communication). We estimated Max(C_{TCA}) values for mice during the first half of the study, when daily water ingestion averaged ~15% of BW. A typical pattern of daily water consumption was assumed to comprise a brief, equal-sized drink once per hour over a 10-hr period during a 12-hr dark cycle and an 11th drink of the same size during the light cycle (Duffy *et al.,* 1990, 1991). Max(C_{TCA}) was calculated as the last peak in predicted plasma concentration during the dark cycle after attainment of dynamic equilibrium.

Malignant liver-tumor incidence data for bioassays of TCE- and TCA-exposed B6C3F$_1$ mice (Table 1) were used to calculate increased tumor probability, *P*, assuming background independence. Data from these bioassays were then assigned to two groups: one (Oil gavage) group exposed to TCE in oil by gavage, and the other (Nongavage) group exposed either to TCE in air or to TCA in drinking water. To facilitate comparison of these groups, a lognormal dose–response model, $P = \Phi\{\log_{10}[\text{Max}(C_{TCA}) - \mu]/\sigma\}$ was fit to the Nongavage data; a similar fit was obtained to the Oil gavage data by assuming that $\sigma \leqslant$ the Nongavage σ value (which allowed convergence using the Oil gavage data).

To model effective dose for end points 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, which, when $\geqslant 1$, indicates saturation of oxidative TCE metabolism (see Table 2). As was done for mice, care was taken to use only rat and human values obtained after virtual dynamic equilibrium was attained (1 week for rats, 6–7 weeks 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/week) (Maltoni *et al.,* 1986) and in male F344 rats exposed to TCE by gavage in corn oil (5 day/week) (NTP, 1990). These are the only two studies of TCE-exposed rats that indicate statistically significant ($P < 0.05$) induction of malignant tubular-

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
	Occupationa	108.3	L/hr
P_b	Blood/air partition coefficient for TCE	9.2	—
P_o	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 TCE and 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.

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. This approach was used to calculate PBPK-based MCLs, both for continuous exposure to TCE in air and for dynamic oral and respiratory exposures to TCE in drinking/household water. These MCLs 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

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

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

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

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,

$$f_{deq} = \frac{1 - e^{-kt}}{1 - e^{-kt_p}}, \quad (3)$$

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/nonexposure cycle period ($t_p = 24$ hr), and k is the first-order rate constant governing loss (in this case, of TCA from plasma).

RESULTS

Target tissues for carcinogenesis. The bioassay results and data analysis summarized in Table 1 indicate clear evidence that TCE induces tumors in some rodent tissues. Target sites differ in rats and mice, and no tumors were induced in hamsters. For both rats and mice, results are inconsistent across experiments, and at each site some tests are negative. 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. 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 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 ($P = 0.11$, Table 1). Increases in benign tumors of the testis occurred in some rat strains, but not in others. Malignant tumors of the mouse lung were significantly increased ($P = 0.017$) in only 1 of 12 experiments, and the only significant result for combined malignant and benign lung tumors was in a different experiment ($P = 0.001$, Table 1). When adjusted for the effect of multiple tests, neither malignant nor combined benign and malignant lung tumors were significantly increased ($P > 0.05$, Table 1).

Epidemiological evidence concerning possible carcinogenic effects of TCE in humans (see Methods) is generally considered inadequate, negative, or limited/inconsistent, but includes some data that (albeit inconclusively) suggest a positive effect for cancer in tissues that are clearly positive in rodent bioassays. This analysis of rodent bioassay results and the human evidence supports the selection of liver and kidney as target sites to illustrate our proposed simplified PBPK method of deriving MCLs designed to protect exposed humans.

Consistency of dose metrics with data on liver and kidney end points. For seven B6C3F$_1$ mouse bioassays, Fig. 2 plots the normalized increased probability of hepatocellular carcinoma incidence as a function of estimated effective dose, Max(C_{TCA}). All rates of significantly increased liver cancer correspond to Max(C_{TCA}) values >130 mg/liter, the NOAEL identified by Larson and Bull (1992a) for *acute* liver cytotoxicity. The lowest such value, 153 mg/liter, is for dosed males in the NTP (1990) gavage study. Figure 2 also shows

