Development of a Physiologically Based Pharmacokinetic Model of Trichloroethylene and Its Metabolites for Use in Risk Assessment

Harvey J. Clewell III,¹ P. Robinan Gentry,¹ Tammie R. Covington,¹ and Jeffery M. Gearhart²

¹The K.S. Crump Group, Inc., ICF Consulting, Ruston, Louisiana USA; ²Procter & Gamble Company, Cincinnati, Ohio USA

A physiologically based pharmacokinetic (PBPK) model was developed that provides a comprehensive description of the kinetics of trichloroethylene (TCE) and its metabolites, trichloroethanol (TCOH), trichloroacetic acid (TCA), and dichloroacetic acid (DCA), in the mouse, rat, and human for both oral and inhalation exposure. The model includes descriptions of the three principal target tissues for cancer identified in animal bioassays: liver, lung, and kidney. Cancer dose metrics provided in the model include the area under the concentration curve (AUC) for TCA and DCA in the plasma, the peak concentration and AUC for chloral in the tracheobronchial region of the lung, and the production of a thioacetylated intermediate from dichlorovinylcysteine in the kidney. Additional dose metrics provided for noncancer risk assessment include the peak concentrations and AUCs for TCE and TCOH in the blood, as well as the total metabolism of TCE divided by the body weight. Sensitivity and uncertainty analyses were performed on the model to evaluate its suitability for use in a pharmacokinetic risk assessment for TCE. Model predictions of TCE, TCA, DCA, and TCOH concentrations in rodents and humans are in good agreement with a variety of experimental data, suggesting that the model should provide a useful basis for evaluating cross-species differences in pharmacokinetics for these chemicals. In the case of the lung and kidney target tissues, however, only limited data are available for establishing cross-species pharmacokinetics. As a result, PBPK model calculations of target tissue dose for lung and kidney should be used with caution. Key words: dichloroacetic acid, dichlorovinylcysteine, metabolism, model, PBPK, pharmacokinetics, risk assessment, trichloroacetic acid, trichloroethanol, trichloroethylene. — Environ Health Perspect 108(suppl 2):283-305 (2000).

http://ehpnet1.niehs.nih.gov/docs/2000/suppl-2/283-305cleveland/abstract.html

Introduction

Physiologically based pharmacokinetic (PBPK) modeling is widely held to be a useful methodology for improving the accuracy of chemical risk assessment (1–6). The goal of PBPK modeling is to simulate the uptake, distribution, metabolism, and elimination of a chemical in an organism, using as realistic a description of the relevant physiology and biochemistry as is necessary and feasible (7–10). For its use in risk assessment, PBPK modeling attempts to describe the relationship between external measures of exposure (e.g., amount administered or concentration in air) and internal measures of biologically effective dose (e.g., amount metabolized or concentration of an active metabolite in the tissue displaying the toxic response) in both the experimental animal and the human (11,12).

Simple pharmacokinetic approaches have occasionally been used by regulatory agencies in risk assessment; for example, the most recent U.S. Environmental Protection Agency (U.S. EPA) cancer risk estimates for trichloroethylene (TCE) were derived using estimates of metabolized dose (13,14). The recent U.S. EPA guidelines for the application of inhalation dosimetry in the derivation of inhalation reference concentrations (15) also make use of pharmacokinetic principles. However, the only case to date where a regulatory agency has used a full PBPK approach in a published risk assessment was in the U.S. EPA’s latest revision of its inhalation risk assessment for methylene chloride (16). The decision to use the PBPK approach in this case was made only after a period of considerable controversy, including a workshop sponsored by the National Academy of Sciences at which the usefulness of PBPK modeling for chemical risk assessment was discussed. The scientific consensus following the workshop was that “relevant PBPK data can be used to reduce uncertainty in extrapolation and risk assessment” (1). In 1989, after a detailed multigeneration evaluation of the available PBPK information and a review by the U.S. EPA Scientific Advisory Board, the U.S. EPA revised the inhalation unit risk and risk-specific air concentrations for methylene chloride in its Integrated Risk Information System (IRIS) (17) database, citing the PBPK model of Andersen et al. (18). The resulting risk estimates were lower than those obtained by the default approach by nearly a factor of 10. Application of the PBPK model for methylene chloride in a cancer risk assessment for occupational exposure has also been described (19–21), and a modified version of the model was used by the Occupational Safety and Health Administration (OSHA) in their rulemaking for methylene chloride (22).

Risk assessments using PBPK models have also been proposed for many other chemicals, including not only TCE (23–26), but also perchloroethylene (PERC) (24,27,28), ethylene dichloride (29), vinyl chloride (26,30–32), dioxane (33,34), chloroform (35), benzene (36), and ethyl acrylate (37). However, apart from the case with methylene chloride described above, there still have been no risk assessments published by a regulatory agency in which a PBPK model was used for estimating target tissue dose. Part of the reason for the slow progress of incorporating PBPK modeling in cancer risk assessment is the concern of regulatory agency risk assessors about uncertainties in its implementation. The potential impact of uncertainty in pharmacokinetic risk assessment has been a subject of some controversy (19–21,38–43). The purpose of the study reported here was to develop a PBPK model for TCE that included as complete a description as possible of all of the metabolites and target tissues that are relevant to the toxicity and carcinogenicity of TCE and to evaluate the suitability of the resulting model to provide dosimetry for each of the target tissues in support of a comprehensive pharmacokinetic risk assessment for TCE.

For completeness, aspects of both the cancer and noncancer risk assessment contexts pertinent to the development and evaluation of the PBPK model are discussed in this article. However, a companion article in this same issue provides a detailed description of the application of the PBPK model in a noncancer risk assessment for TCE (44). Therefore, the discussions in this article will focus primarily on the cancer end points.

Toxicity and Carcinogenicity of TCE

TCE produces noncancer toxicity in a variety of tissues; principal noncancer end points include neurological, hepatic, renal,
immunological, and developmental effects (45–47). The American Conference of Government Industrial Hygienists (ACGIH) threshold limit value (TLV) for TCE is currently 50 ppm as an 8-hr time-weighted average (TWA), based on central nervous system (CNS) effects (headaches, fatigue, irritability), with a short-term exposure limit of 200 ppm to protect against its anesthetic effects (48). In 1989, as part of a major rulemaking that promulgated standards for more than 200 chemicals, OSHA adopted a permissible exposure level (PEL) for TCE of 50 ppm as an 8-hr TWA based on the ACGIH TLV (49). However, since the entire 1989 rulemaking has now been overturned by the courts, the PEL for TCE has returned to its previous value of a 100-ppm 8-hr TWA, also based on CNS effects.

TCE was widely used in industry for many years because of its relatively low toxicity, its excellent solvent properties, and its nonflammability. In recent years, however, use of TCE has been greatly reduced due to concerns regarding its carcinogenicity (46,47). Nevertheless, the question of the human carcinogenicity of TCE remains controversial. Although a large number of studies have demonstrated tumors in animals following exposure to TCE, the relevance of these animal results to the question of the human carcinogenicity of TCE has frequently been challenged (50–52). The ACGIH has classified TCE into Group A5, not suspected as a human carcinogen, based on a well-conducted, negative epidemiological study performed in an aircraft maintenance facility at Hill Air Force Base (Ogden, UT) (53,54). The International Agency for Research on Cancer (IARC), on the other hand, classifies TCE into Group 2A, probably carcinogenic to humans, based on their assessment of sufficient data in animals and limited data in humans (55). The human evidence considered significant by IARC was the consistency of an association of TCE exposure with slightly increased incidences of liver/biliary tract tumors and non-Hodgkin lymphoma in studies of three cohorts in the United States (53), Sweden (56), and Finland (57), despite the fact that all three studies were characterized as negative by the original investigators because the increases were not statistically significant.

Requirements for a PBPK Model to Support TCE Risk Assessments

Quantitative cancer risk estimates for TCE are currently based on animal bioassays, specifically liver and lung tumors in mice. In 1983, the U.S. EPA calculated unit risks for TCE of $4.1 \times 10^{-6}$ (µg/m³⁻¹) for inhalation and $0.54 \times 10^{-6}$ (µg/L⁻¹) for drinking water using data on the incidence of liver tumors in male B6C3F₁ mice given TCE in an oil vehicle by gavage (58,59); the linearized multistage model (60) was used with a calculation of absorbed TCE dose scaled by body surface area (BSA) to obtain these estimates (61). In 1985, lower unit risks of $1.3 \times 10^{-6}$ (µg/m³⁻¹) for inhalation and $0.32 \times 10^{-6}$ (µg/L⁻¹) for drinking water were recalculated on the basis of the same oral bioassays, using the results of pharmacokinetic studies (50,62,63) to calculate total metabolized dose in both animals and humans, rather than absorbed dose. The BSA adjustment was still applied to obtain the human equivalent dose (13). In 1987, the U.S. EPA calculated a new inhalation unit risk of $1.7 \times 10^{-6}$ (µg/m³⁻¹) based on the incidence of mouse liver and lung tumors in inhalation bioassays (64–66), again using a calculation of metabolized dose and the BSA adjustment (16).

Statistically significant increases in the incidence of renal tubular cell adenoma and carcinoma have also been observed in male Fischer 344 (F344) rats exposed to TCE by gavage (67). Although not yet used in a quantitative risk assessment for TCE, the incidences of these kidney tumors in rats have raised concern, since they represent a rare tumor that has also been associated with human occupational exposure to TCE (68).

For each of these three target tissues—liver, lung, and kidney—there is evidence that the carcinogenicity of TCE may be associated with one or more of its metabolites: trichloroacetic acid (TCA) and dichloroacetic acid (DCA) in the liver (69,70), chloral (CHL) in the lung (71), and 1,2-dichlorovinylcysteine (DCVC) in the kidney (72). Thus, to be useful in a comprehensive cancer risk assessment for TCE, a PBPK model should include at least three target tissues—liver, lung, and kidney—along with a description of the kinetics of the metabolites imputed to play a role in the carcinogetic activity.

Several target tissues have also been identified for the noncancer toxicity of TCE, including the liver (73), kidney (65,66), CNS (74), immune system (75,76), and developing fetus (77). As in the case of the carcinogenicity of TCE, several of these noncancer end points appear to be associated with exposure to the metabolites of TCE rather than to the parent chemical itself (73,78). In addition to the metabolites mentioned above with regard to the carcinogenicity of TCE, it was felt (43) that a PBPK model developed to support a noncancer risk assessment for TCE should also include a description of the kinetics of trichloroethanol (TCHO), a major metabolite of TCE that has been suggested to be responsible for the observed neurological effects of chloral hydrate (79).

Previous PBPK Modeling of TCE

A number of PBPK models have been developed for TCE. However, most are only parent chemical models; that is, they provide a pharmacokinetic description of TCE itself, but do not include an explicit description of the pharmacokinetics of any of the metabolites (23,24,80–86). These models have been used successfully for predicting TCE concentrations in the blood and tissues (86), for calculating the respiratory input from inhalation exposures (81,82,85), and for investigating the impact of variations in the physiological or biochemical parameters on the kinetics of TCE (80,83,84).

Parent chemical models have also been employed to calculate total metabolized dose in support of a cancer risk assessment for TCE (23,24). However, these parent chemical models cannot be used for predicting tissue exposure to specific metabolites.

Models of both TCE and its metabolites have also been developed. In a series of publications, Sato and co-workers have described the use of a simple PBPK model to study the kinetics of TCE and its metabolites in humans (87), to evaluate the impact of changes in physiological factors (88) and environmental factors (89) on the kinetics of TCE in the human, and to predict the effects of interactions with ethanol consumption on TCE kinetics (90). However, the structure of these models would not support the animal-to-human extrapolation of pharmacokinetic dose metrics needed for risk assessment.

Fisher and co-workers developed a PBPK model for TCE and its principal metabolite, TCA, in the pregnant (91) and lactating (92) rat, as well as in the mouse (93). These rodent models, together with a similar model of TCE and TCA in the human (94), served as the basis for a PBPK-based risk assessment for TCE liver carcinogenicity (25) based on either total metabolism or AUC for TCA. These models provided the first successful cross-species pharmacokinetic description for a metabolite of TCE. The model development performed in the current study builds on the work of Fisher and Allen (25) by adding limited descriptions of additional metabolites (TCHO, DCA, CHL, DCVC) and target tissues (lung and kidney).

Metabolism of TCE

The following discussion summarizes the experimental evidence for the nature of the pharmacokinetics and metabolism of TCE, which formed the basis for the decisions that were made regarding the structure and parameterization of the PBPK model. TCE is a volatile, lipophilic chemical that distributes readily throughout all tissues, including the brain, but partitions preferentially into fat tissue. In contrast, its major metabolite, TCA, is a water-soluble chemical that preferentially
distributes into the plasma and richly perfused organs and is found only in relatively lower concentrations in the muscle and fat. The properties of TCOH are somewhat intermediate between the other two compounds (95). Clearance of TCE occurs both by exhalation and by metabolism. A schematic of the metabolic pathways for TCE is shown in Figure 1. The major oxidative pathway, which takes place primarily (but not exclusively) in the liver, is shown to the right of the diagram; the minor glutathione-dependent pathway, which involves several locations including the liver and kidney, is shown to the left.

Oxidative metabolism. The primary route of metabolism for TCE, shown on the right side of the diagram in Figure 1, is oxidation via the microsomal mixed-function oxidase (MFO) system, now referred to as cytochrome P450, or CYP (96–102). A minor pathway for TCE metabolism involving conjugation with glutathione (GSH) by glutathione transferase (GST) has also been observed (103). This pathway, which is shown on the left side of the diagram, will be described in the section on conjunctive metabolism.

The principal oxidative metabolite formed in vitro is CHL (96,97,100), which is subsequently reduced to TCOH in the cytosol or oxidized to TCA in either the cytosol or mitochondria (96). CHL is not stable in vivo, and circulating concentrations are relatively low compared to its breakdown products, TCA and TCOH (50). Within a few hours of the administration of 50 mg/kg chloral hydrate to a child, the rapid initial clearance of CHL was essentially complete. Subsequent blood concentrations parallel the time course for TCOH but are approximately an order of magnitude lower, suggesting a continuing production of CHL from TCOH (104).

The principal circulating metabolite of TCE in the blood is TCA, which accumulates in the body due to protein binding (105) and slow excretion (106), whereas TCOH is readily excreted (107,108). TCA appears to be derived both directly from CHL and indirectly from TCOH (107–109). TCE is much more extensively metabolized in the mouse than in the rat, whether TCE is administered orally (50) or by inhalation (91).

Based on both in vitro and in vivo studies, the metabolism of TCE has been suggested to consist of oxidation of TCE to CHL by the MFO system, followed by either oxidation of CHL to TCA by an aldehyde dehydrogenase (also known as chloral dehydrogenase) or reduction to TCOH by alcohol dehydrogenase (ADH) with subsequent glucuronidation. Oxidation of TCOH to TCA by the MFO system has also been proposed (110). Consistent with this proposed metabolic description, oral co-administration of ethanol inhibited the metabolism of inhaled TCE to TCA by more than 50% and the production of TCA was essentially abolished while ethanol was present (110). A similar study in rats demonstrated qualitatively similar, but quantitatively much less remarkable, effects of ethanol co-administration on the kinetics of orally administered TCE (111).

Inhalation exposures of human volunteers to TCE concentrations from 27 to 201 ppm showed no evidence of metabolic saturation or of a change in the proportion of TCA to TCOH (112). Saturation of TCE metabolism has been observed in mice, rats, and dogs (66,113). The relative proportion of the major metabolites does not appear to be a strong function of dose. However, repeated dosing does appear to increase the production of TCA at the expense of TCOH (114), and the relative production of CO₂ increases with increasing dose in mice (115).

