Biologically Based Dose–Response Model for Liver Tumors Induced by Trichloroethylene

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The existing extensive laboratory data on trichloroethylene (TCE) and its two metabolites, dichloroacetic (DCA) and trichloroacetic (TCA), are used to explore the relationship among these three compounds. Under the hypothesis that these compounds induce liver tumors in mice through promotion of preexisting initiated cells, it is demonstrated that DCA alone could be responsible for all the response of carcinomas in liver of $B6CF_1$ mice. The focus of this paper is on how a plausible biological assumption could impact on low-dose risk estimates, rather than on the risk estimate per se. The findings suggest that low-dose risk estimates to humans would be overestimated unless the different background rates between mice and humans are properly accounted for. *Key words:* DCA, dose–response model, liver tumor, TCA, TCE. — Environ Health Perspect 108(suppl 2):335–342 (2000).
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Although there is a large data gap before a true biologically based dose–response (BBDR) model can be constructed for mouse liver tumors, it is desirable to use the existing extensive bioassay data on trichloroethylene (TCE) and its two metabolites, dichloroacetic (DCA) and trichloroacetic (TCA), to explore the relationship among these three compounds and to evaluate its potential impacts on low-dose extrapolation. Data available include *a*) mid- to long-term DCA bioassays from different investigators (*1–5*), with water concentrations ranging from 0.05 to 5 mg/L (considered as one data set); *b*) mid- to long-term TCA bioassays from different investigators (*1,3–5*), with water concentrations ranging from 0.05 to 5 mg/L (considered as one data set); *c*) two TCE bioassays (*6,7*) with gavage doses ranging from 1,000 to 2,400 mg/kg/day; *d*) one TCE inhalation bioassay (*8*) with air concentrations ranging from 100 to 600 ppm; and *e*) an initiation–promotion (IP) study (*3*) with ethylnitrosourea (ENU) as initiator and TCE, DCA, and TCA as promoters (considered as three data sets). Data from all these studies include incidence rates of carcinomas and average number of carcinomas per animal. There is also supplemental laboratory information such as labeling index on normal hepatocytes, and c-*Jun*-positive cells by DCA, as reported in the mode of action paper by Bull (*9*). Given such an extensive data set, it is desirable to use these data to investigate the feasibility of a biological hypothesis and to investigate their implications on low-dose extrapolation. In addition to mouse liver tumors, TCE also induces other tumors (e.g., lung and kidney) in rats. However, the existing database is not sufficient to develop BBDR models for such tumors.

Specific Objectives

On the basis of bioassay data on TCE and its metabolites (DCA and TCA), it is possible to perform statistical analyses to investigate the feasibility of a biological hypothesis proposed by researchers: TCE induces liver tumors mainly via its metabolites, DCA and/or TCA. Both metabolites (DCA and TCA) act through the clonal expansion of preexisting initiated cells.

Under this hypothesis, animals with a higher background tumor incidence are expected to be more susceptible to tumor induction from TCE exposure, assuming that higher background tumor incidence is due to higher frequence of spontaneously induced initiated cells. Therefore, confirmation of this hypothesis would have profound implications on low-dose extrapolation. It should be emphasized, however, that the conclusions reached from our statistical investigations can at most serve as tentative guidance for future research to improve low-dose extrapolation; mathematical analyses alone cannot replace the need for actual laboratory investigations.

Our analyses focus on carcinomas in male mice because these data are more complete in male than in female mice. We recognize that other plausible hypotheses about liver tumors in mice can be postulated. For instance, hypotheses about interrelationships among preneoplastic and neoplastic lesions (foci, hyperplastic nodules, adenomas, and carcinomas) may be postulated. However, it requires more biological insight than presently available to support a particular hypothesis. It should be emphasized that the purpose of this paper is not to provide unit risk estimates; it is to demonstrate how a biological assumption could impact risk estimates at low doses even if the exact shape of the dose–response function is not known.

Preliminary Considerations

In this article, we focus on analysis of tumor incidence data, using other information (i.e., labeling index [LI] of c-*Jun*-positive cells, and averaged numbers of tumors/animal) to evaluate the reasonableness of statistically estimated parameters related to mitotic rate of initiated cells in the model. Since the LI for c-*Jun*-positive cells is available only for an age of about 45 weeks, it can only be used to check the reasonableness of statistically estimated parameters related to cell division rate of initiated cells around comparable ages. Our model allows the rate to vary over the animal's lifespan in a piecewise constant manner.

As mentioned earlier, if all lesions (e.g., foci, hyperplastic nodules, adenomas, and carcinomas) are considered together, a model of multiple-pathway carcinogenesis may be more appropriate. This approach is not adopted here because it would involve more assumptions requiring more data, and thus make any meaningful inference impossible. However, these types of data are very useful for constructing a BBDR model.

Because TCE may affect the growth of neoplastic lesions, the model should include a parameter reflecting such an effect. Furthermore, the model should allow for piecewise constant parameters because some parameters (e.g., cell mitotic rate) are known to be age dependent (*10*), and the data available have different exposure levels over time. For instance, in the low-dose group of the National Cancer Institute (NCI)TCE bioassay (*6*), animals were exposed to 1,000 mg/kg/day of TCE by gavage from age 5 weeks to 17 weeks, 1,200 mg/kg/day from age 17 weeks to 83 weeks, and followed up to age 95 weeks; in the high-dose group, exposure was 2,000 mg/kg/day from age 5 weeks to 17 weeks, 2,400 mg/kg/day from age 17 weeks to 83 weeks, and followed up to age 95 weeks. Under this experimental condition, the dose-affected parameters would have different values over different subintervals, with cutoff points at ages 5, 17, and 83 weeks. Exact exposure pattern over ages is used in all modelings considered in this report.