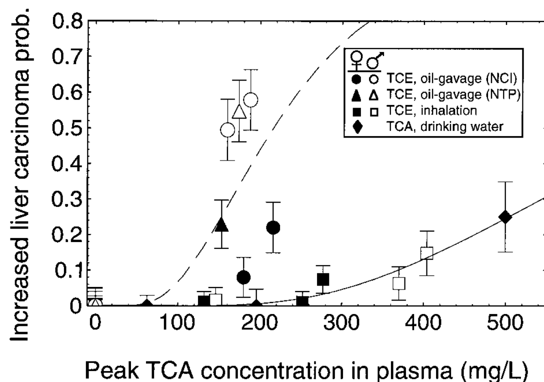

FIG. 2. Increased probability of hepatocellular carcinoma plotted as a function of Max(C_{TCA}), a PBPK-based estimate of weekly peak plasma concentration of TCA, calculated for female and male B6C3F$_1$ mice chronically exposed to TCE or TCA for ~2 years. Mice were exposed to TCE in oil 5 day/week by gavage (NCI, 1976; NTP, 1990), to TCE by inhalation 7 hr/day and 5 day/week (positive studies by Maltoni *et al.,* 1986), and to TCA in drinking water (Pereira, 1996) (see Table 1). Error bars denote ±1 SD assuming binomial sampling error. A lognormal fit (solid curve) is shown for the nongavage data involving respiratory and drinking-water exposures; a corresponding fit (dashed curve) is shown for the data involving exposure to TCE in oil by gavage.

lognormal fits to Oil gavage and Nongavage data. Parameter estimates obtained for the Nongavage data were $\mu = 710$ mg/liter and $\sigma = 0.22$; for the Oil gavage data, the corresponding estimates were $\mu = 220$ mg/liter and $\sigma = 0.22$.

For kidney end points, Table 3 lists PBPK-based estimates of toxicity-related effective dose 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, for rats shown in this table 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 calculated values of effective liver dose listed in Table 3 for rats (in which liver is not a TCE-related target site) are all substantially below 130 mg/liter, the NOAEL for acute liver toxicity in B6C3F$_1$ mice discussed above.

Prediction of occupational hazard. Table 3 also refers to various human occupational (8 hr/day, 5 day/week) scenarios involving time-weighted average respiratory exposures to ≤100 ppm TCE, which is the OSHA (1993) permissible exposure limit (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/liter acute NOAEL in mice (i.e., without safety factors), except that for the smallest TCE concentration considered (20 ppm—the mean exposure of 1670 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

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

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

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

here the parenthesized quantity in Eqs. (4) and (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) under Methods and the reference Q_a value listed in Table 2). The steady-state solution to Eq. (5) for small C_{in} (such that $B(\infty) \ll K_m$) is therefore

$$C_{TCA}(\infty) = 7.38\ \text{hr liter}^{-1}\ Q_a C_{in} f_{mr} = 1450\ C_{in}. \quad (6)$$

We obtained a corresponding MCL for cytotoxicity-induced liver cancer by substituting the NOAEL TCA concentration of 130 mg/liter for $C_{TCA}(\infty) = \text{Max}(C_{TCA})$ in Eq. (6) and dividing this concentration by a 1000-fold safety factor. The MCL thus obtained is ~0.090 μg/liter (16 ppb) for TCE in continuously respired air. At this air concentration, it is easily shown analytically (Bogen, 1988) that in this case $B(\infty) < 10^{-4} K_m$, which satisfies the $B(\infty) \ll K_m$ 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

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] Liver $Max(C_{TCA})$ (mg/L)	Kidney $Max(B)/K_m$ (unitless)	Toxicity[d] Liver (%)	Kidney (%)	Cancer[d] Liver (%)	Kidney (%)
Rat (SD)	0.575	Maltoni et al. (1986)	Inhalation, 0 ppm	0	0	—	0	—	0
			Inhalation, 100 ppm	17	1.4	—	0	—	0
			Inhalation, 300 ppm	35	59	—	22	—	0
			Inhalation, 600 ppm	43	190	—	82	—	3.5
Rat (F344)	0.350	NTP (1990)	Gavage, 0 mg/kg	0	0	—	0	—	0
			Gavage, 500 mg/kg	35	13	—	98	—	0
			Gavage, 1000 mg/kg	42	97	—	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/week) and by gavage in corn oil (5 day/week) and the following occupational (8 hr/day, 5 day/week) scenarios: 20 ppm, mean exposure of 1670 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 $Max(B)/K_m$ (which, when $\geqslant 1$, indicates saturated oxidative TCE metabolism), reflect assumed effective doses for cytotoxic end points in liver and kidney, respectively (see Methods).

[d] Listed values refer to animals with tubular cytomegaly/megalonucleocytosis (toxicity) and malignant tumors (cancer); —, not applicable, no data, 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.

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

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

in which f_{mo} (=0.908) and f_{deq} were defined in Eqs. (1) and (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 $t \leqslant 2$ 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/liter 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 liter/day. Again, it is easily shown analytically that $B(\infty) \approx 10^{-3} K_m$ in this case (see Bogen, 1988), which satisfies the $B(\infty) \ll K_m$ 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 $B(\infty) \ll K_m$ constraint (by a factor of $\sim 10^3$ or more), the corresponding values of the ratio $\text{Max}(B)/K_m$ are similarly $\ll 1$. The MCLs therefore also apply to kidney cancer under our assumption that this end point results from cytotoxicity caused by reactive metabolites produced when TCE metabolism is relatively saturated.