Human in vivo studies with TCE (116) have identified the major urinary metabolites to be TCOH (50% of the administered TCE dose), primarily as the glucuronide, and TCA (19%). Monochloroacetic acid (MCA) was also identified as a minor metabolite (4%) in these studies. Another minor metabolite, N-(hydroxyacetyl)-aminoethanol, has also been identified in human (and rodent) urine following TCE exposure, and TCE-derived oxalic acid has been detected in the rodent (117). A study of TCE metabolism in nonhuman primates (118) found that TCA was partially excreted as the glucuronide, particularly at longer times after dosing. The authors suggest that since the detection of TCA glucuronide had not been reported previously, TCA excretion may have been under-reported in earlier studies (such as the human study cited above). The glucuronidation of TCA is supported by the observation that TCA is excreted in the bile of rats and mice (114). Urinary excretion represents the major route of elimination of the metabolites; fecal excretion, in the form of TCOH glucuronide, accounts for less than 5% of the total (118). The low fecal excretion is apparently associated to some extent with enterohepatic recirculation of TCOH (i.e., biliary excretion of the glucuronide, followed by hydrolysis and reabsorption of TCOH), which has also been suggested to occur in rats (119).

DCA has been identified as a minor urinary metabolite of TCE (on the order of 1%) in both rats and mice (114,115, 117,120), whereas MCA appears to be present at less than 0.1% (114). A recent in vitro study with mouse and rat liver tissues concluded that unlike most other chlorinated compounds, which are metabolized primarily by the microsomal enzymes of the MFO system, DCA degradation appears to occur primarily in the cytosol in a process that requires

![Figure 1. Metabolism of TCE. Abbreviations not given in text: (right pathway) CDH: chlorohydrogenase (aldheyde dehydrogenase); EHR: enterohepatic recirculation; FA: formic acid; GA: glyoxylic acid; OA: oxalic acid; TCE-O-P450: oxygenated TCE-cytochrome P450 transition state complex; TCOG: TCE glucuronide; UGT: UDP glucuronyltransferase; (left pathway) BL: cysteine conjugate β-lyase; CGDP: cysteinyl-glycine dipeptidase; DCVG: dichlorovinyl glutathione; DCVSHE: dichlorovinyl mercaptan; GGT: γ-glutamyl transpeptidase; NADCVC: N-acetyl dichlorovinylcytsteine, NAT: N-acetyltransferase.](image-url)
AUC of DCA in the administration of 0.25-1.87 μg/kg. This artifact may have compromised some of the data reported on DCA plasma concentrations following administration of TCA or TCE (66,67).

Metabolism in the lung. As with most chemicals, the preponderance of the metabolic clearance of TCE appears to take place in the liver. It has been demonstrated, however, in studies with an isolated, ventilated perfused lung preparation (130), that the male F344 rat lung also possesses a limited oxidative metabolic capability for TCE. Although the affinity (Km) for the lung metabolism observed in that study was similar to the affinity observed in the liver, the capacity (Vmax) of the lung metabolism was less than 1% of the capacity of the liver. These results suggest that lung metabolism is not an important contributor to total in vivo metabolism in the rat and that the rat lung does not possess a significant first-pass (presystemic) clearance capability for inhaled TCE. However, these results do not rule out the possibility that metabolism in the lung could produce sufficient local exposure to metabolites to produce toxicity and/or carcinogenicity.

Conjugative metabolism. A small proportion of TCE appears to be metabolized by enzymatic conjugation with GSH, principally by GST in the liver, followed by further metabolism in the kidney to the cysteine conjugate DCVC (131). The GST metabolic pathway is shown on the left side of Figure 1. Delivery of DCVC to the kidney may also be mediated by enterohepatic recirculation, in which GSH conjugate excreted in the bile is converted by gut bacteria to the cysteine conjugate, which is then reabsorbed (132). The GSH conjugate has been identified both in vitro, with rat liver microsomes, and (at 5 nmol/L) in the bile of rats given 2.2 g/kg TCE in corn oil (103). The cysteine conjugate also has been identified in the urine of animals dosed with TCE (72).

The bioactivation of DCVC to a reactive and mutagenic thiocarbamylating intermediate is performed by cysteine conjugate β-lyase in the kidney (133). Although similar β-lyase activity has been measured in the kidney and liver, the two enzymes are distinct (134). Detoxification and clearance of DCVC takes place by urinary excretion of the N-acetyl derivative (103,135). In a study with PERC (136), it was determined that the excretion of the N-acetyl derivative was dose related (a higher fraction of N-acetyl derivative was excreted at doses where the oxidative pathway was saturated) and was significantly greater in the rat than in the mouse. However, measurements of acid-labile protein adducts associated with DCVC suggest that the activation of DCVC in the kidney may be as much as 12-fold greater in mice than in rats and that the kidney tissue exposure to DCVC-derived reactive species from oral dosing with TCE may be twice as great in the mouse as in the rat (137,138).

The activity of β-lyase has been measured in the liver and kidney of both humans and rats. One research group has reported a specific activity in human kidney on the order of 2.0-3.6 nmol/min/mg cytosol (139,140), compared to 6.45-7.6 nmol/min/mg cytosol in the rat (134). Another research group, however, has reported a maximum velocity (Vmax) of only 0.8 nmol/min/mg cytosol in the human, with an affinity (Km) of 0.29 mM, compared to Vmax = 7.5 nmol/min/mg cytosol and Km = 1.6 mM in rat kidney cytosol in the same study (141). Data for PERC on the relative activity of liver cystosolic GST and kidney cytosolic cysteine conjugate β-lyase suggest that the human activity of both enzymes is roughly 10-fold lower than that in the rat (136). On the other hand, the specific activity of N-acetyltransferase in kidney cytosol appears to be very similar across species: 0.41 nmol/min/mg cytosol in the human, compared to 0.35-0.61 in the rat and 0.94 in the mouse (142).

The fact that N-acetyl-DCVC has been identified in the urine of humans exposed to TCE both occupationally (142) and in controlled exposures (143) indicates that exposure of the kidney to DCVC does occur in the human. In the occupational study (142), the concentrations of N-acetyl-DCVC in the workers’ urine was about one-third of that measured in rats dosed orally with 50 mg/kg TCE. The ratio of N-acetyl-DCVC to TCA in the workers’ urine ranged from 0.03 to 0.3, while in rats it ranged from 0.025 to 0.045, and in mice from 0.014 to 0.065. However, more recent data (143) obtained in controlled studies with both rats and human subjects suggest that relative urinary excretion of GST-pathway metabolites from TCE is actually somewhat lower in humans than in rats.

Description of the PBPK Model for TCE

PBPK Model Structure
A diagram of the PBPK model developed for TCE and its metabolites is shown in Figures 2 and 3. The model was written in the Advanced Continuous Simulation Language (ACSL, Mitchell and Gauthier, Concord, MA). The parent chemical portion of the model (Figure 2) includes individual tissue compartments for the liver, gut tissue, fat, and tracheobronchial region of the lungs. All other tissues are lumped into rapidly perfused
weight to the negative one-quarter power) to simplify inraspecies and interspecies extrapolation. Parent chemical dose metrics provided in the model include the concentration of TCE in blood and tissues, as well as the AUC for TCE in the blood.

**Lung submodel.** The model includes three target tissue submodels in which metabolism takes place: lung, kidney, and liver (Figure 3). Michaelis-Menten kinetics are assumed for all metabolic processes. The tracheobronchial region of the lungs, which receives its own arterial blood supply, is described separately to support the modeling of \textit{in situ} metabolism in this region by the Clara cells. This approach for describing metabolism in the cells lining the airways of the lung was felt to be more biologically accurate than the sequential gas exchange and lung tissue compartments used in the methylene chloride model (18). However, as long as metabolism in the lung is unimportant for presystemic elimination, as is the case for TCE and methylene chloride, the two descriptions should yield similar results. The dose metrics provided for the lung are the instantaneous concentration and AUC for CHL in the tracheobronchial region, which is assumed to be produced by saturable production and clearance of CHL in Clara cells. No systemic circulation of CHL is considered in the model.

**Oxidative metabolism.** Apart from the limited metabolism occurring in the lung, the model assumes that all oxidative metabolism takes place in the liver. The dose metric provided to describe metabolism is the total amount of TCE metabolized divided by the body weight. The model does not actually calculate the formation and metabolism of CHL in the liver, but instead assumes that TCA and TCOH are formed in a fixed yield from the oxidative metabolism of TCE. In the model, TCOH can subsequently be oxidized to TCA, conjugated with glucuronic acid, or reduced to DCA. DCA can also be produced from the reduction of TCA.

**PBPK Model Parameters**

The parameters for the model are listed in Table 1, with the parameters for the parent chemical portion of the model listed first, followed by the parameters for each of the metabolites in turn.

**Parameters for the parent chemical.** The physiological parameters, with two exceptions,
### Table 1. Parameter values used in the PBPK model for TCE.

| Parameter                          | Abbreviation | Units  | Mouse       | Rat       | Human     |
|------------------------------------|--------------|--------|-------------|-----------|-----------|
| Body weight                        | BW           | kg     | 0.035* (0.02–0.035) | 0.39* (0.19–0.33) | 70        |
| Alveolar ventilation               | QPC          | L/hr*  | 30          | 24        | 24* (18)  |
| Cardiac output                     | QCC          | L/hr*  | 18          | 15        | 16.5* (13) |
| Fractional blood flows to tissues  |              |        |             |           |           |
| All rapidly perfused               | QRC          | –      | 0.594       | 0.594     | 0.699     |
| Gut                                | QGC          | –      | 0.141       | 0.153     | 0.181     |
| Liver                              | QLC          | –      | 0.02        | 0.03      | 0.046     |
| Tracheobronchial                   | QTBC         | –      | 0.005       | 0.021     | 0.025     |
| All slowly perfused                | QSC          | –      | 0.406       | 0.406     | 0.301     |
| Fat                                | QFC          | –      | 0.07        | 0.07      | 0.052     |
| Fractional volumes of tissues      |              |        |             |           |           |
| All rapidly perfused               | VRC          | –      | 0.165       | 0.106     | 0.101     |
| Gut                                | VGC          | –      | 0.042       | 0.03      | 0.017     |
| Kidney                             | VKC          | –      | 0.017       | 0.007     | 0.004     |
| Liver                              | VLC          | –      | 0.057       | 0.034     | 0.026     |
| Tracheobronchial                   | VTBC         | –      | 0.0007      | 0.0007    | 0.0007    |
| All slowly perfused                | VSC          | –      | 0.638       | 0.718     | 0.851     |
| Fat                                | VFC          | –      | 0.072       | 0.124     | 0.214     |
| Partition coefficients             |              |        |             |           |           |
| Blood/air                          | PB           | –      | 14          | 18.5      | 9.2       |
| Fat/blood                          | PF           | –      | 36          | 27.5      | 73        |
| Fat/blood                          |              |        |             |           |           |
| Glucose oxoidation to TCA          |              |        |             |           |           |
| Capacity                           | VMC          | mg/hr* | 39* (39–60.) | 12* (12–20.) | 10* (6–10.) |
| Affinity                           | KM           | mg/L   | 0.25        | 0.25* (0.25–18.) | 1.5* (1.5–3.) |
| Fraction TCA                       | PO           | –      | 0.035* (0.035–0.1) | 0.02* (0.02–0.06) | 0.08 |
| TCOH reduction to DCA              |              |        |             |           |           |
| Capacity                           | VMRC         | mg/hr* | 1* (0.5–1.5) | 0.12* (0.08–0.25) | 25 (15–25.) |
| Affinity                           | KMR          | mg/L   | 0.25        | 0.25      | 250       |
| Affinity                           |              |        |             |           |           |
| TCOH glucuronidation               |              |        |             |           |           |
| Capacity                           | VMGC         | mg/hr* | 100         | 100* (35–150.) | 5         |
| Affinity                           | KM5          | mg/L   | 25          | 25        | 25        |
| Kinetics of glucuronide            |              |        |             |           |           |
| Biliary excretion                  | KHEBC        | /hr*b  | 1            | 1         | 1         |
| ReabForeign absorption             | KEHRC        | /hr*b  | 0            | 0* (0–0.3) | 0         |
| Urinary excretion                  | KUGC         | /hr*b  | 0.5          | 0.5       | 3         |
| TCA reduction to DCA               |              |        |             |           |           |
| Capacity                           | VMTC         | mg/hr* | 0* (0–0.1)  | 0.1* (0–0.1) | 0         |
| Affinity                           | KMT          | mg/L   | 10          | 10        | 10        |
| Urinary excretion                  | KUTC         | /hr*b  | 0.035* (0.035–0.1) | 0.05       | 0.023     |
| DCA reduction/elimination          |              |        |             |           |           |
| Capacity                           | VMDC         | mg/hr* | 100         | 50        | 1730      |
| Affinity                           | KMD          | mg/L   | 1,000       | 1,000     | 1,000     |
| Urinary excretion                  | KUDEC        | /hr*b  | 0.035       | 0.05      | 0.023     |
| DCV kinetics in kidney             |              |        |             |           |           |
| Production                         | KFC          | /hr*b  | 0.015       | 0.015     | 0.015     |
| Activation                         | KBlC         | /hr*b  | 0.4         | 17        | 37        |
| Clearance                          | KNATC        | /hr*b  | 0.5         | 1.1       | 19        |
| Chloral kinetics in lung Clara cells|              |        |             |           |           |
| Production capacity                | VMTCB        | mg/hr* | 3           | 0.3       | 0.0045    |
| Affinity                           | KMTB         | mg/L   | 0.25        | 0.25      | 1.5       |
| Clearance capacity                 | VMTCB        | mg/hr* | 250         | 250       | 250       |
| Affinity                           | KMTB         | mg/L   | 250         | 250       | 250       |
| Volumes of distribution (fraction of body weight) | | | | | |
| TCA                               | VOTCAC       | –      | 0.238       | 0.25      | 0.1       |
| DCA                               | VDDCAC       | –      | 0.4         | 0.2       | 0.1       |
| DCOH                              | VDBWC        | –      | 0.65        | 0.85      | 0.65      |
| Fraction of lung containing Clara cells | FCLARA      | –      | 0.1         | 0.1       | 0.1       |

*Default value used for calculation of risk assessment dose metrics; different values (in parentheses) were used for comparison with pharmacokinetic studies (see text). *Scaled by body weight to the 3/4 power. *Scaled by body weight to the −1/4 power.

Environmental Health Perspectives • Vol 108, Supplement 2 • May 2000
were based on the recommendations of the International Life Sciences Institute Risk Science Institute Working Group on Physiological Parameters (145). The exceptions were the cardiac output (QCC) in the mouse, based on the recommendations of Arms and Travis (146), and the alveolar ventilation (QPC) in the human, obtained from Astrand and Rodahl (147). In the model, the tissue volumes and blood flows for the gut, liver, and tracheobronchial region are subtracted from the values shown for all rapidly perfused tissues to obtain the parameters for the rapidly perfused tissue compartment shown in Figure 2; those for the fat are subtracted from the values shown for all slowly perfused tissues to obtain the parameters for the slowly perfused tissue compartment. The kidney volume shown in the table is used only in calculations for the kidney dose surrogate; as shown in Figure 2, the kidney is not described separately in the parent chemical model.

The partition coefficients for TCE were obtained from the work of Fisher and Allen (25,94) and Fisher et al (93); the partition coefficients for the gut and tracheobronchial tissues were assumed to be the same as those reported for the richly perfused tissues. The oral uptake parameters were estimated from data on the appearance of TCE and its metabolites in the blood following corn oil gavage in mice and rats (50). For some parameters, identified in Table 1, values chosen for calculating risk assessment dose metrics were different from those chosen to reproduce pharmacokinetic data. For example, human dose metrics were calculated using a value for QPC of 24, which corresponds to the U.S. EPA's standard assumption of a total ventilation rate of 20 m3/day; the corresponding value for QCC of 16.5 was estimated from Astrand and Rodahl (147). Similarly, animals used in pharmacokinetic studies tend to have lower average body weights than animals used in cancer bioassays, so body weights appropriate to each case were used in the model.