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Model Construction

Since DCA is a metabolite produced in the liver after exposure to TCA, a logical approach to determining the contribution of DCA and TCA toward tumor induction from TCE is first to develop a dose–response model for DCA based only on DCA bioassays. Once DCA dose response is determined, the next step is to determine the effect of TCA. Since TCA produces DCA as a metabolite, the effect of TCA can be determined once DCA dose response is known. As discussed later, this approach is feasible only when the amount of DCA in the liver can be estimated. When DCA in blood is used as dosimetry, more uncertainty is introduced in the risk assessment process of liver tumors, making it more difficult to interpret modeling results.

In this report, we adopt the biological framework (but not the deterministic formulation) suggested by Cohen and Ellwein (*10*), as well as the stochastic formulation by Chen and Farland (*11*) and its extended version (*12*). Three special features of this model are *a*) the mitotic rate is explicitly incorporated in the model, *b*) unlike most other two-stage models, a single malignant cell may not necessarily become a tumor, and *c*) the model allows for piecewise constant parameters. As in all two-stage models, the parameters cannot be uniquely estimated using a statistical optimization procedure (*13*).

An approach preferred by statisticians is to reparametertize parameters. Although this approach is statistically appropriate, it defeats the main objective of our investigation; namely, to evaluate the implication of the resultant model on low-dose extrapolation. It should be noted that it is important to keep each parameter as originally defined because the essential purpose of BBDR modeling is to study the behavior of dose response at low doses, and because the shape of the dose–response function at low doses is a function of the shape of each individual exposure-related parameter in a model (*14*). An advantage of keeping all model parameters as originally defined is that the impact of a biological parameter can be easily interpreted. For instance, a compound can induce cell population growth either by increasing cell birth rate b (i.e., b is dose dependent), or by decreasing cell death rate d (i.e., d is dose dependent). If the two parameters b and d are reparameterized as a single parameter $r = b/d$, it would be difficult to differentiate the biological implication described above. Therefore, a meaningful question to ask now is: Is there a set (unique or not) of DCA- and TCA-related parameters that could be used to predict dose–response data in all different studies available? Our approach is to seek such a set of parameters by maximizing the likelihood function (without actually attaining the absolute maximum from DCA (and TCA if necessary) bioassays by computer simulations to be explained below. To reduce the number of parameters to be estimated from bioassay data, NTP historical background data are used to estimate background parameters in the model. Without relying on the reparameterization method, an ad hoc approach to obtain parameter estimation is by *a*) obtaining a set of parameters corresponding to near optimal level (near maximum likelihood) and *b*) by alternately treating some (one or two, depending on the number of unknown parameters to be estimated) of parameters as known constants and then optimizing other parameters, with a goal to maximize likelihood function. This process can be repeated as many times as one desires. This approach would eventually yield a set of parameters that can then be used to predict response in other data sets, recognizing that the parameters may not be unique (in some statistical sense). It is interesting to point out that it is relatively easy to obtain the final parameters for the DCA model as presented in Table A-1 in the Appendix without going through as many iterative processes as one may expect; one reason may be that all those theoretically different sets of near-optimal parameters really make no practical difference in terms of predictability of other data sets.

Median areas under curve (AUC) of DCA and TCA in livers from the posterior distribution of a Bayesian statistical analysis (*15*) are used as dosimetrics in our dose–response analyses. These dosimetrics are provided by Bois (*15*), who has performed a Bayesian statistical analysis of the parameters in physiologically based pharmacokinetic (PBPK) models for TCE and its metabolites on the basis of a PBPK model developed by Fisher et al. (*16*), using Markov chain Monte Carlo simulation. Another PBPK model by Clewell (*17*) has not been adopted for modeling liver tumors because it does not provide AUC in liver for DCA and TCA; it provides only AUC in blood. As discussed later, if Clewell's PBPK model is used for calculating dosimetry, the conclusion about the role of tumor induction in TCE bioassays by DCA and TCA could be different from that when Fisher's model is used, and with more uncertainty.

A dose–response model for DCA is constructed by pooling all available bioassays (Table 1). Since TCA also induces formation of metabolite DCA, there are two dosimetrics (AUC–DCA and AUC–TCA) for the TCA dose–response model. Using the DCA-related parameters obtained previously from DCA bioassays, the next step is to construct a dose–response model for TCA on the basis of pooled data from TCA bioassays. The original plan was to use the dose–response models constructed for DCA and TCA to evaluate the dose–response relationships of other studies. It turns out that DCA alone could account for the carcinomas observed in all the other bioassays: TCE, TCA, ENU–TCE, ENU–TCA, and ENU–DCA. An attempt to consider both AUC–DCA and AUC–TCA in the dose–response model of TCA leads to an

Table 1. Goodness of fit for DCA-induced carcinomas in male mice.

Exposure (g/L)	Dose (mg/kg/day)	Duration (weeks)	AUC–DCA[a]	Incidence rate (95% CI)[b]	Predicted tumor incidence rate	Source (ref.)
0.5	77	104	14.01	15/24 (0.63) (0.43, 0.81)	0.61	(*1*)
0.05	7.6	75	1.38	3/21 (0.14) (003, 0.36)	0.16	(*2*)[c]
0.5	77	75	14.01	1/18 (0.06) (0, 0.27)	0.33	(*2*)[c]
3.5	350	60	63.66	8/12 (0.66) (0.30, 0.90)	0.59	(*2*)[c]
5.0	486	60	88.40	25/30 (0.83) (0.51, 0.94)	0.73	(*2*)[c]
5.0		61	88.40	21/26 (0.80) (0.61, 0.93)	0.76	(*3*)
1	122	52	22.19	0/11 (0.0) (0, 0.28)	0.20	(*4*)
2	213	52	38.74	5/24 (0.21) (0.01, 0.41)	0.30	(*4*)
2	213	37[d]	38.74	0/11 (0.0) (0, 0.28)	0.27	(*4*)
1	122	104	22.19	9/13 (0.69) (0.46, 0.99)	0.75	(*5*)
3.5	350	104	63.66	33/33 (1.00) (0.89, 1.00)	0.99	(*5*)

[a]AUC: Average daily area under curve of DCA in liver tissue. [b]95% confidence intervals are calculated from Rohlf and Sokol (*25*). [c]Tumor incidence data are taken directly from the original pathology report (*26*). [d]Exposed to DCA for 37 weeks and followed up to 52 weeks.

unreasonable dose–response prediction of other studies.