DISCUSSION

Our results indicate the general consistency of our calculated measures of effective dose with observed patterns of cytotoxicity and tumor induction in mice and rats. The comparison of calculated effective kidney doses vs kidney toxicity incidence in rats (Table 3) shows that the measure of effective kidney dose used, $\text{Max}(B)/K_m$, is consistent with relevant rat toxicity data. Effective liver doses we calculated are likewise consistent with data on increased liver-cancer rates observed in $B6C3F_1$ bioassay mice (Fig. 2), insofar as our predicted NOAEL of $\text{Max}(C_{\text{TCA}}) = 13$ mg/liter 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 studies involving exposure by gavage to TCE in oil (NCI, 1976; NTP, 1990) vs exposure either to TCE by inhalation (Maltoni *et al.,* 1986) or to TCA via drinking water (Pereira, 1996). The Nongavage data in Fig. 2 (inhalation, squares; drinking water, diamond) are described approximately by a relatively gradually inclining continuum of carcinoma probability vs $\text{Max}(C_{\text{TCA}})$ (solid curve), which departs substantially from background where $\text{Max}(C_{\text{TCA}}) > 250$ mg/liter. In contrast, the Oil gavage data (circles, triangles) reflect a steeper elevation in increased risk, departing substantially from 0 where $\text{Max}(C_{\text{TCA}}) > 150$ mg/liter. Figure 2 also indicates that if $\text{Max}(C_{\text{TCA}})$ is indeed a meaningful common measure of dose for liver toxicity and cancer, there is variability in susceptibility by sex within both the Oil gavage and the Nongavage groups of $B6C3F_1$ bioassay mice. Nevertheless, this figure appears to indicate a distinction between the Oil gavage and the Nongavage groups beyond any differences in sex-specific susceptibility.

The relatively greater risk per unit effective dose among oil-gavage $B6C3F_1$ 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). Such apparent enhancement of the "potency" of our calculated $\text{Max}(C_{\text{TCA}})$ values resulting from TCE administered in oil, as opposed to water, may be due to a lack of realism in the PBPK model we used. According to this model, TCE is transferred to liver and kidney by blood perfusion upon its partition into blood, without accounting for higher TCE concentrations expected in blood-borne oil droplets delivered to metabolizing and/or target hepatocytes after administration of TCE by oil gavage. (Note that a fat:blood partition coefficient of ~30–40 was used in the model for TCE in mice.) The $\text{Max}(C_{\text{TCA}})$ values we calculated for Oil gavage bioassay mice might thus underestimate those corresponding to TCA concentrations that actually arose in target hepatocytes after exposure. If so, a more refined analysis might predict larger $\text{Max}(C_{\text{TCA}})$ values for Oil gavage mice, such that the dashed curve shown in Fig. 2 would be closer to the solid curve corresponding to Nongavage data. Other explanations of the discrepancy between the Oil gavage and the Nongavage are possible. Resolution of this question would be facilitated by data comparing TCA and TCE concentrations in liver under different TCE-exposure conditions.

Liver cytotoxicity was not observed to be elevated in mice given an acute dose of 100 mg dichloroacetic acid (DCA)/kg BW (Larson and Bull, 1992a), whereas hepatocellular toxicity and increased liver cancer were observed in male $B6C3F_1$ mice exposed for 104 weeks 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).

The results discussed here may have important implications concerning how best to manage any risk posed by TCE exposures. If our assumption of cytotoxic

mechanisms for potential carcinogenic action of TCE in humans is correct, then the U.S. EPA and California MCLs of 5 ppb for TCE in water (United States, 1996; CDHS, 1994) may be overly stringent by a factor of ~40. This possibility 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 tumorigenic doses in rodents (in mg/kg/day) to permitted respiratory exposures in the workplace (in mg/kg/day), that OSHA PELs may fail to protect workers from risks posed by carcinogenic chemicals.

CONCLUSIONS

Previous PBPK-based estimates of human cancer risk from TCE have relied on cumulative or average measures of dose, based on the hypothesis that TCE induces cancer by virtue of its ability to cause DNA damage. Cancer risk assessments for TCE based on linear extrapolation from bioassay results are questionable in light of data indicating a more likely cytotoxic mechanism of action, which has spurred regulatory interest in methods to define safe TCE exposures under this alternative mechanistic assumption (EPA, 1996). We have shown here how the algebraic PBPK method applied by Bogen (1988) to TCE under a genotoxic-mechanism assumption can be extended to the case in which TCE is assumed to induce cancer via cytotoxic mechanisms that correlate better with peak metabolite concentration than with total metabolite yield or average metabolite concentration. The methods allow a simplified, algebraic estimation of environmentally safe concentrations based on PBPK-based extrapolation from rodent toxicity data, instead of iterative numerical computation methods typically applied when peak concentrations are used as measures of biologically effective dose.