**Parameters for oxidative metabolism.**

Initial values for the metabolic parameters for TCE were obtained from the work of Fisher and Allen (25,94) and Fisher et al (93); however, the metabolic and clearance parameters for TCA, TCOH, and DCA were derived primarily on the basis of fitting the pharmacokinetic data depicted in Figures 4–17. When possible, parameters were also estimated from independent studies; for example, data from rodents and humans dosed with TCA were used to estimate the volumes of distribution and urinary excretion of TCA (93,109). Since the model contains a large number of metabolic and clearance parameters, many of which are highly correlated, the parameter values estimated by this process (i.e., the kinetic parameters for TCA, TCOH, and DCA) cannot be considered to be unequivocally identified. However, an additional biological constraint was applied during the modeling by demanding that all parameters be essentially constant across exposure scenarios within a given species and (to the extent justified by the experimental data) across species. This constraint greatly reduces the likelihood that alternative parameterizations could demonstrate equivalent success in reproducing the entire body of data. Another constraint on the parameterization not obvious from the figures is that of the total TCOH extractable from the blood, roughly 80% is present as free TCOH in the human (110), whereas roughly 70–85% is present as glucuronide in the rodent (113,126). In the figures in this article, the model concentrations shown represent either free TCOH (in rodents) or total (in humans), corresponding to the experimental data provided.

It is informative to note the departures from simple allometric expectations that were required on the basis of the experimental data across species. As with most other xenobiotics, the mouse shows a relatively greater and more variable capacity (VMC) for oxidative metabolism of TCE than the rat and human. Also in keeping with evidence from other P450 substrates, the affinity for oxidative metabolism of TCE in the human is roughly an order of magnitude less (i.e., the value of KM is larger) than in the rodents. A striking difference between humans and rodents, which was clearly demanded by the experimental data, was that the oxidation of TCOH to TCA appears to be a relatively high-affinity (small value of KMO), low-capacity (small value of VMOC) process in the rodent but low affinity, high capacity in the human. It may be that this disparity reflects the involvement of different enzymes (e.g., MFO in the rodent vs ADH in the human). The result of this species difference is that although the model uses a similar value across species for PO (based on the initial split of TCA and TCOH from CHL), the apparent ratio of TCA to TCOH predicted (and observed) over the entire time frame of an exposure to TCE is much higher in the human than in the rodent. The capacity (VMG) for glucuronidation of TCOH in the human, on the other hand, is much lower than in the rodent, as reflected in the greatly different ratios of free TCOH to glucuronide in the blood, mentioned above. The prolonged time courses of TCOH in the human provide clear evidence of biliary excretion (KEHBC) and enterohepatic recirculation (KEHRC). Evidence for enterohepatic recirculation was equivocal in the rodents, however, with recirculation being required to reproduce some data, but being contradicted by other data in the same species.

The least well-characterized portion of the oxidative metabolism pathway is the description of the kinetics of DCA. The only species in which DCA has been reproducibly detected in the blood following TCE exposure is the mouse, and these data were used to obtain values for the production (VMRC and KMR) and clearance (VMDC and KMDC) in the mouse. (The artificial production of DCA from TCA in blood samples, noted earlier in this report, may have compromised some of the data on DCA plasma concentrations used to parameterize the production and clearance of DCA in the mouse.) Assuming that the affinities (KMR and KMD) are constant across species, the capacities in the other species were estimated (for VMDC) from the reported half-lives of DCA across species (122), or (for VMRC in the human) from data on peak DCA concentrations in human subjects exposed to TCE by inhalation (148). Since the clearance of DCA in the rat is actually slower than in the mouse (70), the capacity for production of DCA (VMRC) in the rat was set to the lower human value rather than that of the mouse to be consistent with the failure of investigators to observe DCA in the plasma of the rat following administration of TCE (69). The renal clearance of DCA (KUDC) was assumed to be the same as that observed for TCA (KUTC) in the same species. As mentioned earlier, the most striking departure from allometric expectations for the kinetics of DCA is the extremely high clearance in the human compared to the other species.

**Parameters for lung metabolism.**

The parameters in the PBPK model for predicting the lung dose metric are the capacity and affinity for the production of CHL (VMTBC and KMTB) and the capacity and affinity for its clearance (VMCTBC and KMCCTB). In the model, the production of CHL in the tracheobronchial region was assumed to be associated with the P450 activity in that tissue. This is the assumption that was made in the pharmacokinetic risk assessment for methylene chloride (18). The approach used in that risk assessment was also used to obtain the parameters in this case: the affinity in the lung was assumed to be the same as in the liver for the same species, and the relative capacity of the lung compared to the liver was determined on the basis of the P450 activity measured with standard substrates (18). Based on these data, P450 activity falls off much more rapidly with body weight than would be expected from allometric considerations. No data were available on the clearance of CHL in the lung across species; therefore, it was assumed to be a low-affinity, high-capacity enzyme system such as ADH. The parameters in the PBPK model were chosen such that concentrations of CHL in the lung of the mouse predicted by
the model were consistent with those observed in recent studies (149). It was further assumed that the clearance of CHL in the lung scales across species according to allometric expectations (i.e., by body weight to the 3/4 power). This assumption leads to much lower CHL concentrations in the lungs of rats and humans compared to mice for the same TCE exposure conditions. An alternative assumption would have been that the activity of the enzyme responsible for the clearance of CHL scales in the same way as P450. This assumption would lead to similar concentrations of CHL in the lungs of mice, rats, and humans for the same TCE exposure conditions.

Parameters for conjugative metabolism.

The parameters in the PBPK model for predicting the kidney dose metric are the production of DCVC by the GST pathway (KFC), its activation by β-lyase (KBLC), and its clearance by N-acetyltransferase (KNATC). First-order rate constants are used because the production of metabolites by the GST pathway is quite low, and saturation of enzyme capacity is unlikely. As discussed earlier, the capacity and affinity of β-lyase in the kidney have been measured in both rats and humans (141). These data were used to estimate the apparent first-order rate constants (KBLC) used in the model. No data were available on the activity of β-lyase in the mouse, so the relationships between β-lyase metabolic parameters in mice and rats reported for trichloro vinylcysteine derived from PERC (136) were assumed to apply for DCVC as well. For N-acetyltransferase, only specific activity data across species are available (142). These data were converted to the corresponding rate constants (KNATC) by assuming the affinity of N-acetyltransferase for DCVC is the same as that measured for β-lyase in the same species. This assumption is supported by the similarity of the affinities of N-acetyltransferase and β-lyase for DCVC in the rat: 3.3 mM and 1.6 mM, respectively (141,150).

Finally, measurements of oxidative and conjugative metabolites in the urine following TCE exposure (143) were used to obtain estimates of the GST pathway rate constant (KFC). The oxidative pathway was represented by total excretion of TCA plus TCOH, while the conjugative pathway was represented by excretion of 1,2-DCVC. Data from the same study on excretion of 2,2-DCVC were not used. Unlike 1,2-DCVC, there was no evidence of a dose response for 2,2-DCVC as a function of TCE exposure in humans or rodents; similar amounts of 2,2-DCVC were excreted for TCE exposures ranging from 40 to 160 ppm. Ignoring 2,2-DCVC is unlikely to significantly affect the risk assessment for TCE, since 1,2-DCVC is clearly the more toxic and mutagenic of the two isomers (151).

The results of this analysis are shown in Table 2. In performing this analysis, all of the parameters in the model were set at the default values except VMC, KM, and KFC (in particular, KBLC and KNATC were set to the values calculated as described above). The values of VMC, KM, and KFC in the model were then varied to bring the model into agreement with the data for both the oxidative (MFO) and conjugative (GST) pathways. It can be seen that the model could be made to agree quite well with the urinary data when allometric scaling was assumed for conjugative metabolism (i.e., using the same value of the scaled parameter KFC in rat and human). Although it was necessary to adjust KM to obtain the best agreement with the MFO pathway data, allometric scaling of conjugative metabolism also gave the best agreement with the GST pathway data when the default values for KM were used. This result is consistent with the observed allometric scaling of the GST pathway for methylene chloride (152).

Additional data on urinary metabolite concentrations following oral gavage of rats with 50 mg/kg TCE (142), although not suitable for comparing with the model, were consistent with the inhalation data, suggesting that there is not an effect due to route of exposure. Therefore, the value estimated from inhalation was used to obtain the kidney dose metrics for the rat for both inhalation and oral exposures.

PBPK Model Validation

In the strictest sense of the word, validation of a PBPK model would require testing the predictions of the model against data not used in the development and parameterization of the model (153). Ideally, each of the model parameters would have been estimated from separate experiments and the performance of the model could then be tested against pharmacokinetic data such as shown in Figures 4–17. In practice, there are simply not enough experimental data to separately identify all of the parameters in a model as complex as the PBPK model for TCE described in this article. Moreover, as in this case, there are often no data available with which to validate important components of the model. Therefore, the validity of the model for its intended purpose must be evaluated on the basis of the comprehensiveness of its predictive power and the reasonableness of the parameters used to fit the various data sets. The parameterization of the PBPK model for TCE has already been discussed. The ability of the model described to reproduce data on TCE and TCA kinetics in the mouse, rat, and human for both inhalation exposure and oral gavage is shown in Figures 4–17. In addition, these figures demonstrate the successful expansion of the Fisher and Allen model of TCE and TCA to describe TCOH kinetics in mice, rats, and humans, as well as DCA in the mouse. No data suitable for plotting were available for validation of the model predictions for CHL in the lung, DCVC in the kidney, or DCA in the human.

Figures 4–7 demonstrate the ability of the PBPK model to simulate the kinetics of TCE and its metabolites in mice. Figure 4 compares the predictions of the model with experimental data on the concentrations of TCE in the blood and TCA in the plasma of male and female B6C3F1 mice exposed to 110 and 368 ppm, respectively, of TCE by inhalation for 4 hr (93). The model overpredicts the blood concentrations of TCE during the exposure by about 50%, but provides a good description of the time course for TCA in the plasma. Figure 5 shows the ability of the model to simultaneously reproduce experimental data on the blood concentrations of TCE, TCA, and TCOH in mice exposed to 1,000 mg/kg TCE by oral oil gavage (50). The model is also able to simulate (Figure 6) the time course of TCE, TCA, TCOH, and DCA in the B6C3F1 mouse following an oral dose of 499 mg/kg in an aqueous vehicle (126). The DCA data at 6 and 9 hr in Figure 6 are suspect due to problems with the analytical method (129). Figure 7 shows the predictions of the model for DCA data collected under the same experimental conditions but for oral administration of 99 and 1,791 mg/kg TCE.

| Total urinary excretion | MFO pathway* | GST pathway* | Model parameters |
|-------------------------|--------------|--------------|------------------|
| of metabolites          | measured     | predicted    | VMC/KM/KFC       |
| Rat, 8-hr inhalation    |              |              |                  |
| 40 ppm                  | 6.9          | 8.9          | 0.001            | 12/18/0.015 |
| 80 ppm                  | 13.0         | 16.9         | 0.002            | 12/18/0.015 |
| 160 ppm                 | 33.3         | 30.6         | 0.006            | 12/18/0.015 |
| Human, 6-hr inhalation  |              |              |                  |
| 40 ppm                  | 923          | 943          | 0.074            | 10/3/0.015  |
| 80 ppm                  | 1,775        | 1,762        | 0.161            | 10/3/0.015  |
| 160 ppm                 | 3,080        | 3,029        | 0.223            | 10/3/0.015  |

*Metabolites collected for 48 hr after exposure (143), Tables 1 and 2; reported as total micromoles TCA + TCOH excreted. **Metabolites collected for 48 hr after exposure (143), Figures 5 and 7; reported as total micrograms N-acetyl-1,2-DCVC excreted.
Figures 4–13 show the results of exercising the model against similar data in the rat. Figure 8 compares the predictions of the model with experimental data on the concentrations of TCE in the blood and TCA in the plasma of male and female F344 rats exposed to TCE by inhalation for 4 hr (93). The model overpredicts the blood concentrations of TCE in the female rats during the exposure by about 50%, but provides a good description of the time course for TCA in the plasma of the female rats, and for both TCE and TCA in the male rats. Figure 9 shows the ability of the model to simultaneously reproduce experimental data on the blood concentrations of TCE, TCA, and TCOH in rats exposed to 1,000 mg/kg TCE by corn oil gavage (49). As shown in Figures 10–13, the model is also able to simulate the time courses of TCE, TCA, and TCOH in the F344 rat following oral doses of 100, 197, 591, and 3,000 mg/kg in an aqueous vehicle (69,113).
Finally, Figures 14–17 demonstrate the ability of the model to describe the human kinetics of TCE and its metabolites, TCA and TCOH. Figure 14 compares the model predictions with experimental data collected on two different occasions for TCE in the blood, as well as for TCA and TCOH in the plasma and urine, following a 6-hr exposure of human subjects to 100 ppm TCE (109,110). The model provides a reasonable simulation of the time course of TCE in the blood during the exposure and for several hours afterward but underpredicts the long-term concentration of TCE in the blood. Model predictions for TCA in plasma and urine are close to the experimental data throughout the experiment. The model overpredicts the early concentrations of TCOH in the plasma while underpredicting the later concentrations; however, the model predictions of TCOH glucuronide in the urine are very close to the data throughout the experimental period. Figure 15 demonstrates the ability of the model to reproduce data on multiple exposures (in this case, 4-hr exposures of human subjects to 70 ppm TCE repeated daily for 5 days) (154). The model underpredicts the peak concentration of TCE in the blood in this experiment by a factor of 2 but does reproduce the progressive failure of TCE concentrations to return to zero at the end of the day after repeated exposure. The model also provides a reasonable simulation of the accumulation and excretion of TCA in the plasma and urine, as well as TCOH glucuronide in the urine. Figures 16 and 17 show similar results for TCE in exhaled air as well as TCA and TCOH in the urine of human subjects exposed 7 hr per day for 5 days to 200 ppm TCE (155), and for TCA and TCOH in the plasma and urine of human subjects exposed 5 days for 6 hr to 50 ppm TCE (110).

It can be readily seen from Figures 4–17 that it was not possible to obtain complete agreement between the model and each of the studies investigated using a single set of parameters in each species. This failure undoubtedly results from a combination of variation across individuals and animal strains, experimental error, and model error. Nevertheless, given the general agreement of the model with a variety of data on TCE, TCA, and TCOH concentration time courses in both rodents and humans, there can be relatively high confidence in dose metrics based on the

Figure 8. Comparison of predicted and experimental concentrations of TCE in blood and TCA in plasma in F344 rats exposed to TCE by inhalation. The figures show (A) TCE blood concentrations in male rats exposed for 4 hr to 529 ppm TCE vapors and TCA plasma concentrations in male rats exposed for 4 hr to 505 ppm TCE vapors; (B) TCE blood and TCA plasma concentrations in female rats exposed for 4 hr to 600 ppm TCE vapors. Kinetic data are taken from Fisher et al. (50).

Figure 9. Mean observed and predicted blood concentrations of TCE (A), TCA (B), and free TCOH (C) following corn oil gavage with 1,000 mg/kg TCE in rats. Kinetic data are taken from Prout et al. (50).

Figure 10. Mean observed and predicted blood concentrations of TCE (A), TCA (B), and free TCOH (C) following an oral dose of 100 mg/kg TCE in F344 rats. Kinetic data are taken from Templin et al. (113).
predictions of the PBPK model for these chemicals. Similarly, model predictions for the total amount of TCE metabolized per kilogram body weight were generally within a factor of 2 of data on inhalation and oral exposures of mice, rats, and humans (50,62, 63,114,117). Unfortunately, as mentioned earlier, there is a lack of similar data to provide confidence in the model predictions for DCVC in the kidney, CHL in the lung, and DCA in the human.