Modeling Results

As discussed previously, there are eight sets of dose–response data including TCE and its two metabolites, DCA and TCA, involving conventional bioassays and IP design of experiments (Tables 1–4). Although attempts were made to fit dose–response models on the basis of both DCA and TCA, no meaningful results could be obtained. That is, when we assumed that both DCA and TCA were responsible for TCE-induced tumors, we failed to obtain a set of parameters that can also be used to predict tumor response in other studies. We found that amount of DCA metabolite alone could adequately predict tumor response of TCA and TCE under either a conventional bioassay or IP study. Parameters for DCA-based dose response are given in Table A-1 in the Appendix. The goodness of fit of the DCA dose–response curve is given in Table 1. Since data from DCA were used as a base for fitting a dose–response model, it is not surprising to see a good fit for a DCA model. What is significant is that DCA alone can be used to predict tumor response in all other studies, as shown in Tables 2–4.

Goodness of Fit for the DCA Dose–Response Relationship

Data from five DCA studies are pooled as a data set (Table 1) to fit a dose–response model for DCA. These studies have two common features: *a*) sample size for each study was very small, ranging from 11 to 33 mice; *b*) most studies had a duration of less than 75 weeks. A group with 2 g/L of DCA in drinking water, from the study of Bull et al. (*4*), had the shortest duration of exposure at only 37 weeks and was followed up to 52 weeks. Among 11 dose groups, only 1 predicted value for the dosed group of 0.5 g/L from DeAngelo et al. (*2*) falls outside the 95% confidence interval (CI). We note that this dose group has unusually low observed incidence when compared to a much lower dosed group of 0.05 g/L; 14% (3/21) of tumor incidence in the lower dosed group (0.05 g/L) versus 6% (1/18) in the higher dosed group (0.5 g/L).

Predicting TCA Tumor Response on the Basis of Its DCA-Metabolized Dose Alone

TCA bioassays in Table 2 are a collection of TCA studies from four investigators. As shown in Table 2, the prediction of TCA studies by its DCA metabolite is surprisingly good. The TCA prediction is accomplished by using the metabolically generated DCA from TCA, the TCA bioassay tumors, and the model parameters obtained previously from the DCA bioassay. Only one predicted value from the 0.5 g/L group in Daniel et al. (*1*) falls outside the 95% CI. Note, however, that the observed tumor incidence of 0.38 in the 0.5 g/L group in the study of Daniel et al. seems unusually large compared to an incidence of 0.32 at a 10-fold higher concentration of 5.0 g/L in Bull et al. (*4*). For these two particular data points, the model appears to better predict the data from Bull et al. (*4*) than from Daniel et al. (*1*).

Predicting TCE Tumor Response on the Basis of Its DCA-Metabolized Dose Alone

There are three TCE bioassays available: gavage studies by NCI (*6*) and National Toxicology Program (NTP) (*7*), and an inhalation study by Bell et al. (*8*). For the NCI study (*6*), animals in the low-dose group were exposed to 1,000 mg/kg/day of TCE by gavage from age 5 weeks to 17 weeks, 1,200 mg/kg/day from age 17 weeks to 83 weeks, and followed up to age 95 weeks; animals in the high-dose group were exposed to 2,000 mg/kg/day from age 5 weeks to 17 weeks, 2,400 mg/kg/day from age 17 weeks to 83 weeks, and followed up to age 95 weeks. For the NTP study (*7*), animals were exposed to 1,000 mg/kg/day of TCE by gavage from age 5 weeks to 110 weeks. Animals (male mice) in the study of Bell et al. (*8*) were exposed to TCE by inhalation for 6 hr/day, 5 days/week, 104 weeks.

For both NCI and NTP studies in Table 3, observed values are Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality. However, only crude incidences are given for Bell's inhalation study because the time-to-event data are not available. Since crude incidence tends to underestimate true tumor rate because of intercurrent mortality, it is expected that predicted incidence is higher than the crude incidence as given in Table 3. Because the background incidences differ significantly among studies, comparison of predicted versus observed responses are made in terms of excess incidence = $P(t,d) - P(t,0)$, where t is the age at end of study. As discussed later, the excess risk is not the best way to remove

Table 2. Comparison of observed and predicted TCA-induced tumor response on the basis of its DCA metabolite alone.

Exposure (g/L)	Dose (mg/kg/day)	Duration (weeks)	AUC–DCA[a]	Observed incidence rate (95% CI)	Predicted incidence rate	Source (ref.)
0.05	7.6	60	0.70	2/9 (0.22) (0.03, 0.47)	0.11	(*1*)[b]
0.5	77	60	7.12	8/21 (0.38) (0.18, 0.61)	0.16	(*1*)[b]
4.5	441	95	40.80	21/24 (0.87) (0.68, 0.97)	0.85	(*1*)[b]
4.5	441	104	40.80	8/11 (0.73) (0.41, 0.94)	0.73	(*5*)
5.0	486	61	44.97	7/22 (0.32) (0.10, 0.54)	0.47	(*3*)
1	122	52	11.29	2/11 (0.18) (0.02, 0.51)	0.14	(*4*)
2	213	52	19.71	4/24 (0.16) 0.05, 0.36	0.19	(*4*)
2	213	37	19.71	3/11 (0.27) (0.06, 0.59)	0.17	(*4*)

[a]AUC: average daily area under curve of TCA in liver tissue. [b]The number of animals used in Daniel et al. (*1*) is estimated; only percentages of animals with tumors were reported.

Table 3. Predicted tumor response in TCE bioassays on the basis of its metabolite DCA alone.