Based on a new analysis of rodent-bioassay data, as well as a review of human data indicating little definitive additional information, we conclude that liver and kidney are appropriate target tissues for assessing risk of TCE-induced cancer. When applied to malignant tumor-incidence and acute-toxicity data, our measures of PBPK-based cytotoxic effective dose were found to be consistent with the assumption that increased tumor incidence is unlikely to occur in the absence of chronic cytotoxicity—an assumption also supported by a variety of experimental evidence (see Appendix). Our proposed method used with a 1000-fold margin of exposure yielded MCLs for TCE of 16 ppb (87 μg/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.

Cytotoxic effective doses were also estimated for occupational respiratory exposures. These estimates indicate that the current OSHA PEL for TCE would produce metabolite concentrations that exceed an *acute* no observed adverse effect level for hepatotoxicity in mice without *any* safety factors. On this basis, the OSHA PEL for TCE is not expected to be protective.

APPENDIX: CYTOTOXICITY AND TCE-INDUCED CANCER

TCE is relatively unreactive, but is metabolized to a reactive epoxide which is decomposed in microsomes to chloral hydrate (CH) and in cytosol to 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 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 tightly and extensively bound to plasma protein. Roughly 50–80% of metabolized TCE is excreted in urine as TCA and as free plus glucuranide-conjugated trichloroethanol (Bogen *et al.,* 1988; Davidson and Beliles, 1991). In both rats and mice, TCA is 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, TCE-induced cytotoxicity occurs primarily in liver, the principal site of TCE metabolism. Liver toxicity in mice correlates 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). Liver and kidney weights were significantly increased, and liver-related serum enzyme levels were significantly perturbed, in different strains of mice (including $B6C3F_1$) 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/week for 6 weeks 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 $B6C3F_1$ 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, personal communication).

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, Pereira and Phelps, 1996; Pereira, 1996). TCE is hepatocarcinogenic in mice but not rats, which is consistent with the higher peak-plasma concentrations of TCA and DCA measured for $B6C3F_1$ 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 $B6C3F_1$ and Swiss mice, but is only a trace urinary metabolite in NMRI mice (Dekant, 1986b). Liver and lung tumors were induced in TCE-exposed $B6C3F_1$ 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) were observed in $B6C3F_1$ 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 minor urinary TCE metabolite in rats (in which TCE is not hepatocarcinogenic) and mice (in which TCE is hepatocarcinogenic). In male $B6C3F_1$ mice administered approximately 2000 mg (15 mmol) TCE/kg BW in 1–2% aqueous Tween 80, the measured area under the blood concentration vs time curve 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 by 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 chemicals such as 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 analyses presuming 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 hepatotoxicity in male and female B6C3F_1 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 exhibit liver histopathology (as noted above), but radiolabeled hepatocytes were also significantly increased in all treated groups. In a parallel mouse study using 100–1000 mg TCA/kg BW in corn oil by gavage for 11 days, all treated groups appeared healthy but had significantly elevated liver weights and hepatocellular [^{3}H]thymidine levels (Dees and Travis, 1994). In B6C3F_1 mice exposed to either TCA (at 2, 6.67, and 20 mmol/liter) or DCA (at 6.67 or 20 mmol/liter) administered in drinking water, hepatocellular DNA-labeling index (indicating increased cell proliferation) was observed to be significantly elevated after 5 days of exposure, but not after 12 or 33 days of exposure (Pereira, 1996). Ferreira-Gonzalez *et al.* (1995) showed that hepatocellular carcinomas induced by chronic lifetime exposure to DCA (at 1 or 3.5 g/liter) or TCA (at 4.5 g/liter) in drinking water have a *ras* oncogene mutation frequency and (in the case of TCA) spectrum approximately equal to those seen in spontaneous tumors, indicating that TCE metabolites probably accelerate growth of spontaneous tumors via cytotoxic mechanisms, rather than create new malignant cells via genotoxic mechanisms.

Based on data like those summarized above, 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) made a similar point regarding CH, the 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 TCE-induced kidney cancer in male rats (Table 1 and NCI, 1976; NTP, 1988, 1990) and an apparent cluster of renal-cell tumors in highly exposed German factory workers (Henschler *et al.,* 1995). In rats, renal cytotoxicity is central to one set of hypotheses. Most rats chronically exposed to TCE in NCI and NTP bioassays involving F344 and other rat strains developed toxic nephrosis and $\geq$90% of rats (and mice) developed cytomegaly, which generally was most pronounced in male rats; however, kidney tumors were increased only in some male and no female rats (Table 1 and 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/liter DCV-Nac was measured in urine obtained 16 hr postexposure 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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