**Sensitivity and Uncertainty Analysis**

In order to evaluate the level of confidence that could be given to the calculations performed with the PBPK model, a series of quantitative and qualitative analyses were performed to characterize the uncertainty in the model structure, parameterization, and dose metric selection. Since the intended use of the PBPK model is to calculate target tissue dose metrics, any evaluation of the model should focus on this aspect of the model capabilities. Therefore, both the sensitivity analysis and the uncertainty analysis were conducted with respect to model predictions of the target tissue dose metrics. These dose metrics are calculated as a lifetime average daily dose (LADD). The most obvious (and most time-consuming) way to calculate a LADD with the PBPK model is to run the model for the entire lifetime of the animal or human, reproducing the entire exposure scenario. For example, the dose metrics for the inhalation bioassay performed by Maltoni et al. (65,66) could be obtained by simulating repeated exposures at each concentration for 7 hr per day, 5 days per week, until 78 weeks, at which point the exposures would be terminated and the simulation continued until 104 weeks. Dividing the resulting dose metrics by 728 would produce the LADDs. In practice it is faster and sufficiently accurate to run the exposure scenario only until the weekly increase in the dose metric is constant, multiply the weekly result by the fraction of lifetime over which the exposures were conducted, and divide by 7 to obtain the LADD. Except for the AUC for TCA in the human, which can sometimes take more than 7 weeks to reach steady state, simulations usually need to be run for only 2 or 3 weeks to obtain dose metrics in this fashion.

**Figure 11.** Mean observed and predicted blood concentrations of TCE (A), TCA (B), and free TCOH (C) following an oral dose of 197 mg/kg TCE in F344 rats. Kinetic data are taken from Larson and Bull (69).

**Figure 12.** Mean observed and predicted blood concentrations of TCE (A), TCA (B), and free TCOH (C) following an oral dose of 591 mg/kg TCE in F344 rats. Kinetic data are taken from Larson and Bull (69).

**Figure 13.** Mean observed and predicted blood concentrations of TCA (A), TCA (B), and free TCOH (C) following an oral dose of 3,000 mg/kg TCE in F344 rats. Kinetic data are taken from Larson and Bull (69).
PBPK Model Parameter Sensitivity

Table 3 shows the normalized analytical sensitivities for the TCE and TCA parameters in the PBPK model described above. The normalized analytical sensitivity coefficient represents the fractional change in output associated with a fractional change in the input parameter. For example, if a 1% change in the input parameter results in a 2% change in the output, the sensitivity coefficient would be 2.0. In Table 3, the outputs evaluated are the dose metrics for the total amount of TCE metabolized per kilogram body weight and the AUC for TCA. The parameters in the table are defined in Table 1. Sensitivity coefficients of less than 0.1 in absolute value were omitted from the table for clarity, and coefficients above 0.5 are outlined.

It can be seen that of the 29 parameters in the TCE/TCA portion of the model, 12 have essentially no impact on risk predictions based on the two dose metrics, and only 5 have a significant impact: the alveolar ventilation (QPC), the capacity for metabolism of TCE (VM), the fraction of TCA produced from the metabolism of TCE (PO), the volume of distribution (VDTCAC) and rate of excretion (KUTC) of TCA. None of the parameters are associated with sensitivities greater than 1.0, indicating that there is no amplification of error from the inputs of the model to the outputs. This is, of course, a desirable trait in a model to be used for risk assessment.

Sensitivities for the other metabolites in the model are not shown, but the results were similar to those for TCA; that is, none of the parameters were associated with sensitivities greater than 1 in absolute value, and (except for QPC) only the parameters directly related to the production and clearance of a metabolite were associated with significant sensitivities (close to 1) for dose metrics based on that metabolite.

PBPK Model Parameter Uncertainty

There are a number of ways of characterizing the uncertainty associated with use of a PBPK model. The best approach depends on the level of uncertainty. In the case of the TCE model, the level of uncertainty varies considerably from one portion of the model to another. Some parameters in the model, such as those for TCE, TCA, and TCOH, are relatively well established by data, and the uncertainties can be addressed fairly quantitatively. Under these conditions the preferred method for characterizing the overall model uncertainty is to perform Monte Carlo analysis, as discussed below. On the other hand, other parameters in the model, such as those associated with the production and clearance of DCVC in the kidney and CHL in the lung, are based on inadequate
and often conflicting data, and the uncertainties cannot be adequately quantified to support such a rigorous analysis. For these aspects of the model, an appropriate method for characterizing uncertainty is to simply calculate the range of dose metrics that could reasonably be expected given the existing data.

**Liver dose metric: Monte Carlo analysis.**
The sensitivity analysis described above does not consider the potential interactions between parameters; the parameters are tested individually. Also, sensitivity analysis does not reflect the uncertainty associated with each parameter. For example, the fact that the output is highly sensitive to a particular parameter is not important if the parameter is known exactly. To estimate the combined impact of uncertainty regarding the values of the various parameters, a Monte Carlo analysis was performed on an early version of the PBPK model for a characteristic dose metric, the average daily AUC for TCA in the plasma. The version of the model tested in this analysis was essentially identical to that of Fisher and Allen (25) and did not include the description of TCOH and DCA kinetics provided in the current version of the model. In support of the Monte Carlo analysis, the distributions of possible values for each of the input parameters were estimated, as shown in Table 4. The Monte Carlo software (PBPK_SIM, K.S. Crump Group, Ruston, LA) randomly selects a set of parameter values from the distributions for the bioassay animal and runs the PBPK model to obtain dose metric values for each of the bioassay dose groups. It then selects a set of parameter values from the distributions for the human and runs the PBPK model to obtain a dose metric value for a specified human exposure scenario. This process is repeated the specified number of times (400 in this case) until the distributions of dose metrics have been obtained.

Table 4 lists the means and coefficients of variation (CV) used in a Monte Carlo uncertainty analysis of the AUC–TCA dose metric. Truncated (greater than zero) normal distributions were used for all parameters except the kinetic parameters, which were assumed to be log-normally distributed. The CVs for the physiological parameters were estimated from data on the variability of published values (146, 156). In order to maintain mass balance in the PBPK model, after sampled parameter values for cardiac output and fractional tissue blood flows were used to calculate blood flows to each of the tissues, total cardiac output was recalculated as the sum of the individual tissue blood flows. The CVs for the partition coefficients were based on repeated determinations for two other chemicals, PERC (28) and chloropenafurobenzene (6). The CVs for the metabolic and kinetic constants were estimated from a comparison of reported values in the literature and by exercising the model against various data sets to determine the identifiability of the parameters estimated from pharmacokinetic data. It should be understood, however, that the most uncertain part of uncertainty analysis is quantifying uncertainty.

The results of the Monte Carlo analysis are shown in Table 5, which lists the means and 5th and 95th percentiles for the animal and human dose metrics. For dose metrics

![Figure 17. Mean observed and predicted kinetics of TCE and its metabolites during and after 8-hr exposures of human subjects to 50 ppm TCE for 5 days. Kinetic data are taken from Muller et al. (110). (A) TCA plasma concentrations (mg/L); (B) cumulative urinary TCA excretion (mg); (C) total TCOH plasma concentrations (mg/L); (D) cumulative urinary TCOH excretion (mg).](image)

**Table 3.** Normalized analytical sensitivity coefficients for PBPK model predictions of amount TCE metabolized per kg body weight (AMET) and area under the curve for trichloroacetic acid (AUC–TCA). *(0.1)*

| Parameter | Mouse gavage (1,000 mg/kg) | Mouse inhalation (1,000 ppm) | Human inhalation (1 ppm) | Human ingestion (1 mg/kg/day) |
|-----------|-----------------------------|-----------------------------|---------------------------|-------------------------------|
| AMET      | AUC–TCA                     | AMET                        | AUC–TCA                   | AMET                          |
| BW        | -0.1                        | 1.1                         | -0.2                      | 1.0                           |
| QCC       | -                           | -                           | 0.1                       | 0.1                           |
| OPC       | -0.3                        | -0.3                        | 0.1                       | 0.1                           |
| DPC       | -                           | -                           | -0.1                      | -0.1                          |
| GCC       | -                           | -                           | 0.3                       | 0.3                           |
| QLC       | -                           | -                           | 0.1                       | 0.1                           |
| QRC       | -                           | -                           | -                         | -                             |
| GCC       | -                           | -                           | -                         | -                             |
| QTC      | -                           | 0.3                         | 0.3                       | 0.3                           |
| VSC       | -                           | 0.3                         | 0.1                       | 0.1                           |
| VCK       | -                           | -                           | -                         | -                             |
| VLC       | -                           | -                           | -                         | -                             |
| VRC       | -                           | -                           | -                         | -                             |
| VSB       | -                           | -                           | -                         | -                             |
| VTB       | -                           | -                           | -                         | -                             |
| VDB       | -                           | -                           | -                         | -                             |
| PB        | 0.3                         | 0.3                         | 0.3                       | 0.3                           |
| PF        | 0.3                         | 0.3                         | 0.1                       | 0.1                           |
| PL        | -                           | -                           | -                         | -                             |
| PR        | -                           | -                           | -                         | -                             |
| PS        | -                           | -                           | -                         | -                             |
| PTB       | -                           | -                           | -                         | -                             |
| KAS       | -0.4                        | -0.4                        | -                         | -0.9                          |
| VMR       | 0.6                         | 0.7                         | 0.7                       | 0.7                           |
| KM        | -                           | -                           | -                         | -0.2                          |
| PO        | 1.0                         | 1.0                         | 1.0                       | 1.0                           |
| KUTC      | -1.0                        | -0.9                        | -                         | -0.1                          |

*Units: AMET (mg/kg body weight); AUC–TCA (mg-hr/L). – Less than 0.1 in absolute value.
Table 4. Parameter values and coefficients of variation (CV) for Monte Carlo analysis of the TCE/TCA portion of the PBPK model.4

| Parameter | Mouse (M/F) | Human (M/F) |
|-----------|-------------|-------------|
| BW        | 0.031/0.027 | 70.0        |
| QPC       | 29.0        | 24.0        |
| QCC       | 16.5        | 16.5        |
| Tissue blood flows (fraction of cardiac output) | | |
| QGC       | 0.165       | 0.195       |
| QLC       | 0.035       | 0.07        |
| QFC       | 0.03        | 0.05        |
| QSC       | 0.25        | 0.24        |
| QRC       | 0.47        | 0.395       |
| VTC       | 0.06        | 0.06        |
| Tissue volumes (fraction of body weight) | | |
| VGC       | 0.031       | 0.045       |
| VLC       | 0.046       | 0.023       |
| VFC       | 0.04/0.1    | 0.16        |
| VSC       | 0.553/0.513 | 0.48        |
| VRC       | 0.049       | 0.039       |
| VTC       | 0.0007      | 0.0007      |
| Partition coefficients | | |
| PB        | 13.2/14.3   | 9.2         |
| PG        | 2.0/1.6     | 6.8         |
| PL        | 2.0/1.6     | 6.8         |
| PF        | 41.3/31.4   | 73.0        |
| PS        | 1.0/0.5     | 2.3         |
| PR        | 2.0/1.6     | 6.8         |
| PTB       | 2.0/1.6     | 6.8         |
| Kinetic parameters | | |
| VMC       | 39.0/27.6   | 12.0        |
| KM        | 0.25        | 1.5         |
| VDTCAC    | 0.238/0.176 | 0.1         |
| KAS       | 1.2/1.0/1.0 | 50.0        |
| PO        | 0.06/0.07   | 0.33        |
| KUTC      | 0.035/0.125 | 0.023       |

*Based on an earlier version of the model before descriptions of TOCH and DCA were added. Definitions of parameters are the same as those given in Table 1. Coefficient of variation (%) = 100 x standard deviation/mean. *Dose-dependent values (25) used in this earlier version of model.

Table 5. Estimated variation9 in mouse and human dose metrics for area under the curve for trichloroacetic acid (mg-hr/L).

| Mouse inhalationb | 100 ppm | 300 ppm | 600 ppm |
|-------------------|---------|---------|---------|
| Mean              | 495     | 532     | 811     |
| 5th percentile    | 148     | 167     | 271     |
| 95th percentile   | 1,133   | 1,179   | 2,033   |

| Mouse gavagec   | 1,163 mg/kg | 2,333 mg/kg |
|-----------------|-------------|-------------|
| Mean            | 1,197       | 1,519       |
| 5th percentile  | 384         | 481         |
| 95th percentile | 2,576       | 3,381       |

| Human inhalation | 1 ppm | 1 ppm |
|------------------|-------|-------|
| Mean             | 0.233 | 0.233 |
| 5th percentile   | 0.087 | 0.087 |
| 95th percentile  | 0.630 | 0.630 |

| Human drinking water | 1 ppm |
|----------------------|-------|
| Mean                 | 0.012 |
| 5th percentile       | 0.003 |
| 95th percentile       | 0.026 |

*Based on 400-iteration Monte Carlo analysis. *Concentrations used in inhalation bioassay (65,66). *Doses employed in oral bioassay (59).

Based on the average daily AUC for TCA in the plasma, the 5th and 95th percentiles of the dose metric distributions are generally within a factor of 2 to 3.3 of the mean, and all of the 90% confidence intervals for the dose metrics range over somewhat less than an order of magnitude. These results are similar to those reported for a PBPK model of methylene chloride (21) and are probably representative of the uncertainty associated with other dose metrics in the validated portions of the model such as AUC–TCOH, AUC–DCA (in the mouse), and total metabolism of TCE.

**Lung dose metric.** The greatest source of uncertainty regarding the calculation of the lung dose metric is the lack of data on the metabolic clearance of CHL in the lung. Table 6 shows the predicted lung dose metrics for the principal inhalation bioassays providing a dose response for lung tumors (64–66), for the highest oral exposure of the mouse, for the highest rat exposures, and for several human exposure scenarios. The different exposures have been ordered according to the predicted value of the LADD based on the AUC for CHL. In the case of the rat and human, two dose metric values are shown. The first number represents the dose metric calculated based on the assumption that the clearance of CHL scales across species according to allometric expectations (proportional to body weight to the 3/4 power).

Kidney dose metric. The overriding source of uncertainty regarding calculation of the kidney dose metric is the inadequate and often conflicting data in the literature for the conjugative pathway. Specific data gaps include the affinity of kidney N-acetyltransferase for DCVC in the human and the activities of the GST pathway in the rat and human. Table 7 shows the predicted kidney dose metrics for the principal bioassays providing a dose response for kidney tumors (65–67), for the highest oral exposure of the mouse, and for several human exposure scenarios. The different exposures have been ordered according to the predicted value of the LADD based on the production of the toxic thiol per gram of kidney tissue (KTOX). The human dose metrics shown in Table 7 are those calculated assuming allometric scaling of the GST pathway rate constant. There is, of course, additional parameter uncertainty associated with the limited data on the other enzymes involved in the production, intoxication, and clearance of DCVC. As with the lung target tissue, it will not be possible to provide an accurate assessment of the overall uncertainty in the kidney dose metric until reproducible data are collected.