Study (ref.)	AUC–DCA	Observed excess tumor incidence rate (95% CI)	Predicted excess tumor incidence rate
NCI, 1976 (*6*)			
1,000/1,200 mg/kg/day	66.45–67.53	0.61 (0.46, 0.75)	0.71
2,000/2,400 mg/kg/day	70.34–71.26	0.86 (0.72, 0.99)	0.73
NTP, 1990 (*7*)			
1,000 mg/kg/day	66.45	0.65 (0.51, 0.78)	0.76
Bell et al., 1978 (*8*)			
100 ppm	2.180	0.11[a]	0.12
300 ppm	6.214	0.13[a]	0.24
600 ppm	10.82	0.26[a]	0.36

[a]These are crude incidence without adjusting for intercurrent mortality and thus may seriously underestimate the true incidence. The observed (predicted) excess risk for dose d is calculated as the difference of tumor incidence between the exposed groups and control groups. The mortality-adjusted observed tumor incidence for the control groups (and 95% CI) are, respectively, 0.08 (0, 0.21), 0.24 (0.12, 0.37); and 0.18 (not available) for NCI (*6*), NTP (*7*), and Bell et al. (*8*).

the effect of differential background rates. Excess risk is used in Table 3 because it shows more transparently what is being done and because the difference is not as drastic as when cross-species extrapolation is involved.

Predicting Tumor Responses in an Initiation–Promotion Study Based on the DCA-Metabolized Dose Alone

In the IP study (*3*) male mice 15 days of age were administered ENU as an initiator by intraperitoneal (i.p.) injection of 2.5 or 10 g/g body weight. When animals were 4 weeks old, promoters (TCE, TCA, or DCA) in drinking water were added, and continued until 65 weeks of age. The effect of ENU is assumed to increase the initiation rate parameters during the day of injection and for 2 days afterward (i.e., during 15–17 days of age). Although ENU may also have a promotion and/or second mutation effect, it seems reasonable to assume that a single injection of ENU affects only initiation because promotion usually requires longer exposure, and it is not expected to have much effect on the second mutation because the injection occurs at a very early stage of life. The predicted values in Table 4 were calculated by adjusting the initiation rate parameter upward over the 15- to 17-day interval so that the predicted background tumor incidence was comparable to the observed incidence in the control groups, in which only ENU (without active promoters) was administered. All predicted values were within 95% confidence intervals of the observed incidence.

Evaluating the Model against Supplemental Data

On the basis of tumor incidence data alone, the modeling results suggest that DCA could be responsible for most of the tumor (carcinoma) response in TCE and TCA bioassays. There are two available data sets, labeling indices and number of carcinomas/animal, which were not used to reach this conclusion. Therefore, it is of interest to evaluate whether these data are consistent with the DCA dose–response model developed.

Data on Labeling Indices

Labeling indices on cells with c-*Jun*+ taken from within altered foci and tumors (*18*) are useful for checking reasonableness of cell division rates that are statistically estimated from incidence data. Stauber and Bull (*18*) measured replication rates within hyperplastic nodules and tumors in mice induced by 2 g/L DCA for 38 weeks and then mice were transferred to the indicated concentration of DCA in drinking water for an additional 2 weeks. Cell replication rates were measured by quantifying BrdU incorporation into DNA of dividing cells over a 3-day time interval.

As shown in Table 5, the model-based estimates of cell division rate around age 45 weeks appear to be comparable to the laboratory-based values, which are converted from LI to cell division rates by using a formula proposed by Moolgavkar and Luebeck (*19*). Note that these formula-converted rates are themselves subject to uncertainty because the formula can only be considered an approximation of reality.

Data on Number of Carcinomas per Animal

The number of tumors per animal is very useful data for constructing a biologically based dose–response model. However, these data cannot be used in modeling because the individual animal data are not available; for each dosed group, only average number of tumors per animal is available. To use these data, we summarized in Table 6 all available bioassays that consist of groups of animals exposed to both DCA and TCA at the same dose level and duration, with a sample size of 20 or more. Sample size is of concern here because no formal statistical test will be done. Data from DeAngelo et al. (*2*) and Daniel et al. (*1*) are not included in the table because there are no sample sizes provided in the TCA bioassay in Daniel et al. (*1*). In order to conclude that DCA is responsible for most of the tumor response in TCA bioassays, one should observe greater averaged number of tumors/liver in the DCA bioassays than in the TCA bioassays under the same experimental conditions. The numbers in Table 6 appear to support this expectation.

Role of DCA and TCA in TCE-Induced Carcinomas

We have demonstrated that it is possible to find a set of parameters that adequately describes the tumor (carcinoma) response

Table 4. Observed versus predicted tumor response in an initiation–promotion study of male mice on the basis of DCA dose alone.[a,b]

ENU (mg/g)	Promoter	Dose (mg/L)	AUC–DCA	Observed incidence rate (95% CI)	Predicted incidence rate
10	TCE	40	0.17	7/19 (0.37) (0.17, 0.58)	0.39
2.5		40	0.17	1/25 (0.04) (0.01, 0.20)	0.18
2.5		3	0.013	3/27 (0.11) (0.02, 0.29)	0.18
0		40	0.17	3/32 (0.09) (0.02, 0.25)	0.10
2.5	DCA	5	88.40	25/32 (0.78) (0.60, 0.91)	0.77
2.5		2	38.74	19/29 (0.66) (0.47, 0.82)	0.48
0		5	88.40	21/26 (0.81) (0.62, 0.93)	0.75
10.0	TCA	5	44.97	15/28 (0.54) (0.33, 0.73)	0.66
2.5		5	44.97	11/23 (0.48) (0.23, 0.72)	0.53
2.5		2	19.71	16/33 (0.48) (0.28, 0.69)	0.34
0		5	44.97	7/22 (0.32) (0.14, 0.54)	0.47

[a]Observed data from Herren-Freund et al. (*3*). [b]Effect of ENU is assumed to increase initiation parameter during 15–17 days of age such that the predicted and observed background tumor incidence rates are comparable.