**Dose Metric Selection Uncertainty.** The pharmacokinetic dose metrics most commonly applied to characterize the exposure of a tissue to a chemical are the peak concentration and the AUC, and these are the principal types of dose metrics provided in the PBPK model. However, there are other possible forms for dose metrics that might be useful for describing nonlinear processes. For example, time above a critical concentration (TACC) has been suggested as an appropriate dose metric for the effects of metothexate, whose toxicity demonstrates a strong dependence on dose rate (158). The following discussion provides a rationale for
**Table 6. Lung tumor dose metrics.**

| Choral in Lung | AUC–LADD* | C_{MAX}^b |
|---------------|-----------|-----------|
| Mouse***, 600 ppm² | 9.4       | 2.6       |
| Mouse*, 450 ppm² | 7.9       | 1.6       |
| Mouse 1000 mg/kg | 5.9       | 3.4       |
| Mouse**, 300 ppm² | 3.7       | 1.0       |
| Rat, 600 ppm² | 2.8 (28)¹  | 0.3 (3.4)  |
| Mouse*, 100 ppm²  | 1.6       | 0.3       |
| Mouse, 50 ppm²   | 0.8       | 0.2       |
| Human, 100 ppm²  | 0.016 (10.5) | 0.002 (2.2) |
| Human, 50 ppm²   | 0.01 (7.0) | 0.002 (1.6) |
| Human, 1 ppm²    | 0.002 (1.3) | –         |
| Human, 1 mg/L    | 2 × 10^{-5}  | (0.01)   |

### Significant increased lung tumors in more than one study.

*Increased tumors in at least one study (not statistically significant).

**Lifetime average daily area under the curve in the tracheobronchial region (mg·h·m²). Maximum concentration achieved in the tracheobronchial region (mg/L).**

### Inhalation, 7 hr/day, 5 days/week, 78/104 weeks (56,65).

### Inhalation, 7 hr/day, 5 days/week, 104 weeks (64). OIL gavage, 5 days/week, 103 weeks (65). Occupational exposure (9 hr/day, 5 days/week, 45 years)–current PEL. Occupational exposure–current TLV–TWA. Continuous inhalation over a lifetime. Drinking water—lifetime continuous. Alternate (worst-case) calculation—see text.

**Table 7. Kidney tumor dose metrics.**

| Reactive thiol in kidney LADD(KTOX)³ |
|-------------------------------------|
| Rat**, 1,000 mg/kg² | 73.6 |
| Rat, 500 mg/kg² | 32.0  |
| Rat*, 600 ppm² | 19.6  |
| Mouse, 1,000 mg/kg | 13.5  |
| Rat, 300 ppm² | 6.3   |
| Human, 100 ppm² | 0.23  |
| Human, 50 ppm² | 0.23  |
| Human, 1 ppm² | 0.09  |
| Human, 1 mg/L² | 0.0004 |

**Significantly increased tumors in at least one study.**

- Increased tumors in at least one study (not statistically significant).
- Lifetime average daily amount (mg) reactive metabolite generated per gram of kidney. OIL gavage, 5 days/week, 103 weeks (65).
- Inhalation, 7 hr/day, 5 days/week, 78/104 weeks (65,66).
- Inhalation, 7 hr/day, 5 days/week, 104 weeks (64). OIL gavage, 5 days/week, 103 weeks (65). Occupational exposure (9 hr/day, 5 days/week, 45 years)—current PEL. Occupational exposure—current TLV–TWA. Continuous inhalation over a lifetime. Drinking water—lifetime continuous. Alternate (worst-case) calculation—see text.

the pharmacokinetic dose metrics provided in the PBPK model and considers other possible dose metrics that could be selected.

**Liver dose metric.**

If, as was once thought, reactive species produced during the metabolism of TCE were responsible for its liver carcinogenicity, an appropriate dose metric would be total daily metabolism divided by the volume of the liver (13,18). However, current information suggests that two stable metabolites, TCA and DCA, are primarily responsible for the liver tumor incidence observed in mice dosed with TCE (69,70). The commonly accepted form of the dose metric for the chronic interaction of a stable metabolite with a tissue is the AUC in the tissue. This mathematical form implicitly assumes that the cumulative effect of the metabolite on the tissue is linear over both concentration and time. In this case, the most appropriate dose metric would reflect liver tissue exposure (AUC) to both TCA and DCA (69,70). If it is assumed that both DCA and TCA contribute to the carcinogenicity of TCE in the liver, the proportion of the observed tumor risk to assign to each metabolite could be based on their relative potencies when dosed directly. However, as mentioned earlier, data on the AUCs for DCA resulting from exposures to TCA [e.g., (70)] may have been compromised by a sampling artifact that could lead to overestimates of DCA concentrations in the presence of TCA (129), making it impossible to estimate the individual potencies of TCA and DCA. As a simplifying assumption, all of the tumorigenicity of TCE can simply be ascribed to TCA, as was assumed by Fisher and Allen (25). Since DCA has been detected in the mouse to a much greater extent than in the human following TCE exposure, the use of the AUC for TCA alone as the dose metric is almost certainly safe-sided (in the direction of overestimating the human risk estimate) compared to including potency-weighted AUCs for both TCA and DCA.

Strictly speaking, the AUC for TCA should actually be calculated for the concentration in the liver. However, the use of the AUC in the plasma provides a surrogate for the liver AUC that can be validated more readily against experimental data. Since risk estimates are based on the ratio of animal and human dose metrics, this effectively amounts to an assumption that the ratios of the plasma concentrations of the acids to their concentrations in the liver are constant across species. In fact, data on the binding of TCA in the plasma of rats and humans (113) suggest that TCA in plasma is bound to a much greater extent in the human (~ 20%) than in the rodent (~ 50%). Based on these data, it can be estimated that the liver-to-plasma TCA concentration ratio in the human is about 40% of the ratio in the rodent. This estimate is also consistent with the ratio of reported relative volumes of distribution of TCA in the two species, which are on the order of 10 and 25% of body weight in the human and rodent, respectively. Thus, using the AUC of TCA in the plasma as the dose metric provides a conservative estimate of the cross-species relationship for the AUCs in the liver, tending to overestimate liver exposure to TCA in the human by about 2-fold.

Table 8 shows the predicted liver dose metrics for the principal animal bioassays providing a dose response for liver tumors (59,65–67,159), for the highest rat exposures in these same studies, and for several human exposure scenarios. The different exposures have been ordered according to the predicted value of the LADD based on the AUC for TCA. Bioassays exposures associated with LADDs for AUC–TCA of greater than 1,150 were uniformly positive, whereas bioassays exposures with LADDs less than 700 were uniformly negative. The highest exposures of rats produced AUC–LADDs considerably less than those producing tumors in the mouse, consistent with the negative results in the rat bioassays.

The most striking feature of the results for this target tissue compared to the lung and kidney that two of the three highest dose metrics were obtained for the human occupational exposure scenarios. The relatively high dose metrics for AUC–TCA in the human reflect the much slower clearance of TCA compared to the rodent. It is interesting to note that the rank ordering in Table 8 would be different if it were based on AUC–DCA. In that case, all of the human dose metrics would be uniformly below the positive animal bioassay dose metrics, reflecting the rapid clearance of DCA in the human.

Although AUC is a standard metric for tissue exposure, other forms of the dose metrics for DCA and TCA might be more appropriate for their modes of action. If it is possible that the tumorigenic effects of these chemicals are related to some aspect of their interaction with a receptor, peak concentrations (C_{MAX}), or TACC, might actually be more appropriate than AUCs. Another nonlinear dose metric recently discussed for receptor-mediated effects is based on average receptor occupancy (160). Unfortunately, the more an attempt is made to include pharmacodynamic events in a dose metric, the more difficult it becomes to collect the data necessary for its use. In the case of TCE, there are currently no experimental data available to evaluate the use of such alternative pharmacodynamic dose metric approaches. Of the possible dose metrics, only AUC, C_{MAX}, and TACC can be estimated from the data currently available. All three of these metrics are available for TCA in the PBPK model.

**Lung dose metric.** As described earlier, tumors have been observed in the lungs of mice exposed to TCE by inhalation. The mechanism in this case appears to be entirely different from that in the liver. In a well-designed experimental effort (71), investigators at ICI combined in vivo and in vitro experiments to elucidate the mechanism of TCE carcinogenicity in the mouse lung.

In the in vivo studies, female mice and rats were exposed to TCE at a range of inhaled concentrations at and below the concentrations at which lung tumors have been observed in mice, and the effects of TCE in the lung were determined. A specific lesion, characterized by vacuolization of lung Clara
cells, was observed in mice, but not rats. There was evidence of a threshold for the Clara cell effects at about 20 ppm. The majority of Clara cells were unaffected at 20 ppm and all enzyme markers were normal, whereas at 200 ppm most of the Clara cells showed marked vacuolization accompanied by marked loss of CYP450 activity. Mice exposed to 100 ppm CHL by inhalation displayed Clara cell lesions similar to those observed with 1,000 ppm TCE. In contrast to these results, only mild effects were observed with TCOH inhaled at 100 ppm, and none were observed with 500 mg/kg TCA given intraperitoneally. (The effects had been observed with intraperitoneally administered TCE at 2,000 mg/kg.) These results suggested that CHL was responsible for the toxicity.

In the in vitro studies conducted by Odum et al. (71), mouse lung Clara cells metabolized TCE to CHL, TCOH, and TCA, with CHL being the major metabolite. Significantly, no TCOH glucuronide was detected. In comparison with mouse Clara cells, mouse hepatocytes produced primarily TCOH and its glucuronide. In both cell preparations, a steady-state concentration of CHL was achieved. Separate in vitro studies demonstrated that mouse Clara cells possess a relatively low activity for the glucuronidation of TCOH compared either to the glucuronidation of other substrates in the lung or to the glucuronidation of TCOH in the liver. It has also been determined that ADH, the enzyme that converts CHL to TCOH, has low activity in the mouse lung (162), consistent with the relatively low production observed in the Clara cells. On the basis of this evidence, the investigators concluded that the observed acute toxicity in the lung was a result of accumulation of CHL in Clara cells, resulting from a limitation in the formation of TCOH and its glucuronide. The specificity of this lesion for the Clara cells can be rationalized in terms of their relatively high CYP450 activity, coupled with a limited ADH and glucuronosyltransferase activities.

The implications of these results for the lung tumorigenicity of TCE are 2-fold. First, the accumulation of CHL, if it does occur in vivo, has clear carcinogenic implications, since CHL was genotoxic in a number of studies (71). Second, the recurrent toxicity observed with intermittent exposure could produce increased cell proliferation, exacerbating the genotoxic effect. In terms of the requirements for a lung dose metric in the PBPK model of TCE, it would appear that the model should include, at minimum, a description of the in situ metabolism of TCE in the Clara cell, to the extent of providing dose measures based on achieved CHL concentrations. Although a number of significant qualitative and quantitative uncertainties remain concerning the carcinogenicity observed in the lung, the use of the PBPK model could provide insights on the quantitative consistency of various mechanistic hypotheses with experimental data. There do not appear to be sufficient data at this point to support a quantitative description of the species-dependent pharmacokinetic dose response for the lung carcinogenicity.

The lung dose metric calculations shown in Table 6 can be used to evaluate the consistency of the CHL dose metrics with the bioassay results. As mentioned previously, the metrics in the table are arranged in decreasing order of LADD for AUC–CHL. Bioassay exposures associated with LADDs for AUC–CHL greater than 1.5 and $C_{max}$ for CHL greater than 0.3 tended to be positive, whereas bioassay exposures with AUC–CHL LADDs less than 1.5 and $C_{max}$ for CHL less than 0.3 were uniformly negative. The daily peak concentration dose metric ($C_{max}$ for CHL) appears to be more consistent with the negative response of the rats. Neither metric explains the fact that the oral mouse bioassays were negative for lung tumors, suggesting the possibility of a portal-of-entry effect.

**Kidney dose metric.** A variety of mechanisms have been identified for the kidney effects of halogenated hydrocarbons (162). The fact that tumors are observed only in the rat might suggest that they are associated with the male rat nephropathy described for many hydrocarbons, in which the accumulation of a male-rat-specific $\alpha_1$-globulin in proximal tubular cells leads to hyaline droplet accumulation, necrosis, increased cell proliferation, and cancer (163). However, a study designed specifically to evaluate this possibility showed evidence of the hyaline droplet accumulation and increased cell replication with PEC but not with TCE (163). It was also felt that the oxidative metabolism of TCE was unlikely to explain the kidney carcinogenicity in rats, since the rate of metabolism in the liver greatly exceeds that in the kidney, and no liver tumors are seen in the rat (72). An alternative mechanism was proposed, in which direct conjugation of TCE with GSH in the liver was followed by further metabolism in the kidney to a cysteine conjugate that could then be cleaved to a reactive intermediate in the kidney tubular cells (131). The cysteine conjugate formed from TCE, DCVC, has been shown to be highly nephrotoxic (78) and mutagenic in the Ames test (115).

As with the lung carcinogenicity of TCE, more than one mechanism may play a role in the kidney tumors. The tumors produced in the kidney by TCE are very rare tumors in control animals and do not appear to be associated with the exacerbation of spontaneous processes, suggesting that a genotoxic mechanism may be responsible. On the other hand, in the only bioassay that reported a significant increase in kidney tumors from TCE (67), cytotoxicity was observed in the kidney at both the low and high doses, whereas tumors were observed only at the high dose. Kidney cytotoxicity was also reported in association with a nonstatistically significant incidence of kidney tumors in another study (65,66). However, dosing of mice with DCVC in drinking water for 46 weeks produced clear evidence of toxicity at 87 weeks but no evidence of tumors (164). The significance of this result is enhanced by the observation that activation of DCVC in the kidney appears to be much greater in the mouse than in the rat, and the mouse also appears to be more
responsive to the induction of cell proliferation by DCVC than the rat (138). Moreover, measurements of acid-labile protein adducts in the kidney associated with DCVC suggest that the production of DCVC-derived reactive species in the kidney resulting from an oral dose of 1,000 mg/kg TCE may actually be greater in mice than in rats (137,138), and mice but not rats showed increased cell proliferation in the kidney in response to treatment with TCE at 1,000 mg/kg. Other studies in the rat also fail to support the suggestion that significant hyperplasia is produced in the kidney from exposure of rats to TCE (163). Thus, whether a genotoxic or cytotoxic mechanism involving DCVC is proposed, it is difficult to explain either the negative results of the DCVC bioassay in the mouse or the greater sensitivity of the rat compared to the mouse with regard to kidney tumors from TCE. Nevertheless, a mode of action for TCE in the kidney involving mutagenicity and cytotoxicity from DCVC is the most supportive choice at present, especially since no suggestion for an alternative source of the observed tumorigenicity has been provided in any of the studies just described.

The kidney dose metric calculations shown in Table 7 can be used to evaluate the consistency of the KTOX dose metric with the bioassay results. Bioassay exposures associated with KTOX LADDs greater than 15 tended to be positive, whereas bioassay exposures with KTOX LADDs less than 15 were negative. The mouse dose metric is well below that of the rat, consistent with bioassay results. The lower dose metric values in the mouse result from the higher ratio of clearance (KNATC) to intoxication (KBLC) in the mouse as compared to the rat.

Noncancer dose metrics. The issues associated with the selection of dose metrics for the noncancer toxicity of TCE are discussed in a companion article in this same issue (44). Therefore, only a short summary of the rationale for the selection of noncancer dose metrics is included here. The relationship of various noncancer dose metrics across species is shown in Table 9. In this table, the values of the dose metrics are shown for equal administered dose. Clearly, the human equivalent dose or concentration for a given animal study depends on both the route of exposure and the dose metric chosen, which in turn depend on the mode of action assumed for the specific end point being considered.

Neurological effects. The neurological effects of short-term exposure to solvents such as TCE are rapidly reversible, suggesting that they result from a physicochemical effect of the parent chemical on the proper function of lipophilic cellular membranes. Appropriate dose metrics for these effects would be the peak concentration or AUC for the parent chemical in the brain. Since tissue–blood partition coefficients are relatively uniform across species, the peak concentration in the blood can be used as a surrogate. Alternatively, in the case of TCE, an appropriate dose metric might be the peak concentration for TCOH, which has been suggested to be responsible for the observed neurological effects of chloral hydrate (79).