Table 5. Comparison of laboratory-based versus model-based estimations of cell division rate for initiated cells at age of 45 weeks.

Concentration (g/L)	LI in c-*Jun*+ cells[a]	Laboratory-based cell division rate derived from LI[b]	Model-based cell division rate around the comparable age when LI was taken[c]
0.02	18	0.033	0.034
0.1	18	0.033	0.034
0.5	22	0.041	0.035
2	27	0.052	0.036

[a]Data adapted from Bull (*9*). Development of cell kinetic model for liver cancer induced by dichloroacetate. [b]Calculated by $[\log(1/(1 + LI/100))]/(2 \times t)$ where $t = 3$ days, proposed by Moolgavkar and Luebeck (*19*). [c]Calculated by $\alpha[\gamma_0 + \gamma_1 \times \log(1 + AUC\text{–}DCA)] = 0.523[6.631 \times 10^{-2} + 1.026 \times 10^{-3} \times \log(1 + AUC\text{–}DCA)]$, with parameters taken from Table A-1.

observed in DCA bioassays. Based on this DCA dose–response model, it in turn is capable of adequately predicting tumor response observed in seven other datasets. This suggests that DCA metabolites may be responsible for most, if not all, TCE-induced carcinomas. It should be noted, however, that this conclusion is based on carcinomas alone, as well as dosimetry calculated from Fisher's PBPK model. TCA may also play an important role in TCE-induced tumors if other neoplastic lesions (e.g., adenomas, hyperplastic nodules) are considered. The conclusion may also depend on the choice of PBPK model.

Uncertainty from Use of Different PBPK Models

There is a significant "numerical" difference with respect to the ratio AUC–DCA/(AUC–DCA + AUC–TCA) between Clewell's and Fisher's PBPK models. On the basis of Fisher's model, DCA (AUC–DCA in liver) accounts for about 1% of total metabolites (TCA and DCA combined) when TCE or TCA is administered as a parent compound. Conversely, if the Clewell model is used, DCA (AUC–DCA in blood) accounts for about 3% of total metabolites when TCE is administered as a parent compound, and about 0.1–10% of total metabolites, depending on administered dose level of TCA. However, one should view these numerical differences carefully by recognizing that two very different dosimetries are involved here: one is AUC in liver, another is AUC in blood. The same amount of DCA in blood, but that originated from different routes of exposure (e.g., inhalation and oral), may have very different implications in terms of its effects on liver tumor induction.

It is of interest to determine whether a different conclusion would be reached if dosimetry (AUC in blood) is calculated by the Clewell PBPK model. This question appears to be more difficult than it first appears because AUC–DCA in blood may have very different implications with respect to induction of liver tumors, depending on whether DCA, TCA, or TCE is administered as a parent compound. Since the liver is the target tissue, it is reasonable to assume that AUC–DCA in the liver has the same effect on liver tumor induction regardless of the route of exposure, but the answer to the question is not so straightforward when AUC–DCA in blood is used as a dosimeter. With this consideration in mind, a compromise for dose–response analysis is to use AUC–TCA as a biomarker to represent effect of TCA (which could include effects from both TCA and TCA-induced DCA). That is, AUC–DCA induced by TCA in the body is not explicitly considered (but it is represented by AUC–TCA) in dose–response modeling. We recognize that the choice of AUC–TCA as a biomarker to represent the effect of TCA and TCA-induced DCA in blood may result in overprediction of risk due to TCE exposure, as part of DCA in blood may come from TCA. However, the bias is expected to be small because TCA-induced DCA accounts for only a small proportion of the total dose (AUC–TCA + AUC–DCA) on the basis of the Clewell model, and because TCA is responsible for more than 70% of tumor response, as shown in Table 7. The analysis performed in this section is more to provide insight into the problem than to solve the problem itself.

If AUC in blood derived from Clewell's PBPK model is used as a dosimeter, it is possible to find a set of parameters from DCA and TCA that are capable of predicting tumor response in TCE oral bioassays (even though the predicted value for the high-dose group in the NCI study falls slightly below the 95% CI), but overpredict the response in the inhalation study (Table 7). One may argue, however, that the inhalation study's failure to predict could be explained by the fact that the AUC of DCA and TCA in blood after inhalation exposure to TCE differs (overestimated) from the AUC after gavage administration, which is the basis of data used to construct dose–response models for DCA and TCA (used to predict tumor response in TCE bioassays). If this argument is accepted, one could conclude that both TCA and DCA are responsible for TCE-induced tumors, even though the overall prediction result is not as good as the results when Fisher's model was used. As can be seen from Table 7, TCA appears to account for more than 70% of TCE-induced carcinomas.

Implications of Results for Risk Assessment

The conclusion that DCA (and possibly TCA also) is responsible for TCE-induced tumors by acting on spontaneously induced initiated cells has a profound implication on extrapolating risk from mice to humans. As demonstrated in Table 8, the excess risk at a given dose is more than 90-fold higher for a group with higher tumor background rate (0.23 for male mice) than for a group with lower background rate (0.002 for humans).

To extrapolate risk to humans from animal-based models, it is assumed that lifetime risk to humans can be calculated from the lifetime risk of animals by adjusting (reducing) the initiation parameter in the animal model so that the background lifetime risk is reduced from 0.23 for male mice to 0.002 for humans. On the basis of the Fisher model, AUC–TCA in liver is estimated to be 3.8×10^{-3} when humans are exposed to 1 g/L of TCE in water. The model does not provide an estimate for AUC–DCA. The lack of DCA in the human model is because there is no clear evidence of its formation in humans. In the absence of better data, one may assume that the ratio of AUC–DCA/(AUC–DCA + AUC–TCA), which is about 1% in mice, is identical with that of humans. Under this assumption, we see from Table 8 that the unit risk is reduced from 2.486×10^{-6} to 2.447×10^{-8} if the background tumor rate is reduced from 0.23 to 0.002, a 100-fold reduction of excess risk.