Hepatotoxicity. Pharmacokinetic studies (73) have demonstrated that the relationship between the acute hepatotoxicity of TCE and the total production of urinary metabolites is linear, and it has been suggested that this result is consistent with the hypothesis that the toxicity is produced by reactive intermediates (13). Based on this assumption, the most reasonable dose metric for the hepatic toxicity of TCE would be the total amount of metabolism divided by the volume of the liver (18). On the other hand, a comparison of the toxic potency for TCE and PERC (73) suggests that TCA, rather than total metabolism, is responsible for the liver effects of these chemicals (44).

Nephrotoxicity. As already discussed, the toxicity observed in the kidney appears to be due to metabolism of DCVC. Therefore, the cancer dose metric (KTOX) provides a useful metric for the kidney toxicity as well.

Immunological and developmental effects. Significant uncertainty exists regarding the appropriate dose metric for immunological and developmental effects. Possible metrics include the peak concentrations and AUCs for TCE and TCA. In the case of fetal effects, the dose metric would most properly be calculated using a PBPK model with a description of the fetus. However, dose metrics based on maternal blood should provide a reasonable surrogate for the effects of TCE, since TCE and its metabolites appear to move readily across the placenta (91).

Pharmacodynamic Dose Metrics

The discussion in this article has been restricted primarily to pharmacokinetic issues. However, the line between pharmacokinetics and pharmacodynamics is ill defined, and a pharmacokinetic model can often be extended somewhat into the pharmacodynamic realm. The following discussion touches on some of the areas where there is potential to develop improved dose metrics for the PBPK model and that include some level of pharmacodynamics.

Liver. Evidence regarding the mode of action of TCA and DCA could potentially be used to develop a dose metric more closely associated with tumorigenicity. In principle, if information on the differential response across species to mitogenic effects from TCA and DCA were obtained, it could be incorporated into a pharmacodynamic tissue dose metric. Possible pharmacodynamic metrics in the case of the liver carcinogenicity of TCE might be the expression of TGF-β or a measurable suppression of cell proliferation in hepatocytes as a marker of an early response to a presumed mitogenic signal. The use of a similar approach, based on the observed dose dependence of hormonal response, has been proposed for an analogous carcinogenic mechanism associated with follicular cell carcinoma in the thyroid (165).

Lung and kidney. If the kidney and lung carcinogenicity of TCE is considered to result primarily from enhanced cell proliferation secondary to recurrent toxicity, possible dose metrics would include measures of cytotoxicity, cell death, or cell division, as has been proposed for the liver carcinogenicity of chloroform (35,166). In the case of chloroform, fairly complicated metrics involving the instantaneous rates of metabolism and distributions of cellular sensitivity have been suggested (35,55). To apply these approaches to

Table 9. Noncancer daily dose metrics for continuous exposure.

| Metric                        | Mouse | Rat | Human |
|-------------------------------|-------|-----|-------|
| 1 ppm inhalation              |       |     |       |
| TCE, peak concentration       | 0.028 | 0.028 | 0.020 |
| AUC                           | 0.77  | 0.80 | 0.56  |
| Total metabolism              | 69    | 79   | 16    |
| TCA, peak concentration       | 6.9   | 1.0  | 9.7   |
| AUC                           | 164   | 24   | 230   |
| TCOH, peak concentration      | 0.013 | 0.022 | 0.13  |
| AUC                           | 0.31  | 0.52 | 3.2   |
| KTOX, production of reactive mercaptan | 0.0012 | 0.006 | 0.006 |
| 1 mg/kg/day drinking water    |       |     |       |
| TCE, peak concentration       | 0.00007 | 0.00048 | 0.009 |
| AUC                           | 0.0016 | 0.011 | 0.19  |
| Total metabolism (per gram liver) | 17.4 | 28.1 | 33    |
| TCA, peak concentration       | 1.36  | 0.38 | 20.13 |
| AUC                           | 12.6  | 9.15 | 483   |
| TCOH, peak concentration      | 0.0024 | 0.008 | 0.275 |
| AUC                           | 0.057 | 0.19 | 6.6   |
| KTOX, production of reactive mercaptan | 0.0002 | 0.002 | 0.013 |

*Concentration units: mg/L. **AUC unit: mg·h/L. ATotal metabolism units: mg/kg liver. **KTOX units: mg/kg kidney.
the kidney and lung carcinogenicity of TCE would require extensive studies similar to those that have recently been conducted with chloroform at the Chemical Industry Institute of Toxicology (CIIT) (167–170). Of particular note, the dose response for cytotoxicity in these subchronic studies with chloroform is markedly different from that observed in acute and \textit{in vivo} studies (35). It appears that caution must be used when the dose response for a surrogate measure of tissue response is derived from \textit{in vitro} or short-term \textit{in vivo} experiments. If exposure to a genotoxic metabolite is also considered to be quantitatively important in the lung and kidney target tissues, an even more complicated dose metric would be required. The use of a dose metric based on the product of DNA-protein cross-links and cell-labeling index has been suggested for evaluating the incidence of nasal tumors from formaldehyde exposure, assuming a carcinogenic mode of action involving both genotoxicity and cytotoxicity (171).

Again, extensive studies paralleling CIIT’s efforts with formaldehyde would be required to apply this approach to TCE.

**Target Tissue Correspondence**

Another aspect of uncertainty relevant to the incorporation of pharmacokinetic modeling in risk assessment is the question of how to deal with a lack of correspondence of target tissues across species. For example, none of the tumors observed in TCE bioassays were produced in both mice and rats. This behavior can be contrasted with that of a trans-species carcinogen such as vinyl chloride, which produces tumors in the same target tissue (the liver) in all species tested, as well as in humans, with similar potency (26). The lack of site correspondence for TCE in different animal species clearly has important implications for the expectation of site correspondence between animals and humans (which is implicit in the pharmacokinetic approach for risk assessment). Nevertheless, the assumption necessarily underlying the application of pharmacokinetic modeling in a risk assessment for TCE would be that the human target tissues of concern would be the same tissues identified in the animal studies. As pointed out earlier, there is at least some suggestion from epidemiological studies that another target tissue for TCE in the human could be the lymphatic system. If sufficient evidence of a link between TCE exposure and lymphoma were obtained from epidemiological studies, but without sufficient dose–response information to support a potency estimate, it is unclear how pharmacokinetic modeling could be used to provide an animal-based estimate unless a statistically significant dose response for lymphoma could be demonstrated in the rodent. Even then, data would also be required on the metabolism and mode of action of TCE in this target tissue.

**Human Variability**

Standard cancer risk assessment practice estimates the risk for an average individual, whereas noncancer risk assessment typically applies an uncertainty factor of 10 to account for human variability and the possibility of sensitive subpopulations. Human variability plays an important role in determining the actual risk to an individual compared to the average risk to a population. Part of this variability is pharmacokinetic and is subject to quantification. For example, the variation in the human dose metrics for TCA presented in Table 5 primarily reflects the impact of variability, as opposed to uncertainty, in human pharmacokinetics on target tissue dose (in this case, for tissue exposure to TCA resulting from environmental exposure to TCE).

Pharmacokinetic factors affecting the response of an individual to the toxicity and carcinogenicity of a chemical such as TCE include size, weight, condition, fat content, and level of physical activity. These factors modify the uptake, distribution, and elimination of TCE associated with a given exposure (172). For example, an individual with a large proportion of fat will absorb more of a chemical such as TCE and retain it longer than a lean individual. This longer storage increases the opportunity for metabolism to the active species. Studies on normal human volunteers have shown significant variation in individual pharmacokinetic behavior, and it is clear that this variability in pharmacokinetic factors is an important component of the overall interindividual variability of susceptibility to the toxic effects of chemicals (173).

By far the most important variability impacting target tissue dose is in metabolism. Four different isozymes of CYP450 have been found to play a role in the oxidative metabolism of TCE in rodents: 1A1/2, 2B1/2, 2C11/6, and 2E1. Of these, only 2C11/6 is not found in humans. CYP 2E1 appears to have the highest affinity for TCE, although the other isozymes can become important at higher concentrations (100). Sex, pregnancy, and age-related differences in metabolism can result from normal variations in CYP 2E1 content (101); increased metabolism can result from the inducibility of CYP 1A1/2 (e.g., by aromatics), 2B1 (e.g., by phenobarbital), or 2E1 (e.g., by ethanol) (102,115). Studies of human populations have shown that the activity of the CYP enzymes can vary by more than a factor of 10 between individuals (152,174,175), and that there is a genetic difference (polymorphism) between individuals with high activity and low activity that is associated with a different susceptibility to cancer (176). Genetic polymorphisms of the CYP enzymes across racial and ethnic groups have been observed (177), as have quantitative differences in metabolic capacity (178).

Sex differences in the excretion of TCE metabolites have also been noted in the human (179), with females excreting a larger proportion of TCA and a smaller proportion of TCOH than males. The difference between females and males in the ratio of TCA to TCOH excreted is greatest initially (as much as a factor of 5.5 during the first 24 hr after exposure), suggesting that the difference derives from a relatively greater rate of the production of TCA from CHL rather than from TCOH. The production of TCA in humans appears to be highly variable and generally somewhat higher than in other animals. For example, in one study the production of TCA from chloral hydrate in different individuals varied from 5 to 47% (107).

There are still other factors such as disease and hormonal status that could also affect the individual risk from exposure to a TCE, either because of an impact on pharmacokinetics or metabolism, or due to other interactions. Estrogens, for example, have been associated with both increased risk (for breast cancer) and decreased risk (for colon cancer) and are also metabolized by the CYP system (175). Therefore, the possibility of interaction with TCE exposure includes metabolic inhibition or induction as well as tumor promotion or repression.

Pharmacokinetic and metabolic differences alone cannot explain the overall interindividual variation in susceptibility observed in exposed populations (173,180). Clearly there are other, less well understood interindividual differences, both acquired (due to environmental exposures or disease states) and inherited (due to genetic differences) that are also important determinants of the individual risk for development of toxicity from exposure to a chemical. However, to the extent that we can quantitatively describe and evaluate pharmacokinetic and metabolic variation, it will become increasingly possible to estimate the range of risks in an exposed population and to identify the factors that put individuals at the greatest risk.

**Conclusions**

The PBPK model described in this article provides reasonably accurate and precise estimates of dose metrics based on TCE and its major metabolites, TCA, TCOH, and DCA, in both experimental animals and humans. Tissue dose metrics calculated with the model should therefore be useful in risk assessments for end points where the mode of action involves tissue exposure to these chemicals. Other target tissue dose metrics that can be calculated with the model, including CHL in the lung and DCVC in the kidney, are highly uncertain due to a lack
of adequate pharmacokinetic data across species. Additional studies could greatly reduce the uncertainty associated with these dose metrics and make their use in risk assessments more viable. However, it must be understood that pharmacokinetics is only one dimension of the process of estimating human risk from animal studies; the other potentially more important dimension is pharmacodynamics. Species differences in pharmacodynamics may lead to wide differences in susceptibility to tumors or to other toxic outcomes at the same target tissue doses.

Appendix

Equations for TCE PBPK Model

Equations for Main (Parent Chemical) Model

Concentration of TCE in inhaled air (m/L):

\[ C_{\text{inh}} = \frac{MW_{\text{TCE}}}{24450.0} \times C_{\text{inh}}(ppm) \]

Rate of exhalation of TCE (mg/hr):

\[ \frac{dA_{\text{inh}}}{dt} = Q_P \times \frac{C_{\text{inh}} - C_A}{P_b} \]

Concentration of TCE in exhaled air (ppm):

\[ C_{\text{ex}} = \frac{C_A \times 24450.0}{MW_{\text{TCE}}} \]

Rate of change in amount of TCE in duodenum (mg/hr):

\[ \frac{dA_D}{dt} = k_D \times A_D - k_D \times A_D - k_D \times A_D \]

Rate of change in amount of TCE in gut (mg/hr):

\[ \frac{dA_G}{dt} = Q_G \times (C_{AB} - CV \times k_D) + k_G \times A_D \]

Rate of change in amount of TCE in liver (mg/hr):

\[ \frac{dA_L}{dt} = Q_l \times (C_{AB} - CV \times k_D) + k_G \times A_D \]

Rate of metabolism in liver (mg/hr):

\[ RAM_L = \frac{dA_L}{dt} = k_G \times A_D \]

Rate of change in amount of TCE in stomach (mg/hr):

\[ \frac{dA_{\text{st}}}{dt} = TOTAL - k_A \times A_S - k_A \times A_S \]

Rate of change in amount of TCE in other tissues (mg/hr):

\[ \frac{dA_{\text{other}}}{dt} = Q_{\text{other}} \times (C_{AB} - CV_{\text{other}}) \]

Concentration of TCE in tissue venous blood (mg/L):

\[ CV_{\text{other}} = \frac{A_{\text{other}}}{V_{\text{other}} \times P_{\text{other}}} \]

Rate of excretion of TCE (mg/hr):

\[ \frac{dA_{\text{ex, TCE}}}{dt} = k_D \times A_D \]

Concentration of TCE in arterial blood (mg/L):

\[ C_A = \frac{QC \times CV_A + OP \times C_{\text{inh}}}{QC + OP} \]

Concentration of TCE in mixed venous blood (mg/L):

\[ CV_B = \frac{Q_B \times CV_B + Q_P \times C_{\text{inh}} + Q_{GB} \times CV_{GB} + Q_{RP} \times CV_{RP}}{QC} \]

Liver Metabolism Submodel

Rate of change in amount of TCA (mg/hr):

\[ \frac{dA_{\text{TCA}}}{dt} = PO \times \frac{MW_{\text{TCA}}}{MW_{\text{TCE}}} \times RAM_L + k_{art, TCA} \times A_{\text{TCA}} - k_{heb, TCA} \times A_{\text{TCA}} \]

Concentration of TCA (mg/L):

\[ C_{\text{TCA}} = \frac{A_{\text{TCA}}}{VD_{\text{TCA}}} \]

Rate of reduction of TCA to DCA (mg/hr):

\[ RAM_{\text{TCA}} = \frac{dA_{\text{TCA}}}{dt} - \frac{V_{\text{Max}, TCA}}{KM_{\text{TCA}} + C_{\text{TCA}}} \]

Concentration of TCA (mg/L):

\[ C_{\text{TCA}} = \frac{A_{\text{TCA}}}{VD_{\text{TCA}}} \]

Rate of enterohepatic recirculation of glucuronide (mg/hr):

\[ \frac{dA_{\text{Glu}}}{dt} = k_{heb, TCA} \times A_{\text{TCA}} \times \frac{MW_{\text{TCA}}}{MW_{\text{TCOG}}} \]

Rate of change in amount of DCA (mg/hr):

\[ \frac{dA_{\text{DCA}}}{dt} = \frac{MW_{\text{DCA}}}{MW_{\text{TCA}}} \times \frac{RAM_{\text{TCA}}}{RAM_{\text{TCA}}} + \frac{MW_{\text{DCA}}}{MW_{\text{TCA}}} \times \frac{RAM_{\text{TCA}} - RAM_{\text{DCA}}}{RAM_{\text{TCA}} - RAM_{\text{DCA}}} \]

Rate of change in amount of TCOH (mg/hr):

\[ \frac{dA_{\text{TOH}}}{dt} = 1.0 - PO \times \frac{MW_{\text{COH}}}{MW_{\text{TCE}}} \times \frac{RAM_L}{RAM_L} + k_{heb, TCOH} \times A_{\text{GL}} - k_{heb, TCOH} \times A_{\text{GL}} - RAM_{\text{COH}} - RAM_{\text{COH}} \]

Concentration of TCOH (mg/L):

\[ C_{\text{TCOH}} = \frac{A_{\text{TCOH}}}{VD_{\text{TCOH}}} \]

Rate of excretion of TCOH (mg/L):

\[ \frac{dA_{\text{ex, TCOH}}}{dt} = \frac{MW_{\text{TCE}}}{MW_{\text{TCA}}} \times k_{heb, TCOH} \times A_{\text{TCOH}} - k_{heb, TCOH} \times A_{\text{TCOH}} \]