Table 6. Averaged number of carcinomas per liver in DCA and TCA bioassays with identical experimental conditions, and with at least 20 animals in a group.

Exposure (mg/L)	Duration (weeks)	AUC–DCA	AUC–DCA induced by TCA	DCA studies No. carcinomas/liver	TCA studies, No. carcinomas/liver	Source (ref.)
2	52	38.74	19.71	0.25	0.17	(4)
5	61	88.4	44.97	1.7	0.5	(3)

Table 7. Predicted tumor response in TCE bioassays when dosimeters derived from Clewell's PBPK model are used.

Study	Observed excess tumor incidence (95% CI)	Predicted excess tumor incidence rate		
		TCA only	DCA only	TCA + DCA[a]
NCI, 1976 (6)				
1,000/1,200 mg/kg/day	0.61 (0.46, 0.75)	0.52	0.07	0.66
2,000/2,400 mg/kg/day	0.86 (0.72, 0.99)	0.58	0.08	0.71
NTP, 1990 (7)				
1,000 mg/kg/day	0.65 (0.51, 0.78)	0.6	0.13	0.67
Bell et al., 1978 (8)				
100 ppm	0.11[b]	0.52	0.10	0.62
300 ppm	0.13[b]	0.59	0.13	0.67
600 ppm	0.26[b]	0.63	0.14	0.70

[a]Values in the last column, TCA + DCA, are calculated from the dose–response model with two dosimeters, TCA and DCA. These values need not equal the sum of values under TCA and DCA because different portions of the dose–response curve are involved at the given doses of DCA, TCA, and TCA + DCA. [b]These are crude incidences without adjusting for intercurrent mortality, and thus may seriously underestimate the true incidence. The excess risk for dose d is calculated as the difference of tumor incidences between exposed groups and control groups. The mortality-adjusted observed tumor incidences for controls (and 95% CI) are, respectively, 0.08 (0, 0.21), 0.24 (0.12, 0.37), and 0.18 (not available) for NCI (6), NTP (7), and Bell et al. (8).

Table 8. Lifetime excess risk—effect of DCA on species with different background tumor incidence rates.

AUC–DCA in liver (mg-hr/L)	Lifetime background tumor incidence rates 0.23	0.016	0.002
0.0000388	0.000002	0	0
0.0003871	0.0000245	0.000002	0
0.001935	0.000124	0.00001	0.000001
0.03871	0.002452	0.0001923	0.0000242
0.3817	0.02203	0.001749	0.00022
3.871	0.1517	0.01315	0.001663
5	0.1847	0.01641	0.002077

Research Needs

Biological Data

As demonstrated in this article, dosimetry plays an important role in our conclusion. Admittedly, the importance of PBPK models would be greatly reduced if the objective were not to explore the relationship among TCE and its two metabolites, DCA and TCA. If the relationship among these three compounds were known, any dose surrogate probably would do a reasonably good job of dose–response modeling. Nevertheless, the need to understand interaction between these compounds (or any other reactive metabolites) and the host tissue is obvious. Even if we accept a simple (yet significant for risk assessment) hypothesis that DCA and TCA promote the clonal expansion of initiated cells, there still would be a need to understand the dose–response relationship of these two compounds with respect to cell dynamics of initiated cells at all dose levels. Conceivably, to understand such a relationship would require extensive laboratory research at a molecular level, as discussed by Bull in this volume (*9*). The importance of knowing dose–response relationships for cell dynamic parameters in a dose–response model has been discussed by Crump (*14*). Crump (*14*) has shown that, at low doses, the shape of tumor dose–response function is completely dependent on the dose–response function of dose-dependent model parameters.

A potentially useful knowledge may be gained if the relationship between different preneoplastic and neoplastic lesions is understood. These end points, which include various types of foci, hyperplastic nodules, adenomas, and carcinomas, suggest that different mechanisms may induce liver tumors; some may be compound dependent and some may be spontaneously induced. It is difficult to conceive that these lesions are linearly related, meaning that one is a prerequisite for the others in linear sequence. This problem suggests the need to develop a more flexible modeling procedure than the two-stage models now used.

More Flexible Modeling Procedures

To understand the need for new modeling approaches, we have to discuss the shortcomings of existing approaches. Most existing models of carcinogenesis and the approaches used to develop them suffer from the following shortcomings:

- Only tumor incidence data can be adequately incorporated into the two-stage model. For instance, there have been attempts to use preneoplastic data such as foci (or nodules) in the liver to estimate rates of initiation and proliferation under the assumption that cells in foci (or nodules) are preneoplastic lesions. The problem with such an approach is that one cannot confidently decide which cells in foci or nodules represent the initiated cells defined in a two-stage model, even though it is reasonable to assume that foci and nodules are preneoplastic lesions. The dilemma of choosing either foci or nodules, but not both, to represent initiated cells in a two-stage model mandates the development of an alternative approach for BBDR modeling.
- In addition to the shortcoming above, the most serious drawback of the existing BBDR modeling approach is extreme difficulty in modifying the existing models to adopt a biological hypothesis beyond the framework of a two-stage, single-pathway model. For instance, in considering the shortcoming above when both foci and nodules are available, a natural question to ask is, "Why not construct a model with both foci and nodules as two intermediate stages of carcinogenesis?" Or even better, one could construct a stochastic model of carcinogenesis including both foci and nodules as two intermediate steps, along with a statistical (or observational) model with foci and nodules as observations. It is very difficult to accomplish even such a modest goal using the approach currently used to develop the two-stage models. However, this can be easily accomplished by using the state-space approach proposed in Tan and Chen (*20*), in which two systems of models are constructed: one consists of a set of stochastic differential equations consistent with the underlying hypothetical mechanism of carcinogenesis, and the other consists of an observational model that reflects both actual observations and the underlying mechanism. It is interesting to point out that this modeling approach is conceptually similar to PBPK modeling, except that PBPK modeling uses deterministic differential equations, not stochastic differential equations. Tan and Chen (*20*) have proposed a statistical approach and numerical algorithms for efficiently estimating model parameters under various scenarios of data that may become available in the future.

The above discussions should serve as motivations to develop more flexible biologically based dose–response models, which should be computationally simple yet biologically realistic.

Appendix: A Tumor Growth Model

Brief Description of Model and Its Parameters

Model Parameters and Notations

The following parameters are incorporated in the dose–response model, including initiation rate (μ_1), proliferation rate ($\gamma\alpha$), conversion rate ($\gamma\nu_2$), and probability of tumor progression (q). The death rate for the initiated cells is implicitly defined by $\beta = \gamma(1 - \nu_2 - \alpha)$. Some of these parameters are dose dependent.

- d dose of DCA, in terms of AUC–DCA, which may vary over time.
- D dose of TCA, in terms of AUC–TCA, which may vary over time.
- μ_1 initiation rate (per cell per day), which is assumed to be independent of dose.
- ν_2 probability of producing a malignant cell at the end of an initiated cell (I-cell) lifetime. ν_2 is assumed to be linearly related to dose.
- α probability that an I-cell divides into two daughter cells at the end of its lifetime. The parameter α is assumed to be dose independent.
- q probability that a single malignant cell will develop into a malignant tumor. The value q is assumed to be linearly related to dose.
- γ $1/\gamma$ is the mean I-cell lifetime in days; a cell's lifetime ends if it goes into mitosis, or with cell death. Note that if one assumes that the probability for a cell to enter into mitosis is about the same as cell death, then the mean cell lifetime can be conveniently interpreted as time to mitosis (i.e., cell turnover time); thus, shorter cell lifetime implies more frequent cell division. This cell turnover rate is assumed to be related to dose by $\gamma(d,D) = \gamma_0 + \gamma_1\log(1 + d) + \gamma_2\log(1 + D)$, which may be age dependent.
- $N(t)$: number of (normal) target cells in the population at age t.

It should be noted that the assumption that μ_1 is independent of dose is reasonable because it is not known that DCA either initiates or promotes proliferation of cells in normal liver tissue. The tumor growth model with piecewise constant parameters is from Tan and Chen (*12*) and is an extension of a stochastic model developed by Chen and Farland (*11*). This model has a biological motivation similar to the two-stage model proposed by Greenfield et al. (*21*), which has been used by Cohen and

Ellwein (*22*) to analyze bladder tumors. However, the two models differ from each other with respect to their mathematical formulations; the one adopted in this report is a stochastic model, whereas the other is a deterministic model that does not allow for parameter estimation because it does not have complete mathematical expression.

Although its most general form will not be used here because of the lack of data, it is worthwhile to note that the stochastic model of Chen and Farland (*11*) has two desirable features: *a*) it allows for any cell growth distributions (e.g., Gompertz) rather than being limited to the exponential distribution as in other existing models, and *b*) it incorporates the birth and death of tumor cells rather than assuming that a tumor is born once a single tumor cell occurs, as in the MVK model [proposed by Moolgavkar and Venzon (*23*) and Moolgavkar and Knudson (*24*)]. Therefore, if information on cell lifetime distribution and the progression phase of tumor development is available, a reasonably realistic model can be constructed.

For completeness of this article, a brief description of the model used in this report will be presented here. We assume that the number of normal cells initiated by an agent in any given interval $(x, x + t)$ is a Poisson random variable with expectation $\int_x^{x+1} \mu_1(y)N(y)dy$. Let $f(t)$ be the probability density function for the lifetime of an initiated cell (I-cell). For an I-cell, at the end of its lifetime it either divides (mitosis) or dies (programmed or nonprogrammed death). If it enters into mitosis, it either divides into two I-cells with probability a, or divides into one I-cell and one malignant cell (M-cell) with probability ν_2. Note that at the end of a cell's lifetime the probability for the cell to die is $\beta = 1 - \alpha - \nu_2$. A similar setup (i.e., to allow for any cell lifetime distribution) can be made for an M-cell. However, we will confine ourselves to a simpler version assuming that an M-cell lifetime follows an exponential distribution. Thus, we can simply assume that an M-cell follows a simple birth–death process; it can either divide into two M-cells with a rate α_m or die with a rate β_m.

When parameters are constant over time (ages), the hazard function is given by

$$h(t) = \mu_1 \nu_2 q \int_0^t a(t-s)N(s)m(t-s)ds$$

where

$$m(t) = \frac{(y_2 - y_1)^2 \exp[A(t)\alpha(y_2 - y_1)]}{\langle (1-y_1) + (y_2 - 1)\exp[A(t)\alpha(y_2 - y_1)]\rangle^2};$$

where $y_1 < y_2$ are two real roots of $\alpha y^2 - (\alpha + \beta + \nu_2 q)y + \beta = 0$; $\alpha + \beta + \nu_2 = 1$, $q = 1 - \beta_m/\alpha_m$, $A(t) = \int_0^t a(x)dx$, where $a(t) = f(t)/[1 - F(t)]$ is the hazard function of the cell lifetime, and $F(t)$ is the cumulative function of $f(t)$. Two special cases of interest here are $a(t) = g$, when the exponential distribution is assumed, and $a(t) = \exp(-\gamma t)$, when the Gompertz distribution is assumed.

When exponential distribution (i.e., $a(t) = \gamma$ or $A(t) = \gamma t$) and $q = 1$ are assumed, the model is equivalent to the MVK model. A special case that may be more appropriate than the exponential distribution is when the Gompertz distribution is assumed; i.e., $A(t) = [1 - \exp(-\gamma t)]/\gamma$.