Rate of change in amount of TCOH (mg/hr):

\[ \frac{dA_{\text{COH}}}{dt} = \frac{MW_{\text{COH}}}{MW_{\text{TCA}}} \times \frac{A_{\text{COH}}}{VD_{\text{TCOH}}} \]

Rate of change in amount of TCA (mg/hr):

\[ \frac{dA_{\text{TCO}}}{dt} = \frac{MW_{\text{TCA}}}{MW_{\text{TCA}}} \times \frac{A_{\text{TCO}}}{VD_{\text{TCA}}} \]

Rate of excretion of TCA (mg/hr):

\[ \frac{dA_{\text{ex, TCA}}}{dt} = k_{heb, TCA} \times A_{\text{TCA}} \times \frac{MW_{\text{TCA}}}{MW_{\text{TCA}}} \]

Rate of change in amount of TCOH (mg/hr):

\[ \frac{dA_{\text{TOH}}}{dt} = 1.0 - PO \times \frac{MW_{\text{COH}}}{MW_{\text{TCE}}} \times \frac{RAM_L}{RAM_L} + k_{heb, TCOH} \times A_{\text{GL}} - k_{heb, TCOH} \times A_{\text{GL}} - RAM_{\text{COH}} - RAM_{\text{COH}} \]
Rate of clearance of DCA (mg/hr):

\[ \text{RAM}_{\text{DCA}} = \frac{\text{VMax}_{\text{DCA}} \times C_{\text{DCA}}}{\text{KM}_{\text{DCA}} + C_{\text{DCA}}} \]

Concentration of DCA (mg/L):

\[ C_{\text{DCA}} = \frac{A_{\text{DCA}}}{V_{D_{\text{DCA}}}} \]

Rate of excretion of DCA (mg/hr):

\[ \frac{dA_{\text{exDCA}}}{dt} = k_{\text{DCA}} \times A_{\text{DCA}} \]

**Lung Metabolism Submodel**

Concentration of TCE in tracheobronchial venous blood (mg/L):

\[ CV_{TB} = C_{AB} \times \frac{RAM_{TB}}{Q_{TB}} \]

Rate of TCE metabolism in tracheobronchial (mg/hr):

\[ RAM_{TB} = \frac{dA_{\text{TB}}}{dt} = \frac{V_{\text{Max}}_{TB} \times CV_{TB}}{K_{M_{TB}} + CV_{TB}} \]

Concentration of chloral in Clara cells (mg/L):

\[ C_{\text{CM}} = \frac{\frac{RAM_{TB}}{Q_{TB}} \times \frac{MW_{\text{CM}}}{LM_{\text{TCE}}}}{1.0 - \frac{\frac{RAM_{TB}}{Q_{TB}} \times \frac{MW_{\text{CM}}}{LM_{\text{TCE}}}}{\frac{V_{\text{Max}}_{TB}}{K_{M_{TB}}} \times \frac{MW_{\text{TCE}}}{LM_{\text{TCE}}}}} \]

**Kidney Metabolism Submodel**

Rate of change in amount of DCVC (mg/hr):

\[ \frac{dA_{\text{DCVC}}}{dt} = \frac{MW_{\text{DCVC}} \times KF \times CV_{T} \times V_{L}}{(\text{knat} + \text{kb}) \times A_{\text{DCVC}}} \]

Concentration of DCVC (mg/L):

\[ C_{\text{DCVC}} = \frac{A_{\text{DCVC}}}{V_{D_{K}}} \]

Rate of clearance of DCVC (mg/hr):

\[ \frac{dA_{\text{exDCVC}}}{dt} = \frac{MW_{\text{DCVC}} \times \text{knat} \times A_{\text{DCVC}}}{MW_{\text{DCVC}}} \]

Kidney toxicity from DCVC (mg/hr):

\[ K_{\text{TOX}} = \frac{k_{\text{D}} \times A_{\text{DCVC}}}{V_{L}} \]

**Variables**

| Amounts | Description |
|---------|-------------|
| \( A_{\text{tissue}} \) | amount of TCE in compartment (mg) |
| \( A_{\text{chemical}} \) | amount of chemical (mg) |
| \( A_{\text{Exc-chemical}} \) | amount of chemical excreted in urine (mg) |
| \( A_{\text{metabolite}} \) | amount of TCE metabolized in compartment (mg) |
| \( A_{\text{metabolite}} \) | amount of chemical metabolized (mg) |
| \( A_{\text{Ox-TCOH}} \) | amount of TCOH oxidized (mg) |
| \( A_{\text{Red-TCOH}} \) | amount of TCOH reduced (mg) |
| \( A_{\text{Gl-TCOH}} \) | amount of TCOH glucuronidated (mg) |
| \( CV_{\text{tissue}} \) | venous concentration of TCE in compartment (mg/L) |

**Concentrations**

| \( C_{\text{tissue}} \) | concentration of TCE in compartment (mg/L) |
| \( C_{\text{chemical}} \) | concentration of chemical (mg/L) |

**Parameters**

**Flow rates**

- QC = cardiac output (L/hr)
- Ql = blood flow to compartment (L/hr)
- Qp = pulmonary ventilation (L/hr)

**Volumes**

- \( V_{T} \) = volume of compartment (kg)
- \( V_{D_{\text{chemical}}} \) = volume of distribution for chemical (kg)

**Partitions**

- PB = blood to air partition coefficient
- \( P_{\text{tissue}} \) = tissue to blood partition coefficient

**Rate constants**

- \( k_{\text{tissue}} \) = oral uptake rate from compartment (l/hr)
- \( keh_{\text{TCOH}} \) = biliary excretion rate of TCOH (l/hr)
- \( keh_{\text{TCOH}} \) = enterohepatic recirculation rate for TCOH (l/hr)
- \( kp_{\text{zero}} \) = input rate for TCE in drinking water (mg/hr)
- \( k_{\text{tissue}} \) = transfer rate from compartment (l/hr)
- \( k_{\text{urine}} \) = urinary excretion rate of chemical (l/hr)

**Abbreviations Used in Equations**

**Parameters (cont’d.)**

- PO = percent oxidation of chloral
- KF = rate of production of DCVC from TCE (hr)
- kb = rate of metabolism of DCVC by β-lyase (hr)
- knat = clearance rate of DCVC by NAT (hr)
- \( V_{\text{Max}}_{\text{tissue}} \) = capacity for metabolism of TCE in compartment (mg/hr)
- \( V_{\text{Max}}_{\text{tissue}} \) = capacity for chloral clearance in compartment (mg/hr)
- \( V_{\text{Max}}_{\text{chemical}} \) = capacity for metabolism of chemical (mg/hr)
- \( V_{\text{Max}}_{\text{GTCOH}} \) = capacity for glucuronidation of TCOH (mg/hr)
- \( V_{\text{Max}}_{\text{OTCOH}} \) = capacity for oxidation of TCOH (mg/hr)
- \( V_{\text{Max}}_{\text{RTCOH}} \) = capacity for reduction of TCOH (mg/hr)
- \( K_{\text{m}}_{\text{tissue}} \) = affinity for metabolism of TCE in compartment (mg/L)
- \( K_{\text{m}}_{\text{tissue}} \) = affinity for chloral clearance in compartment (mg/L)
- \( K_{\text{m}}_{\text{chemical}} \) = affinity for metabolism of chemical (mg/L)
- \( K_{\text{MG}_{\text{TCOH}}} \) = affinity for glucuronidation of TCOH (mg/L)
- \( K_{\text{MO}_{\text{TCOH}}} \) = affinity for oxidation of TCOH (mg/L)
- \( K_{\text{MR}_{\text{TCOH}}} \) = affinity for reduction of TCOH (mg/L)

**Other**

- \( K_{\text{Tox}} \) = metric for kidney cytotoxicity from DCVC
- \( MW_{\text{chemical}} \) = molecular weight of chemical (mg/mole)
- \( TOTAL \) = total oral dose of TCE (mg)

**Compartment**

- AB = arterial blood
- Du = duodenum
- Exh = exhaled air
- F = fat
- G = gut tissue
- GL = gut lumen
- Inh = inhaled air
- L = liver
- RP = rapidly perfused tissues
- SP = slowly perfused tissues
- St = stomach
- TB = tracheo-bronchi
- VB = venous blood

**Chemicals (denoted by “chemical”)**

- Chl = chloral
- DCA = dicloroacetic acid
- DCVC = dichlorovinylcysteine
- NADC = N-acetyl-DCVC
- TCA = trichloroacetic acid
- TCE = trichloroethylene
- TCOH = TCOH glucuronide
- TCOH = trichloroethanol
REFERENCES AND NOTES
1. National Research Council. Pharmacokinetics in Risk Assessment. Drinking Water and Health, Vol 8. Washington, DC: National Academy Press, 1997.
2. U.S. EPA. Biological Data for Pharmacokinetic Modeling and Risk Assessment. EPA/800/0-90/019, Washington, DC: U.S. Environmental Protection Agency, 1989.
3. Geelhoed TR, Henry PJ. Concepts of Route-to-Route Extrapolation for Risk Assessment. New York: Elsevier, 1990.
4. Frederick CB. Limiting the uncertainty in risk assessment by the development of physiologically based pharmacokinetic and mechanistic models in cancer risk assessments for environmental contaminants: examples with vinyl chloride and trichloroethylene. Chemosphere 31:2591–2578 (1995).
5. Frieman CW, Blinks D, Finan CW, and Friesen GW. Filling the gap: From molecular pharmacokinetics in risk assessment: perchloroethylene as an example. In: Pharmacokinetics in Risk Assessment. Drinking Water and Health, Vol 8. Washington, DC: National Academy Press, 1997:389–399.
6. Clewell HJ, Jaront BM. Incorporation of pharmacokinetics in non-carcinogenic risk assessment: example with chlortoform-fluorobenzene. Risk Anal 14:265–276 (1994).
7. Himmeleit JJ, Lutz RJ. A review of the application of physiologically based pharmacokinetic modeling. J Pharmacokin Biopharm 7:127–145 (1979).
8. Gerlofs-Kleij EJ, Jain RK. Physiologically based pharmacokinetic modeling: principles and applications. J Pharm Sci 72:1107–1126 (1983).
9. D’Souza RW, Bloebaum H. Physiological pharmacokinetic models: some aspects of theory, practice and potential. Toxicol Ind Health 4:151–171 (1988).
10. Leung HW. Development and utilization of physiologically based pharmacokinetic models for toxicological applications. Toxicol Environ Health 21:247–267 (1991).
11. Clewell HJ, Andersen AM. Risk assessment extrapolations and physiological modeling. Toxicol Ind Health 1:111–131 (1985).
12. Clewell HJ, Andersen AM. Biologically motivated models for chemical risk assessment. Health Phys 57(supp 1):129–137 (1989).
13. U.S. EPA. Health Assessment Document for Trichloroethylene. EPA/600/8-89/055, Washington, DC: U.S. Environmental Protection Agency, 1989.
14. U.S. EPA. Addendum to the Health Assessment Document for Trichloroethylene: Updated Carcinogenicity Assessment for Trichloroethylene. EPA/600/8-90/026F, Washington, DC: U.S. Environmental Protection Agency, 1987.
15. U.S. EPA. Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. EPA/600/8-90/066F, Washington, DC: U.S. Environmental Protection Agency, 1987.
16. U.S. EPA. Update to the Health Assessment Document and Addendum for Dichloromethane (Methylene Chloride). Pharmacokinetics, Mechanism of Action, and Epidemiology. EPA/600/8-93/007A, Washington, DC: U.S. Environmental Protection Agency, 1997.
17. U.S. EPA. Integrated Risk Information System (IRIS) Database, Dichloromethane. http://www.epa.gov/iris. Cincinnati, OH: U.S. Environmental Protection Agency, 6 April 2000.
18. Andersen M, Clewell H, Gargason M, Smith FA, Beal WE. Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. Toxicol Appl Pharmacol 195:205–197 (1999).
19. Dankovic D, Baier AJ. The impact of exercise and intersubject variability on dose estimates for dichloromethane derived from a physiologically based pharmacokinetic model. Fundam Appl Toxicol 22:205–209 (1994).
20. Clewell HJ. Coupling of computer modeling with in vitro methodologies to reduce animal usage in toxicity testing. Toxicol Lett 80:101–119 (1996).
21. Clewell HJ. The use of physiologically based pharmacokinetic modeling in risk assessment: a case study with methylene chloride. In: Low-Dose Extrapolation of Cancer Risks: Issues and Perspectives (Olin S, Farland W, Park C, Rombouts L, Schuller R, Starr T, Wilson J, eds). Washington, DC: ILSI Press, 1995.
22. Occupational Safety and Health Administration (OSHA). Occupational Exposure to Methylene Chloride: Final Rule. Fed Reg 60:61940–61956 (1995).
23. Begon KT. Pharmacokinetics for regulatory risk analysis: the case of trichloroethylene. Regul Toxicol Pharmacol 8:447–466 (1988).
24. Bortland RA. Potential of physiologically based pharmacokinetics to ameliorate kinetic data of trichloroethylene and tetrachloroethylene obtained in rats and man. Br J Ind Med 46:239–240 (1989).
25. Fisher JW, Allen BC. Evaluating the risk of liver cancer in humans exposed to trichloroethylene using physiological models. Risk Anal 13:87–95 (1993).
26. Clewell HJ, Gentry PR, Gearhart JM, Allen BC, Andersen AM. Considering pharmacokinetic and mechanistic information in cancer risk assessments for environmental contaminants: examples with vinyl chloride and trichloroethylene. Chemosphere 31:2591–2578 (1995).
27. Frieman CW, Blinks D, Friesen GW, Friesen GW. Filling the gap: From molecular pharmacokinetics in risk assessment: perchloroethylene as an example. In: Pharmacokinetics in Risk Assessment. Drinking Water and Health, Vol 8. Washington, DC: National Academy Press, 1997:389–399.
28. Gearhart JM, Mahe DL, Greene RJ, Seckel CS, Flemming CD, Fisher JW. Clewell HJ. Variability of physiologically based pharmacokinetic (PBPK) model parameters and their effect on PBPK model predictions in a risk assessment for perchloroethylene (PCE). Toxicol Lett 88:131–144 (1995).
29. D’Souza RW, Francis WR, Bruce RD, Andersen AM. Physiologically based pharmacokinetic model for ethylene dichloride and its application in risk assessment. In: Pharmacokinetics in Risk Assessment. Drinking Water and Health, Vol 8. Washington, DC: National Academy Press, 1997:286–301.
30. Chen GW, Biancano JN. Incorporation of biological information in cancer risk assessment: example - vinyl chloride. Cell Biol Toxicol 5:417–444 (1989).
31. U.S. EPA. Vinyl chloride. Support document. Integrated Risk Information System (IRIS), Washington, DC: U.S. Environmental Protection Agency, 1998.
32. Reitz RH, Gargason M, Andersen AM, Provan WF, Green T. Predicting cancer risk from vinyl chloride exposure with a physiologically based pharmacokinetic model. Toxicol Appl Pharmacol 136:1–15 (1996).
33. Reitz RH, McCloskey FS, Park CN, Andersen AM, Gargason ML. Development of a physiologically based pharmacokinetic model for risk assessment with 1,4-dioxane. Toxicol Appl Pharmacol 105:57–54 (1990).
34. Leung HW, Puwanantachai J. Cancer risk assessment for dioxane based upon a physiologically-based pharmacokinetic approach. Toxicol Lett 51:147–162 (1990).
35. Reitz RH, Mendrala AL, Corley RA, Quast JT, Gargason ML, Andersen AM, Staats DA, Connolly RL. Estimating the risk of liver cancer associated with human exposures to chlorofom using physiologically based pharmacokinetic modeling. Toxicol Appl Pharmacol 105:443–459 (1990).
36. Cox LA, Ricci PF. Reassessing benzene cancer risks using internal dose doses. Risk Anal 11:401–410 (1991).
37. Frederick CB, Potter DW, Chang-Mateu ML, Andersen AM. A physiological pharmacokinetic and pharmacodynamic model to describe the oral dosing of rats with ethyl acrylate and its implications for risk assessment. Toxicol Appl Pharmacol 114:248–290 (1992).
38. Porter CJ, Kaplan NL. Variability of safe dose estimates when using complicated models of the carcinogenic process. A case study: methylene chloride. Fundam Appl Toxicol 13:533–544 (1990).
39. Bois FY, Zwie L, Tocier TN. Precision and sensitivity of pharmacokinetic models for cancer risk assessment: tetrachloroethylene in mice, rats, and humans. Toxicol Appl Pharmacol 102:308–315 (1990).
40. Hattis D, White P, Marmorstein L, Koch P. Uncertainties in pharmacokinetic modeling for perchloroethylene. I. Comparison of model structure, parameters, and predictions for low-dose metabolism rates for models derived by different authors. Risk Anal 10:445–459 (1990).
41. Hattis D, White P, Koch P. Uncertainties in pharmacokinetic modeling for perchloroethylene. II. Comparison of model predictions with data for a variety of different parameters. Risk Anal 13:599–610 (1993).
42. Spear RC, Bois FY, Woodwell T, Auslander D, Parker J, Salvin S. Modeling benzene pharmacokinetics across three sets of animal data: parametric sensitivity and risk implications. Risk Anal 14:641–654 (1994).
43. Barton HA, Clewell HJ. Evaluating noncancer effects of trichloroethylene: dosimetry, mode of action, and risk assessment. Environ Health Perspect 108(supp 2):323–334 (2000).
44. Buckingham JR, Bickley NJ. Pharmacokinetic and mechanistic toxicology, and carcinogenicity of trichloroethylene. CRC Crit Rev Toxicol 20:51–100 (1990).
45. Davison TF, Bellis RP. Consideration of the target organ toxicity of trichloroethylene in terms of metabolism and pharmacokinetics. Drug Metab Rev 23:493–599 (1991).
46. ATSDR. Toxicological Profile for Trichloroethylene. TP-92/19. Atlanta, GA:Agency for Toxic Substances and Disease Registry, 1993.
47. American Conference of Governmental Industrial Hygienists (ACGIH). Notice of intended change — trichloroethylene. Appl Occup Environ Hyg 7:788–792 (1992).
48. Occupational Safety and Health Administration (OSHA). Air cont...
Cleveland et al. 304

Environmental Health Perspectives 108: Supplement 2 • May 2000

89. Nakajima T, Okino T, Ohtsuka S, Kanku T, Yonekura I, Sato A. Ethanol-induced enhancement of trichloroethene metabolism and hepatotoxicity: difference from the effect of phenol. Toxicol Appl Pharmacol 94:227–237 (1989).

90. Nakajima T, Wang R-S, Elsowa E, Park SS, Gelboum HV. Vainio H. Cytochrome P450-related differences between rats and mice in the metabolism of benzene, toluene and trichloroethene in liver microsomal fractions. Toxicol Appl Pharmacol 45:15–23 (1978).

91. Nakajima T, Wang R-S, Elsowa E, Park SS, Gelboum HV. A comparative study on the contribution of cytochrome P450 isoforms to metabolism of benzene, toluene and trichloroethene in the liver of male and female B6C3F1 mice. Drug Metabol Dispos 16:390–395 (1988).

92. Nakajima T, Wang R-S, Kataoka Y, Kishi E, Elsowa E, Park SS, Gelboum HV. Vainio H. Sex-, age- and pregnancy-induced changes in the metabolism of toluene and trichloroethene in rat liver in relation to the regulation of cytochrome P450 at the transcriptional level. J Pharmacol Exp Ther 261:689–694 (1992).

93. Nakajima T, Wang R-S, Murayama N, Sato A. Three forms of trichloroethylene-metabolizing enzymes in rat liver induced by ethanol, phenobarbital, and 3-methylcholanthrene. Toxicol Appl Pharmacol 102:546–552 (1991).

94. Dekant W, Kook M, Henschler D. Metabolism of trichloroethylene in vivo and in vitro evidence for activation by glutathione conjugation. Chem Biol Interact 72:99–101 (1991).

95. Meyers DJ, Hindmarsh KW, Kankun K, Gorecki DJK, Kasiun GF. Chloral hydrate disposition following single-dose administration to critically ill neonates and children. Dev Pharmacol Ther 16:711–717 (1990).

96. Muller G, Spassovski M, Henschler D. Trichloroethylene exposure and metabolites in urine and blood. Arch Toxicol 29:335–340 (1972).

97. Payzow J, Powell J. The excretion of sodium trichloroacetate. J Pharmacol Exp Ther 157:257–263 (1967).

98. Marshall E, Owens A. Absorption, excretion and metabolic fate of chloral hydrate and trichloroethanol. Johns Hopkins Hosp Rep 95:1–18 (1954).

99. Owens A, Marshall E. Further studies on the metabolic fate of chloral hydrate and trichloroethanol. Johns Hopkins Hosp Rep 97:320–325 (1956).

100. Muller G, Spassovski M, Henschler D. Metabolism of trichloroethylene in the rat: Biopharm Sci 1:293–295 (1983).

101. Muller G, Spassovski M, Henschler D. Metabolism of trichloroethylene in man. III. Interaction of trichloroethylene and its metabolites. Environ Health Perspect 109:183–195 (2001).

102. Larson JL, Bull RJ. Effect of ethanol on the metabolism of trichloroethylene. J Toxicol Environ Health 28:390–406 (1990).

103. Nomiyama K, Nomiyama H. Dose-response relationship for trichloroethylene in man. Int Arch Occup Environ Health 39:237–248 (1977).

104. Template MV, Stevens DK, Stenner RD, Bonate PL, Tuman D, Bull RJ. Factors affecting species differences in the kinetics of metabolites of trichloroethylene. J Toxicol Environ Health 44:435–449 (1994).

105. Green T, Oudem J, Nash JA, Fostert JS. Perchloroethylene-induced rat kidney tumors: an investigation of the mechanism involved and their relevance to humans. Toxicol Appl Pharmacol 120:73–78 (1993).

106. Ely RJ, Stevens DK, Parkin J, Bull RJ. Acid-labile additives to protein can be used as indicators of the cytochrome S-conjugate pathway of trichloroethylene metabolism. J Toxicol Environ Health 46:443–464 (1995).

107. Ely RJ, Stevens DK, Parkin J, Bull RJ. Renal activation of trichloroethylene and S-(1,2-dichlorovinyl)lysine and cell proliferative responses in the kidneys of F344 rats and B6C3F1 mice. J Toxicol Environ Health 46:485–481 (1995).

108. Buckberg JD, Blagbrough IS, Borroff SW, Shah PN. Human renal C-S lyases: two cystolic isoenzymes. Toxicol Lett 53:253–259 (1990).

109. Blagbrough IS, Buckberg LD, Borroff SW, Shah PN. Human renal C-S lyase: structure-activity relationships of cysteine and mitochondrial enzymes. Toxicology 53:257–259 (1990).

110. Lash WH, Nelson RM, Van Dyke RA, Anders MW. Purification and characterization of human kidney cysteine conjugate lyase activity. Drug Metab Dispos 18:55–64 (1990).

111. Bimer G, Vamvakas S, Dekant W, Henschler D. Nephrotic and genotoxic N-acetyl-S-dichlorovinyl-L-cysteine is a urinary metabolite after occupational 1,1,2-trichloroethylene exposure in humans: implications for the risk of trichloroethylene exposure. Environ Health Perspect 99:281–284 (1993).

112. Bernauer U, Birner G, Dekant W, Henschler D. Bio- transformation of trichloroethylene: dose-dependent excretion of 1,1,2-trichloroethylene and metabolites in rats and humans after inhalation. Arch Toxicol 70:238–246 (1997).

113. Staats DA, Fisher JW, Conolly RB. Gastrointestinal absorption of xenobiotics in physiologically based pharmacokinetic models. Drug Metab Dispos 18:1055–1059 (1990).

114. Brown RP, Delp MD, Lindstedt SL, Rhomberg LR, Bellies RP. Physiological parameter values for physiologically based pharmacokinetic models. Toxicol Ind Health 13:407–408 (1997).

115. Arms AD, Travis DC. Reference Values for Parameters in Pharmacokinetic Modeling. EPA/600/68/004. Washington, D.C.U.S. Environmental Protection Agency, 1988.
147. Astrand P, Rodahl K. Textbook of Work Physiology. New York: McGraw-Hill, 1970.

148. Fisher JW, Peil JD, MacMahan KL, Abbas RR. A physiologically based pharmacokinetic model for inhalation of trichloroethylene in human volunteers. Toxicologist 30:22 (1970).

149. Abbas R, Fisher JW, Black RK, Janicki TJ, MacMahan K. Development of a physiologically based pharmacokinetic (PBPK) model for trichloroethylene and its metabolites in B6C3F1 mice. Toxicologist 30:248 (1996).

150. Ellifar AA, Lash LH, Andrus MW. Metabolic activation and detoxication of naphthoquinone and homocysteine 5-conjugates. Proc Natl Acad Sci USA 83:2667–2671 (1986).

151. Commandeur JNM, Boogaard PJ, Mulder GJ, Vermeulen NPE. Mutagenicity and cytotoxicity of two regiosomeric mercuric acids and cysteine 5-conjugates of trichloroethylene. Arch Toxicol 65:373–380 (1991).

152. Reitz RH, Mendrala AL, Guengerich FP. In vitro metabolism of ethylene chloride in human and animal tissues: use in physiologically-based pharmacokinetic models. Toxicol Appl Pharmacol 97:230–238 (1989).

153. Andersen ME, Clewell HJ, Frederick CB. Applying simulation modeling to problems in toxicology and risk assessment — a short perspective. Toxicol Appl Pharmacol 132:181–187 (1995).

154. Monaster A, Boerrama G, Duba W. Kinetics of trichloroethylene in repeated exposure of volunteers. Int Arch Occup Environ Health 20:239 (1970).

155. Stewart R, Dodd H, Gay H, Erley DS. Experimental human exposure to trichloroethylene. Arch Environ Health 20:246 (1970).

156. Lundstedt S. Personal communication.

157. Bogdan MS, Randall HW, Morgan KT. Histological localization of aldehyde dehydrogenase in the respiratory tract of the Fischer 344 rat. Toxicol Appl Pharmacol 82:569–587 (1986).

158. Dedrick RL. Pharmacokinetic and pharmacodynamic considerations for chronic hemodialysis. Kidney Int 31:515–517 (1975).

159. Bell Z, Olson K, Beny T. Final Report of Audit Findings of the Manufacturing Chemists Association (MCA): Administered Trichloroethylene (TCE) Chronic Inhalation Study at Industrial Bio-Test Laboratories, Inc., Decatur, IL, Unpublished data, 1978.

160. Mills JJ, Andersen ME. Dioxin hepatic carcinogenesis: biologically motivated modeling and risk assessment. Toxicol Lett 68:177–188 (1993).

161. Sorkin SP. The cells of the lung. In: Morphology of Experimental Respiratory Carcinogenesis (Netterfield P, Hanna MG Jr, Beecham JW, eds). Oak Ridge, TN:U.S. Energy Commission, 1970:3–43.

162. Lock EA. Studies on the mechanism of naphropoxygenation and naphropocarcinogenicity of halogenated alkenes. CRC Crit Rev Toxicol 19:23–42 (1988).

163. Goldsworthy TL, Light O, Bumett VL, Popp JA. Potential role of ox-2-glutathione, protein droplet accumulation, and cell replication in the renal carcinogenicity of rats exposed to trichloroethylene, perchloroethylene, and pentachlorothereane. Toxicol Appl Pharmacol 96:307–379 (1989).

164. Jaffe DR, Gandolfi AJ, Nagle RB. Chronic toxicity of S-tras-1,2-dichlorovinyl-l-cysteine in mice. J Appl Toxicol 4:315–318 (1984).

165. Cook JC, Murray SM, Frame ST, Hurlt ME. Induction of Ledyig cell adenomas by ammonium perfluorooctanoate: a possible endocrine-related mechanism. Toxicol Appl Pharmacol 113:309–317 (1992).

166. Butterworth BE, Templin MV, Borghoff SJ, Conolly RB, Kedders GL, Wolf DC. The role of regenerative cell proliferation in chloroform-induced cancer. Toxicol Lett 62:63–85 (1995).

167. Larson JL, Templin MV, Wolf DC, Jamison KC, Meyr S, Lenzinger J, Morgan KT, Wong BA, Conolly RB, Butterworth BE. A 90-day chloroform inhalation study in male and female B6C3F1 mice: implications for cancer risk assessment. Fundam Appl Toxicol 30:118–137 (1996).

168. Larson JL, Wolf DC, Butterworth BE. Acute hepatic and naphropoxygenation effects of chloroform in male F-344 rats and female B6C3F1 mice. Fundam Appl Toxicol 20:302–315 (1993).

169. Larson JL, Wolf DC, Butterworth BE. Induced cytotoxicity and cell proliferation in the hepatocarcinogenicity of chloroform in female B6C3F1 mice: comparison of administration by gavage in corn oil vs. SC injection in drinking water. Fundam Appl Toxicol 22:90–102 (1994).

170. Templin MV, Templin JL, Butterworth BE, Jamison KC, Wong BA, Wolf DC. A 90-day chloroform inhalation study in F-344 rats: profile of toxicity and relevance to cancer studies. Fundam Appl Toxicol 32:109–125 (1996).

171. Conolly RB, Morgan KT, Andersen ME, Monticello TM, Clewell HJ. A biologically-based risk assessment strategy for inhale formaldehyde. Comm Toxicol 4:269–283 (1992).

172. Wallace LA, Pellizzari ED, Hartwell TD, Davis V, Michael LC, Whitmore RW. The influence of personal activities on exposure to volatile organic compounds. Environ Res 50:37–55 (1989).

173. Hatto D, Endrich L, Bellum M. Human variability in susceptibility to toxic chemicals—a preliminary analysis of pharmacokinetic data from normal volunteers. Risk Anal 7:415–426 (1987).

174. Sabadie N, Malavieille C, Camus A-M, Bartsch H. Comparison of the hydroxilation of benz[a]pyrene with the metabolism of vinyl chloride, N-nitrosomorpholine, and N-nitroso-N'-methylurea to mutagens by human and rat liver microsomal fractions. Cancer Res 40:119–126 (1980).

175. Guengerich FP. Interindividual variation in biotransformation of carcinogens: basis and relevance. In: Molecular Dosimetry and Human Cancer: Analytical, Epidemiological, and Social Considerations (Gropman JD, Skipper RL, eds). Boca Raton, FL:CRC Press, 1989;27–51.

176. Uematsu F, Kikuchi H, Motomiya M, Abe T, Sagami I, Ohmachi T, Wakiu A, Kanamori R, Watanabe M. Association between restriction fragment length polymorphism of the human cytochrome P450E1 gene and susceptibility to lung cancer. Jpn J Cancer Res 82:254–256 (1991).

177. Stephens EL, Taylor JA, Kappel N, Yang CH, Heisel LL, Lucifer GW, Bell DA. Ethnic variation in the CYP2E1 gene: polymorphism analysis of 695 African-Americans, European-Americans and Taiwanese. Pharmacogenetics 4:185–192 (1994).

178. Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 60 Caucasians. J Pharmacol Exp Ther 270:414–423 (1994).

179. Nomiyama K, Nomiyama H. Metabolism of trichloroethylene in human. Sex difference in urinary excretion of trichloroacetic acid and trichloroethanol. Int Arch Arbeitsmed 28:37–48 (1971).

180. Hatto D, Silver K. Human interindividual variability — a major source of uncertainty in assessing risks for noncancer health effects. Risk Anal 14:421–431 (1994).