For the model with time-dependent parameters, assume that the study begins at time t_0. Divide time scale $(t_0, t]$ into k subintervals $L_j = (t_{j-1}, t_j]$, $j = 1,2,\ldots k-1$, and $L_k = (t_{k-1}, t_k]$, where $t_k = t$. The parameters that vary over subintervals $(t_{i-1}, t_i]$, $i = 1,2,\ldots,k$ are μ_{1j}, α_j, β_j, ν_{2j}, N_j, and those parameters related to $f(t)$. The hazard function is given by

$$h(t) = \sum_{j=1}^{k}\left[\mu_{1j}\nu_{2j}q_j N_j \int_{t_{j-1}}^{t_j} a_j(t_j - s)m_j(t_j - s)ds\right] \prod_{i=j+1}^{k} m_j(t_i - t_{i-1}),$$

where

$$\prod_{i=j+1}^{k} m_j(t_j - t_{j-1}) = 1, \text{ when } j = k$$

and

$$m_j(t) = \frac{(y_{2j} - y_{1j})^2 \exp[A_j(t)\alpha_j(y_{2j} - y_{1j})]}{\langle (1-y_{1j}) + (y_{2j} - 1)\exp[A_j(t)\alpha_j(y_{2j} - y_{1j})]\rangle^2}$$

where $y_{1j} < y_{2j}$ are two real roots of $\alpha_j y^2 - (\alpha_j + \beta_j + \nu_{2j}q_j)y + \beta_j = 0$; $\alpha_j + \beta_j + \nu_{2j} = 1$; $q_j = 1 - \beta_{mj}/\alpha_{mj}$, $j = 1,2,\ldots,k$. The formulation of m_j is not exact for the case where a, ν_2 (and thus β also) are not independent of time.

When exponential distribution [i.e., $A_j(t) = \gamma_j(t - t_{j-1})$] and $q_j = 1$ are assumed, the model is equivalent to the MVK model with piecewise constant parameters. A special case that may be more appropriate than the exponential distribution is when the Gompertz distribution is assumed, i.e., when $A_j(t) = \{1 - \exp[-\gamma_j(t - t_{j-1})]\}/\gamma_j$.

An alternative formulation that may be more suitable for developing a computer program is given in Tan and Chen (*12*) under the assumption of exponential cell lifetime distribution and constant α and ν_2. The tumor free distribution function, $S_x(t)$, can be written as

$$S(t) = \exp\left\{-\sum_{j=1}^{k}\left[A_{jj}(t_{j-1}, s_j) + \sum_{i=1}^{j-1} A_{ij}(t_{i-1}, s_j)\right]\right\}$$

where $s_j = t_j$ if $j < k$ and $s_j = t$ if $j = k$ and

$$A_{ij}(t_{j-1}, s_j) = 2N_j\mu_{1j}\nu_{2j}\frac{1}{w_I + z_I}\left\{-(s_j - t_{j-1}) + \frac{2}{\gamma_{Ij}(w_I - z_I)}\log\left[1 + \frac{w_I - z_I}{2_{w_I}}\left(e^{w_I\gamma_{Ij}(s_j - t_{i-1})} - 1\right)\right]\right\},$$

Table A-1. Estimated parameters for DCA dose–response model.

Parameters	Age in days		
	< 35	35–365	365+
μ_1:			
Background	2.446×10^{-8}	4.415×10^{-8}	0
DCA	0	0	0
ν_2:			
Background	7.463×10^{-7}	7.463×10^{-7}	7.463×10^{-7}
DCA	5.324×10^{-8}	5.324×10^{-8}	5.32×10^{-8}
α:			
Background	0.523	0.523	0.523
DCA	0	0	0
q:			
Background	6.694×10^{-1}	6.694×10^{-1}	6.694×10^{-1}
DCA	3.740×10^{-3}	3.740×10^{-3}	3.740×10^{-3}

	Age in days				
	< 7	7–15	15–75	75–426	426+
γ^*:					
Background	0	5.99×10^{-20}	1.219×10^{-1}	6.631×10^{-2}	2.041×10^{-2}
DCA			7.760×10^{-11}	1.026×10^{-3}	6.492×10^{-3}

$$A_{ij}(t_{i-1}, s_j) =$$
$$4N_i\mu_{1i}v_{2j}\left[\frac{1}{\gamma_{1i}(w_1^2 - z_1^2)}\right]x$$
$$\left\langle \log\left[\frac{w_1 + z_1 + (w_1 - z_1)\exp(w_1\Delta_{i+1,j-1}(t_i, t_{j-1}))}{w_1 + z_1 + (w_1 - z_1)\exp(w_1\Delta_{i,j-1}(t_{i-1}, t_{j-1}))}\right]\right.$$
$$\left. - \log\left[\frac{w_1 + z_1 + (w_1 - z_1)\exp(w_1\Delta_{i+1,j}(t_i, s_j))}{w_1 + z_1 + (w_1 - z_1)\exp(w_1\Delta_{ij}(t_{i-1}, s_j))}\right]\right\rangle$$

where
$w_1 = [(\alpha + \beta + v_2q)^2 - 4\alpha\beta]^{1/2}$ and
$z_1 = \alpha - \beta - v_2q$.
$\Delta_{ii}(s,t) = \gamma_i(t - s)$ if both s and t are in the same closed subinterval $[t_{i-1}, t_i]$ and

$$\Delta_{ij}(s,t) = \gamma_i(t_i - s) + \sum_{r=i+1}^{j-1}\gamma_r(t_r - t_{r-1}) + \gamma_j(t - t_{j-1})$$
$$\text{if } s \in L_i,\ t \in L_j \text{ with } t_i < t_j$$

Table A-1 gives estimated parameters for the DCA dose–response model, which is used to predict tumor response in other studies as given in Tables 2–4 and 8. For ease of presentation, the parameter g that is related to mitotic rate separately under the table because it has more time divisions. This time division is based on the work of Cohen and Ellwein (*10*), who observed that the mitotic rate in mouse liver varies with age.

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